ROLE OF SULFUR IN IMPROVING MORPHOLOGICAL, PHYSIOLOGICAL AND YIELD PERFORMANCE OF RICE PLANT (*Oryza sativa* L.) UNDER SALT STRESS

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This is to certify that the thesis entitled **"ROLE OF SULFUR IN IMPROVING MORPHOLOGICAL, PHYSIOLOGICAL AND YIELD PERFORMANCE OF RICE PLANT (*Oryza sativa* L.) UNDER SALT STRESS"** submitted to the **DEPARTMENT OF AGRICULTURAL BOTANY**, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE** in **AGRICULTURAL BOTANY**, embodies the results of a piece of bona-fide research work carried out by **SWARNA SULTANA JENNI**, Registration No. **13-05253** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: June-2020
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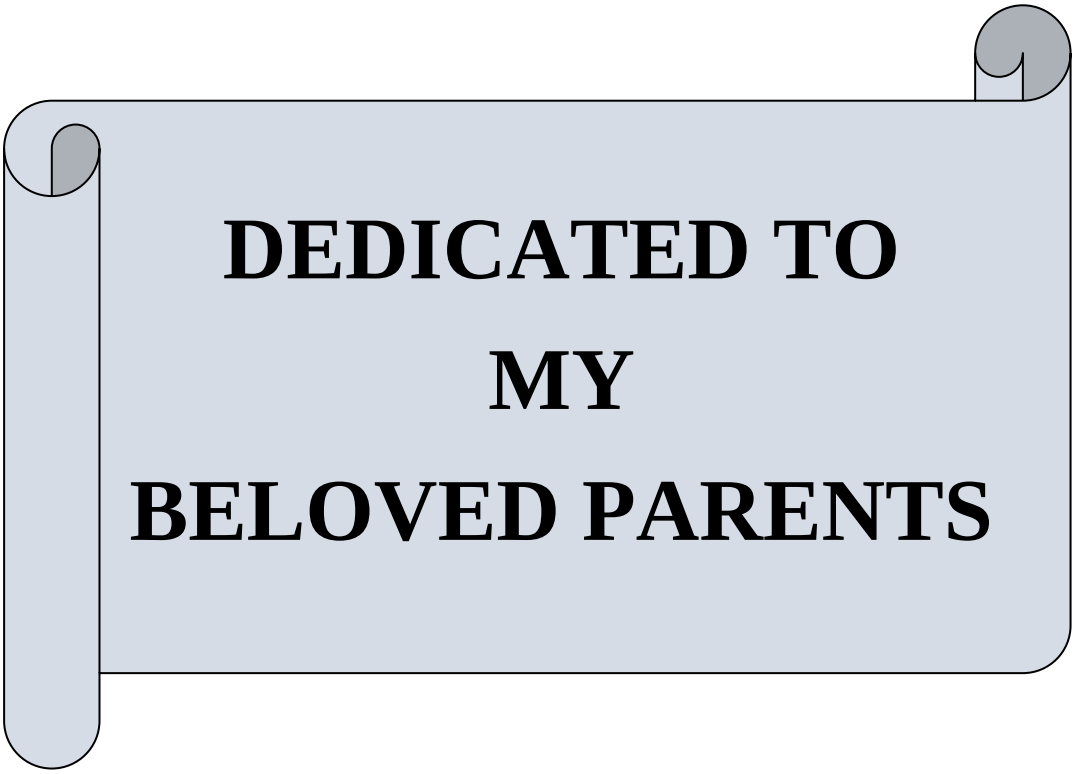
DEDICATED TO
MY
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ABSTRACT

Salt stress adversely affects the growth and development of rice plants. Sulfur plays diversified roles to regulate plant physiology. Sulfur application can be helpful in inducing salt tolerance in plants. An experiment was carried out at the net house and Plant Physiology Laboratory of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from November 2018 to May 2019 to study the effects of different salinity levels on the morphological, physiological and yield performance and the role of sulfur in improving the above mentioned traits of rice plant under salt stress. *Oryza sativa* L. cv. BRRI dhan67 plants were subjected to various levels of salinity (0, 8, 12 dSm^{-1}) and sulfur (0, 3, 6 Kg S ha^{-1}). In this experiment, the treatments consisted of three different salinity levels viz. N_0 = without salt (0 dSm^{-1}), N_1 = 8 dSm^{-1}, N_2 = 12 dSm^{-1}, and three different levels of sulfur viz. S_0 = 0 Kg S ha^{-1}, S_1 = 3 Kg S ha^{-1} and S_2 = 6 Kg S ha^{-1}. A randomized complete block design was followed as experimental design with three replications. Treatments were given at 15 days after transplanting (DAT). Data were recorded at different DAT following the standard procedure. The results showed that all the morphological (plant height, no. of tillers, leaf area), physiological (relative water content, K^+ content, chlorophyll content indicated by SPAD value, dry weights), yield attributes (panicle length, spikelet fertility, no. of effective and non-effective tiller, no. of filled and unfilled spikelets $panicle^{-1}$, 1000 grain wt.) and yield (grain and straw yield) parameters were reduced significantly ($P \leq 0.05$) under salt stress except membrane stability index (MSI%), Na^+ content and, no. of non-effective tillers, days to flowering, no. of unfilled spikelets $panicle^{-1}$. Under salt stress plants grown in control condition (N_0S_0) performed best whereas worst performance of all parameters was recorded from N_2S_0 treated plants. Supplementary sulfur fertilization (3, 6 Kg S ha^{-1}) improved all the morphological, physiological and yield contributing characters significantly with the best performance found in N_0S_2 treatment. Supplemental S treatment decreased the root and shoot Na^+ content and improved the K^+ content which reduced the toxic effects of salt stress. Supplemental S also improved the relative water content and leaf MSI% and chlorophyll content in salt affected rice plants. The combinations of salinity and sulfur significantly influenced almost all the morphological, physiological and yield contributing characters, compared to salt affected plants alone. In every case N_1S_1, N_1S_2 gave better result than N_1S_0 and N_2S_1, N_2S_2 gave better result than N_2S_0. In most of the parameters, S_2 treatment (6 Kg S ha^{-1}) showed better salinity mitigating potential than S_1 treatment (3 Kg S ha^{-1}) even at higher level of salt stress (N_2 treatment). Therefore, supplemental sulfur induced improvement of physiology, growth and developmental processes contributed to improve the grain yield which are the indications for improved salt tolerance in rice plants.

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
^{0}C	Degree Celsius
ABA	Abscisic Acid
AEZ	Agro-Ecological Zone
APX	Ascorbate Peroxidase
AsA	Ascorbate
BARI	Bangladesh Agriculture Research Institute
BAU	Bangladesh Agricultural University
BRRI	Bangladesh Rice Research Institute
Cm	Centimeter
Ca	Calcium
CAT	Catalase
CV%	Percentage of Coefficient of Variation
Cys	Cysteine
DAT	Days After Transplanting
DNA	Deoxyribonucleic Acid
DHAR	Dehydroascorbate Reductase
dSm^{-1}	DeciSiemens per metre
EC	Electrical Conductivity
e.g	As for example
et al.	and others
FAO	Food and Agriculture Orzanization
g/gm	Gram
GB	Glycine Betaine
Gly I	Glyoxalase I
Gly II	Glyoxalase II
GPX	Glutathione Peroxidase
GR	Glutathione Reductase
GST	Glutathione S-transferase
GSH	Glutathione
GSSG	Glutathione Disulfide
ha	Hectare
i.e.	that is
kg	Kilogram
Kg ha^{-1}	Kilogram per hectare
LSD	Least Significant Difference
L	Liter
M	Meter
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate Reductase
MDHA	Monodehydroascorbate

LIST OF ABBREVIATIONS (Cont'd)

MPa	Megapascal Pressure Unit
ml/L	Milliliter per Liter
MG	Methylglyoxal
Mg/L	Milligram per Liter
MoP	Muriate of Potash
N	Nitrogen
NaCl	Sodium Chlloride
NR	Nitrate Reductase
nm	Nano Meter
Ng	Nano Gram
P	Phosphorus
pH	Hydrogen ion concentration (Negative Logarithm)
PI	Panicle Initiation
Pn	Net Photosynthetic Rate
Pro	Proline
POD	Peroxidase
PS I/II	Photo System I/II
qP	Photochemical Quenching
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
RWC	Relative Water Content
ppm	Parts Per Million
S	Sulfur
SAU	Sher-e-Bangla Agricultural University
SOD	Superoxide Dismutase
TSP	Triple Super Phosphate
µg/kg	Microgram per kilogram
Zn	Zinc

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.), as one of the world's most important cereal crops, feeds about half of the world's population and constitutes 30% to 80% of the daily caloric requirements in Asia (Lafitte *et al*., 2004; Khush, 2005). More than 90 per cent of rice is grown in Asia. It is the main source of food for the people of Bangladesh. Rice, the dominant food crop of Bangladesh is covering about 75% of agricultural land and contributing 28% of GDP (Salam *et al*., 2019).

Global climate change is increasing gradually and Bangladesh is exceptionally defenseless around the effect of climate change. Due to the effects of climate change, salinity is increasing day by day and out of 2.86 million hectares of coastal and offshore areas, about 1.056 million hectares are affected by varying degrees of salinity that normally covers 30% of cultivable land (Khatun *et al*., 2019). In Bangladesh, rice is found to be one of the most widely grown crops in coastal lands inundated with sea water during high tidal period, though it is known as salt sensitive crop (Akbar *et al.*, 1972; Korbe and Abdel-Aal, 1974; Mori and Kinoshita, 1987). Among the various factors that limits rice yield, salinity is considered to be the oldest and the most serious environmental problems in the world (McWilliam, 1986). In fact, salinity is one of the most constraining natural variables for crop production around the world. In vulnerable regions, particularly in deltaic costal zones, its occurrence and severity are anticipated to increase by around 25% by 2050 (Dasgupta *et al*., 2014) where rice growing areas account for more than 65% of global production, making salinity one of the major threats to food security.

Salinity causes complex interactions among different morphological, physiological and biochemical processes in rice. Salt stress is dominated by sodium (Na^+) and chloride (cl^-) ions, affect plant growth and development through: 1. Low osmotic potential of soil solution (water stress), 2. Nutritional imbalance, 3. Specific ion effect (salt stress) or 4. A combination of these factors (Ashraf 1994). But at the preliminary level, two main phases can be described as the response of plants to salinity: firstly, the shoot ion-independent response occurs within minutes to days which is thought to be related to Na^+ sensing and signaling (Gilroy *et al*., 2014; Roy *et al*., 2014). During the initial phase, effects of salinity on water relations can be important, causing stomatal closure and the inhibition of leaf expansion (Munns and Termaat, 1986). It also causes various physiological changes, such as interruption of membranes, nutrient imbalance, differences in the antioxidant enzymes, decreased photosynthetic activity, and decrease in stomatal aperture and impairs the ability to detoxify reactive oxygen species (ROS). The problem is compounded by nutritional imbalance (especially K^+ deficiency because it inhibits K^+ uptake that causes leakage from the cells), mineral deficiencies (Zn, P) and toxicities (Fe, Al, organic acids) (Gregorio *et al*., 2002). Thus photosynthetic area become reduced and reduced photosynthesis with increasing salinity is attributed to either stomatal closure, leading to a reduction in

intracellular CO_2 partial pressure, or non-stomatal factors (Bethke and Drew, 1992). Salt stress also changes photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content (RWC). During long term exposure (days to weeks) to salinity, plants experience ionic stress which involve the build-up of ions in the shoot to toxic concentrations, particularly in old leaves, causing pre mature senescence of leaves and ultimately reduced yield or even plant demise (Munns and Tester, 2008a).

Rice plant responses to salt stress are obviously very complex in nature and depend on duration and type of salt stress, development stage of rice, day length, and other factors (Bernardo *et al*., 2000; Cramer *et al*., 2001). Salt stress decreased the ability of rice plants to uptake water and nutrients, promoted metabolic alterations and ultimately reduced growth rate (Munns, 2002). There are so many growth inhibiting effects of salt stress on rice plants and the outcome of this effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death (Mahajan and Tuteja 2005, Hasanuzzaman *et al*. 2012). Moreover, owing to high pH, nutrient deficiency is very common under salt stress and this deficiency limit productivity trends observed in rice growing countries (Zhu *et al*., 2004). According to FAO (2009), by 2050, 70% more food is required for 2.3 billion people in the world. So, it is urgent to increase food production in future especially rice production under salt stress (Heong and Hardy 2009).

There are various ways to mitigate salt stress damage and improving salt tolerance in plants. An economical way to overcome salt stress conditions is to supply the essential nutrients to the plant, so the plant can properly carry out the physiological and biochemical processes inside the plant cell and tolerate the damages caused by salinity. Many inorganic nutrients, plant hormones and osmoprotectants are used for this purpose (Epstein and Bloom, 2005). Among the macronutrients, sulfur not only plays essential roles in normal plant development but also useful in reversing the adverse effects of abiotic stress because of their free radicals scavenging property. Sulfur also improves the chemical properties of salt affected soils, like pH, electric conductivity (EC) and Sodium absorption rate (SAR) by decreasing their values and subsequently yield attribute of rice crop. Under salt stress, sulfur- containing metabolites such as, amino acids (cysteine and methionine), vitamins (biotin and thiamine), thioredoxin system, glutathione lipoic acid and glucosinolats have potential to promote or modify physiological and molecular processes in plants (Khan *et al*., 2014). Through glutathione, a sulfur metabolite, sulfur helps in improving nutrient assimilation and in stimulating the anti-oxidative defense system of plants (Gondent and Ullman, 2000). Sulfur supplementation in saline soils is helpful to counteract the uptake of toxic elements (Na^+ and Cl^-), which encourage K/Na selectivity and ability of calcium ion to decrease the harmful impacts of sodium ions in plants (Zaman *et al*., 2002). Sulfur is not only necessary for the formation of chlorophyll and aiding photosynthesis but also plays a vital role in the activation of enzymes, nucleic acids (Kaur *et al*., 2013). Thus, application of sulfur decreased the deleterious effect of salinity and had desirable effect on growth and

nutrient contents of rice. Moreover, sulfur fertilization is low cost and used as a soil amendment for enhancing the rice productivity (Helmy *et al*., 2013).

Considering the above mentioned points in view, the present study was undertaken with the following objectives-

- To evaluate the morphological, physiological, yield contributing characters of rice plant under salt stress
- To observe the role of sulfur in mitigating salinity induced injuries in rice crop
- To determine the effective dose of sulfur for mitigating salt stress in rice crop

CHAPTER II

REVIEW OF LITERATURE

2.1 Salt Stress: an overview

Stress is defined as any abiotic (temperature, drought, salinity, flooding, metal toxicity, ozone, UV-radiations, etc.) or biotic (herbivores) factors that affects the rate of photosynthesis and reduces the ability of plants to convert energy to biomass (Grime, 1977). Environmental stresses like extreme temperature, salt stress, drought, high wind, flood etc. have affected the production of agricultural crops, among these, salt stress is one of the most deleterious environmental stresses, causing reduction of cultivated area and crop productivity (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013).

Salt stress is an abiotic stress that represents the presence of soluble salts in excessive amount in the soil which adversely affects plant growth altering plant's normal physiological processes. Normally soils contain some water-soluble salts and plants absorb essential nutrients from these soluble salts, but excessive accumulation of salts negatively affects plant growth. According to FAO report (FAO 2009), salt affected soils have excessive amount of soluble salts like sodium (Na^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), and anions chloride (Cl^-), sulphate (SO_4^{2-}), bicarbonate (HCO_3^-) with exceptional amounts of potassium (K^+), carbonate (CO_3^{2-}), and nitrate (NO_3^-) (Bohn *et al*. 1985; Manchanda and Garg 2008). But mostly, the adverse effects of salt stress have been attributed to Na^+ and Cl^- ions in different plants, consequently these ions create critical conditions for plants through altering plant's own mechanisms. Although both Na^+ and Cl^- are the leading ions that generate physiological disorders in plants, Cl^- is the most dangerous (Tavakkoli *et al*. 2010). Saline soil is defined by USDA saline soil laboratory as soil that have an electrical conductivity of solution extracted from the water-saturated soil paste ECe (Electrical Conductivity of the extract) of 4 dS m^{-1} (Deci siemens per meter), where 4 dS m^{-1} or 40 mM NaCl or more with an osmotic pressure of approximately 0.2 MPa. However, the conductivity of saturated extract can vary provoking severe crop damage (Table 1). Normal soil having pH=4.5–7.5, electrical conductivity (EC)< 4 dS m^{-1}, exchangeable sodium percentage (ESP)<15, and sodium absorption ration (SAR)<15, are most favorable for nutrient availability and normal plant growth whereas saline soil has ESP<15, and SAR<15 (Bohn *et al*. 1985). The pH of saline soils generally ranges from 7-8.5 (Mengel *et al*. 2001). There is another category of soil affected by salt, which has EC less than 4 dS m^{-1}, ESP≥15, and SAR≥15 is defined as sodic soil (Bohn *et al*. 1985).

On the basis of nature, characteristics, and plant growth relationships in salt stressed soils, two main types of soils have been described by Szabolcs (1974). They are (a) saline soils—the soluble salts are mainly NaCl and Na_2SO_4 but sometimes also contain appreciable amounts of Cl^- and SO_4^- of Ca^{2+} and Mg^{2+}; these soils contain plenty of neutral soluble salts which pose adverse effects on growth of plants, and (b) sodic soils—these soils

contain Na^+ salts which is capable of alkaline hydrolysis, specially Na_2CO_3; previously these soils have also been termed as 'Alkali'. According to FAO, in the world more than 6% land is affected by salinity or sodicity, and more than 20% of irrigated land has become salt stressed (Pitman and Lauchli 2002; Munns 2005a).

Table 1. Soil salinity classes based on electrical conductivity (dSm^{-1}) and response of crops

Soil Salinity Class	Conductivity of saturated extract (dS m^{-1})	Effect on Crop Plants
Non-saline	0-2	Salinity effects negligible
Slightly saline	2-4	Yield of sensitive crops may be restricted
Moderately saline	4-8	Yield of many crops are restricted
Strongly saline	8-16	Only tolerant crops yield satisfactorily
Very strongly saline	>16	Only a few very tolerant crops yield satisfactorily

Source: Chabra (2004)

On the basis of source of salinization, salinity has been classified into primary and secondary salinity. The main source of primary salinity is the deposition of sand stones, alluviam in the arid and semi-arid lands, decay of rocks that releases variety of salts, intrusion of oceans into the coastal areas followed by evapo-transpiration and high tidal intrusion of sea water into rivers. Secondary salinity is caused by anthropogenic activities i.e. irrigation, poor quality of water usage, overgrazing by domestic animals, deforestation, contamination of river waters with industrial chemicals, intensive cropping etc. (Ashraf, 1994; Omami *et al*., 2006).

Plants can be classified into two groups based on the effect of salt stress on plant growth: Crop species sensitive to soil salinity are defined as glycophytes, whereas plants grown in water of high salinity or which can tolerate high salt concentrations are defined as halophytes. Most of the terrestrial plants including agricultural crops are glycophytes and cannot tolerate high concentration of salt (Tuteja *et al*, 2011).

2.2 Global Scenario of Salt Stress

Saline soils have gained global concern. The world population is increasing rapidly while the world's irrigated land is decreasing by 1-2% every year (FAO, 2004). Soil salinity is principal issue of irrigated areas. Irrigation is practiced on approximately 17% of the total

cultivated land which adds to 30% of global agricultural production (Hillel, 2000). Globally, salt affected area accounts for about 1125 million hectares, of which approximately 76 million hectares of land are affected by human induced salinization and sodification (Wicke *et al*., 2011). More than 45 million hectares of irrigated land which account to one fifth of total land have been affected by salt worldwide and 1.5 million hectares are becoming unsuitable for production every year due to high levels of salinity in the soil (Pitman and Läuchli 2002; Munns and Tester 2008a). According to another estimate, about 50% of the arable land will be prone to damages by salt stress up to the middle of the twenty-first century (Manchanda and Garg, 2008). However, the statistics varies depending on different sources. According to the FAO Land and Nutrition Management Service (2008), 6.5% of the total land in the world is salt affected (either salinity or sodicity) which accounts for approximately 831 million hectares of land (Appendix I).

The countries where salt affected soils exists include but not limited to Australia, Bangladesh, China, India, Egypt, Iran, Iraq, Mexico, Pakistan, the former USSR, Syria, Turkey and the United States (Hossain, 2019). The global distribution of salt affected land area is shown in Appendix II.

The dried areas with high temperature and evapo-transpiration but little rainfall, are facing the more adverse salinity problem (Neto *et al*., 2006). The use of low-quality water and poor soil management practices is further increasing the problem of salinity throughout the world (Misra *et al*., 1997; Pitman and Lauchli, 2002).

2.3 Scenario of Salt Stress in Bangladesh

Bangladesh is predominately an agricultural country and thus, agriculture is an important economic sector in Bangladesh. Bangladesh comprises of 147, 570 km^2 of total geographical area out of which the coastal area covers about 20% of the country and over 30% of the net cultivable area. A part of the coastal area is a reserve natural mangrove forest, the Sundarbans, which covers about 4,500 km^2 area and the remaining area is used in agriculture. The cultivable lands in coastal areas are affected with different degrees of salinity (Petersen & Shireen, 2001). Distribution of areas with varying degrees of salinity is shown on Soil Salinity map, 2009 (Appendix III).

Out of 1.689 million hectares of coastal area, about 1.056 million hectares are affected by varying degrees of soil salinity. More precisely about 0.328, 0.274, 0.189, 0.161 and 0.101 million hectares of land are affected by very slight, slight, moderate, strong and very strong salinity respectively and agricultural land use in these areas is very poor. A comparative study of the salt affected land during the last four decades (1973-2009) showed that about 0.223 million ha (26.7%) new land was salt affected. Along with 19 costal districts some of the new area of Satkhira, Patuakhali, Borguna, Barisal, Jhalakathi, Pirojpur, Jessore, Narail, Gopalganj and Madaripur districts are affected by varying degrees of soil salinity, which suppresses agricultural productivity remarkably (SRDI 2010). About 50% of the

coastal areas face varying degrees of inundation, which limits effective use of these areas. Due to climate change this situation may become worse (Islam, 2006). In accordance to the above facts and keeping in mind the present alarming scenario, mitigation of salt stress is definitely an urgent need of the hour.

2.4 Mechanism of Salt Stress on Plants

Three potential mechanisms of salt stress on plants are osmotic stress due to low water potential, specific ion effect by sodium and/or chloride, or nutritional imbalance due to interference with the uptake and transport of essential nutrients (Flowers and Flowers, 2005).

2.4.1 Osmotic Effect

Due to salt stress the osmotic pressure in the soil solution exceeds the osmotic pressure in the plant cells (Hasanuzzaman *et al*., 2012; Munns *et al*.,2006). In osmotic or water deficit condition, soluble salts reduce the water potential making water unavailable for plant's uptake as well as plant also loses its ability to uptake minerals, especially the uptake of K^+ and Ca^{2+} (Hasanuzzaman *et al*., 2012; Nawaz *et al.,* 2010). Germination process is affected by salt stress as it alters the imbibition of water by seeds due to lower osmotic potential of germination media (Khan and Weber 2008). Root and shoot growth are also disturbed because of water stress than salt specific effect during the initial stage of salt stress (Munns, 2002). At moderate osmotic stress though root growth is not much affected but shoot growth reduction is maximum (Hsiao and Xu, 2000). As rapidly growing cells have the ability to store salts in their expanding vacuoles, so the growth of the new leaves is not hampered due to accumulation of salts in the cytoplasm (Munns, 2005b). Osmotic stress in the early stage of salt stress causes different physiological changes, such as interruption of membranes, nutritional imbalance, differences in the antioxidant enzymes, decreased photosynthetic activity, and decrease in stomatal aperture and weaken the ability of plants to detoxify reactive oxygen species (ROS) (Cuin *et al*.,2008; Munns and Tester,2008b; Parihar *et al*.,2015). Due to variation of different plant species, stage of stress, types of cells and tissues, damage due to osmotic effect may vary (Munns *et al*., 2000).

2.4.2 Specific Ion Effect

The presence of excessive soluble salts like sodium and chloride in the soil competes with the uptake of mineral nutrient that are essential to plants. But for proper growth and development appropriate ion ratios is essential (Wang *et al*. 2003). However, the uptake of excessive salts causes specific ion toxicities like Na^+ or Cl^- which also decrease the uptake of essential nutrients like potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and manganese (Mn^{2+}) whereas chloride (Cl^-) ions limits the absorption of nitrate (NO^{3-}), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) ions (Zhu, 2001). The accumulation of toxic ions like Na^+ perturb the plant's ability to uptake k^+ and disturbs stomatal regulation causing water loss and necrosis. Besides this, Na^+ appears to accumulate to toxic levels before Cl^-

most of the cases. Though Cl^- is a co-factor in photosynthesis and required for the regulation of some enzyme activities and pH but it is toxic to plants at high concentrations. Higher concentration of Cl^- inhibits photosynthesis as well as causes significant reduction in growth and water use efficiency in plants (Flowers and Yeo, 1988; Xu *et al*. 2000). High concentrations of sodium and chloride ions inside leaf sap, causes the reduction of root and shoot fresh weight up to 50% (Parveen and Qureshi, 1992). Most of the higher plants especially agricultural crops are highly susceptible to ionic toxicity stress (Abrol *et al*., 1988). When the salt concentration become higher it is accumulated in the older leaves but not inside the vacuoles rather in the cytoplasm and affect the normal enzyme action. When salt is accumulated in the cell wall, cell dehydration occurs (Munns, 2005a). To overcome specific ion effect, plants employ some mechanisms like restriction of the salt entry in their bodies, control of long-distance transport of salt, compartmentalization of salt, extrusion of salt from the plant, and prioritization of the maintenance of K^+: Na^+ ratio in the cytosol (Zhu 2001; Parida *et al.,* 2005).

2.4.3 Nutritional Imbalance

In salt stressed plants water relation is disturbed resulting limited uptake and utilization of important nutrients which affects metabolic activities of the cell and enzyme function (Lacerda *et al.,* 2003). Interaction of nutrients and salts cause deficiencies and imbalances of the important nutrients (McCue and Hanson, 1990). Under salt stress due to interaction between Na^+ and NH_4^+ and/or between Cl^- and NO^{3-} uptake of N reduced resulting in reduced growth and yield of crops (Rozeff 1995). With the increase of salinity, the phosphate availability was reduced in saline soils due to (a) ionic strength effects that reduced the activity of PO_4^{3-}, (b) phosphate concentrations in soil solution was tightly controlled by sorption processes and (c) low solubility of Ca-P minerals thus phosphate concentration in crops also decreased (Qadir and Schubert, 2002). Excessive uptake of Na^+ causes reduction in the uptake of both K^+ and Ca^{2+} concentrations in plant tissues of many plant species (Hu and Schmidhalter 1997, 2005; Asch *et al*., 2000). K^+ along with Ca^{2+} are essential for the maintenance of integrity and functioning of the cell membranes (Wenxue *et al.,* 2003). It has been reported that, Mg^{2+} concentrations of all plant organs decreased due to external NaCl salinity (Hussin *et al*., 2013). In saline soils micronutrients availability dependents on the solubility of micronutrients, the pH of soil solution, redox potential of the soil solution, and the nature of binding sites on the organic and inorganic particle surfaces. Micronutrient concentrations in plants can also vary due to different crop species and salinity levels (Oertli 1991). As the pH of saline soils is high micronutrient deficiencies are very common here (Zhu *et al*., 2004).

2.4.4 Oxidative Stress

Along with the direct impact of salt stress on plants, a major effect of salinity is induction of excessive reactive oxygen species (ROS) i.e., superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH*) and singlet oxygen ($^1O_2$) production which is highly reactive and causes peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage, and/or interact with other vital constituents of plant cells (Parida and Das

2005 ; Ahmad and Sharma 2008 ; Ahmad *et al*. 2010a, 2011). Reduction of oxygen causes generation of these reactive oxygen species (ROS) which alters plant metabolic routes (Asada, 1999). Salt stress can inhibit the stomata opening and decrease the CO_2 availability for photosynthesis in the leaves which inhibits carbon fixation, exposing chloroplasts to excessive excitation energy which actually increase the generation of reactive oxygen species (ROS) and this ROS promotes the photoinhibation and photooxidation in plant cells (Parida and Das 2005; Ahmad and Sharma 2008; Ashraf, 2009; Ahmad *et al*., 2010a, 2011). Osmotic effects due to salt stress creates water deficit condition that leads to the formation of ROS (Halliwell and Gutteridge 1985; Elstner, 1987). Hasegawa *et al*. (2000) observed that ROS production is increased under salt stress in many plants. ROS-mediated membrane damage is a major cause of the cellular toxicity by salinity in many crop plants such as rice, tomato, citrus, pea and mustard (Gueta-Dahan *et al*., 1997; Dionisio-Sese and Tobita 1998; Mittova *et al*., 2004; Ahmad *et al*., 2009, 2010b). Therefore, regulation of ROS is a very important process to avoid unwanted cellular toxicity and oxidative damage (Halliwell and Gutteridge, 1989).

2.5 Effects of Salt Stress on Plant Growth

Salt stress not only decreases the agricultural production of most crop plants, but also affects soil physicochemical properties, and ecological balance of the area (Hu and Schmidhalter, 2002). As salt stress is complex, salinity effects are the results of interactions among morpho-physiological, and biochemical processes including seed germination, plant growth etc. (Akbarimoghaddam *et al*., 2011; Singh and Chatrath, 2001). These effects of salt stress on plant growth are discussed under separate headings.

2.5.1 Germination Stage

Seed germination is the most important stage for successful establishment of healthy seedlings that determine the vigorous growth and yield of the crops. Salt stress has its detrimental effects on both glycophytes and halophytes especially at germination stage (Sosa *et al*., 2005). In fact, germination stage is more sensitive to salt stress than other vegetative stages (Khan and Weber, 2008). As seeds remain in close association to the soil surface, they are more susceptible to salinity (Dodd and Donovan, 1999). Higher level of salinity retards the germination of seeds while lower level of salt stress induces seed dormancy (Khan and Weber, 2008). Salt stress disturbs the germination process in several ways. It lowers the osmotic potential of soil that alters the imbibition of water by seeds posing toxic effects to the developing embryo, resulting in delayed germination process (Khan and Ungar, 1984; Khan and Weber 2008). Salinity causes ionic toxicity that alters the activity of enzymes involved in nucleic acid metabolism (Gomes-Filho *et al*., 2008). Other impacts of soil salinity on seed germination includes change in protein metabolism (Rasheed, 2009), hormonal imbalance (Khan and Rizvi, 1994) and lower utilization of seed reserves (Promila and Kumar 2000; Othman *et al*. 2006). But there are also a number of internal (plant) and external (environmental) factors which affect the germination of seeds

under salt stress such as nature of seed coat, seed dormancy, seed age, seed polymorphism, seedling vigor, temperature, light, water and gasses (Wahid *et al*., 2011). However, there is always decreasing rate of germination with increasing trend of salt stress. The average time of seed germination varies considerably on strength of salt stress and genotypes (Ditommaso, 2004).

Based on the available literature it is now well established that salinity has its deleterious effect on the seed germination of various crop plants like *Oryza sativa* (Xu *et al*., 2011) , *Triticum aestivum* (Akbarimoghaddam *et al*., 2011), *Zea mays* (Carpıcı *et al*., 2009; Khodarahmpour *et al*., 2012), *Brassica spp*. (Ibrar *et al*., 2003; Ulfat *et al*., 2007), *Glycine max* (Essa, 2002) , *Vigna spp*., (Jabeen *et al*., 2003) *Helianthus annuus* (Mutlu and Buzcuk, 2007) and *Posidonia oceanica* (L.) (Fernández-Torquemada and Sánchez-Lizaso, 2013).

In *Oryza sativa*, germination percentage and the time required to reach a same level of germination was negatively affected by the increase of NaCl concentration (Fogliatto *et al*., 2019). Rahman *et al*. (2001) reported among various concentration of salt (0-3.0% NaCl) salinities up to 0.3% delayed germination but did not reduce final germination percentage; it was reduced significantly at 1.0% NaCl. Rice cultivars at the stage of seed germination were more tolerant to salinity than at the early seedling stage. A significant reduction in germination rate was observed when exposed to various concentration of salt (30–150 mM) and among four cultivars the sensitive cultivars were more prone to germination reduction under salt stress (Hasanuzzaman *et al*. 2009). Narale *et al*. (1969) found rice seeds could germinate normally in medium of ECe up to 4.5 mmho/cm began to be adversely affected from ECe of 8.9 mmoh/cm. Salt stress negatively affect the vigor index by raising salt concentration in the growing medium (Djanaguiraman *et al*., 2003). According to Farooq *et al*. (2006) salts toxicity on rice seedlings and time required for 50% germination are reduced if seedlings are treated with ethanol treatment.

Carpici *et al*. (2009) reported due of salt stress there was reduction in germination index of maize cultivars (*Zea mays*). Salinity (240 mM NaCl) caused 32% reduction in germination rate, 80% in length of radicle, 78% in plumule length, 78% in seedling length and 95% in seed vigor of *Zea mays* (Khodarahmpour *et al*., 2012). Gremination of *Hordeum secalinum* was progressively retarded and decreased with an increase in NaCl concentration, where 10-day treatment with 400 and 500 mM NaCl caused 40% and 38% reductions in germination rate, respectively (Lombardi and Lupi, 2006).

In chickpea, salinity exerts very pronounced effect on the germination index and seed size (Kaya *et al*., 2008). Nahar and Hasanuzzaman (2009) reported that germination percentage of *Vigna radiata* was decreased up to 55% when irrigated with 250 mM NaCl. In *Brassica napus* germination percentage significantly reduced at 150 and 200 mM NaCl. With increasing concentration of salinity levels germination rate was decreased. Along with this germination percentage and germination speed were also decreased by 38% and 33%, respectively at 200 mM NaCl (Bordi, 2010).

The germination percentage and seeding growth of 20 wheat (*Triticum aestivum* L.) genotypes declined under salinity stress (Singh *et al*., 2000). Akbari *et al*. (2007) reported that increasing concentrations of NaCl reduced germination percentage, radicle length, hypocotyl length, seedling fresh and dry weights, radicle and hypocotyl dry weight of *Triticum aestivum*. An increase in NaCl concentrations (0-12.5 dSm^{-1}) progressively delayed and decreased germination in six bread wheat cultivars. Increasing salt concentrations often cause osmotic and/or specific ion toxicity which may reduce germination percentage (Akbarimoghaddam *et al*., 2011).

In *Solanum lycopersicum* a significantly negative correlation between salt stress and germination rate and percentage was found that caused delayed germination and reduced germination percentage (Kaveh *et al*., 2011). Foolad and Lin (1997, 1998) observed that high concentrations of salt (150 mM NaCl) in the germination media significantly delayed onset and reduced the germination rate of *Solanum lycopersicum*. According to Cuartero and Fernandez-Munoz (1999) seeds of *Solanum lycopersicum* need 50% more days to germinate at 80 mM NaCl and about 100% more days at 190 mM NaCl than control.

Small sized seeds normally show high value of germination index as compared to large size seeds under salt stress. Salt stress delays the time to get 50% germination in citrus (Zerki, 1993). Germination of soybean (*Glycine max* L.) decreased at NaCl concentrations of 330 mMolal (81% germination) and above. At 420 mMolal NaCl, only 40% of seeds germinated, and at 500 mMolal NaCl there was no germination (Hosseini *et al*., 2002).

2.5.2 Plant Morphology

After seed germination, next stage of plant growth is crop establishment. Salt stress hinders crop establishment through reducing shoot growth, blocking leaf development and expansion, reducing growth of internodes and promoting leaf abscission (Ziska *et al*., 1990; Zekri, 1991). Salt stress negatively affects plant morphology in several ways. However, these modifications vary considerably on different cultivars, intensity and duration of salinity (Khan *et al*., 2003; Munns and James, 2003).

In *Oryza sativa,* reduction in plant height, tiller number and leaf area index were observed in saline soil by Hasanuzzaman *et al*. (2009). Salt stress decreased biomass and leaf area in rice (Ashraf and Bhatti, 2000). Salt stress increased the number of sterile florets and viability of pollen with an increase in salinity and seed set was also reduced by 38% when female plants were grown in as low as 10 mM NaCl (Khatun and Flowers, 1995). With the increase of salt stress (3, 6, 9, 12 and 15 dSm^{-1}) there was reduction in number of leaves (Islam, 2004a). Similarly, Khan *et al*. (1997) reported that there was a significant reduction in leaf number and leaf area under salt stress in rice.

While studying morphological attributes of Tomato, (cv. riogrande) Mohammad *et al*. (1998) observed that increasing salt stress (0, 50, 100 and 150 mM NaCl) was accompanied by significant reductions in shoot weight, plant height, number of leaves per plant.

Akbarimoghaddam *et al*. (2011) reported that increase of NaCl concentrations adversely affected shoot dry weight, shoot dry weight of six bread wheat cultivars (*Triticum aestivum* L). Another study on wheat revealed that increasing levels of chloride (0-12 dSm^{-1}) and sulfate salinity decreased leaf number (Angrish *et al*., 2001).

Salt stress significantly decreased shoot and root weight, total biomass, plant height and leaf number but leaf area was not affected in *Glycine max* (Dolatabadian *et al*., 2011). Growth and leaf area expansion of sugar beet was reduced even at very low NaCl concentration (Terry and Waldren, 1984).

While studying the morphogenetic parameters of germplasm of 15 guar ecotypes under varying degrees of salinity Ashraf *et al*. (2002) reported that salinity poses a significant reduction in various vegetative parameters. The toxic effects were observed like burning of the leaves (Plate VI), chlorosis, reduction in the leaf area and the necrosis in different plants and the salt affected plants have dark green, thicker and succulent leaves.

Evlagon *et al*. (1992) observed that leaf bio-mass of stem was reduced due to salt stress. Leaf area, root and shoot dry weights were also reduced with increasing salt levels (Ashrafuzzaman *et al*., 2002). While studying the interactive effects of salinity, calcium and potassium on physio-morphological traits of sorghum (*Sorghum biclolor* L.) in a green-house experiment it was observed that salinity substantially reduced the plant growth as reflected by a decrease in the plant height, shoot and root weight (Jafari, 2009).

In Coriander (*Coriandrum sativum* L.), plant height, number of leaves, roots number and length were reduced with the increase of NaCl concentration and Coriander plants were found to resist salinity up to the concentration of 3000 ppm NaCl only (Alaa El-Din Sayed Ewase, 2013).

In *Suaeda salsa*, a significant reduction in the dry biomass was observed when exposed to different concentration of NaCl under different water regimes (Liu *et al*., 2008). Salt stress i.e., increased content of Na^+ and Cl^- significantly decreased plant height, number of branches, length of branches and diameter of shoot of *Suaeda salsa.* Salt stress increases plant's osmotic effect, ionic toxicity and nutritional imbalance; these are identified as most important causes of reduction in crop growth that ultimately lead to crop failure (Guan *et al*., 2011). Besides this, to cope with osmotic stress, plants reduce the leaf area and increase the rooting density also (Guo *et al*., 2002; Han and Wang, 2005).

The number of branches of nine *Brassica juncea* varieties along with one *Brassica carinata* variety decreased gradually with the increase of salt level (Uddin *et al*., 2005). Another study revealed that higher NaCl concentration affected the morphological characters of *Brassica* species and leaf number as well as leaf area decreased significantly at 75 mM NaCl (Javaid *et al*., (2002). Sesame grown under 9 dSm^{-1} salinity produced lower number of leaves per plant compared to control (Chakraborti and Basu, 2001).

Under salt stress growth was adversely affected by increasing salinity but leaf number and leaf area were mostly affected (72%) followed by plant height (67%) in case of sunflower

(El-Midaoui *et al*., 1999). Increased salinity is found to affect root, stem and shoot developments, fresh wt. & dry wt. of stem and root; leaf area and number (Sixto *et al*., 2005).

Increase of salt concentration inside the plant causes accumulation of salts inside the older leaves that ultimately causes the death of the leaves (Munns, 2002). The death of the older leaves reduces the capacity of plants to supply the carbohydrate to the younger leaves (Munns *et al*., 2006) which is marked by the appearance of some specific symptoms of plant damage in the leaves like color change, tip burn, marginal necrosis and succulence (Munns and Tester, 2008a).

2.5.3 Plant Anatomy

Salt stress has pronounced effect on anatomical characters of crop plants. Under salt stress plants adopt various strategies to deal with the problem. Generally, plants grown in salt stress conditions have more thickness of leaves, epidermis, cell walls and cuticles (Waisel, 1991). Increase in salinity increases mesophyll cell layers and cell size, may be because of more extension in cell wall at high turgor pressure (Munns and Termaat, 1986; Zekri and Parsons, 1990). Though salt stress reduces number of cells per leaf, it increases leaf thickness and the density of stomata at lower side of leaves with increased palisade tissues (Raafat *et al*., 1991; Hussein *et al*., 2012). Cavisoglu *et al*., 2007 reported that salt stress reduces the number of stomata on the surface of epidermis. Total leaf area and leaf plastochron index also reduced at salt stress (Awang *et al*., 1993; Bray and Reid, 2002). Salt stress is recognized to stimulate suberization of the root hypodermis and endodermis (Kozlowski, 1997). Length of vascular, xylem rows, number of vessels have been reported to decline due to salt stress (Hussein *et al*., 2012). Plants have large in number but narrow xylem vessels in salt stress condition than in normal condition (Walker *et al*., 1985).

Stem diameter was reported to be reduced in rice while trichome and stomata density increased under salt stress (Pimmongkol *et al*., 2002). In wheat, cortical and pith region was found to be decreased under salinity (Akram *et al*., 2002). The reduction of xylem vessel diameter was reported under saline conditions in cotton and tomato plants (Strogonov, 1962) and in wild barley (Huang and Redmann, 1995). Hameed *et al*. (2009) reported that gradually decrease in vascular bundles area, metaxylem area and phloem area observed with increasing salinity level in the growth media. In another study, the average area of xylem (protoxylem + metaxylem) within a leaf was reduced by 55% in *Imperata cylindrica* under saline conditions (Hu and Schmidhalter, 2001). Rashid *et al*. (2004) reported that salt stress lowered the xylem development and width of vascular bundle in mungbean.

A reduction in thickness and area of mesophyll tissue around the axis of leaves along with leaf rolling was observed by increasing salinity in kallar grass. High levels of salinity 200 or 300 mM increased the number of stomata, decreasing in stomata area and mesophyll area (Ola *et al*., 2012). Bulliform cells have a prominent role in leaf rolling to avoid water loss during drought stress (Alvarez *et al*., 2008) but in high salinity, well-developed

bulliform cells are found in *Deschampsia antarctica* plants (Gielwanowska *et al*., 2005). Salinity increased volume density of the palisade mesophyll but intercellular spaces and abaxial epidermis was reduced. In *Phaseolus vulgaris*, salt stress increased the numbers of epidermal and palisade cells per unit area and the stomatal density of the abaxial epidermis but reduced the numbers of cells per leaf (Bray and Reid, 2002).

Salinity is recognized to play a vital role to reduce cell size, epidermal thickness of leaves, apical meristem, diameter of cortex and central cylinder (Javed *et al*., 2001). Salt stress caused prominent thickening of endodermis and exodermis (Gomes *et al*., 2011) and increased development of sclerenchymatous tissues (Javed *et al*., 2001). In *Vracbiaria decumbens* lignification of intercellular spaces in exodermis decumbens was observed (Degenhardt and Gimmler, 2000; Gomes *et al*., 2011).

2.5.4 Plant Physiology

In salt stress condition, reduction in crop productivity is usually interlinked with a number of biochemical, physiological, and molecular characteristics (Shahbaz *et al*., 2008, 2011, 2012; Akram *et al*., 2009; Ashraf, 2009; Kanwal *et al*., 2011; Perveen *et al*., 2011). Salt stress has its devastating effects on different physio-biochemical attributes like protein synthesis (Ashraf *et al*., 2010), phytohormone regulation (Ashraf *et al*., 2010), respiration (Moud & Maghsoudi, 2008), photosynthetic capacity (Saleem *et al*., 2011), efficiency of photosystems (Perveen *et al*., 2011), stomatal regulation (Saleem *et al*., 2011, 2012), water relations (Akram *et al*., 2012), activities of enzymatic antioxidants, and levels of nonenzymatic compound (Ashraf, 2009) as well as inorganic nutrition (Flowers *et al*., 2010; Akram *et al*., 2011). Reactive oxygen species (ROS) are generated in plants in response to salt stress are one of the key secondary effects of salt stress on plants (Dat *et al*., 2000; Ashraf, 2009; Akram *et al*., 2011). These reactive oxygen species (ROS) damages cellular ultrastructure and organic compounds as well as impair different metabolic reactions (Asada 1997; Ashraf 2009).

Almost all metabolic activities within the cell are dependent on the availability of sufficient amount of water but excessive salt concentration in root zone of plant causes change in plant water relations. Salt stress is found to cause a significant decrease in relative water content (RWC) in sugar beet varieties (Ghoulam *et al*., 2002). To cope with the stress, the osmotic potential decreases (Ashraf *et al*., 2011; Kaymakanova and Stoeva, 2008) which causes reduction in water uptake by the plant reducing turgor in plant cells. It also reduces cell division and regulates stomatal aperture which ultimately leads to low photosynthesis and finally death of plant tissues (Marschner, 1995; Munns, 2002). Reduced turgor pressure causes stomatal closure reducing gaseous exchange through transpiration (Munns and Tester, 2008a). In sunflower, Akram *et al*. (2012) recorded a significant decrease in osmotic and water potentials under salt stress. Similarly, Noreen *et al*. (2010a) reported reduction in osmotic potential in various pea cultivars and suggested that this reduction in leaf osmotic potential in pea cultivars might be due to loss of water or an increased uptake of dissolved solutes (Munns and Tester 2008a; Noreen et al. 2010a, 2010b).

Other physiological processes under salt stress include changes in membrane permeability leading to destabilization of membrane proteins (Gupta *et al*., 2002; Grattan and Grieve, 1992) and reduction in the process of photosynthesis (Sayed, 2003; Ashraf and Shahbaz, 2003). Salt stress cause damage to thylakoid membrane, the site where all different types of photosynthetic pigments are accumulated. The available literature revealed that there is a close association between photosynthetic pigments (mainly chlorophyll a and b) and rate of photosynthesis in most plants subjected to salt stress. For example, salt-induced reduction in chlorophyll a and b has been observed in turnip (Noreen *et al*., 2010b), sunflower (Akram & Ashraf, 2011), pea (Noreen *et al*. 2010a), radish (Noreen *et al*. 2012), and safflower (Siddiqi *et al*. 2009). In safflower, a positive correlation between chlorophyll a and b and net photosynthetic rate was observed suggesting that reduction in net photosynthetic rate might be partly due to decrease in chlorophyll contents (Siddiqi *et al*. 2009). However, there are also some other factors that reduced photosynthetic rates under salt stress such as enhanced senescence, changes in enzyme activity, induced by alterations in cytoplasmic structure and negative feedback by reduced sink activity (Iyengar and Reddy 1996).

Nutritional imbalance is one of the most important responses of plants to salt stress. Salt-stress induced increase in tissue Na^+ and decrease in K^+ is a very common phenomenon in most of the crop plants (Akram *et al*., 2011). High accumulation of osmoprotectants, especially of proline and GB, is also a common feature of most plants under salt stress as they can scavenge free radicals (Akram *et al*. 2007; Banu *et al*., 2010). Salt stress also causes oxidative stress in most crop plants, though it is considered as one of the secondary effects of salt stress on plants (Ashraf, 2009). Akram *et al*. (2012) reported that in sunflower, salinity induced high accumulation of leaf H_2O_2 and MDA, and improved the activities of CAT, POD, and SOD which are considered to be the key enzymes to play an effective role in plants' oxidative defense mechanism. However, to mitigate salt stress plants employ different strategies. If these strategies fail to cope with the increased salinity, the plant may exhibit programmed cell death (PCD) as the last effort (Greenberg, 1996). Programmed cell death (PCD) is a physiologically and genetically controlled process where plants sacrifice cells under stress condition may be to prevent uncontrolled death and the release of toxins to protect and keep other cells growing (Fomicheva *et al*., 2012).

2.5.5 Yield

The above mentioned effects of salt stress ultimately lead to the reduction of crop yield. As discussed above, salt stress affects various physiological growth parameters and as a result of change in normal plant metabolism there is a reduction in yield (Reddy and Vora, 1986). But yield loss varies greatly depending on various salinity levels and the degree of tolerance (Mass 1986). Salt stress caused reduction in the growth and yield of barley by 65% and increased ash content (Isla *et al*., 1998). Reduction of grain yield observed in *Oryza sativa* varieties. An application of 150 mM salinity reduced grain yield 50%, 38%, 44% and 36% over control for the cultivars BR11, BRRI dhan41, BRRI dhan44 and BRRI dhan46, respectively (Hasanuzzaman *et al*., 2009). In different wheat cultivars, grain yield

decreased 69% and straw yield decreased by 64% with increase of salinity in the root zone (Khan *et al*., 1999). Different yield components like number of pods per plant, seeds per pod and seed weight of *Vigna radiata* were severely affected by salt stress. In different cultivars of *Vigna radiata* yield loss was observed 77%, 73% and 66% for BARI mung-2, BARI mung-5 and BARI mung-6, respectively with 250 mM NaCl salinity over control (Nahar and Hasanuzzaman, 2009). Ahmad *et al*., (1995) reported that salt stress significantly reduced the dry matter content and seed cotton yield. High salinity stress reduced the number of grains at 31% in barley and 22% in wheat, grain size of both crops also reduced which ultimately caused yield reduction (Harris *et al*., 2010). Semiz *et al*. (2012) observed that yield of *Foeniculum vulgare* was affected significantly by increasing irrigation water salinities. In rice, grain yield reduction due to salt stress is also observed by Linghe and Shannon (2000) and Gain *et al*. (2004). In 200 mM NaCl, a salt-tolerant species like sugar beet might have a reduction of only 20% in dry weight, a moderately tolerant species like cotton might have a 60% reduction, and a sensitive species like soybean might be dead (Greenway and Munns, 1980). Hamayun *et al*. (2010) reported that 1000 seed weight and yield of soybean significantly decreased in response 70 mM and 140 mM concentrations of NaCl. 15 dSm^{-1} salinity is found to decrease forage dry yield 11.33 g plant^{-1} than control in Sorghum (Saberi *et al*., 2011). Salt stress poses a severe problem in vegetative and reproductive stage in crop plants (Kafi and Goldam, 2000).

2.6 Rice, the Major Food Crop

Rice is the major food for about 156 million people of Bangladesh. It is a member of the genus *Oryza* in the family Poaceae and the *Oryza* genus has many species, of which two diploid species - *Oryza sativa* L. and *Oryza glaberrima* L. are cultivated. In Asia, *Oryza sativa* is most commonly cultivated (Vaughan *et al*., 2008). The basic chromosome number of rice is n=12 and there is both diploid (2n=24) and tetraploid species (4n=48) (Brar and Khush, 2003). As the agroclimatic conditions of the country are favorable for year-round rice cultivation, Bangladesh has a long history of growing rice. That's why rice is cultivated throughout the country except in the southeastern hilly areas (Shelley *et al*., 2016). Rice covers a global area of 162.62 million hectares of land producing about 499.37 million tons of crop. This crop is being cultivated across an area of 11.77 million ha yielding about 34.91 million tones in Bangladesh (USDA, 2020). However, the national average rice yield is much lower than that of other rice-growing countries of the world.

Rice, considered as a staple food across major countries of the world, feeds more than half of the world's population (IRRI 2006). The population growth rate is approximately 2 million per year, and if the population growth rate remains same, the total population will reach 238 million by 2050 (Shelley *et al*., 2016). With the expanding population, the increase in rice production is very important in order to keep in accord to the national food requirement. Due to increased population total cultivable area is decreasing at a rate of more than 1% per year because of construction of houses, roads, industries, etc. Moreover because of climate change like drought, flood, salt stress, extreme temperature stress,

agriculture is facing different kinds of adverse conditions and among them salt stress is the most important one. Rice is very salt sensitive cereal crop with a threshold of 3 dSm^{-1} for most of the cultivated varieties (USDA, 2016), whereas, a soil is considered salt affected only if it has an ECe (electrical conductivity of its saturation extract) above 4 dSm^{-1} (Rengasamy, 2006). But in case of rice even at ECe as low as 3.5 dSm^{-1}, it loses about 10% of its yield whereas at ECe 7.2 dSm^{-1}, about 50% yield loss was recorded (Umali, 1993). However, soil salinity is a natural phenomenon occurring near sea shores due to sea water flooding. As the coastal area covers about 20% of the country, which is about 30% of the net cultivable area (Haque, 2006), therefore, it is crucial to mitigate salt stress to enable this staple crop to provide enough food for rice-consuming communities of the world.

2.7 Effects of Salt Stress on Rice Plant

Rice is economically and socially dominant over all other crops in Bangladesh. But it is susceptible to salinity as compared to other main cereal crops (Joseph *et al*., 2010). The available literature revealed that rice is sensitive to salinity, especially at the early vegetative and later reproductive stages (Mass and Hoffman, 1977). The effects of salt stress on the growth and yield of rice have been well studied.

2.7.1 Effect of Salinity on Morphological Characters of Rice

Salt stress severely affects the morphology traits of rice in various ways, leading to inhibition of germination, difficulties in crop area establishment, leaf area development, decrease in dry matter production, delay in seed set and even sterility can also occur (Khatun and Flowers, 1995). Abundant literature revealed that the effect of salt stress on seedling growth, seedling establishment, yield components like number of tillers, number of spikelets has gradually led to a reduction in grain yield. Moreover, salt stress also caused decrease of the spikelet number per panicle, 1000 grain weight and increased sterility, regardless of the season and development stage (Khatun *et al*., 1995). However, there is a wide range of variation between and within different rice varieties in response to salinity (Yeo *et al*., 1990).

Rice is more sensitive to salinity at early seeding stage than tillering stage (Shereen *et al*., 2005). There was a potential reduction in germination rate at different concentration of salt (30–150 mM) (Hasanuzzaman *et al*., 2009). Moreover, seedling growth and fresh weight of rice was found to decrease with increased salt stress from 5 to 7.5 dS m^{-1} (Kazemi and Eskandari, 2011). In another study, NaCl induced salt stress reduced the germination percentage gradually with increasing salt stress from 0–300 mM NaCl though up to 50 mM NaCl, 100% germination was observed (Rajakumar, 2013). Germination of both weedy rice and cultivated rice showed a significant difference in germination at 50, 100, 200, 350 and 400 mM NaCl and salinity influenced not only the germination level of seeds, but also the time required to reach a same level of germination (Fogliatto *et al*., 2019).

Growth reduction in *Oryza sativa* L. was observed immediately after the exposure of 12.5 dS m^{-1} salinity but no significant variation was seen at lower levels (8.5 and 4.5 dS m^{-1}) (Alam *et al*., 2004). Different growth parameters like plant height, green leaf area, leaf weight, shoot and root growth were significantly affected by salt stress (Khan *et al*., 2004). Rice seedlings in salinized conditions expressed various visual symptoms of physical injury. Minh *et al*. (2016) observed that decrease in root growth, stunted shoot growth and thickened stem caused a complete reduction of growth and dying of seedlings under salt stress. Shoot dry weight of wild-type Nippobare rice reduced upto 57.14% at 300 mM NaCl salinity (Ishak *et al*., 2015).

Increasing salt stress resulted in gradual decrease in shoot and root length with more negative effects on shoots growth (Rajakumar, 2013). Significant reduction in mean root length, mean root numbers per plant, and shoot length was found to occur under increased salinity (Jamil *et al*., 2006; Jiang, 2010). Therefore, root and shoot lengths are two indicators of rice plant response to salinity. Rice cell division and cell elongation are severely affected by salt stress, that ultimately lead to reduction of root, shoot growth, and yield (Munns, 2002). Under salt stress a significant reduction in plant height and tiller number and leaf area were observed by Hasanuzzaman *et al*. (2009) in *Oryza sativa* plants. The magnitude of reduction in shoot and root length and dry weight in twelve rice varieties increased with increasing salinity levels from 0-20 dSm^{-1} (Hakim *et al*., 2010). An exposure of 200 mM salinity reduced plant height 7.67%, 7.18%, 6.52%, 5.55%, 5.06%, 4.16%, 3.99%, 1.50%, 0.91% for the cultivars BRRI dhan56, BRRI dhan40, BRRI dhan41, BRRI dhan53, IR29, Nona Bokra, FL-478, Binadhan-8, Binadhan-7 respectively, whereas Binadhan-10 was not affected (Hussain *et al*., 2016).

Shereen *et al*. (2005) reported that at early seedling stage of rice, leaf mortality increased with increased level of salinity in all rice cultivars. After 1 week of salt stress exposer leaf mortality was about 0-300% and after few months' salt stress showed reduction of growth and development (Munns 2005a). Salt stress has also been reported to decrease leaf area tremendously and showed profound changes in leaf anatomy in rice grown in-vitro (Bahaji *et al*., 2002) or in greenhouse (Wankhade *et al*., 2010). Leaf injury and death are attributed to the high salt accumulation in the leaf that exceeds the capacity of salt compartmentation in the vacuoles and causes accumulation of salt in the cytoplasm to toxic levels (Munns *et al*., 2006). Decrease of leaf area and death of leaves ultimately reduced photosynthesis rate of plant (Amirjani, 2011). Wankhade *et al*. (2013) reported that leaves of salt stressed rice (*Oryza sativa* L.) plants exhibited rolled margins with partial or complete chlorosis/necrosis of apical parts in emerging and/or older leaves and these symptoms were evident under 150 mM NaCl during the vegetative growth and under 20 mM NaCl during the reproductive stage.

2.7.2 Effect of Salinity on Physiological Attributes of Rice

The physiological effect of salinity on rice is many fold, leading to decrease in net photosynthesis (Pn), photosynthetically active radiation (PAR), degradation of pigment, transpiration rate (Tr), relative water content (RWC) and stomatal conductance (Gs)

(Cattivelli *et al*., 2008). Salt stress also has tremendous effect on water use efficiency (WUE) of rice plant (Ramezani *et al*., 2012) because water use efficiency (WUE) of rice plant decreased with increased salt concentration (Gholipoor *et al*., 2002). All these factors cause negative effects on rice physiology at the molecular and biochemical levels which induce abnormal growth, development of rice plant and ultimately plant death (Parida and Das 2005; Nishimura *et al*., 2011).

Available literature on response of rice at physiological level indicated chloroplast and mitochondria to be the most vulnerably affected organs among others (Rahman *et al*., 2000). Hence, to understand the negative effect of salt stress on photosynthetic efficiency chlorophyll content, changes in chlorophyll fluorescence (Fv/Fm) and membrane permeability are supposed to be efficient and potential indicators (Baker, 2008). Due to accumulation of Na^+ and Cl^- in rice leaves, chlorophyll contents are reported to be damaged that might interrupt the major electron transport in PSII (Sudhir and Murthy 2004; Munns *et al*., 2006). After 14 days of 200 mM NaCl salinity exposure Amirjani (2011) observed the reduction of Chl a and b contents in *Oryza sativa* leaves whereas reduction of the Chl b content of leaves (41%) was more than that of Chl a content (33%). Similarly, chlorophyll and carotenoids contents in rice leaves were reported to be significantly decreased after imposition of salt stress (Cha-umi *et al*., 2009). In another study, Chutipaijit *et al*., (2011) reported that with 100 mM NaCl salinity Chl a, Chl b and carotenoids (Car) contents of rice decreased 30%, 45% and 36% respectively as compared to control. Decrease of chlorophyll content was observed by 38%, 32%, 42% in BRRI dhan28, BRRI dhan47 and Binadhan-8 respectively under 60 mM NaCl compared to control (Kibria *et al*., 2017). Net photosynthetic rate of *Oryza sativa* L. declined significantly under 150 mM NaCl during the vegetative growth (Wankhade *et al*., 2013).

The salt stress induces two initial deleterious effects on plants: one is osmotic stress characterized by lowered osmotic potential and the other is, ionic effect causing ion toxicity. Under salt stress plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Relative water content (RWC) of rice (cv. BR11) decreased at 25% under 250 mM NaCl salinity as compared to control (Mostofa *et al*., 2015). In another study, Salt treatment (200 mM NaCl, 14 days) reduced the relative water content (RWC) of *Oryza sativa* from 87% in the control plants to 74% in the stressed plants (Amirjani, 2011). Ion imbalance may result from the effect of salt stress on nutrient availability, competitive uptake, transport or distribution within the plant. Generally, high NaCl lead to specific ion (Na^+ and Cl^-) toxicity in plants that decreases the N, P, K, Ca, Mg, and increases Na^+/K^+, Na^+/ Ca^{2+}, and Ca^{2+}/Mg^{2+}, and $Cl^-/NO^{3\ -}$ in plants inducing nutritional imbalance (Grattan and Grieve, 1999a; Abd El- Wahab, 2006; Razzaque *et al*., 2011; Zeinolabedin, 2012). However, it is well established that salt stress reduces nutrient uptake and accumulation of nutrients into the plants (Rogers *et al*., 2003; Hu and Schmidhalter, 2005). High level of salinity potentially affects Na^+, Ca^{2+}, K^+, and Mg^{2+} concentration in root and shoot in rice plant (Abdur *et al*., 2011). The level of K^+ and Ca^{2+} in the salt-stressed rice cells gradually decreased while that of Na^+ was dramatically increased (Jamil *et al*., 2012). Salt stress (200 mM NaCl, 30 days) decreased the uptake of

minerals in rice cultivar BRRI dhan47- Ca (7.32% in root, 29.26% in shoot), Mg (48% in root, 13.63% in shoot), Mn (27.78% in root, 37% in shoot) and Zn (31.15% in root, 31.58% in shoot) (Rahman *et al*., 2016).

2.7.3 Effect of Salinity on Yield and Yield Contributing Characters of Rice

Salt stress severely affects the grain yield of rice which is the ultimate product of yield components. Yield contributing characters like spikelets per panicle, panicle length, number of tillers per plant, number of florets per panicle, fertility, and 1000-grain weight are significantly affected by salt stress (Khatun and Flowers, 1995; Farshid and Hassan, 2012). Among all these yield contributing characters, the fertility of grain is reported to be most significantly affected causing reduction in total grain yield. In addition to fertility, panicle length and panicle numbers are two prominent characters under salt stress that have contribution to yield of grain (Hasamuzzaman *et al*., 2009). The magnitude of salinity induced yield losses could not be attributed to a single factor, there is some other alarming issues in rice grain yield like panicle sterility (Flowers and Yeo, 1981). Panicle sterility is usually seen in some rice cultivars, especially at pollination and fertilization stages perhaps due to some genetic mechanisms and nutrient deficiencies resulting from the effect of salt stress (Khatun and Flowers, 1995; Hasamuzzaman *et al*., 2009). Available literature showed that salinity may induce sterility of panicle, that leads to a decline in grain setting, pollen bearing capacity, and decrease of the stigmatic surface, or both during fertilization (Abdullah *et al*., 2001). The severe inhibitory effects of salts on fertility may be due to lack of transformation of carbohydrates to vegetative growth and spikelets development ((Murty and Murty, 1982). Moreover, reduced viability of pollen under salt stress might result in failure of seed set. Under salt stress rice grain yield reduced because of significant reduction in translocation of soluble sugar contents to superior and inferior spikelets and inhibition of starch synthetase activity during grain development (Abdullah *et al*., 2001).

Zeng and Shannon (2000a) studied the interrelationship among yield contributing characters of rice under different level of salinity. Different yield contributing characters like number of tillers per plant, number of spikelets per panicle, and percent of sterile florets decreased with increased salinity and all these yield contributing characters are linked to each other regarding final grain yield. Highly significant linear responses of grain weight per plant, grain weight per panicle, spikelet number per panicle, and tiller number per plant to salinity were observed in rice and harvest index was significantly decreased when salinity was at 3.40 dS m^{-1} and higher (Zeng and Shannon, 2000b).

The loss of grain yield due to 200 mM salinity are 49.64% over control for the cultivar IR29 (Hussain *et al*., 2016). In another study, number of filled grains per panicle decreased with increased salt concentration from 2-8 dSm^{-1} in rice (Farshid and Hassan 2012). An exposure of four levels of salinity (0, 4, 8, 12 dSm^{-1}) at reproductive stage reduced grain yield of rice (34-96%) with increased salt concentration (Hakim *et al*., 2014a). In different rice cultivars, grain yield reduction under salt stress is also reported by Gain *et al*. (2004).

2.8 Sulfur and Crop Productivity under Salt Stress

Salt stress is a major abiotic stress affecting crop productivity hence the management of salt stress in considered difficult because of its multigenic and quantitative nature. Available literatures show various strategies adopted to counteract the adverse effects of salt stress (Ashraf, 2009; Türkan and Demiral, 2009; Hasanuzzaman *et al*., 2013), but the information on the role of mineral nutrients to mitigate salt stress is scarce (Choi *et al*., 2004; Al-Harbi *et al*., 2008; Khan *et al*., 2009, 2010; Khorshidi *et al*., 2009). Among the mineral nutrients, sulfur (S) is increasingly being recognized as the fourth major macronutrient element after nitrogen (N), phosphorus (P) and potassium (K) which plays vital role not only in growth and development of higher plants but also is associated with salt stress tolerance (Marschner, 1995). Several reports are available which shown that sulfur is fruitful for different abiotic stress tolerance of crop plants as it is an integral part of major metabolic compounds, such as amino acids (methionine; Met and cysteine; Cys), antioxidant (GSH), proteins, and sulfolipids. Sulfur is also a component of Iron-S- clusters, polysaccharides and lipids and a wide variety of biomolecules like vitamins (biotin and thiamine), cofactors (CoA and S-adenosyl-Met), peptides (GSH and phytochelatins) and secondary products (allyl-cysteine sulphoxides and glucosinolates) (Khan *et al*., 2013; Nocito *et al*., 2007).

Sulfur nutrition has been reported to reduce the negative effects of salt stress, specially to protect salt-induced oxidative damage (Nazar *et al*., 2011). Application of sulfur induced enzymes of the S-assimilatory pathway which helped in neutralizing or scavenging ROS under salt stress (Nazar *et al*., 2014, 2015). Assimilation of sulfate is highly regulated in a demand-driven manner (Lappartient and Touraine, 1996; Leustek *et al*., 2000; Kopriva and Rennenberg, 2004; Kopriva, 2006; Davidian and Kopriva, 2010). S in higher plants is taken up by roots in the form of sulfate through sulfate transporter from soil. The primary points of regulation of S assimilation is the uptake of sulfate by roots and transport to shoot. Reduction of this sulfate takes place in leaf chloroplasts which produces sulfide. After sulfate reduction, it either remains in cytosol or transported into the plastid or stored in the vacuole for further metabolic reactions. First step in sulfate assimilation involves the activation of sulfate in cells by ATP-sulfurylase (ATPS) and the reduction of adenosine 5′-phosphosulfate (APS) to sulfite by APS reductase (APR) (Vauclare *et al*., 2002). Sulfite is further reduced to sulfide by sulfite reductase with ferredoxin as a reductant and the sulfide is incorporated into cysteine (Cys) by coupling to O-acetyl serine (OAS) which is controlled by the enzyme OAS thiol lyase (OAS-TL), also known as Cys synthase. Cys is the precursor of glutathione (GSH) (Fatma *et al*., 2016).

Glutathione (GSH) is not only an important S-containing compound but also a strong antioxidant which prevents damage to important cellular components caused by ROS (Pompella *et al*., 2003). The S-containing group thiol is emphatically nucleophilic and preferable for biological redox reactions and plays a significant role in protection against salt stress-induced oxidative damage (Nazar *et al*., 2011). Salt stress is known to affect the rate of S assimilation ultimately affecting root thiol content (Astolfi *et al*., 2010).

According to Nazar *et al*. (2011) external S supply increase the synthesis of glutathione (GSH) via increased Cys synthesis under salt stress which is correlated with salinity tolerance. That's why there is an increase in S assimilation in the plants grown with salt, resulting in higher Cys biosynthesis required for increased GSH production and defense responses to salt stress (Rais *et al*., 2013; López-Berenguer *et al*., 2007; Khan *et al*., 2009; Khan *et al*., 2013). Thus, the regulation of synthesis of S-containing compounds using genetic tools helps in increasing salinity tolerance. S-containing compounds like methionine, thioredoxins, vitamins, and coenzyme-A also play an important role in salt stress tolerance in plants along with Cys and GSH (Khan *et al*., 2013). Methionine, a regulatory molecule is a part of S-adenosyl methionine (SAM) and under salt stress the level of SAM synthase increases significantly which indicates the sensitivity of the methionine pathway to salinity. Supplementation with methionine has been reported to increase salt tolerance (Fatma *et al*., 2013; Khan *et al*., 2013). Fatma *et al*. (2013) reported that thioredoxins are also involved in responses of plants to salinity. They are hydrogen donor and act as signal for plant salt stress responses through participating ROS metabolism and reducing H_2O_2 production (Noctor *et al*., 2012; Khan *et al*., 2013). Application of thiamine also reported to alleviate salt stress by increasing the contents of Cys and methionine (El-Shintinawy *et al*., 2001). Sufficient supply of S improves photosynthesis and growth of plants through regulating N assimilation (Khan *et al*., 2005). The activity of nitrate reductase and the accumulation of N is regulated by the availability of S (Pal *et al*., 1976) and these larger N accumulation maintains high chlorophyll content in plants and enhance the activity of enzymes of Calvin cycle (Lawlor *et al*., 1989), and boost up growth (Khan *et al*., 2005) as S and N have established role in cell differentiation, photosynthetic functions and overall growth of plants (Marschner, 1995). In this manner, S nutrition may provide a good strategy to reduce the deleterious effect of salt stress through increased utilization and synthesis of reduced S compounds like Cys and GSH.

Supplementary S fertilization to high S loving crops such as *Brassica* and legume crops enhanced salt stress defense mechanisms by improving AsA and GSH (Rausch and Wachter, 2005). Foliar spray of S at 5 and $10gL^{-1}$ enhanced the morphological and physiological traits like root and shoot lengths, photosynthesis rate, stomatal conductance, chlorophyll content, stem diameter, leaf area and number of leaves, number of flowers and fruits, fruit weight, root and shoot weights which indicates the improvement in salinity tolerance potential of tested chili plants under salinity level of 5 dS m^{-1}. Hence, S played an important role in conferring salt-tolerance in chillies (Mukhtar *et al*., 2016). In another study, Arshadullah *et al*. (2011) reported that in a saline-sodic soil (EC 5.65 dS m^{-1}, pH 8.57, and sodium adsorption ratio 17.4 in saturated soil pate extract) application of increased levels of S, up to 75 kg ha^{-1} to field-grown wheat improved grain yield and yield component as well as the content of Ca and K in grains was significantly increased and that of Na was decreased, and the yield obtained with 75 kg ha^{-1} of S was 43% higher than the control where S was not added. In Date Palm, supplementation of S at 200g caused not only a significant increase in offshoot height, leaf area, number of leaves and girth of plant with cv. Berhi but also a significant increase in biochemical characteristics such as (Total Chlorophyll, Dry weight, RWC, Carbohydrates, proline concentration soluble protein,

peroxidase enzyme activities and endogenous indol acetic acid (IAA) content of two cultivars compared with control under saline conditions (The average of EC soil of field was (15.93 dS m^{-1}) and to EC water (4.55 dS m^{-1})) (Abbas *et al*., 2015). Application S increased faba bean fresh and dry weight by about 5.2% and 2.3% and improved proline content by about 20.4% and 11.2% relative to the control treatments (Abdelhamid *et al*., 2013).

Ahmed *et al*., (2017) reported that application S @ 125 & 100% of SGR significantly increased vegetative growth and yield attributes of rice and wheat crop than non-amended soil (control) and proved best to improve the vegetative growth of rice and wheat crops in term of plant height, No. of tillers, panicle/spike length.

In Maize, application of sulfur at 60 to 80 mM improved all germination parameters such as germination percentage germination index, coefficient of velocity of emergence, mean emergence time, vigour index, germination energy, germination speed, mean daily germination and germination value and reduced time needed for 50 % seed to germinate through reducing the toxic effects of salinity (Riffat and Ahmad, 2016).

Sulfur application @ 40 mM improved the crop yield by developing salt tolerance in maize plants. Sulfur at 40 mM level not only improved the salt tolerance in two maize varieties by improving yield related attributes, nutrient contents and forage value parameters but also lowered the Na^+ contents to reduce the toxic effects of salinity (Riffat, 2018). In another study on maize Riffat and Ahmad (2018) reported that sulfur at 60 and 80 mM improved shoot and root length, fresh and dry weights, nutrient contents (K^+, Ca^{2+}, NO^{3-}, PO_4^{3-}, SO_4^{2-}, Ca^{2+}/Na^+, K^+/Na^+) and lowered Na^+ ions at all levels of salinity.

Pea (*Pisum sativum* L.) plants grown on reclaimed saline soil (EC = 8.2 - 8.5 dS m^{-1}) has been shown to enhance plant stress-defense responses, to act indirectly by improving general plant performance under stress, and to increase ascorbic acid (AsA) and reduced glutathione (GSH) contents, leading to an increase in photosynthetic efficiency and, subsequently, to an increase in plant growth and crop yield when treated with 200 kg ha^{-1} sulfur (Osman and Rady, 2012).

Increasing elemental sulfur levels promoted reductions in soil pH and electrical conductivity of the saturation extract and the sulfur level of 1.39 t ha^{-1} was sufficient to reduce soil pH and salinity to a level that best promoted sorghum growth (de Andrade *et al*., 2018).

Sunflower plants grown with 4 mM sulfur level showed significant growth whereas interaction between sulfur and salinity (0, 75, and 150 mM NaCl) was highly significant ($P<0.01$) for growth parameters like fresh weight, dry matter yield of shoot and root, diameter and length of stem and root (Badr-uz-Zaman *et al*., 2002).

Ali *et al*. (2012) reported that tillering, number of grains $spike^{-1}$, 1000- grain weight, grain yield significantly ($p\leq 0.05$) increased by enhancing the rate of S application. Wheat grain yield was the maximum (4040 kg ha^{-1}) at the application of 50 kg S ha^{-1} and 26% more

than control treatment. The maximum number of tillers/5 plants (110), number of grains spike^{-1} (63.6) and 1000 grain weight (47 g) were recorded with S application at 50 kg ha^{-1}.

In another study, wheat plants grown under high salinity level 4000 ppm and high rate of sulfur (952 kg S ha^{-1}) showed that the increment in the crop under study and cultivated in calcareous, alluvial and sandy soils were 74, 60 and 46% for wheat grain yield, relative to the control, respectively. Also, sulfur applications significantly increased total uptake of NPK of wheat crop cultivated in different soils, especially calcareous soil (Mohamed *et al*., 2019).

2.9 Effect of Sulfur on Rice under Salt Stressed Condition

Sulfur application decreased the deleterious effects of salinity and had suitable effect on growth and nutrient contents of rice. Sulfur fertilizer used as soil amendments for enhancing the productivity of rice (Helmy *et al*., 2013). Rahman *et al*. (2007) reported that sulfur fertilizer significantly increased grain yield of cereal crop including rice through significant improvement in all yield attributes and rice growth traits like leaf area index and dry matter production.

Increasing sulfur fertilizer significantly increased flag leaf area, LAI, dry matter content, panicle weight and filled grains panicle^{-1} and grain yield whereas decreased sterility % of Giza 178 rice cultivar up to 150 kg S ha^{-1} under salinity level 5.3 and 5.0 dS m^{-1}. On the other hand, plant height and number of panicles were markedly increased by sulfur fertilizer application up to 100 Kg S ha^{-1} (Zayed *et al*., 2011).

Shaban *et al*. (2013) claimed that the application of sulphuric acid as a source of S increased the plant height, number of spikes plant^{-1} and 1000-grain weight of rice by about 29.9, 133 and 72.4%, respectively, compared with untreated plants whereas the maximum straw and grain yields (11.2 and 9.41 Mg ha^{-1}, respectively) were produced in the sulphuric acid treatment, followed by mineral sulphur and gypsum as well as the highest chlorophyll content (2.51 mg g^{-1} fresh weight of leaves) was obtained after treatment with sulphuric acid, and represented an increase of 56.9% over the control. Sulphur application also enhanced the uptake of N, P, K and Zn by the plants.

Under 7.9 and 7.5 dS m^{-1} salinity S treatment of 240 kg S ha^{-1} significantly improved rice growth criteria i.e. leaf area index, chlorophyll content, dry matter production, number of tillers hill^{-1}, plant height, yield components; number of panicles hill^{-1}, panicle length, panicle weight, number of filled grains panicle^{-1} and 1000-grain weight, grain and straw yields and harvest index compared to control treatment (Bassiouni, 2016).

In rice, pooled data (average of four seasons) showed that that varying levels of sulfur and gypsum had significant effect on the vegetative growth and yield attributes of rice crop under saline soil conditions. Data regarding plant height, No. of tillers, panicle length, paddy yield, straw yield and 1000 grain weight depicted that treatment using gypsum @

100% SGR recorded the statistically ($P \leq 0.05$) maximum plant height (109.67 cm), No. of tillers (151.00), panicle length (24.00 cm), paddy yield (4.18 Mg ha^{-1}), straw yield (9.87 Mg ha^{-1}), 1000 grain weight (23.38 g) which was followed by S @ 125 % of SGR and S @ 100 % of SGR however statistically all the treatments were at par but significantly ($P< 0.05$) better over control (Ahmed *et al*., 2017).

S application @ 600 kg S/ ha proved to stimulate no. of panicles/ hill, panicle length, no. of filled grains/ panicle, fertility %, 1000-grain weight and panicle weight of rice as compared with the other tested sulfur rates (0, 200 and 400 kg S/ha) under salt stress. Application of sulfur at a rate of 600 kg S/ ha produced the maximum values of grain yield (3.83 and 4.19 ton/ ha), biological yield (8.86 and 9.07 ton/ ha) and harvest index (43.23 and 46.20 %) though there were no significant differences between 400 and 600 kg S/ ha for grain yield/ ha and harvest index (Zayed *et al*., 2017).

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted from November 2018 to May 2019 comprising of collection of seed, raising of seedlings, growing and experimentation, data collection, compilation, etc. to study the role of S in improving morphological, physiological and yield performance of rice under salt stress. A brief of soil, climate, materials and methods used for conducting the experiment is presented below.

3.1 Location of the experimental site

The experiment was set at the Net House and Plant Physiology Laboratory of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location of the pot experiment at 24^0 75′ N latitude and 90^0 50′ E longitude at the elevation of above 18m of sea level and it was under the Agro-Ecological Zone-28, namely Madhupur Tract. For better understanding the experimental location, the Map of AEZ of Bangladesh has been added in Appendix IV.

3.2 Characteristics of Soil that used in Pot

The soils used in pot were collected from the experimental field of Department of Agricultural Botany, SAU, Dhaka. The pot experiment was conducted by using typical rice growing silty loam soil having noncalcarious properties. The soil was Deep Red Brown Terrace Soil under Tejgaon Series belonging to the Agro-Ecological Zone of Madhupur Tract. The soil for the pot was collected from 0-15 cm depth. The collected soil was pulverized followed by the removal of weeds, stubble, brick pieces, insects, etc. The soil was then sun dried, crushed and passed through a 2 mm sieve. After that the soils were mixed up properly and 400 g soil was taken for initial physical and chemical analysis. The morphological properties of this soil have been presented in Appendix V and the physio-chemical properties in Appendix VI.

3.3 Climate

The site of the study was characterized by a subtropical monsoon climatic zone. Moderately low temperature along with moderate rainfall prevailed during the period from November to April. The cool and dry weather prevailed during November to January with the mean temperature 22.67^{o}C. Temperature during February to April was moderately hot but highly humid along with moderate to high rainfall. Cyclone Fani, the strongest storm has barrelled into Bangladesh after leaving a trail of deadly destruction across the eastern coast of India in the month of April. The detailed meteorological records (monthly) of air temperature, relative humidity, rainfall from November, 2018 to April, 2019 have been presented in Appendix VII.

3.4 Planting Material

Oryza sativa L. cv. BRRI dhan67 was used as test crop which is a salt tolerant rice variety recommended for cultivation in *Boro* season. This variety was developed at Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur and was released for farmers use in 2014. Life Duration is 140-150 Days. Plant height is almost 100 cm. Yield ranges from 3.8 - 7.4 t ha^{-1} depending on various salinity level.

3.5 Treatments

The pot experiment consisted of two factors as shown below:

Factor A: Different levels of salinity (NaCl) with irrigation water

i. N_0 = 0 dSm^{-1}
ii. N_1 = 8 dSm^{-1}
iii. N_2 = 12 dSm^{-1}

Factor B: Different doses of sulfur (S)

i. S_0 = 0 Kg S ha^{-1}
ii. S_1 = 3 Kg S ha^{-1}
iii. S_2 = 6 Kg S ha^{-1}

There were following 9 treatment combinations:

N_0 S_0 = Without Salt + Without Sulfur

N_0 S_1 = Without Salt + 3 Kg S ha^{-1}

N_0 S_2 = Without Salt + 6 Kg S ha^{-1}

N_1 S_0 = 8 dSm^{-1} Salt + Without Sulfur

N_1 S_1 = 8 dSm^{-1} Salt + 3 Kg S ha^{-1}

N_1 S_2 = 8 dSm^{-1} Salt + 6 Kg S ha^{-1}

N_2 S_0 = 12 dSm^{-1} Salt + Without Sulfur

N_2 S_1 = 12 dSm^{-1} Salt + 3 Kg S ha^{-1}

N_2 S_2 = 12 dSm^{-1} Salt + 6 Kg S ha^{-1}

3.5.1 Salinity treatment

There were three salinity levels including control which were prepared by adding respected amount commercial salt (NaCl) to the soil/pot as water dissolved solution. The salinity treatments were N_0 (Control), N_1, and N_2. To spread salinity homogeneously in each pot, salts were dissolved in water and were given to pots as irrigation water for proper salinity imposition. When no salt added it termed as control (C) and for N_1, and N_2 salinity 2.3376

g/L and 3.5064 g/L commercial salts were added respectively with water (Plate V) and after that in concerned pot average salinity was found 8 dSm^{-1} and 12 dSm^{-1} for N_1, and N_2 respectively. The salt treatments were begun at 15 DAT.

3.5.2 Sulfur treatment

Sulfur (S) fertilizer was used to test the role of S in improving morphological, physiological and yield performance of rice under salt stress. There were three sulfur doses including control. The Sulfur treatments were S_0, S_1, and S_2. When no sulfur added it termed as control (C) and for S_1 and S_2, 0.144 g and 0.288 g sulfur fertilizer were added to the pot respectively to get 3 Kg S ha^{-1} and 6 Kg S ha^{-1} (Plate IV).

3.6 Experimental Design and Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) as two factorial arrangements with three replications. The experimental area was divided into three equal blocks. Each contain 9 pots where 9 treatment combinations were allotted randomly. There were total 27 (9×3) pots in the experiment. The layout of the experiment has been shown in Appendix VIII.

3.7 Collection of Planting Material

Seeds of *Oryza sativa* L. cv. BRRI dhan67 were used as planting material, which were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

3.8 Pot Preparation

Plastic pots were used in this experiment. The diameter of each pot was 35 cm (14 inches) at the top and 15 cm (6 inches) at the bottom. The depth of each pot was 30 cm (12 inches). The collected soil was sun dried, crushed and passed through a sieve to remove weeds, stubble, brick pieces, insects, etc. The dry soil was then thoroughly mixed up with well rotten cow dung (75 g for 12 kg soil) before filling the pots. Each pot was filled up with 12 kg soil on 14 December, 2018 and all experimental pots received recommended doses of N, P and K fertilizers. After that the pots were pre-labeled for each treatment combination and placed at the net house of the Department of Agricultural Botany. At last, measured water was added to bring soil at field capacity condition.

3.9 Manure and Fertilizer Application

Well rotten cow dung at the rate of 12.5 t ha^{-1} mixed up with soil before filling the pot. The following fertilizers i.e., urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and $ZnSO_4$ were used as sources of nitrogen, phosphorus, potassium, sulfur and zinc were applied at a rate recommended by BARI for the variety BRRI dhan67 shown in tabular form below.

Table 2. Manures and Fertilizers applied for the experimental pot

Manures and Fertilizers	Dose bigha^{-1}	Dose ha^{-1}	Dose Pot^{-1}	Application (%)			
				Basal	15 DAT	35 DAT	55 DAT
Cow dung	---	12.5 Ton	75 g	100	---	---	---
Urea	36 kg	269 kg	1.62 g	---	33.33	33.33	33.33
TSP	13 kg	97 kg	0.66 g	100	---	---	---
MoP	16 kg	120 kg	0.72 g	100	---	---	---
Zypsum	13 kg	97 kg	0.58 g	100	---	---	---
$ZnSO_4$	1.5 kg	11 kg	0.066 g	100	---	---	---

The weight of 1 ha soil at the depth of 15 cm is considered approximately 2 million kg of soil. According to the above rate, manures and fertilizers were calculated as per pot that contained 12 kg soil. The whole amounts of TSP, MoP, Zypsum and $ZnSO_4$ were applied during the final pot preparation. Urea was applied in three equal splits at 15, 35 and 55 days after transplanting (DAT).

3.10 Seedbed Preparation

Wet seedbed was prepared (Plate I) by November 21, 2018 and sprouted seeds were sown on November 22, 2018 following the recommendation of BRRI (BRRI 1995).

3.11 Seedling Raising

A very common procedure was followed in raising of seedlings i.e., the seeds were soaked for 48 hours and then washed properly in fresh water and after that incubated for sprouting. The sprouted seeds were sown in the wet seedbed on November 22, 2018.

3.12 Uprooting and Transplanting of Seedlings

Healthy and uniform seedlings of thirty days old were uprooted carefully from the seedbed (Plate II) and were transplanted in the experimental pots (Plate III) at the rate of single seedling hill^{-1} on December 21, 2018 maintaining three seedlings in each pot. The seedbed was watered before uprooting the seedlings from the seedbed to minimize the root damage. The seedlings were watered after transplanting in the pot for their better establishment. After one week of transplanting all the experimental pots were checked for any missing hill, which was filled up with extra seedlings.

3.13 Intercultural Operations

After transplantation of seedlings, different intercultural operations like weeding, irrigation, plant protection measures etc. were accomplished for better growth and development of the seedlings.

3.13.1 Weeding and Irrigation

The hand weeding was done as when necessary to keep the experimental pots free from small aquatic weeds. Irrigation was done whenever necessary but the frequency of irrigation became less in harvesting stage. Irrigation was done at evening as salt was applied with irrigation water.

3.13.2 Plant Protection Measures

Leaf blast disease was observed and Trooper 75 WP@ 8g/10 L water was sprayed to effectively control the disease. Beside this, about 1-2inch depth of water was maintained and Muriate of potash (MoP) was also top dressed. During the conduction of experiment there was a severe attack of rats which were effectively controlled by using Zinc Phosphide (2%) and rat trap, where dry fish was used. The holes made by the rats was poured off with water.

3.14 General observation of the experimental pots

The plants were under regular observation and the plants looked normal green except the plants treated with salt. No lodging was observed but the maximum tillering, panicle initiation, and flowering stages were not uniform.

3.15 Detection of maximum tillering and panicle initiation stage

Maximum tillering and panicle initiation stages were detected through regular inspection. When the number of tillers hill^{-1} reached the highest number and after that decreasing in trend, was considered as maximum tillering stage. When a small growth at the top of upper most nodes of main stem was noted like a dome was considered as an indication of the beginning of panicle initiation stage. But these stages were not uniform and were varied with treatments.

3.16 Harvesting

The crops were harvested at maturity when 80-90% were turned into straw colored on April 28, 2019. The crop was cut at the ground level and pot wise crop was bundled separately, tagged and brought to the threshing floor. The grains were then sun dried to a moisture content of 12% and straw was also sun dried properly. The grain and straw yields and different plant physiological parameters were recorded after harvesting.

3.17 Data Collection

The data on the following parameters were collected from each treatment.

A. Morphological parameters

- Plant Height
- No. of Tillers Plant^{-1}
- Leaf Area Index (LAI)

B. Physiological parameters

- Chlorophyll Content (SPAD Value)
- Leaf Membrane Stability Index (MSI)
- Relative Water Content (RWC)
- Dry Weight of Root
- Dry Weight of Stem
- Dry Weight of Leaf
- Total Dry Matter (TDM)
- Na and K content in Roots and Shoots

C. Phenological parameters

- Days to flowering
- Days to grain formation
- Days to maturity

D. Yield contributing and other parameters

- Panicle Length
- No. of Effective Tillers Plant^{-1}
- No. of Non-Effective Tillers Plant^{-1}
- No. of Filled Grains Panicle^{-1}
- No. of Unfilled Grains Panicle^{-1}
- 1000 Grain Weight (g)

E. Yields

- Grain Yield Plant^{-1}
- Straw Yield Plant^{-1}
- Biological Yield (t ha^{-1})
- Harvest Index
- Mitigation

3.18 Detailed Procedures of Recording Data

A brief outline of the data collecting procedure followed during the experiment is given below:

3.18.1 Plant Height (cm)

Plant height was measured in centimeter from 30 days after transplanting (DAT) at 15 days interval up to 120 DAT, beginning from the top surface level of the pot to the tip of the longest leaf at booting and flowering stage and at maturity stage, from the top surface level of the pot to the tip of the tipper end of the longest panicle (Plate VIII).

3.18.2 Number of Tillers Plant^{-1}

Tillers, which had at least one visible leaf were counted from 30 days after transplanting (DAT) at 15 days' interval up to 120 DAT.

3.18.3 Leaf Area (cm^2)

Leaf area was measured in centimeter^2 by non-destructive method at heading stage (Plate XII).

3.18.4 Leaf Membrane Stability Index (MSI%)

Electrolyte leakage was assayed according to Dionisio-Sese and Tobita, (1998) with slight modification. The plasma membrane stability or intactness was estimated through the leakage of electrolytes. Fresh leaf trips (0.2 g) of uniform size were placed in test tubes, containing 10 ml distilled water and kept for 30 minutes in water bath (Plate X) at 40 °C for measuring the initial electrolyte conductivity (C_1). The final electrolyte conductivity (C_2) was measured after boiling the plant samples for 15 minutes at 100 °C. MSI was calculated as-

$$MSI = \left(1 - \frac{C_1}{C_2}\right) \times 100$$

3.18.5 Relative water content (RWC)

Relative water content (RWC) was measured according to the following method suggested by Barrs and Weatherley (1962). From each experimental pot three leaves were randomly selected and cut with scissors. Fresh weight (FW) of leaf laminas were taken (Plate XI) and then immediately floated on distilled water in a Petri dish for 4 hours in the dark. After drying excess surface water with paper towels turgid weights (TW) were measured. Then the sample was oven dried at 80 °C for 48 hours and dry weights (DW) were measured. RWC% was calculated by the following formula:

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

3.18.6 Chlorophyll Content (SPAD Value)

Leaf chlorophyll content was measured by using a hand-held chlorophyll content SPAD meter (SPAD 502, Konica Minolta, Japan). At each evaluation the chlorophyll content was measured five times from three randomly selected leaves at different positions plant^{-1} (Plate IX) and the average was used for analysis.

3.18.7 Dry Weight of Root

After harvesting, roots of the plants were very carefully separated from the soil and then sun dried. Then they were sliced into small pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of root was taken.

3.18.8 Dry Weight of Stem

After harvesting, stems of the plants were separated from the leaf and then sun dried. Then they were sliced into very thin pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of stem was taken.

3.18.9 Dry Weight of Leaf

After harvesting, leaves of the plants were collected and sun dried. Then they were sliced into small pieces and were put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of the sample was taken.

3.18.10 Total Dry Matter (TDM)

The plant parts i.e., roots, stems, leaves and panicles were detached from each other and were kept separately in oven for 72 hours at 70 ℃ (Plate XV). The oven dried samples of these plant parts were weighted for dry matter production. The total dry matter production was calculated from the summation of dry matter produced by the above-mentioned plant parts and grain weight per plants in gram.

3.18.11,12 Measurement of Na and K content in Roots and Shoots

Root and shoot samples were oven-dried at 80^{o} C for 48h. Dried samples were ground and subjected to acid digestion in HNO_3:$HClO_4$ (5:1 v/v) mixture at 80^{o} C. The Na and K contents were measured using flame atomic absorption spectrophotometer (Nahar *et al*., 2016).

3.18.13 Days to Flowering

Days to flowering was recorded from the day the first pistils emergence was seen. Days to flowering varied from plant to plant, so it was checked daily once the change in life cycle was initiated to record the days to flowering at different days after transplanting.

3.18.14 Panicle Length

Panicle length was measured in centimeter from the basal nodes of the rachis to the apex of each panicle. Each observation was actually an average of 5 panicles.

3.18.15 Spikelet Fertility

Spikelet fertility was calculated by dividing the number of filled grains by the total number of grains (i.e., florets) and was described as the per cent.

$$Spikelet\ Fertility\ (\%) = \frac{Number\ of\ Filled\ Grains}{Total\ Number\ of\ Grains} \times\ 100$$

3.18.16, 17 No. of Effective and Non-Effective Tillers Plant^{-1}

The total number of tillers plant^{-1} was counted from the experimental pots at maturity and were grouped into effective (panicle bearing tillers) and non-effective tillers plant^{-1}.

3.18.18, 19 No. of Filled Grains and Unfilled Grains Panicle^{-1}

Each grain was tested for whether it was filled or not by pressing the grain between the forefinger and the thumb. In case of more than 5 effective tillers plant^{-1}, grains of 5 randomly selected panicles of each experimental pot were counted and then the average number of filled and unfilled grains for each panicle was determined. In case of less than 5 effective tillers plant^{-1}, grains of all the panicles plant^{-1} were counted (Plate XIV) and then the average number of filled and unfilled grains for each panicle was determined.

3.18.20 Thousand Grain Weight (g)

200 clean sundried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance and then multiplied by 5.

3.18.21. Grain Yield Plant^{-1}

The grains plant^{-1} was separated by threshing and then properly sun dried and weighed to get grain yield plant^{-1}.

3.18.22 Straw Yield Plant^{-1}

The straw plant^{-1} was separated by threshing and then properly sun dried and weighed to get straw yield plant^{-1}.

3.18.23 Biological Yield Plant^{-1}

The summation of grain yield and straw yield is defined as biological yield of a crop. The biological yield of rice was measured for each experimental pot and expressed in gram per pot.

The biological yield was calculated with the following formula:

Biological yield = Grain yield + Straw yield

3.18.24. Relative Grain Yield (%)

Relative grain yield percent is the ratio of yield value of treatment combination to yield value of control treatment and was calculated by the following formula:

$$Relative\ Grain\ Yield\ (\%) = \frac{Yield\ value\ of\ treatment\ combination}{Yield\ value\ of\ control\ treatment} \times 100$$

3.19 Statistical Analysis

The recorded data of different parameters were statistically analyzed to get the level of significance using the Statistix 10 computer package program. Analysis of variance was calculated following two factors randomized complete block design. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

CHAPTER IV

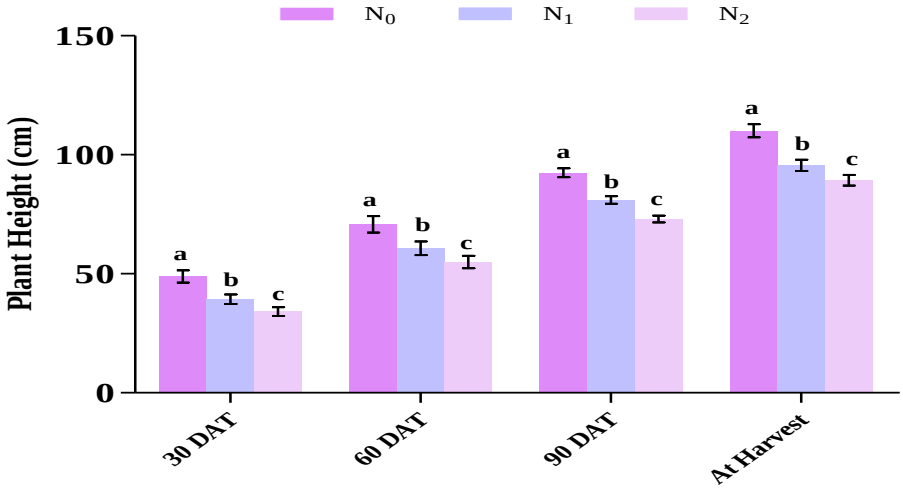
RESULTS AND DISCUSSION

The effect of sulfur in improving morphological, physiological and yield performance of rice plant under salt stress in the present study were presented in the tables and figures and discussed. A summary of the analysis of variance (ANOVA) with regards to all the studied parameters has been shown in Appendices IX to XVII. The results obtained in the experiment were presented and discussed under the following subheadings.

4.1Results

4.1.1 Plant Height

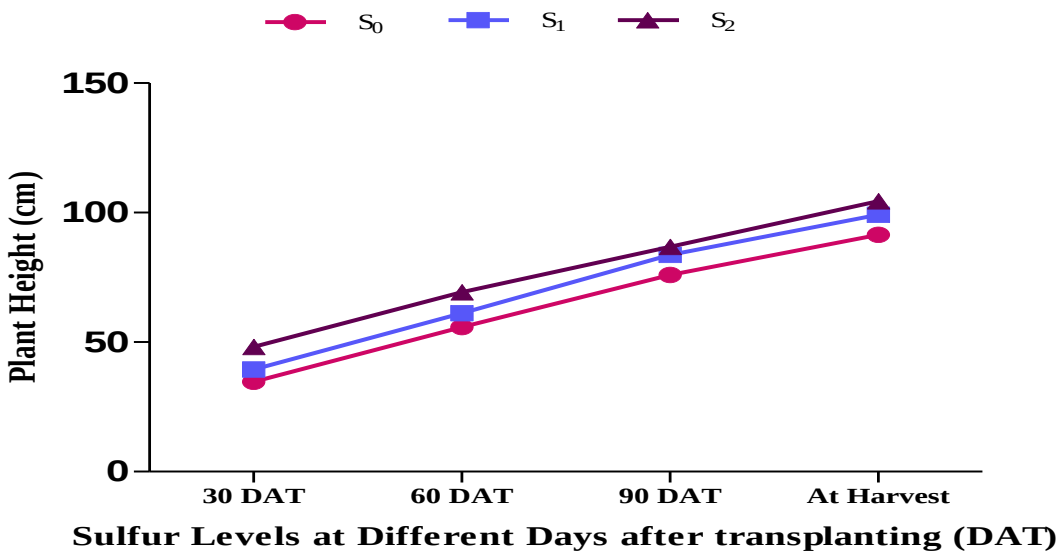
Salt stress caused a significant ($P \leq 0.01$) reduction in plant height (cm) at 30 DAT, 60 DAT, 90 DAT and, at harvest compared to the control treatment without salt stress (Figure 1 and Appendix IX). A clear difference was noticed between the plants grown under salt stress conditions and control conditions. At 30 DAT, 60 DAT, 90 DAT and at harvest with 8 dSm^{-1} salinity levels (N_1 treatment) plant height was decreased by 26.34%, 18.89%, 12.36%, 13.16% and with 12 dSm^{-1} salinity levels (N_2 treatment) by 36.86%, 27.04%, 21.02%, 18.89% respectively, when compared to control treatment. Fig.1 showed that plant height decreased gradually with increasing salinity stress.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 1. Effect of different salt concentrations on the plant height of rice at different days after transplanting (LSD $_{(0.05)}$ = 5.49, 4.98, 2.00 and 2.49 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Supplementary sulfur fertilization (3, 6 Kg S ha^{-1}) improved plant height significantly ($P \leq 0.01$). An increase of plant height at 30 DAT, 60 DAT, 90 DAT and, at harvest with 3 Kg S ha^{-1} (S_1 treatment) was 11.91%, 8.87%, 9.21%, 7.89% and, with 6 Kg S ha^{-1} (S_2 treatment) was 16.31%, 10.96%, 12.59%, 12.44% respectively, when compared to control treatment without sulfur (Figure 2 and Appendix IX). At 30 DAT and 60 DAT, the applied 6 Kg S ha^{-1} (S_2 treatment) increased the plant height but remained statistically at par with S_1 treatment (3 Kg S ha^{-1}). But at 90 DAT and at harvest all three means were significantly different from one another. In every case, the lowest values of the plant height of rice plants were produced when rice plants did not receive any sulfur fertilizer.



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 2. Effect of different sulfur levels on the plant height of rice at different days after transplanting (LSD $_{(0.05)}$ = 5.49, 4.98, 2.00 and 2.49 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Plant height of rice plants decreased significantly under salt stress (Table 3 and Appendix IX). However, the application of sulfur significantly ($P \leq 0.05$) increased the height of rice plants exposed to salt stress, in contrast to salt treatment without Sulfur. It was revealed from statistically significant Salinity × Sulfur interaction (Table 3). At 30 DAT, treatment with 8 dSm^{-1} salinity, the plant height increment at 3 Kg S ha^{-1} was 8.24% when compared to 8 dSm^{-1} salt treatment alone. In contrast, it was 12.57% when sulfur supply was increased from 3 Kg S ha^{-1} to 6 Kg S ha^{-1}. Similarly, at 60 DAT, 90 DAT and, at harvest treatment with 8 dSm^{-1} salinity, the plant height increment at 3 Kg S ha^{-1} was 3.83%, 10.07% and, 9.04% respectively while at 6 Kg S ha^{-1} it was 6.75%, 14.34%, 15.16% respectively when compared to salt treatment alone. On the other hand, at 30 DAT, 60 DAT, 90 DAT and, at

harvest treatment with 12 dSm^{-1} salinity, the plant height increment at 3 Kg S ha^{-1} was 8.92%, 7.05%, 13.28% and, 12.35% respectively while at 6 Kg S ha^{-1} it was 11.73%, 8.79%, 16.71% and, 16.63% respectively when compared to salt treatment alone. Generally, sulfur increased the plant height by developing salt tolerance at both levels (8 dSm^{-1}, 12 dSm^{-1}). However, 3 Kg S ha^{-1} was not so efficient as 6 Kg S ha^{-1} in this respect in improving the plant height but is significant as compared to salt treatment alone.

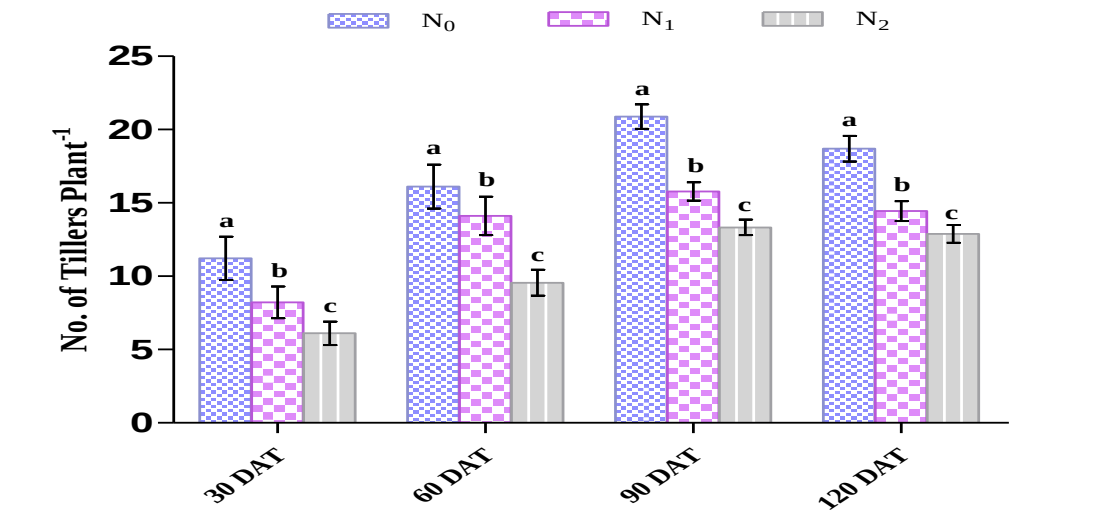
Table 3. Interaction effect of different salt concentrations and sulfur levels on the plant height of rice at different days after transplanting

Treatments	Plant Height (cm)			
	30 DAT	60 DAT	90 DAT	At Harvest
N_0S_0	42.2 b	63.3 b	88.4 b	106.5 bc
N_0S_1	50.5 a	73.7 a	93.2 a	110.0 ab
N_0S_2	53.9 a	75.2 a	95.7 a	113.8 a
N_1 S_0	33.4 de	55.3 cd	74.1 e	87.5 e
N_1 S_1	36.4 cd	57.5 cd	82.4 c	96.2 d
N_1 S_2	38.2 c	59.3 bc	86.5 b	103.13 c
N_2 S_0	28.6 f	48.8 e	65.3 f	80.2 f
N_2 S_1	31.4 ef	52.5 de	75.3 e	91.5 e
N_2 S_2	32.4 e	53.5 de	78.4 d	96.2 d
LSD $_{(0.05)}$	**3.66**	**5.17**	**2.84**	**4.24**
CV (%)	**5.49**	**4.98**	**2.00**	**2.49**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

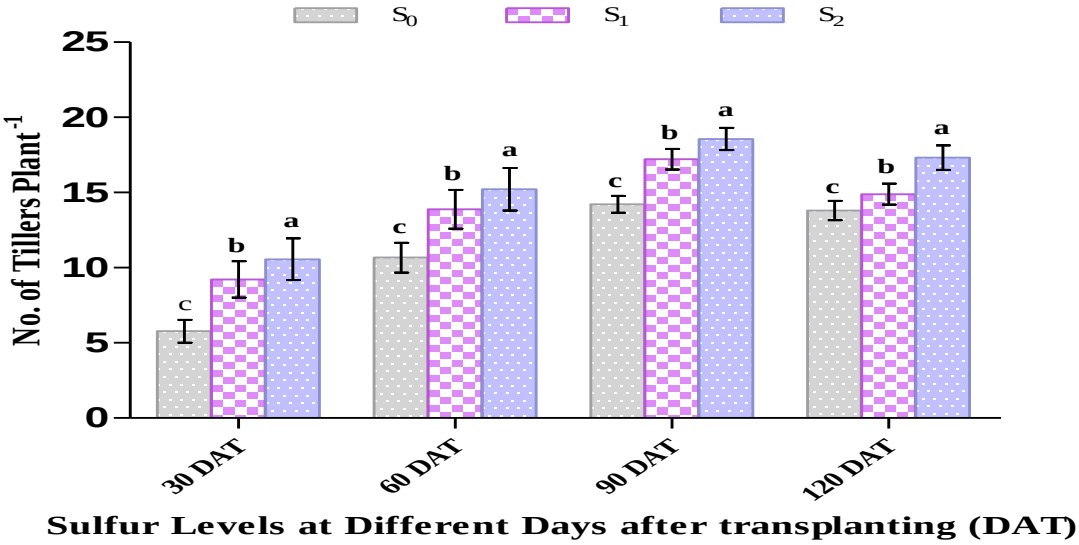
4.1.2 No. of Tillers Plant^{-1}

Tiller formation in rice is a very important agronomic trait for grain production and number of tillers provide valuable information about the stress profile of a plant under abiotic stress (Suzuki *et al*., 2005). The number of tillers plant^{-1} were significantly ($P \leq 0.01$) reduced by increasing level of salinity (Figure 3 and Appendix X). N_1 and N_2 treatment reduced tiller number by 26.75% and 45.54% respectively at 30 DAT, 12.42% and 40.66% respectively at 60 DAT, 24.46% and 36.19% respectively at 90 DAT, 22.78% and 31.12% respectively at 120 DAT when compared to control. In respect of salinity effect, the result showed that the number of tillers plant^{-1} were greatly affected even at N_1 treatment but the maximum reduction in number of tillers plant^{-1} was found at N_2 treatment.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 3. Effect of different salt concentrations on number of tillers $plant^{-1}$ of rice at different days after transplanting ($LSD_{(0.05)}$ = 1.12, 1.23, 0.67 and 0.72 at 30, 60, 90 DAT and 120 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 4. Effect of different sulfur levels on number of tillers $plant^{-1}$ of rice at different days after transplanting ($LSD_{(0.05)}$ = 1.12, 1.23, 0.67 and 0.72 at 30, 60, 90 DAT and 120 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

By exogenous application of sulfur, the number of tillers plant^{-1} was increased significantly ($P \leq 0.01$) at different days after transplanting (Figure 4 and Appendix X). The number of tillers plant^{-1} enhancement over control was 37.31%, 23.18%, 17.42% and 7.25% by S_1 treatment and 45.27%, 29.89%, 23.38% and 20.27% by S_2 treatment at 30 DAT, 60 DAT, 90 DAT and 120 DAT respectively. So, the parameters of plants not receiving supplementary sulfur (control) were still lower than the values of sulfur treatment.

Results showed that total number of tillers plant^{-1} was significantly lowered by increasing the concentration of salinity (Table 4 and Appendix X). However, sulfur supplementation (S_1, S_2) improved the number of tillers plant^{-1} significantly ($P \leq 0.05$) at both levels of salinity (N_1, N_2). It was evident from statistically significant Salinity × Sulfur interaction (Table 4). Number of tillers plant^{-1} was increased by 40.78%, 36.18%, 18.37% and 9.28% after S_1 supplementation of salt stressed (N_1S_1) plant and 48.4%, 40.01%, 24.56% and 18.7% after S_2 supplementation of salt stressed (N_1 S_2) plant when compared to salt treatment (N_1 S_0) alone at 30 DAT, 60 DAT, 90 DAT and at harvest respectively. Whereas, S_1 supplementation during salt stress (N_2) increased number of tillers plant^{-1} by 31.6%, 10.34%, 14.63% and 7.9%, and S_2 supplementation by 43.55%, 16.07%, 20.45% and 18.69% at 30 DAT, 60 DAT, 90 DAT and at harvest respectively when compared to salt treatment (N_2 S_0) alone. In comparison to the group of salinity stress alone (N_1 S_0, N_2 S_0), the number of tillers plant^{-1} increased all the way to the level of S_1 and S_2 treatment, while there was no further significant difference between these two treatments except the treatments at 120 DAT.

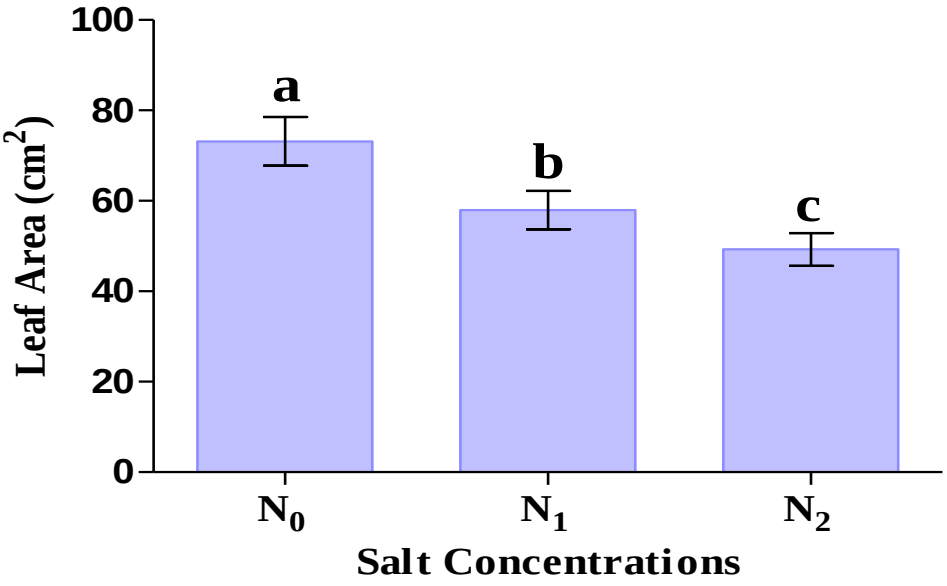
Table 4. Interaction effect of different salt concentrations and sulfur levels on the number of tillers plant^{-1} of rice at different days after transplanting

Treatments	Number of Tillers Plant^{-1}			
	30 DAT	60 DAT	90 DAT	120 DAT
N_0S_0	7.67 cd	13.33 c	17.67 c	16.77 bc
N_0S_1	12.33 a	16.33 b	21.67 b	17.67 b
N_0S_2	13.67 a	18.67 a	23.33 a	21.67 a
N_1 S_0	5.33 ef	10.00 d	13.33 f	13.00 e
N_1 S_1	9.00 bc	15.67 b	16.33 d	14.33 d
N_1 S_2	10.33 b	16.67 ab	17.67 c	15.99 c
N_2 S_0	4.33 f	8.67 d	11.67 g	11.66 f
N_2 S_1	6.33 de	9.67 d	13.67 ef	12.66 ef
N_2 S_2	7.67 cd	10.33 d	14.67 e	14.34 d
LSD (0.05)	**1.94**	**2.14**	**1.15**	**1.24**
CV (%)	**13.17**	**9.31**	**4.00**	**4.67**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.3 Leaf Area

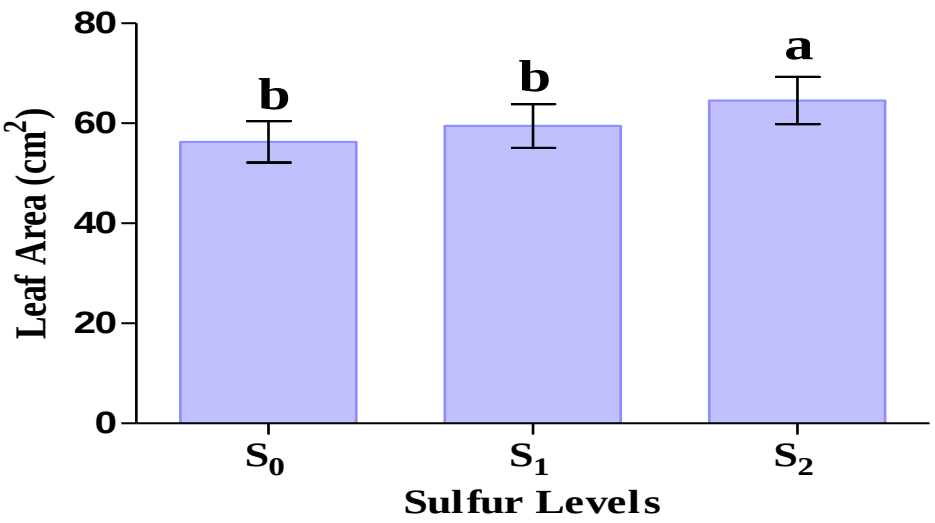
The most immediate response to salt stress was the decrease in the expansion rate of the leaf surface area. The leaf area of rice plants was significantly ($P \leq 0.01$) affected by salinity stress (Figure 5 and Appendix XI). The highest leaf area was observed with control (N_0) treatment while the lowest was observed with N_2 treatment. 20.76% and 32.66% reduction in leaf area was noticed in salt-affected N_1 and N_2 plants respectively when compared to control.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 5. Effect of different salt concentrations on the leaf area of rice ($LSD_{(0.05)}$ = 4.40 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Increasing sulfur fertilizer significantly ($P \leq 0.01$) increased the leaf area of rice plants (5.31% in S_1 and 12.76% in S_2 over control). The highest values of leaf area were produced by the higher level of sulfur (S_2). On the other hand, the lowest values of the leaf area of rice were produced by control (when rice plants did not receive any sulfur fertilizer) without any significant differences with those produced by the sulfur level of S_1 (Figure 6 and Appendix XI).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 6. Effect of different sulfur levels on the leaf area of rice ($LSD_{(0.05)}$ = 4.40 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Statistical analysis has shown that salinity reduced the leaf area of rice plants significantly (Table 5 and Appendix XI). Nevertheless, the sulfur application had a pronounced positive effect on leaf area enhancement as evident from Salinity × Sulfur interaction (Table 5). Leaf area was increased by 6.11% and 16.51% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased leaf area by 5.02% and 14.64% respectively when compared to salt treatment (N_2S_0) alone. Data regarding the leaf area depicted that treatment using S_2 recorded the statistically maximum leaf area which was followed by S_1 treatment at both levels of salinity (N_1 and N_2), however, statistically, all the treatments were at par.

Table 5. Interaction effect of different salt concentrations and sulfur levels on the leaf area, membrane stability index (MSI %) and relative water content (RWC %) of rice

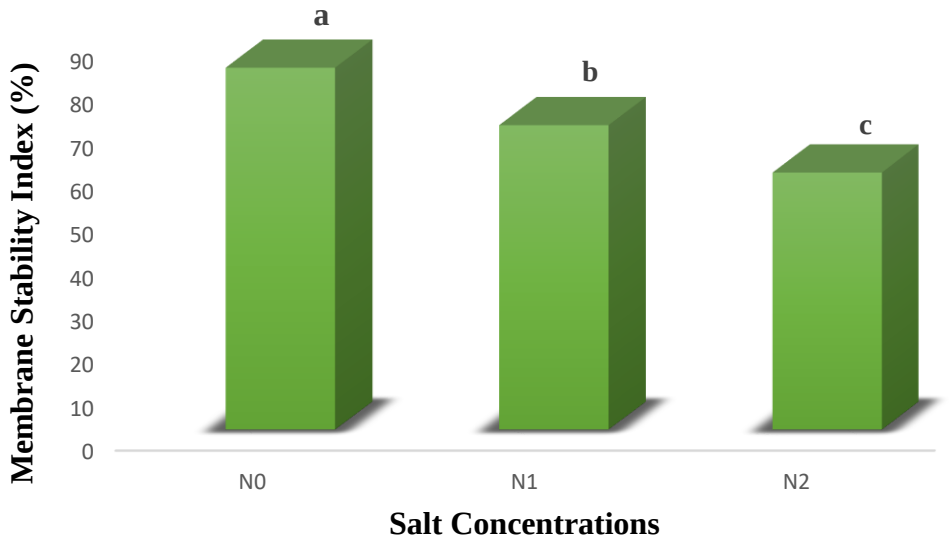
Treatments	Leaf Area (cm^2)	Membrane Stability Index (MSI %)	Relative Water Content (RWC %)
N_0S_0	69.84 ab	77.21 b	83.02 ab
N_0S_1	73.44 a	85.84 a	86.53 a
N_0S_2	76.17 a	87.56 a	89.30 a
N_1 S_0	53.29 d-f	61.31 e	72.72 cd
N_1 S_1	56.76 cd	71.91 c	77.11 bcd
N_1 S_2	63.83 bc	77.78 b	79.30 bc
N_2 S_0	45.83 f	51.25 f	71.78 d
N_2 S_1	48.25 ef	60.91 e	73.19 cd
N_2 S_2	53,69 de	66.19 d	76.67 bcd
LSD $_{(0.05)}$	**7.63**	**2.84**	**6.59**
CV (%)	**7.33**	**2.31**	**4.83**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.4 Leaf Membrane Stability Index (MSI%)

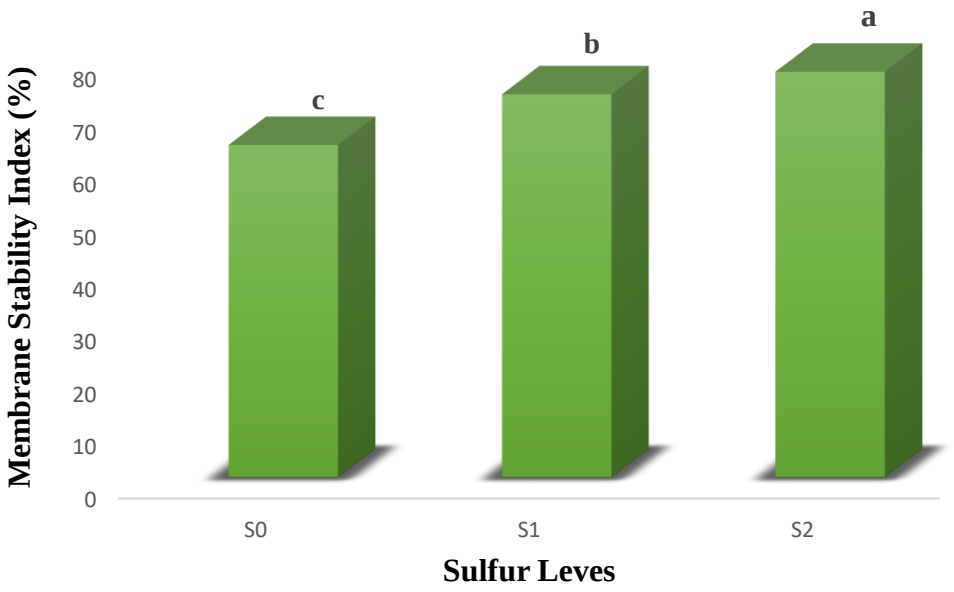
One of the major influence of salt stress include changes in membrane permeability leading to destabilization of membrane proteins. That's why, electrolyte leakage was measured to determine the leaf cell membrane stability index (MSI%). Membrane stability index (MSI%) of plants decreased significantly ($P \leq 0.01$) with the increment of salt stress (Figure 7 and Appendix XI). MSI% decreased by 15.81% and 28.84% under N_1 and N_2 salt stress respectively compared to control. The exposure of rice plants to salt stress reduced the MSI% and higher reduction was found at N_2 treatment.

However, supplied sulfur increased MSI% of rice plants significantly ($P \leq 0.01$) compared untreated plants (Figure 8 and Appendix XI). The application of S_1 and S_2 treatment improved MSI% by 13.21% and 18.04% respectively, when compared to control treatment. Thus, both levels of sulfur (S_1 and S_2) were proved beneficial in improving the MSI % of rice plants.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 7. Effect of different salt concentrations on the membrane stability index (MSI%) of rice ($LSD_{(0.05)}$ = 1.64 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



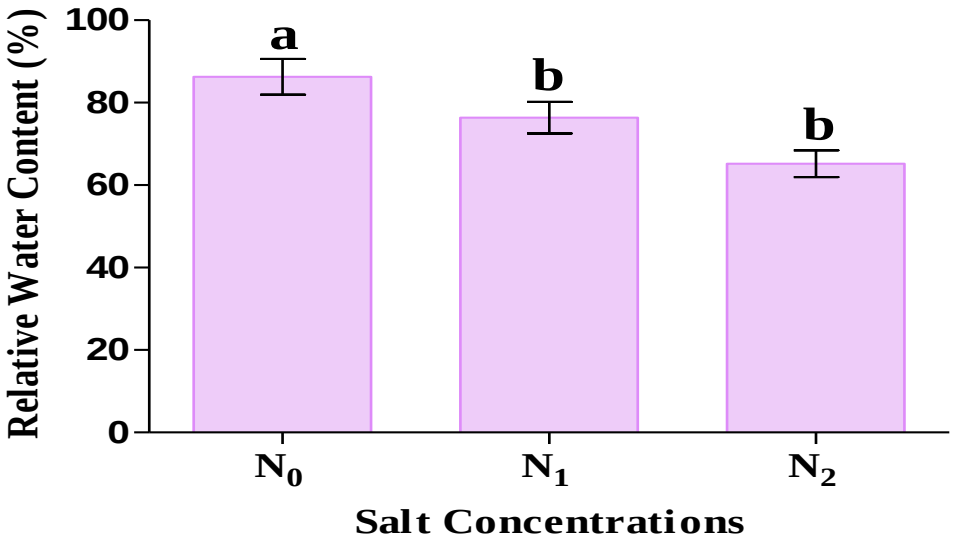
S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 8. Effect of different sulfur levels on the membrane stability index (MSI%) of rice ($LSD_{(0.05)}$ = 1.64 and bars with different letters are significantly different at $p \leq$ 0.05 applying LSD)

The differences in membrane stability index (MSI%) between two sulfur treatments were significant ($P \leq 0.05$) for both level of salinity (N_1 and N_2). In other words, the interaction effect of Salinity × Sulfur treatment had significant effect on membrane stability index (MSI%) which was evident from statistically significant Salinity × Sulfur interactive effect (Table 5 and Appendix XI). Sulfur supplementation at the rate of S_1 was sufficient to improve MSI% significantly, by 14.74% and 15.86% for both level of salinity (N_1 and N_2 respectively) when compared to salt treatment alone. Further, higher sulfur application (S_2) increased MSI% for both level of salinity (N_1 and N_2) by 21.18% and 22.57% respectively compared to sole salt treatment without sulfur. Thus, the results of interaction corresponding to membrane stability index (MSI%) provided that membrane stability index reached its maximum values when rice plants were treated with S_2 treatment.

4.1.5 Relative Water Content (RWC%)

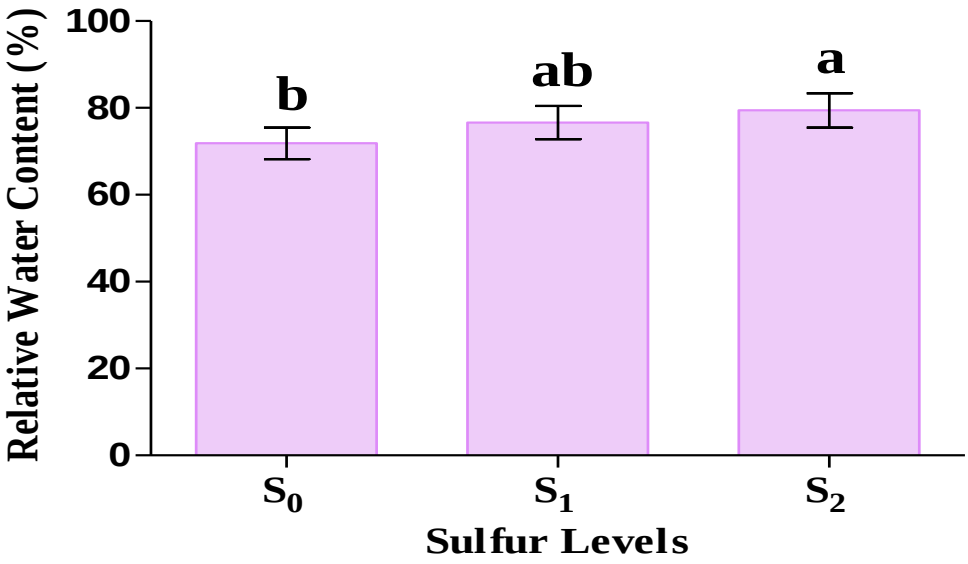
A characteristic symptom of salt stressed rice plants was tissue dehydration and that was exhibited as leaf relative water content (RWC %) reduction, compared with non-salt stress treatment (Figure 9 and Appendix XI). Increased salinity level caused significant ($P \leq 0.01$) reduction in leaf relative water contents (RWC%). RWC% was reduced from 86.28% in the control plants to 76.38% and 73.88% in the plants treated with N_1 and N_2 level of salinity respectively.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 9. Effect of different salt concentrations on the relative water content (RWC %) of rice ($LSD_{(0.05)}$ = 3.81 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Sulfur addition to rice plants resulted in enhancement of the leaf relative water content (RWC %) significantly ($P \leq 0.01$). With an increase of sulfur supply RWC% was increased from 75.84% in the control plants to 78.94% and 81.76% in the S_1 and S_2 treated plants respectively (Figure 10 and Appendix XI). But no significant difference was observed between the treatment S_1 and S_2.



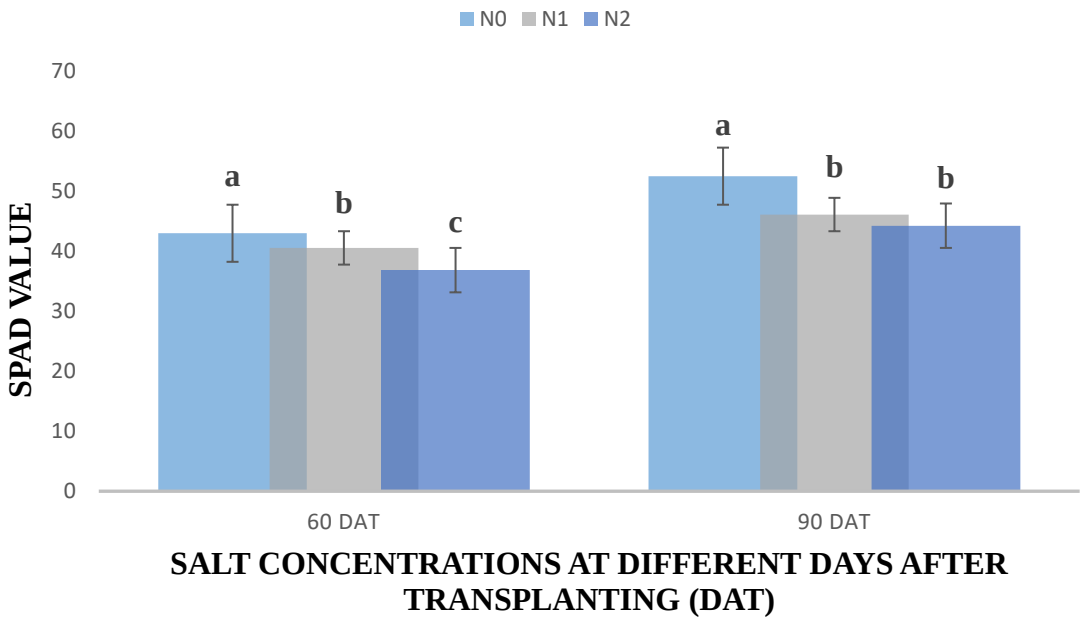
S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 10. Effect of different sulfur levels on the relative water content (RWC %) of rice ($LSD_{(0.05)}$ = 3.81 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Relative water content (RWC %) in leaf was greatly influenced by salinity levels whereas sulfur application (S_1 and S_2) improved the RWC % at both levels of salinity (N_1 and N_2). Salinity × Sulfur interactive effect supported this finding (Table 5 and Appendix XI). Under N_1 salt stressed condition, RWC % increased by 5.69% at S_1 (N_1 S_1) in comparison with N_1 salt treatment alone (N_1 S_0) whilst it was 9.05% at S_2 treatment (N_1 S_2). Whereas, under N_2 treated condition, an increase of 9.68% and 14.19% in RWC % was observed at S_1 and S_2 treatment (N_2 S_1 and N_2 S_2) respectively compared to N_2 S_0 treatment where no sulfur was added. This result suggest that additional sulfur supply ensures more water content in leaf under salt stress condition. Though there were no statistically significant differences among mean values, a significant trend to increase in relative water content with increasing sulfur level was noticeable.

4.1.6 Chlorophyll Content (SPAD Value)

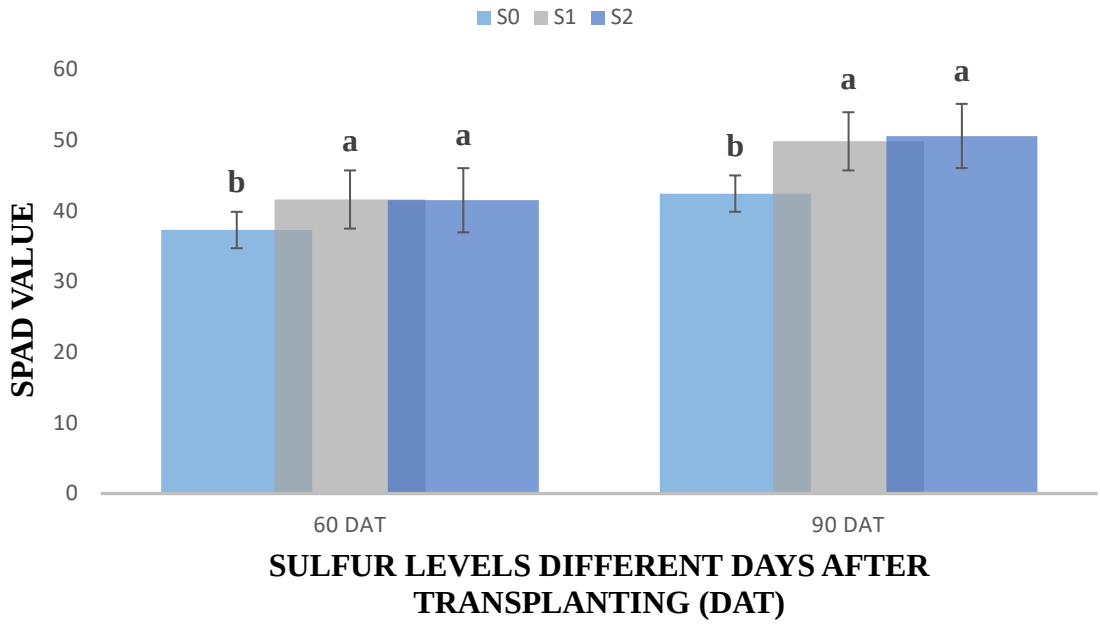
Chlorophyll meter (SPAD) is a convenient tool to estimate the absolute values of chlorophyll per unit leaf area. Salt stress caused a substantial decline ($P \leq 0.01$) for leaf chlorophyll content (Figure 11 and Appendix XII). Leaf chlorophyll content (SPAD) was high in the control both at 60 DAT and 90 DAT. Under N_1 salinity stress, SPAD values reduced by 5.68% and 12.18% at 60 DAT and 90 DAT respectively when compared to unstressed control. But under N_2 salinity stress, there was a 14.29% and 15.72% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. The percent reduction of total chlorophyll content proportionally increased with the increase of salinity.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 11. Effect of different salt concentrations on the chlorophyll content (SPAD value) of rice at different days after transplanting ($LSD_{(0.05)}$ = 1.63 and 2.01 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

When supplemental sulfur was applied, the improvements in leaf chlorophyll content (SPAD) were significant ($P \leq 0.01$). S_1 and S_2 application increased leaf chlorophyll content (SPAD) by 10.39% and 10.15% respectively at 60 DAT, whereas at 90 DAT it was by 14.85% and 16.12% respectively when compared to control (Figure 12 and Appendix XII). In comparison to the control group, leaf chlorophyll content (SPAD) was significantly increased all the way to the level of S_1 and S_2 treatment, while there was no further significant difference between these two treatments.



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 12. Effect of different sulfur levels on the chlorophyll content (SPAD value) of rice at different days after transplanting (LSD $_{(0.05)}$ = 1.63 and 2.01 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Decrement of leaf chlorophyll content (SPAD) might suggest that salt stress enhanced the degradation of chlorophyll. Nevertheless, the Salinity × Sulfur interaction significantly ($P \leq 0.01$) increased SPAD values compared to the saline conditions alone (Table 6 and Appendix XII). At 60 DAT and 90 DAT, under N_1 salinity, the leaf chlorophyll content (SPAD) increment at S_1 (N_1 S_1) was 9.84% and 10.73% respectively while at S_2 (N_1 S_2) it was 13.12% and 3.18% respectively when compared to salt treatment (N_1 S_0) alone. On the other hand, at 60 DAT and 90 DAT under N_2 salinity, leaf chlorophyll content (SPAD) increased by 18.13% and 21.71% at S_1 treatment (N_2 S_1) while it was 21.59% and 24.18% at S_2 treatment (N_2 S_2) respectively when compared to salt treatment (N_2 S_0) alone.

Table 6. Interaction effect of different salt concentrations and sulfur levels on the chlorophyll content (SPAD value) of rice at different days after transplanting

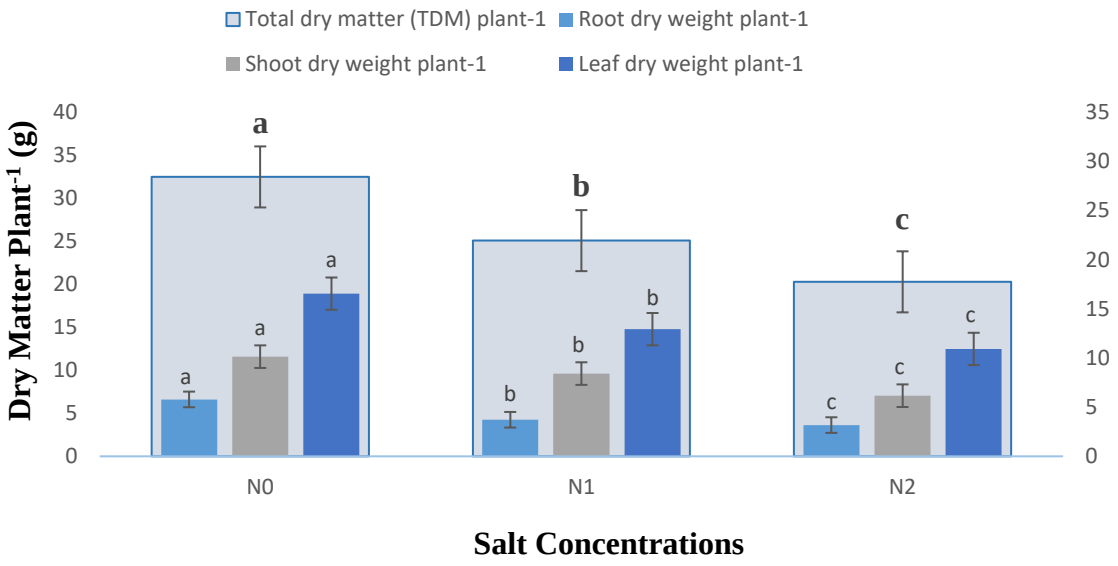
Treatments	SPAD Value	
	60 DAT	90 DAT
N_0S_0	42.90 ab	46.50 c-e
N_0S_1	44.80 a	53.20 b
N_0S_2	41.20 bc	57.73 a
N_1S_0	37.30 d	43.86 e
N_1S_1	41.37 bc	49.13 c
N_1S_2	42.93 ab	45.30 de
N_2S_0	31.60 e	36.90 f
N_2S_1	38.60 cd	47.13 c-e
N_2S_2	40.30 bc	48.67 cd
LSD $_{(0.05)}$	**2.83**	**3.49**
CV (%)	**4.07**	**4.23**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.7 Dry Weights of Root, Shoot, Leaf and Total Dry Matter (TDM) plant^{-1}

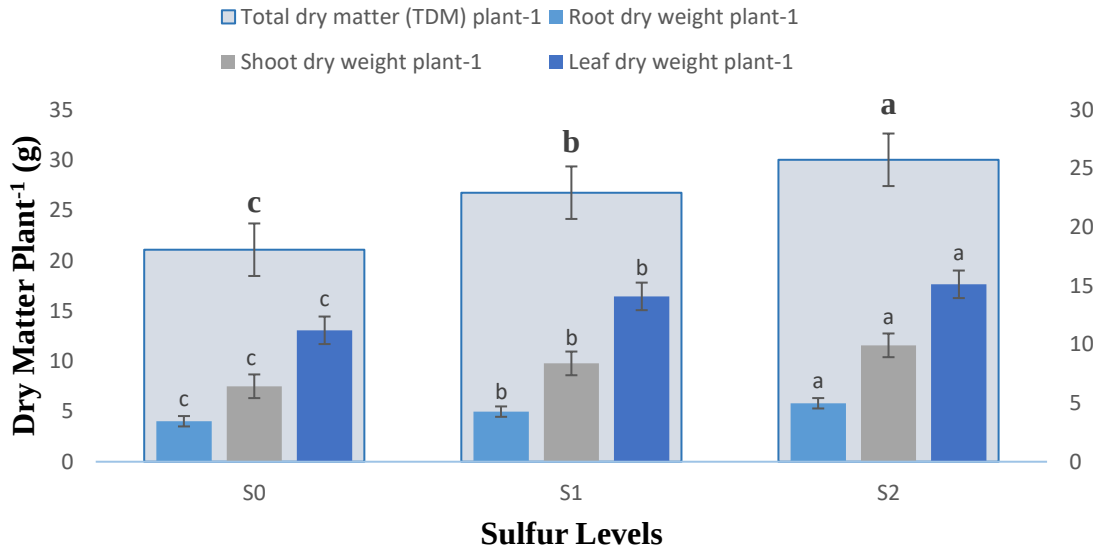
Dry matter estimation is regarded as a valuable index for monitoring vegetative growth of the rice plant (Hakim *et al*., 2014b). Total dry matter (TDM) is defined as the sum total of root, shoot, and leaf dry weight. The total dry matter (TDM) was significantly ($P \leq 0.01$) influenced under different levels of salinity (Figure 13 and Appendix XIII). Root, shoot, leaf dry weights, and TDM decreased by 35.75%, 16.96%, 21.81%, and 22.81% respectively in salt-stressed plants (N_1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with N_2 salt stress was 45.08%, 39.15%, 33.96%, and 37.55% respectively when compared to control plants without salt stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at N_2 treatment.

The supplementation of sulfur revealed a significant ($P \leq 0.01$) increment in root, shoot, leaf dry weights, and TDM of rice plants (Figure 14 and Appendix XIII). 19.2%, 23.27%, 20.51%, and 21.2% increment in the root, shoot, leaf dry weights, and TDM were noticed respectively in S_1 treated plants, when compared to control plants that did not receive any sulfur. Root, shoot, leaf dry weights, and TDM of S_2 treated plants increased by 30.72%, 35.18%, 25.93%, and 29.78% respectively compared to control. A maximum increase in root, shoot, leaf dry weights, and TDM was observed at S_2 treatment.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 13. Effect of different salt concentrations on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice (LSD $_{(0.05)}$ = 0.29, 0.59, 0.73 and 1.06 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 14. Effect of different sulfur levels on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice (LSD $_{(0.05)}$ = 0.29, 0.59, 0.73 and 1.06 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Salt stress decreased the deposit of dry materials. Reduction in total dry matter production under the saline condition and the positive effect of sulfur on total dry matter production are presented in Table 7 and Appendix XIII. The use of sulfur under salt stress revealed a significant ($P \leq 0.01$) enhancement in the root, shoot, leaf dry weights, and TDM. It was revealed from statistically significant Salinity × Sulfur interaction (Table 5). S_1 and S_2 supplementation during N_1 salt stress increased root dry weight plant^{-1} by 10.27% and 19.81% respectively; shoot dry weight plant^{-1} by 11.58% and 23.96% respectively; leaf dry weight plant^{-1} by 17.35% and 19.54% respectively and total dry matter (TDM) plant^{-1} by 14.46% and 21.12% respectively (compared to salt-affected plants without sulfur application). Root, shoot, leaf dry weights and TDM increased by 19.11%, 49.39%, 40.11% and 39.87% respectively after S_1 supplementation and 34.37%, 61.82%, 42.25% and 47.72% after S_2 supplementation of salt-stressed (N_2) plants (compared to salt treatment alone). However, S_2 supplementation was noticed as the most effective dose for the increment of plant root, shoot, leaf dry weights, and TDM under both levels of salinity. Furthermore, the parameters of salt-stressed plants receiving supplementary sulfur were still lower than the values of non-salt stress treatment.

Table 7. Interaction effect of different salt concentrations and sulfur levels on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice

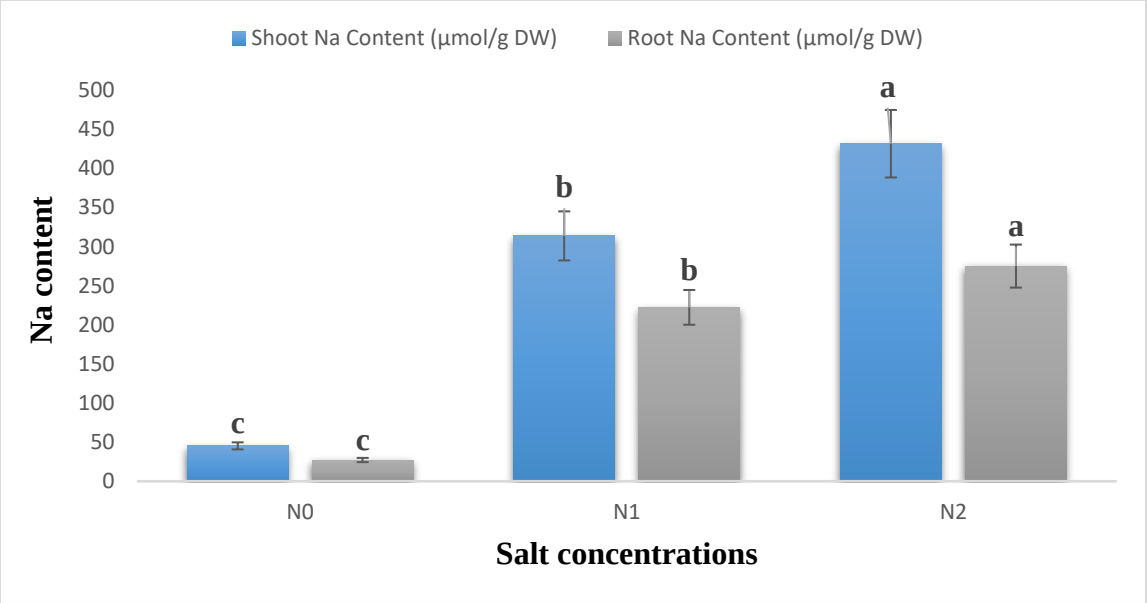
Treatments	Root dry weight plant^{-1} (g)	Shoot dry weight plant^{-1} (g)	Leaf dry weight plant^{-1} (g)	Total dry matter (TDM) plant^{-1} (g)
N_0S_0	4.49 c	8.65 cd	14.92 c	28.06 c
N_0S_1	5.97 b	10.32 b	16.25 b	32.54 b
N_0S_2	6.92 a	11.46 a	18.48 a	36.86 a
N_1 S_0	3.32 ef	7.33 ef	11.24 f	21.89 e
N_1 S_1	3.70 de	8.29 de	13.60 de	25.59 d
N_1 S_2	4.14 cd	9.64 bc	13.97 cd	27.75 c
N_2 S_0	2.54 g	3.31 g	7.45 g	13.30 f
N_2 S_1	3.14 f	6.54 f	12.44 ef	22.12 e
N_2 S_2	3.87 d	8.67 cd	12.90 de	25.44 d
LSD $_{(0.05)}$	**0.49**	**1.03**	**1.26**	**1.83**
CV (%)	**6.78**	**7.23**	**5.40**	**4.07**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.8 Na Content in Roots and Shoots

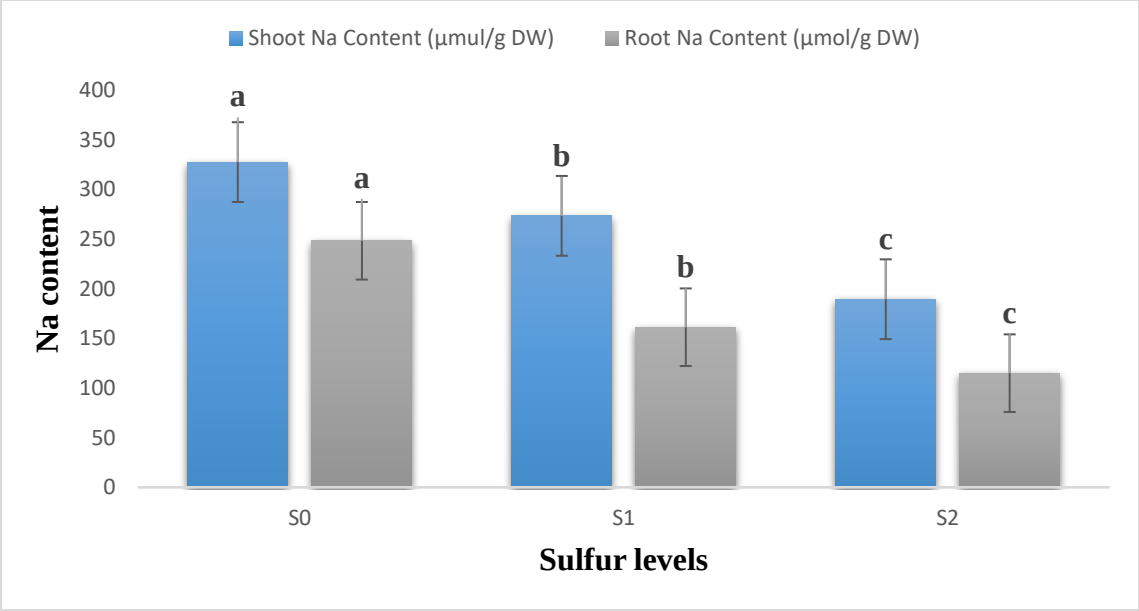
Increase in salt concentrations showed a uniform increase in Na content in the roots and shoots of stressed plants compared to control (Figure 15 and Appendix XIV). Shoot and root Na content increased by 85.57% and 87.78% respectively in salt-stressed plants (N_1) when compared to unstressed control. Further, a dramatic increment of Na content of shoot and root with N_2 salt stress treatment was 89.51% and 90.13% respectively when compared to control plants without salt stress.

The supplementation of sulfur revealed a significant ($P \leq 0.01$) decrease in root and shoot Na content of rice plants (Figure 16 and Appendix XIV). 16.52% and 35.06% reduction in the shoot and root Na content were noticed respectively in S_1 treated plants, when compared to control plants that did not receive any sulfur. Shoot and root Na content of S_2 treated plants decreased by 42.16% and 53.69% respectively compared to control. A maximum decrease in shoot and root Na content was observed at S_2 treatment.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 15. Effect of different salt concentrations on the Na content in shoots and roots of rice ($LSD_{(0.05)}$ = 4.72 and 3.73 for Na content in shoots and roots, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



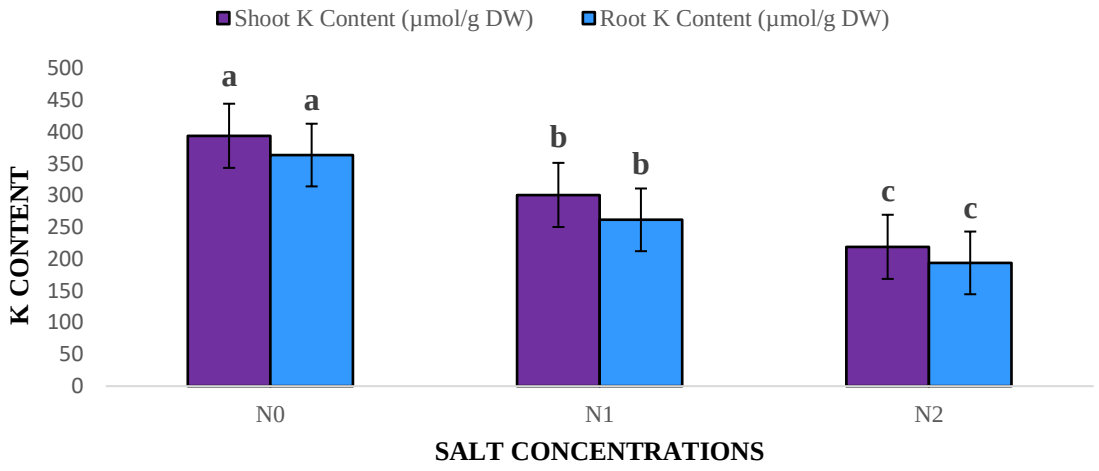
S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 16. Effect of different sulfur levels on the Na content in shoots and roots of rice ($LSD_{(0.05)}$ = 4.72 and 3.73 for Na content in shoots and roots, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

The protective role of sulfur against salt stress was examined by determining the Na content in roots and shoots of rice plants. Salt treatment resulted in a marked increase in Na contents in the roots and shoots when compared to control plants. However, the application of sulfur significantly decreased the Na content in root and shoot. It was revealed from statistically significant Salinity × Sulfur interaction (Table 8 and Appendix XIV). S_1 and S_2 supplementation during N_1 salt stress decreased shoot Na content by 21.19% and 48.59% respectively; root Na content by 32.77% and 57.91% respectively (compared to salt-affected plants without sulfur application). Shoot and root Na content decreased by 14.67% and 40.36% respectively after S_1 supplementation and 42.07% and 55.25% after S_2 supplementation of salt-stressed (N_2) plants (compared to salt treatment alone).

4.1.9 K Content in Roots and Shoots

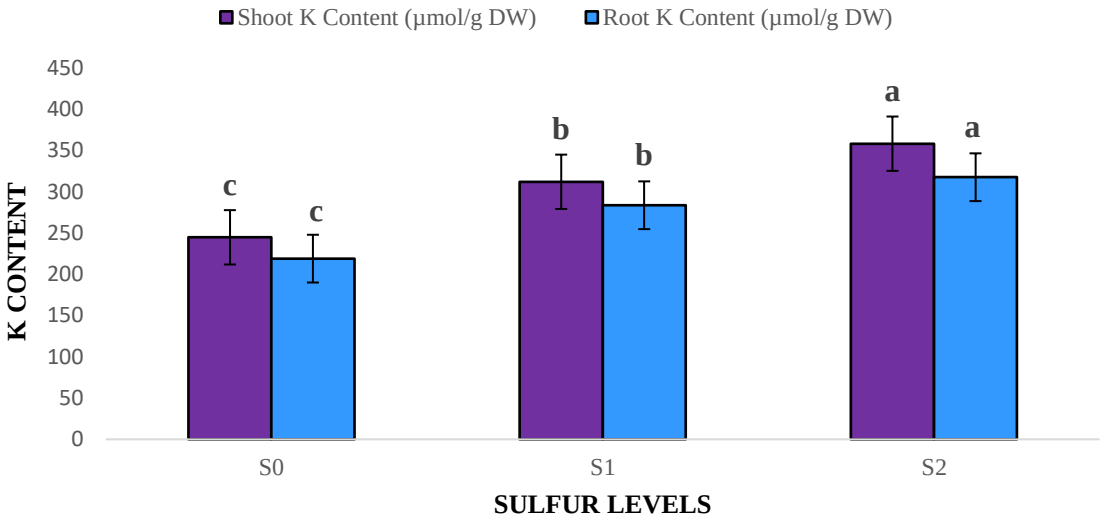
The levels of K in the roots and shoots, decreased significantly ($P \leq 0.01$) under salt stress. A gradual decrease of K content in shoot and root were observed with the increase in salinity concentration (Figure 17 and Appendix XIV) and it was 23.62% and 28.03% with the treatment N_1 and 44.35% and 46.66% with the treatment N_2 respectively compared to non-salt stress control.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 17. Effect of different salt concentrations on the K content in shoots and roots of rice ($LSD_{(0.05)}$ = 4.37 and 3.68 for K content in shoots and roots, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

The application of sulfur significantly ($P \leq 0.01$) increased K level in the shoots and roots (Figure 18 and Appendix XIV) and these increases were 21.55% and 22.81% in S_1 and 31.66% and 31.06% in S_2 respectively compared to control.



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 18. Effect of different sulfur levels on the K content in shoots and roots of rice ($LSD_{(0.05)}$ = 4.37 and 3.68 for K content in shoots and roots, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

K content in the roots and shoots significantly ($P \leq 0.01$) responded to sulfur levels under salt stress to increase the above-mentioned trait. A statistically significant Salinity × Sulfur interaction supported this finding (Table 8 and Appendix XIV). K content in shoots were increased by 28.8% and 41.64% and K content in roots were increased by 30.89% and 33.73% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased the K content in shoots by 43.92% and 57.72% and K content in roots by 47.94% and 59.95% respectively when compared to salt treatment (N_2S_0) alone.

Table 8. Interaction effect of different salt concentrations and sulfur levels on the Na and K content in the shoots and roots of rice

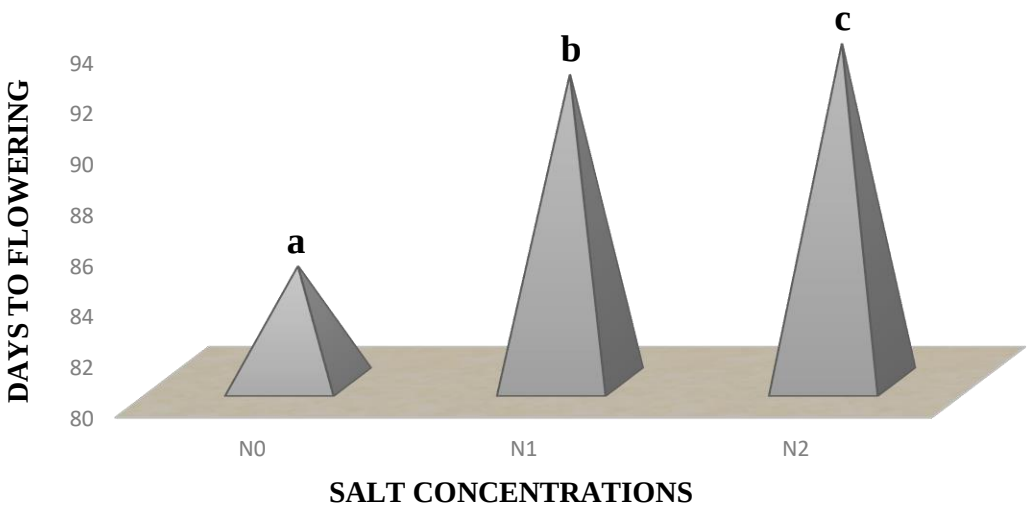
Treatments	Na Content (µmol/g DW) Shoot	Na Content (µmol/g DW) Root	K Content (µmol/g DW) Shoot	K Content (µmol/g DW) Root
N_0S_0	41.78 g	22.58 h	386.90 b	350.54 b
N_0S_1	44.11 g	28.80 gh	399.77 a	356.81 b
N_0S_2	49.95 g	30.14 g	396.03 a	384.37 a
N_1 S_0	408.90 c	318.70 b	219.37 f	198.62 g
N_1 S_1	322.27 d	214.25 d	308.12 d	287.38 d
N_1 S_2	210.22 f	134.15 f	375.89 c	299.72 c
N_2 S_0	532.37 a	403.89 a	127.84 g	107.48 h
N_2 S_1	454.25 b	240.87 c	227.96 e	206.44 f
N_2 S_2	308.39 e	180.76 e	302.36 d	268.37 e
LSD $_{(0.05)}$	8.18	6.46	7.56	6.38
CV (%)	1.79	2.13	1.43	1.35

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.10 Days to Flowering

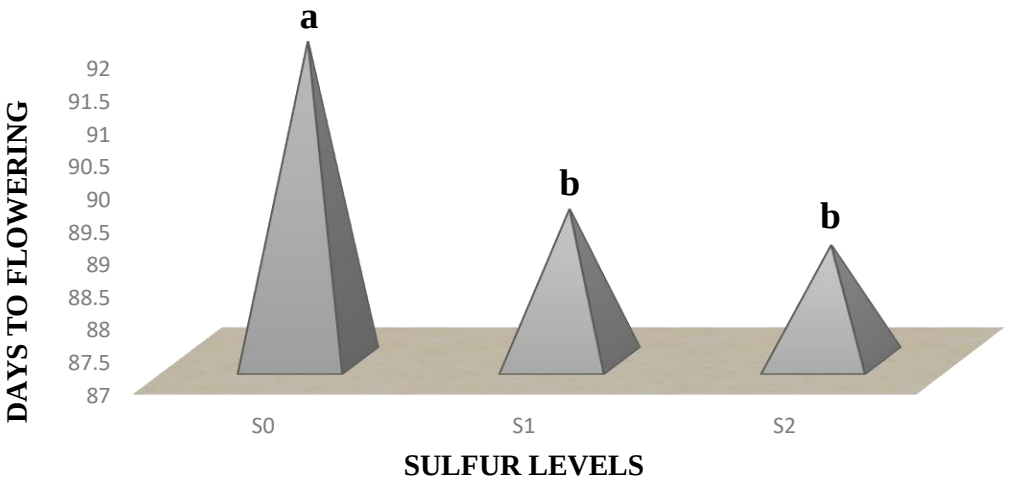
The deleterious effects of salt stress in rice include delay in flowering. Days to flowering among the treatments ranged from 84.56 to 93.33 days where treatment N_0 (control) took the lowest days to flowering (84.56 days) and treatment N_2 took the highest days to flowering (93.33 days). A gradual delay in days to flowering was observed with the

increase in salinity concentration and it was 8.19% and 9.39% with the treatment N_1 and N_2 respectively compared to control condition (Figure 19 and Appendix XV).



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 19. Effect of different salt concentrations on the days to flowering of rice (LSD $_{(0.05)}$ = 1.89 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

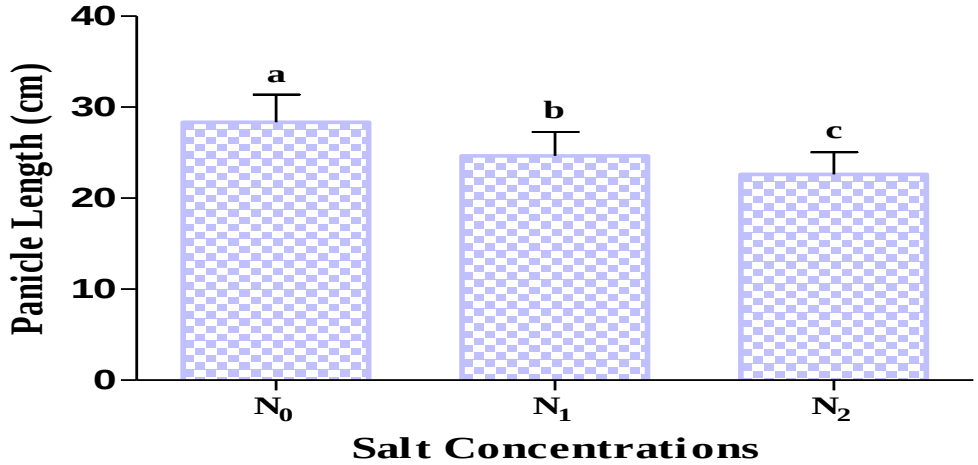
Figure 20. Effect of different sulfur levels on the days to flowering of rice (LSD $_{(0.05)}$ = 1.89 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

In case of the effect of sulfur treatment, it was desired that control treatment will give the maximum days to flowering. Days to flowering were highest in the control treatment (91.89 days) followed by treatments S_1 (89.33 days) and S_2 (88.78 days). So, increasing sulfur fertilizer significantly ($P \leq 0.01$) decreased the days to flowering of rice (2.79% in S_1 and 3.38% in S_2 over control) (Figure 20 and Appendix XV).

The days required to flowering were much earlier in control than salt-treated rice plants, which indicates that salt stress can decelerate the development of the plant. However, sulfur supplementation reduced the delay in flowering with a significant ($P \leq 0.01$) interaction between Salinity × Sulfur treatment (Table 9 and Appendix XV). Days to flowering was decreased by 2.15% and 0.72% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) decreased the days to flowering by 1.41% and 1.76% respectively when compared to salt treatment (N_2S_0) alone. However, statistically, all the treatments were at par.

4.1.11 Panicle Length

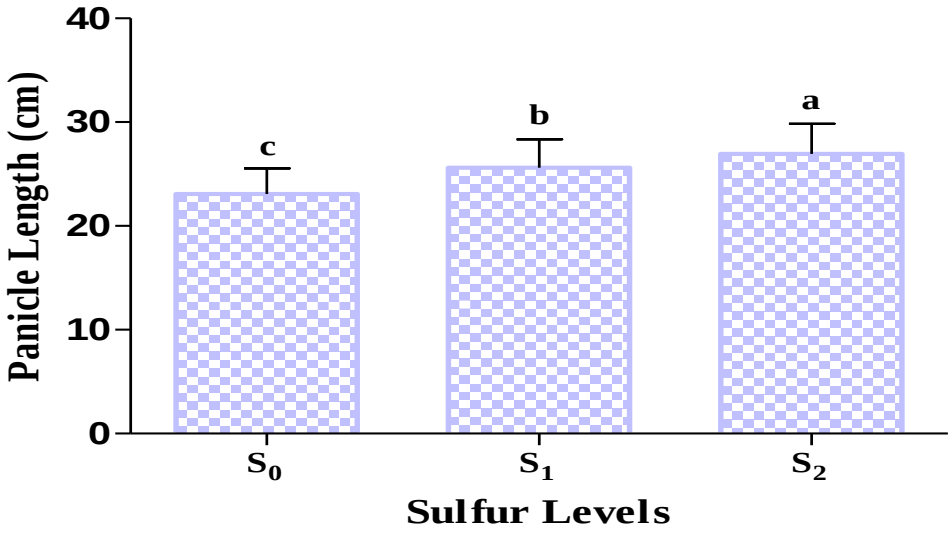
As higher panicle length could provide a higher number of grains, so panicle length is regarded as an important yield contributing character. Results revealed that the panicle length of rice was also significantly ($P \leq 0.01$) affected by various levels of salinity (Figure 21 and Appendix XV). Here, salt stress caused about 13.02% and 20.15% downfall in the length of panicle with N_1 and N_2 treatments respectively when compared to control plants without salt stress.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 21. Effect of different salt concentrations on the panicle length of rice ($LSD_{(0.05)}$ = 0.94 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Panicle length increased significantly ($P \leq 0.01$) after the supplementation of sulfur (Figure 22 and Appendix XV). Panicle length of sulfur treated plants increased by 9.84% and 14.39% with S_1 and S_2 treatments respectively compared to control plants that did not receive any sulfur. The application of both S_1 and S_2 increased the panicle length but the increment in panicle length was more pronounced in S_2 treatment.



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 22. Effect of different sulfur levels on the panicle length of rice ($LSD_{(0.05)}$ = 0.94 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

A marked decrease in panicle length by salt stress (N_1 and N_2) was found in rice plants but the application of sulfur (S_1 and S_2) improved the panicle length at both levels of salinity which was evident from Salinity × Sulfur interaction (Table 9 and Appendix XV). Percent increase of panicle length in the treatment N_1S_1 and N_1S_2 was 11.48% and 15.04% respectively, indicating a remarkable difference with N_1S_0 where no sulfur was applied. Whereas, it was 13.57% and 17.74% with N_2S_1 and N_2S_2 respectively when compared with N_2S_0 with salt treatment only. However, S_2 treatment was more efficient than S_1 treatment though both treatments were statistically at par.

Table 9. Interaction effect of different salt concentrations and sulfur levels on the days to flowering, panicle length, spikelet fertility % of rice

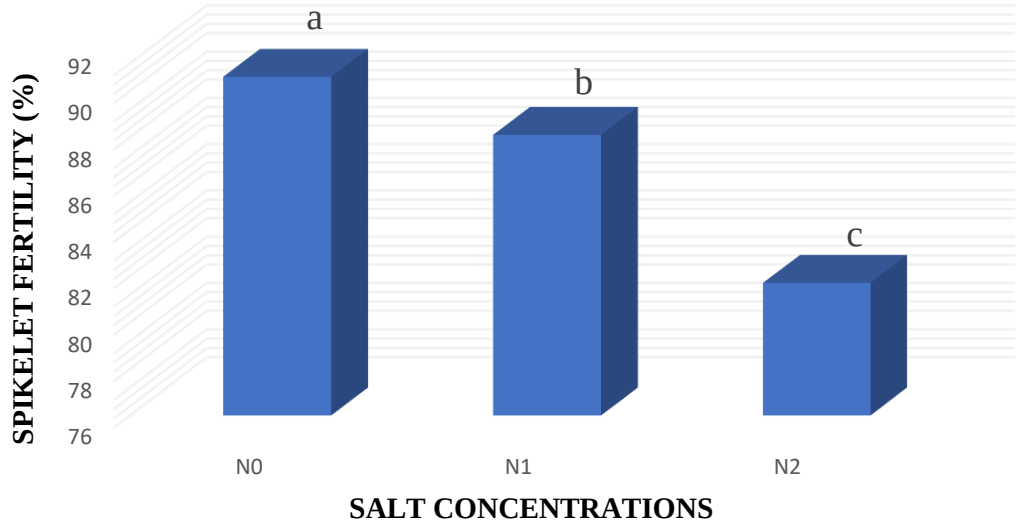
Treatments	Days to Flowering	Panicle Length (cm)	Spikelet Fertility %
N_0S_0	88.33 c	26.73 bc	86.33 d
N_0S_1	84.00 d	28.23 b	90.97 b
N_0S_2	81.33 e	30.07 a	94.57 a
N_1 S_0	93.00 ab	22.37 f	78.02 e
N_1 S_1	91.00 b	25.27 cd	91.34 b
N_1 S_2	92.33 ab	26.33 c	94.98 a
N_2 S_0	94.33 a	20.13 g	71.93 f
N_2 S_1	93.00 ab	23.29 ef	84.56 d
N_2 S_2	92.67 ab	24.47 de	88.63 c
LSD $_{(0.05)}$	**2.06**	**1.63**	**2.26**
CV (%)	**1.32**	**3.75**	**1.50**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.12 Spikelet Fertility

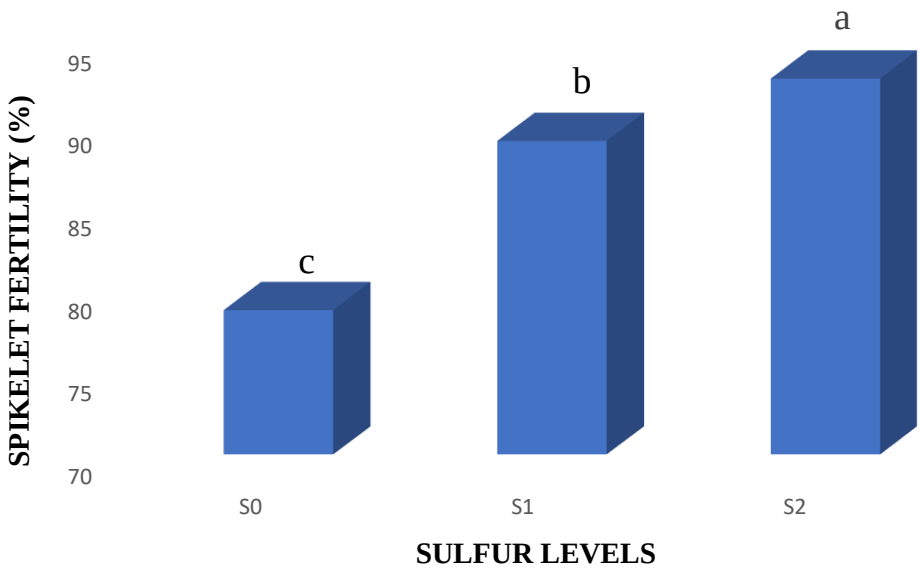
Spikelet fertility% is an important yield contributing character as fertile grain is directly related to grain yield. Spikelet fertility was significantly ($P \leq 0.01$) influenced by salinity level (Figure 23, Appendix XV and Plate VII). 2.77% and 9.83% reduction of spikelet fertility was observed due to N_1 and N_2 salinity compared to control.

Exogenous sulfur significantly ($P \leq 0.01$) increased fertility percentage in rice (Figure 24, Appendix XV). S_1 and S_2 supplementation increased spikelet fertility% by 11.47% and 15.1% respectively compared to control plants without sulfur application.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 23. Effect of different salt concentrations on the spikelet fertility% of rice (LSD $_{(0.05)}$ = 1.30 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)



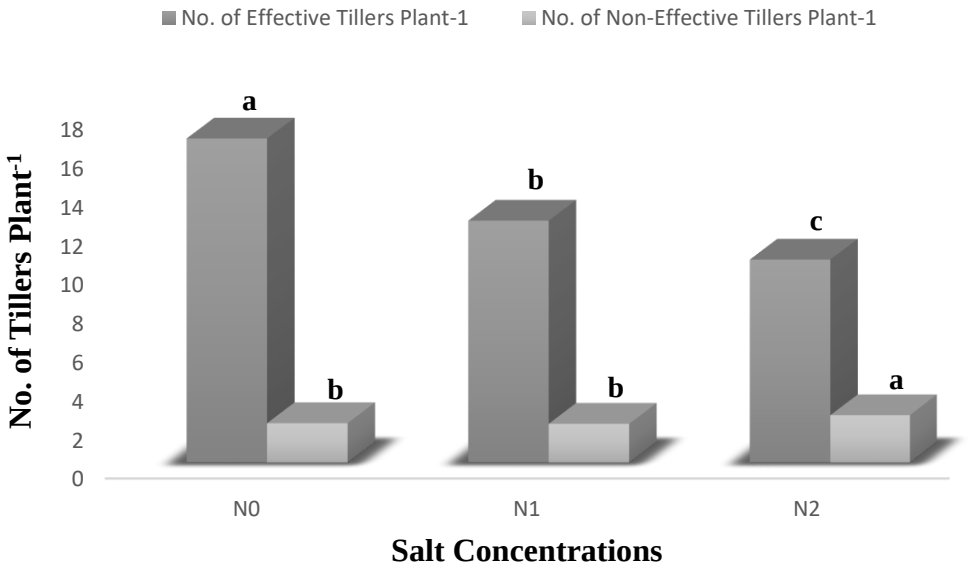
S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 24. Effect of different sulfur levels on the spikelet fertility% of rice (LSD $_{(0.05)}$ = 1.30 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Statistical analysis has shown that salt stress reduced the fertility percentage of grains. Nevertheless, sulfur application improved the fertility percentage under salt stress condition. A significant ($P \leq 0.01$) Salinity × Sulfur interactive effect supported this finding (Table 9 and Appendix XV). Spikelet fertility% was increased by 14.58% and 17.86% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased fertility% by 14.94% and 18.84% respectively when compared to salt treatment (N_2S_0) alone.

4.1.13 No. of Effective Tillers Plant^{-1}

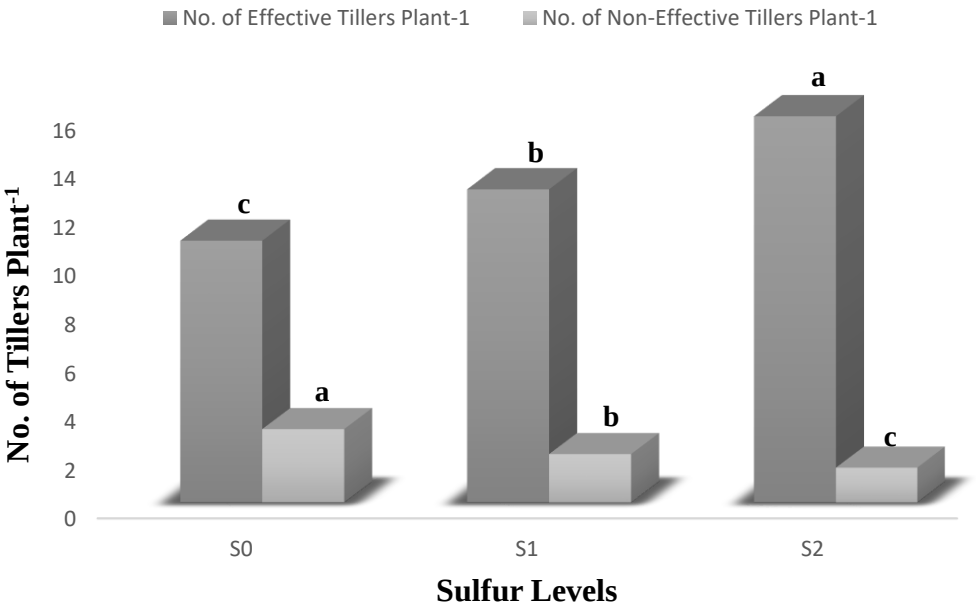
The yield of rice plants is mostly dependent upon the number of effective tillers i.e., panicle-bearing tillers plant^{-1}. Rice plants were significantly ($P \leq 0.01$) influenced by salt stress in terms of effective tiller production. No. of effective tillers plant^{-1} gradually decreased with increased levels of salinity (Figure 25 and Appendix XVI). In case of N_1 and N_2 treatment, 25.37% and 37.37% reduction of effective tillers was observed respectively compared to non-salt stress condition (control).



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 25. Effect of different salt concentrations on the number of effective and non-effective tillers plant^{-1} of rice (LSD $_{(0.05)}$ = 0.68 and 0.15 for number of effective and non-effective tillers, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

No. of effective tillers plant^{-1} increased significantly ($P \leq 0.01$) after sulfur supplementation (Figure 26 and Appendix XVI). The no. of effective tillers plant^{-1} increased by 16.4% and 32.16% due to S_1 and S_2 supplementation respectively compared to control (where no sulfur was applied).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 26. Effect of different sulfur levels on the number of effective and non-effective tillers plant^{-1} of rice (LSD $_{(0.05)}$ = 0.68 and 0.15 for number of effective and non-effective tillers, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Salt stress greatly affected the development and viability of tillers plant^{-1} but it was not surprising that supplementary sulfur application increased this parameter significantly ($P \leq 0.05$) under salt stress condition. It was evident from statistically significant Salinity × Sulfur interaction (Table 10 and Appendix XVI). Under N_1 salinity, the no. of effective tillers plant^{-1} increment at S_1 (N_1 S_1) was 16.22% while at S_2 (N_1 S_2) it was 29.58% when compared to salt treatment (N_1 S_0) alone. On the other hand, under N_2 salinity, the no. of effective tillers plant^{-1} increased by 19.36% at S_1 treatment (N_2 S_1) while it was 34.25% at S_2 treatment (N_2 S_2) when compared to salt treatment (N_2 S_0) alone.

4.1.14 No. of Non-Effective Tillers Plant^{-1}

Less number of non-effective tillers plant^{-1} is a positive attribute towards higher grain yield. But both levels of salinity increased the no. of non-effective tillers plant^{-1} significantly ($P \leq 0.01$). The no. of non-effective tillers plant^{-1} ranged from 2.00 to 2.44. N_2 treatment had the highest no. (2.44) of non-effective tillers plant^{-1} and N_1 had the lowest no. (2.00) of non-effective tillers plant^{-1} (Figure 25 and Appendix XVI). There was no significant difference between N_1 treatment and control.

In comparison to the control group, the no. of non-effective tillers plant^{-1} reduced significantly ($P \leq 0.01$) to the sulfur level of S_1 and S_2 (Figure 26 and Appendix XVI). In comparison with the control, the no. of non-effective tillers plant^{-1} decreased at these sulfur concentrations by 33.99% and 52.48% respectively.

The no. of non-effective tillers plant^{-1} increased with both levels of salinity but the application of sulfur decreased this parameter which was evident from statistically significant Salinity × Sulfur interaction (Table 10 and Appendix XVI). The no. of non-effective tillers plant^{-1} decreased by 25.09% and 50.18% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) decreased by 30.03% and 49.85% respectively when compared to salt treatment (N_2S_0) alone. Here, S_2 supplementation was found more effective than S_1 supplementation.

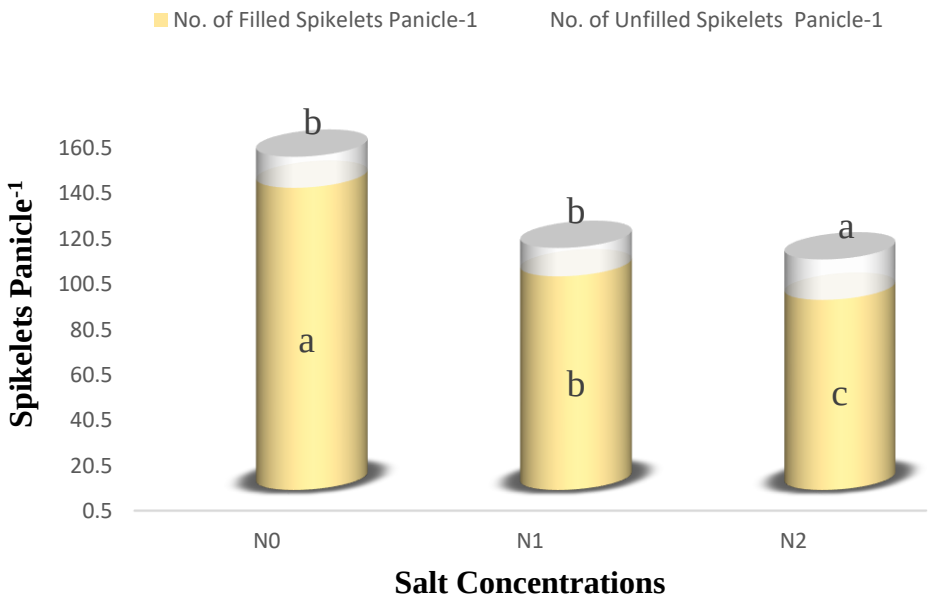
Table 10. Interaction effect of different salt concentrations and sulfur levels on the number of effective and non-effective tillers plant^{-1} of rice

Treatments	No. of Effective Tillers Plant^{-1}	No. of Non-Effective Tillers Plant^{-1}
N_0S_0	13.67 cd	3.10 a
N_0S_1	16.00 b	1.67 e
N_0S_2	20.33 a	1.33 f
N_1 S_0	10.33 f	2.67 b
N_1 S_1	12.33 e	2.00 d
N_1 S_2	14.67 c	1.33 f
N_2 S_0	8.33 g	3.33 a
N_2 S_1	10.33 f	2.33 c
N_2 S_2	12.67 de	1.67 e
LSD $_{(0.05)}$	**1.18**	**0.26**
CV (%)	**5.19**	**6.96**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.15 No. of Filled Spikelets Panicle^{-1}

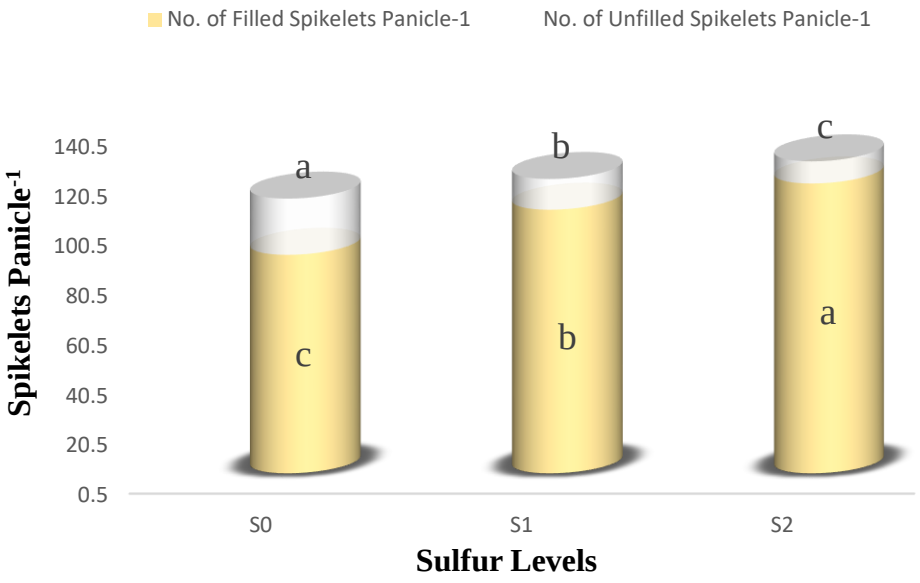
Rice grain yield is closely related to the number of filled grains panicle^{-1}. No. of filled grains panicle^{-1} decreased significantly ($P \leq 0.01$) with the increase of salinity (Figure 27 and Appendix XVII). The highest number of filled grain panicle^{-1} was counted at control condition and the lowest number of filled grain per panicle was recorded at the N_2 level of salinity. A decrease of 29.02% and 36.72% number of filled grains panicle^{-1} was observed with N_1 and N_2 level of salinity respectively compared to control (no salinity).



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 27. Effect of different salt concentrations on the number of filled and unfilled spikelets panicle^{-1} of rice (LSD $_{(0.05)}$ = 1.86 and 1.62 for number of filled and unfilled spikelets, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Significant ($P \leq 0.01$) influence of sulfur on filled grains panicle^{-1} was observed. A gradual increase in the number of filled grains panicle^{-1} was observed with the increase of sulfur level (Figure 28 and Appendix XVII). No. of filled grains panicle^{-1} increased by 16.93% and 24.37% after S_1 and S_2 supplementation when compared to control (without sulfur).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 28. Effect of different sulfur levels on the number of filled and unfilled spikelets $panicle^{-1}$ of rice ($LSD_{(0.05)}$ = 1.86 and 1.62 for number of filled and unfilled spikelets, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

The interaction effect of Salinity × Sulfur treatment had a significant ($P \leq 0.01$) effect on filled grains $panicle^{-1}$ which was evident from statistically significant Salinity × Sulfur interactive effect (Table 11 and Appendix XVII). Sulfur supplementation at the rate of S_1 was sufficient to improve the number of filled grains $panicle^{-1}$ significantly, by 18.18% and 25.47% for both levels of salinity (N_1 and N_2 respectively) when compared to salt treatment alone. Further, higher sulfur application (S_2) increased the number of filled grains $panicle^{-1}$ for both levels of salinity (N_1 and N_2) by 26.85% and 30.22% respectively compared to sole salt treatment without sulfur. Thus, the results of interaction corresponding to the number of filled grains $panicle^{-1}$ provided that number of filled grains $panicle^{-1}$ reached its maximum values when rice plants were treated with S_2 treatment.

4.1.16 No. of Unfilled Spikelets $Panicle^{-1}$

In rice, less number of unfilled spikelets $panicle^{-1}$ is a desirable attribute to get higher grain yield. Due to salt stress, panicles produced sterile spikelets with partial or complete grain loss. A gradual increase in the number of unfilled grains $panicle^{-1}$ was observed in N_1 and N_2 treatment where 8.54% and 23.19% increment was observed respectively compared to unstressed control (Figure 27 and Appendix XVII).

The number of unfilled spikelets panicle^{-1} reduced significantly ($P \leq 0.01$) after sulfur supplementation (Figure 28 and Appendix XVII). No. of unfilled spikelets panicle^{-1} decreased by 45.31% and 60.64% with S_1 and S_2 supplementation when compared to control.

It was revealed that salinity increased the number of unfilled spikelets panicle^{-1} but sulfur application decreased the number of unfilled spikelets panicle^{-1} under saline condition. It was shown by statistically significant ($P \leq 0.01$) Salinity × Sulfur interaction (Table 11 and Appendix XVII). Under N_1 salt-stressed condition, the number of unfilled spikelets panicle^{-1} decreased by 58.8% at S_1 ($N_1 S_1$) in comparison with N_1 salt treatment alone ($N_1 S_0$) whilst it was 74.34% at S_2 treatment ($N_1 S_2$). Whereas, under N_2 treated condition, a decrease of 44.81% and 52.79% in the number of unfilled spikelets panicle^{-1} was observed at S_1 and S_2 treatment ($N_2 S_1$ and $N_2 S_2$) respectively compared to $N_2 S_0$ treatment where no sulfur was added.

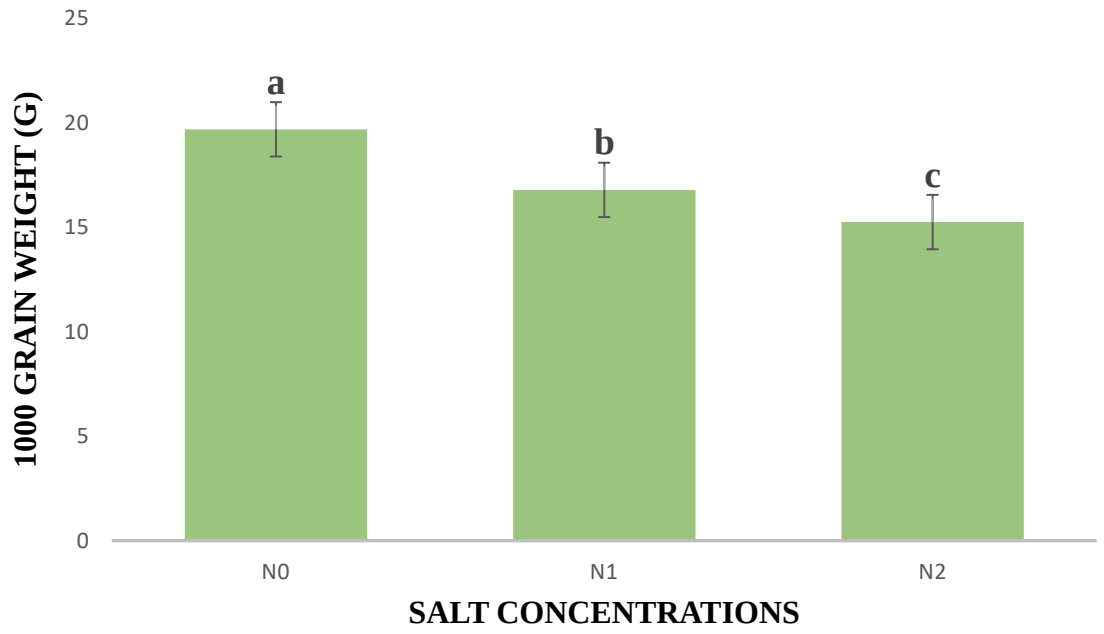
Table 11. Interaction effect of different salt concentrations and sulfur levels on the number of filled and unfilled spikelets panicle^{-1} of rice

Treatments	No. of Filled Spikelets Panicle^{-1}	No. of Unfilled Spikelets Panicle^{-1}
N_0S_0	119.73 c	19.00 c
N_0S_1	133.40 b	13.27 d
N_0S_2	147.33 a	8.47 ef
N_1 S_0	79.20 g	22.33 b
N_1 S_1	96.80 e	9.20 e
N_1 S_2	108.27 d	5.73 f
N_2 S_0	67.13 h	26.20 a
N_2 S_1	90.07 f	14.46 d
N_2 S_2	96.20 e	12.37 d
LSD $_{(0.05)}$	**3.22**	**2.80**
CV (%)	**1.78**	**11.13**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.17 1000 Grain Weight (g)

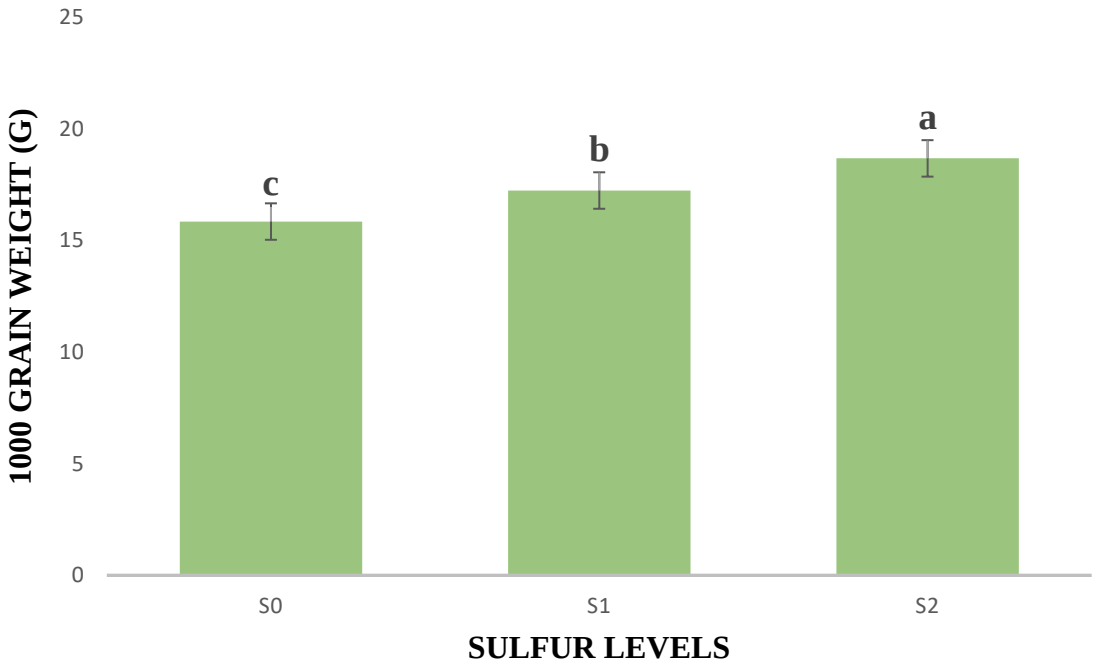
To study the effect of salt stress on rice yield, the average weight of 1000 grains grown under control and, two different salinity levels were measured. 1000 grain weight significantly ($P \leq 0.01$) decreased with the increase of salinity levels (Figure 29 and Appendix XVIII). A gradual decrease in 1000 grain weight was observed with the increase in salinity concentration and it was 14.72% and 22.54% with the treatment N_1 and N_2 respectively compared to non-salt stress control.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 29. Effect of different salt concentrations on the 1000 grain weight of rice (LSD $_{(0.05)}$ = 0.23 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Regarding the effect of sulfur supplementation, it was obvious that sulfur supplementation improved 1000 grain weight significantly ($P \leq 0.01$) and these increases were 8.06% and 15.15% for S_1 and S_2 respectively compared to control (Figure 30 and Appendix XVIII).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 30. Effect of different sulfur levels on the 1000 grain weight of rice ($LSD_{(0.05)}$ = 0.23 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

1000 grain weight significantly ($P \leq 0.05$) responded to sulfur levels under salt stress to increase the above-mentioned trait. A statistically significant Salinity × Sulfur interaction supported this finding (Table 12 and Appendix XVIII). 1000 grain weight was increased by 6.77% and 14.47% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased the 1000 grain weight by 12.62% and 19.79% respectively when compared to salt treatment (N_2S_0) alone.

4.1.18 Grain Yield $Plant^{-1}$

The ultimate desirable product of yield components of rice is grain yield. Salt stress led to significant ($P \leq 0.01$) reduction in the grain yield $plant^{-1}$ with the most drastic reduction being observed at N_2 treatment (Figure 31 and Appendix XVIII). The loss of grain yield due to N_1 and N_2 level of salinity was 23.49% and 36.92% respectively over non-salt stressed control.

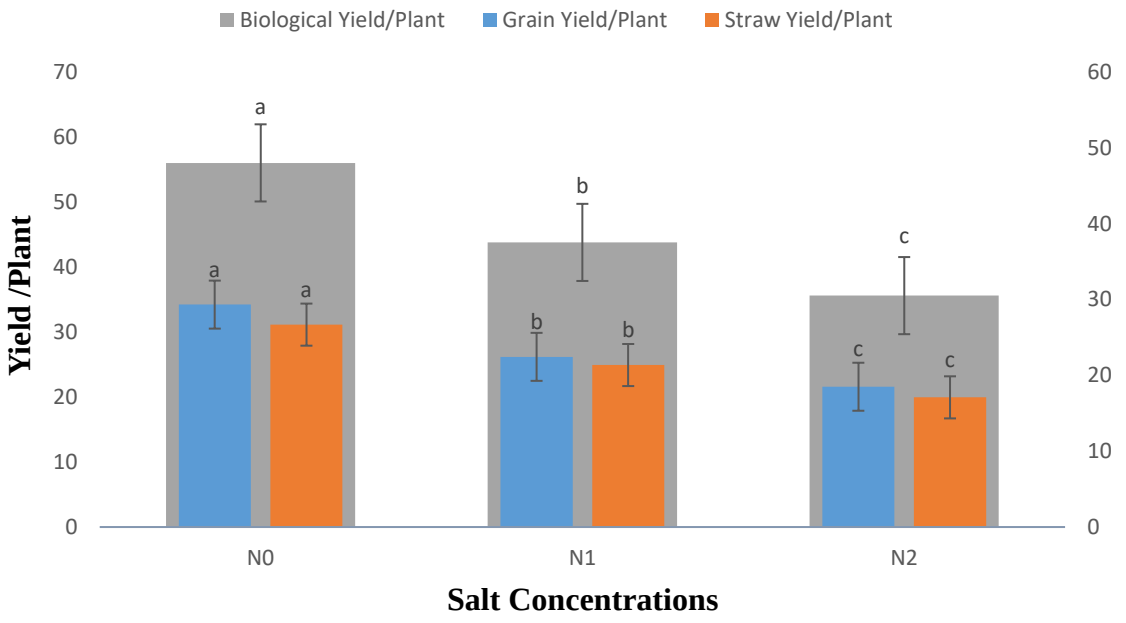
The impact of sulfur application on rice grain yield revealed that sulfur was found to be more fruitful in improving grain yield of rice under salt stress. The sulfur levels had

significant ($P \leq 0.01$) effect on rice grain yield (Figure 32 and Appendix XVIII). The grain yield increased by 13.53% and 22.44% at S_1 and S_2 level of sulfur respectively compared to control.

Salinity reduced the grain yield of rice. By exogenous application of sulfur, the production of grains was increased under saline condition (Plate XIII). It was shown by statistically significant Salinity × Sulfur interaction (Table 12 and Appendix XVIII). Percent increase of grain yield in the treatment N_1S_1 and N_1S_2 was 19.55% and 26.9% respectively, indicating a remarkable difference with N_1S_0 where no sulfur was applied. Whereas, it was 14.28% and 25.09% with N_2S_1 and N_2S_2 respectively when compared with N_2S_0 with salt treatment only.

4.1.19 Straw Yield Plant^{-1}

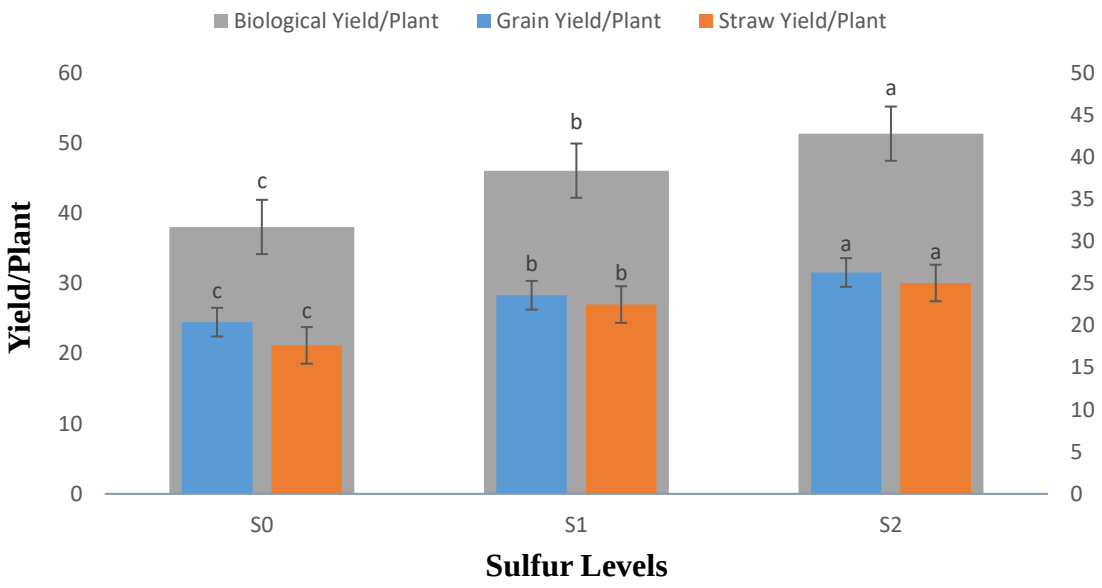
A drastic decline in straw yield plant^{-1} was observed under both levels of salinity. Straw yield was significantly ($P \leq 0.01$) influenced by salinity level where 19.97% and 35.93% reduction of straw yield was observed due to N_1 and N_2 level of salinity compared to unstressed control (Figure 31 and Appendix XVIII).



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 31. Effect of different salt concentrations on the grain yield, straw yield and biological yield plant^{-1} of rice (LSD $_{(0.05)}$ = 0.58, 0.94 and 1.05 for grain yield, straw yield and biological yield, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

With respect to the straw yield plant^{-1}, data revealed that increase of sulfur from S_1 to S_2 resulted an increase of straw yield by about 21.57% and 29.59%, respectively compared to control (Figure 32 and Appendix XVIII).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 32. Effect of different sulfur levels on the grain yield, straw yield and biological yield plant^{-1} of rice (LSD $_{(0.05)}$ = 0.58, 0.94 and 1.05 for grain yield, straw yield and biological yield, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Though salinity reduced the straw yield plant^{-1}, a clear positive response of sulfur was observed under saline condition. It was evident from statistically significant ($P \leq 0.01$) Salinity × Sulfur interactive effect (Table 12 and Appendix XVIII). Straw yield plant^{-1} was increased by 15.17% and 21.35% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased the straw yield plant^{-1} by 43.31% and 50.12% respectively when compared to salt treatment (N_2S_0) alone.

4.1.20 Biological Yield Plant^{-1}

Results showed that salinity reduced the biological yield plant^{-1} significantly ($P \leq 0.01$). The highest biological yield was observed with control (N_0) treatment while the lowest was observed with N_2 treatment (Figure 31 and Appendix XVIII). 21.83% and 36.45%

reduction in biological yield was noticed in salt-affected N_1 and N_2 plants respectively when compared to control (no salinity).

Sulfur application improved the biological yield plant^{-1} (Figure 32 and Appendix XVIII) significantly ($P \leq 0.01$). Increasing sulfur fertilizer gradually increased the biological yield of rice plants (17.43% at S_1 and 25.91% at S_2 over control). The highest values of biological yield were produced by the higher level of sulfur (S_2).

Statistical analysis has shown that salinity reduced the biological yield of rice plants significantly ($P \leq 0.01$). Nevertheless, the sulfur application had a pronounced positive effect on biological yield enhancement which was evident from Salinity × Sulfur interaction (Table 12 and Appendix XVIII). Biological Yield Plant^{-1} was increased by 17.42% and 24.23% after S_1 and S_2 supplementation of salt-stressed (N_1S_1 and N_1S_2) plants respectively when compared to salt treatment (N_1S_0) alone. Whereas, S_1 and S_2 supplementation during salt stress (N_2) increased the biological yield by 28.98% and 37.73% respectively when compared to salt treatment (N_2S_0) alone. Data regarding the biological yield plant^{-1} depicted that treatment using S_2 recorded the statistically maximum biological yield which was followed by S_1 treatment at both levels of salinity (N_1 and N_2).

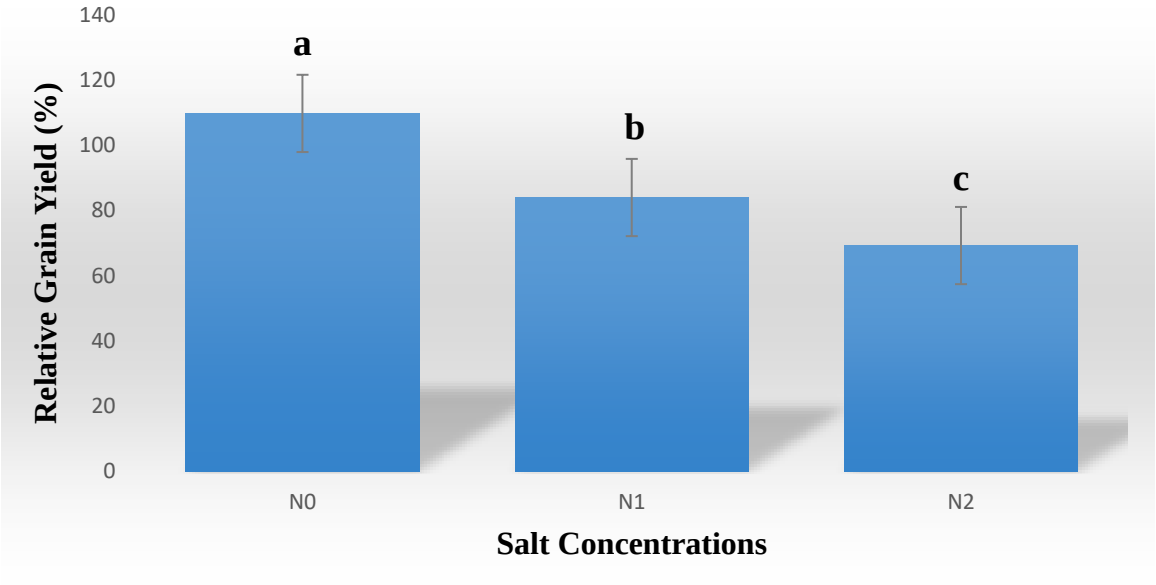
Table 12. Interaction effect of different salt concentrations and sulfur levels on the 1000 grain weight, grain yield plant^{-1}, straw yield plant^{-1}, biological yield plant^{-1} and relative grain yield (%) of rice

Treatments	1000 grain weight (g)	Grain Yield Plant^{-1} (g)	Straw Yield Plant^{-1} (g)	Biological Yield Plant^{-1} (g)	Relative Grain Yield %
N_0S_0	18.5 c	26.70 c	23.57 c	50.27 c	100.00 c
N_0S_1	19.58 b	29.08 b	26.57 b	55.65 b	108.91 b
N_0S_2	21.02 a	32.21 a	29.94 a	62.15 a	120.64 a
N_1 S_0	15.55 e	18.64 g	18.57 e	37.21 f	69.80 g
N_1 S_1	16.68 d	23.17 e	21.89 d	45.06 d	86.79 e
N_1 S_2	18.18 c	25.50 d	23.61 c	49.11 c	95.51 d
N_2 S_0	13.50 f	15.85 h	10.76 f	26.61 g	59.34 h
N_2 S_1	15.45 e	18.49 g	18.98 e	37.47 f	69.25 g
N_2 S_2	16.83 d	21.16 f	21.57 d	42.73 e	79.25 f
LSD $_{(0.05)}$	**0.41**	**1.01**	**1.63**	**1.81**	**2.22**
CV (%)	**1.36**	**2.48**	**4.33**	**2.32**	**1.46**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.21 Relative Grain Yield Plant^{-1}

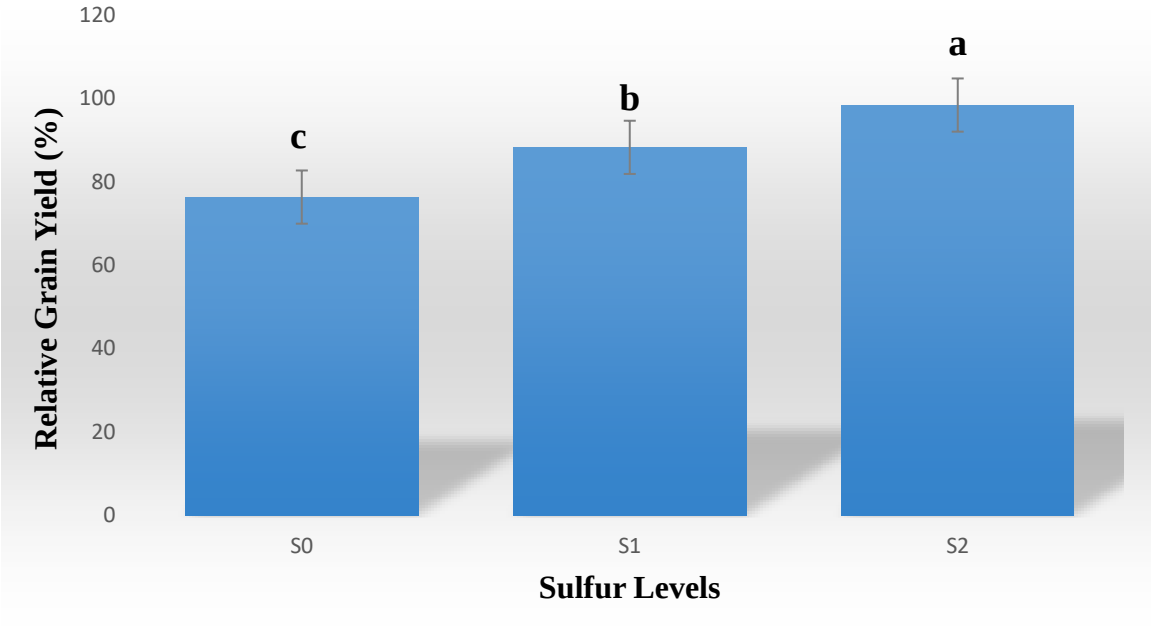
Increased salinity level caused significant ($P \leq 0.01$) reduction in relative grain yield percent of rice plants (Figure 33 and Appendix XVIII). Relative grain yield percent was reduced from 109.85% in the control plants to 84.03% and 69.28% in the plants treated with N_1 and N_2 level of salinity respectively.



N_0=0 dSm^{-1}, N_1=8 dSm^{-1}, N_2=12 dSm^{-1}

Figure 33. Effect of different salt concentrations on the relative grain yield plant^{-1} of rice (LSD $_{(0.05)}$ = 1.28 and bars with different letters are significantly different at $p \leq$ 0.05 applying LSD)

Sulfur addition to rice plants resulted in enhancement of the relative grain yield percent significantly ($P \leq 0.01$). With an increase of sulfur supply relative grain yield percent was increased from 76.38% in the control plants to 88.32% and 98.47% in the S_1 and S_2 treated plants respectively (Figure 34 and Appendix XVIII).



S_0=0 Kg S ha^{-1}, S_1=3 Kg S ha^{-1}, S_2=6 Kg S ha^{-1}

Figure 34. Effect of different sulfur levels on the relative grain yield $plant^{-1}$ of rice ($LSD_{(0.05)}$ = 1.28 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

Relative grain yield % in rice was greatly influenced by salinity levels whereas sulfur application (S_1 and S_2) improved the relative grain yield percent at both levels of salinity (N_1 and N_2). Salinity × Sulfur interactive effect supported this finding (Table 12 and Appendix XVIII). Under N_1 salt stressed condition, relative grain yield percent increased by 19.58% at S_1 (N_1 S_1) in comparison with N_1 salt treatment alone (N_1 S_0) whilst it was 26.92% at S_2 treatment (N_1 S_2). Whereas, under N_2 treated condition, an increase of 14.31% and 25.12% in relative grain yield percent was observed at S_1 and S_2 treatment (N_2 S_1 and N_2 S_2) respectively compared to N_2 S_0 treatment where no sulfur was added.

4.2 Discussion

Among abiotic stresses, salt stress causes substantial disturbance in physiological and biochemical parameters of plant resulting in reduced crop yield (Flowers *et al*., 2010, Ashraf *et al*., 2010). In this experiment, a considerable variation in morphological, physiological and yield performance of rice was noticed under saline conditions (8 and 12 dSm^{-1} salinity). It's might be due to the reason that, the availability of nutrients decreases due to binding with soil particles and reduced the availability of water in the rooting zone of plants. It is well known that nutrients not only take part in growth and development of plants but also take part in tolerance mechanisms against different types of abiotic stresses

including salinity. It is widely accepted that nutrients play meaningful role in inducing resistance in plants against different abiotic stresses like salinity (Shahid *et al*., 2015). That's why, under salinity stress there is a need to supply higher quantities of the required minerals and nutrients to plants.

In the present experiment, Sulfur, an important plant nutrient required for proper growth and development was applied to the rice plants grown under saline conditions. Rice plants grown under salinity were treated with 3 and 6 Kg S ha^{-1} to help the plants coping with salt stress. Plant's reduced response to salt stress impacts might be due to the fact that sulfur played an important role in combating salt stress through maintaining redox state of cell by enhanced sulfur metabolism in plant cells. Moreover, sulfur is considered as an essential nutrient for plants because of its role in metabolic compounds such as amino acids like cysteine, methionine, sulfolipids and proteins under abiotic stress ((Nocito *et al*., 2007; Khan *et al*., 2013). These sulfur containing compounds are linked to antioxidant system of plants which play an important role in alleviating salt stress (Nazar *et al*., 2011; Ashfaque *et al*., 2014).

Results showed that salt stress reduced the plant height of rice plants. This might partially be attributed to high concentration of Na^+ and Cl^- in the rooting zone (Zhang *et al*., 2010) which lower water potential in the growing media of plants and ultimately results in loss of cell turgor. Because of this, photosynthetic rate, cell division and cell elongation become reduced, resulting in shorter plant height (Zekri, 1991; Ali- Dinar *et al*., 1999; Ebert *et al*., 2002). The reduction in plant height due to imposition of salinity has been reported in various studies (Atak *et al*., 2008; Khan and Weber, 2008; Asaadi, 2009; Hasanuzzaman *et al*., 2009; Mahmood *et al*., 2009 and Saha, 2013). However, supplementation with sulfur improved the plant height in the current study. This might be due to the reason that sulfur enhances the cell division and cell elongation in the meristematic region of the plant. On the other hand, Sulfur performs many physiological functions like synthesis of sulfur containing amino acids (cysteine, cystine and methionine), synthesis of vitamins, and metabolism of carbohydrates and proteins. The findings related to increment in plant height under salt stress are in a good harmony with those reported by Rahman *et al*., (2007), Amaraweera (2009), Shehata *et al*., (2009), Zayed *et al*., (2010), Mazhar *et al*., (2011), and Zayed (2012).

Different growth parameters like number of tillers $plant^{-1}$, leaf area, chlorophyll content (SPAD value), membrane stability index (MSI%), leaf relative water content (RWC%) were markedly reduced except days to flowering, under salinity stress. This may be due to the reason that salinity reduced both nutrient uptakes by the roots and also limited mineral transport from the roots to the shoots due to reduced transpiration rates and damaged membrane permeability (Alam, 1999). Under salt stress, the presence of extreme ratios of Na^+/K^+, Na^+/Ca^{2+}, ions also create the toxic and imbalanced ionic environment which reduce plant growth (Grattan and Grieve, 1999b) and finally cause plant cell death (Bhardwaj and Yadav, 2012). Sulfur element might be played vital role in growth and development of rice plants because of their important role in metabolic processes. In plants,

sulfur-containing metabolites, amino acids (cysteine and methionine), vitamins (biotin and thiamine), thioredoxin system, glutathione lipoic acid and glucosinolates have potential to promote or modify physiological and molecular processes under salt stress. Thus, modulation of sulfur metabolites production could alter physiological and molecular mechanisms to provide tolerance against salinity (Khan *et al*., 2014).

Salt stress affected every aspect of plant development including dry weights of different plant parts. The estimation of dry matter is considered as a valuable index for monitoring vegetative growth of the rice plant (Hakim *et al*., 2014b). Reduction of dry weights under salt stress might be due to lower amount of photosynthetic apparatus (chlorophyll), lower stomatal conductance and lower uptake of nutrients from the soil thus led to a decrease in the dry weights of plant root, shoot and, leaf, in a word total dry weight (TDM) (Mondal *et al*., 2013). According to Malik and Srivastava (2005), the reduction in dry weights may be due to suppressing cell enlargement and division and also to the inhibition of enzyme activities by salt, especially Na^+ ions. Reduction in dry weights due to salinity as compared to control was reported by Sultana *et al.,* (1999), Ferdous *et al*., (2018). Sulfur has very important role in formation of proteins and a number of metabolites necessary for the increment of fresh and dry weights of plants (Ali *et al*., 1990; Zhao *et al*., 1993). Gilbert and Robson (1984) reported that use of sulfur improved shoot and root fresh and dry weights.

The immediate and primary responses of plants exposed to salt stress include an influx of Na^+ into root epidermal cells through plasma membrane non-selective channels, followed by a depolarization and activation of the K^+ efflux (Shabala *et al*., 2007, Zhao *et al*., 2007). The present study provided firm evidence that salt stress caused higher Na accumulation and reduced K level. Replacement of Na^+ by K^+, Na^+ exclusion and retention of intracellular K^+ are all important salt tolerance mechanisms i.e., maintaining Na^+ and K^+ homeostasis is crucial for plant survival under salt stress conditions. (Shabala *et al*., 2007). An increase in K and decrease in Na level in sulfur supplemented rice plants indicated the influential roles of sulfur in reducing Na toxicity. Sulfur has significant contribution in ionic homeostasis in the plant (Riffat, 2018). It not only reduces the accumulation of toxic ions in the plants that improve the productivity and quality of crop plants but also maintains the soil condition for production of healthy crops (Zaman *et al.,* 2002). Sulfur application improves the K^+/Na^+ ratio that decrease the toxic effects of salinity (Prasad, 2003).

Under salinity stress, when the yield attributes were compared on the basis of % reduction with their respective controls, a severe reduction in all components was observed. But, among all these components, the fertility of grain was found most severely affected and thus causing significant reduction in total grain yield. In addition to fertility, number of effective tillers $plant^{-1}$ and number of filled grains $panicle^{-1}$ were two important affected characters contributing in grain yield. The reduction of all yield contributing components might be due to the differential competition in carbohydrates supply between vegetative growth and constrained its distribution to the developing panicles (Murty & Murty, 1982), whereas other is probably linked to reduce viability of pollen under stress condition, thus

resulting failure of seed set (Abdullah *et al*., 2001). Sulfur supplementation significantly increased all yield attributes; fertility of grains, effective and non-effective tiller number, panicle length, filled and unfilled grains numbers, and 1000-grain weight. The superiority of most yield attributes obtained herein owing to sulfur application may be due to its effect on improving soil proprieties by reducing pH of saline soil, drainage improvement, encouraging aggregates formation and raising nutrient availability reflecting on plant growth and salinity tolerance of rice (Farook and Khan, 2010 and Chien *et al*., 2011). Sulfur induced higher assimilation and translocation of carbohydrates to panicle might be a reason of improvement in yield attributes. Another reason of increased yield attributes might be synthesis of chloroplast protein resulting in greater photosynthetic efficiency (Biswas and Tewatia, 1992). Similar findings were reported Rahman *et al*. (2007), Amaraweera (2009), Shehata *et al*. (2009), Zayed *et al*. (2010) and Zayed (2012).

The reduction in biological yield (grain yield and straw yield) and relative grain yield under salt stress was manifestation of the cumulative reduction of plant height, no. of effective tillers, leaf area, total dry matter, no. of filled grains, panicle length, 1000 grain weight, etc. The magnitude of salinity induced yield losses could not be attributed to single factor. Different physiological, biochemical factors at different growth stages of rice plants might be involved. Lack of transformation of carbohydrates to vegetative growth and grains development might be the main cause of decreased grain yield under salt stress. According to Abdullah *et al*., (2001), the main reasons behind lower rice grain yield under salt stress is significant reduction in translocation of soluble sugar contents to superior and inferior grains and inhibition of starch synthetase activity during grain development. Decrease of grain yield with increased salinity was also reported by Islam (2004b) and Hossain (2006) in rice. Grain yield was positively correlated with biological yield in rice as reported by Munshi (2005). On the other hand, inhibition of photosynthesis under salt stress caused less amount of nutrient uptake by the plant and because of this plant growth was slow which resulted in shorter plant height and fewer number of leaves plant^{-1} which ultimately reduced the straw yield of rice (Hoque, 2013). However, the superiority of grain yield, straw yield and biological yield plant^{-1} as well as relative grain yield of rice by the application of sulfur under salt stress was apparent because of its vital role in synthesis of proteins and pigments as well as soil reclamation. Moreover, sulfur has a direct role in some amino acids formation, activation of some very important metabolism enzymes and improvement of some soil chemical and physical prosperities like soil pH. So, application of sulfur might be reduced soil pH and improved soil structure resulting in more nutrients availability as well as having beneficial role in plant metabolism might consequently increase the yield. The increase in grain yield by exogenous application of sulfur has been reported in previous studies (Rahman *et al*., 2007; Ali *et al*., 2008; Lunde *et al*., 2008; Amaraweera 2009; Shehata *et al*., 2009, Zayed *et al*., 2010 and Zayed 2012).

CHAPTER V

SUMMARY AND CONCLUSION

To evaluate the role of sulfur under salt stress, an experiment was carried out at the net house of the Department of Agricultural Botany and Plant Physiology Laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh in *Boro* season during the period from November 2018 to May 2019.

The experiment was carried out to assess the role of sulfur in improving morphological, physiological and yield performance of rice plant (*Oryza sativa* L. cv. BRRI dhan67) under salt stress.

The double factor experiment was laid out in a randomized complete block design (RCBD) with three replications and the differences between the means were evaluated by Least Significant Difference (LSD). In this experiment, the treatments consisted of three different salinity levels viz. N_0= without salt (0 dSm^{-1}), N_1= 8 dSm^{-1}, N_2 = 12 dSm^{-1}, and three different levels of sulfur viz. S_0= 0 Kg S ha^{-1}, S_1= 3 Kg S ha^{-1} and S_2 = 6 Kg S ha^{-1}. The salt treatments were begun at 15 DAT. But sulfur was applied during the time of other fertilizers application.

Then data was recorded on plant height (cm), no. of tillers $plant^{-1}$, leaf area (cm^2), leaf membrane stability index (MSI%), leaf relative water content (RWC%), chlorophyll content (SPAD reading), dry weights of root, shoot, leaf and total dry matter (TDM,), Na and K content in shoots and roots, days to flowering, panicle length (cm), spikelet fertility%, no. of effective and non-effective tillers $plant^{-1}$, no. of filled and unfilled spikelets $panicle^{-1}$, 1000 grain weight (g), grain yield and straw yield $plant^{-1}$. The collected data were statistically analyzed for evaluation of the treatment effect and a significant variation among the treatments was found while different salinity levels and sulfur levels were applied in different combinations.

The result of the experiment revealed that almost all the morphological, physiological and yield contributing characters were decreased significantly except no. of non-effective tillers, days to flowering, no. of unfilled spikelets $panicle^{-1}$ due to imposition of salinity. Plants grown in control condition (without salinity) performed best in recording the morphological, physiological and yield contributing characters of rice whereas the lowest data of all parameters was recorded from 12 dSm^{-1} treated plants. In fact, there was a gradual decrease of all the parameters with the increase of salinity.

Supplementary sulfur fertilization (3, 6 Kg S ha^{-1}) improved all the morphological, physiological and yield contributing characters significantly. The maximum data of all the studied parameters was noted in S_2 treatment (6 Kg S ha^{-1}) and the minimum in control treatment (plant that did not receive any sulfur).

The combinations of salinity and sulfur significantly influenced almost all the morphological, physiological and yield contributing characters. In every case it was observed that N_1S_1, N_1S_2 gave better result than N_1S_0 and N_2S_1, N_2S_2 gave better result than N_2S_0. In most of the parameter, S_2 treatment (6 Kg S ha^{-1}) was found to have better salinity mitigating potential than S_1 treatment (3 Kg S ha^{-1}) even at higher level of salt stress (12 dSm^{-1}).

The results indicated that all the morphological, physiological and yield attributes varied to a considerable extent under salinity stresses which render the lower yield. While the no. of non-effective tillers $plant^{-1}$, days to flowering, no. of unfilled spikelets $panicle^{-1}$ was increased in response to salt stress. It was observed that the supplementation of sulfur enhanced all the morphological, physiological and yield performance of rice. The best results were mostly found at sole 6 Kg S ha^{-1} treatment which indicates that sulfur played important roles in different physiological and metabolic processes of rice plants. Under salt stress, the maximum improvement in all the studied parameters by sulfur application was found at 6 Kg S ha^{-1} while lower level of sulfur was not much effective in improving salt tolerance in rice plants. Thus, it can be concluded that application of sulfur significantly ($P \leq 0.05$) decreased the salinity induced damages in rice plants and improved the growth and physiological metabolisms.

Further studies may be needed to ensure the role of sulfur in improving morphological, physiological and yield performance of rice under salt stress along with more growth parameters like grain nutrient content and other quality attributes of rice. Another combination of salinity and sulfur may be included for further study.

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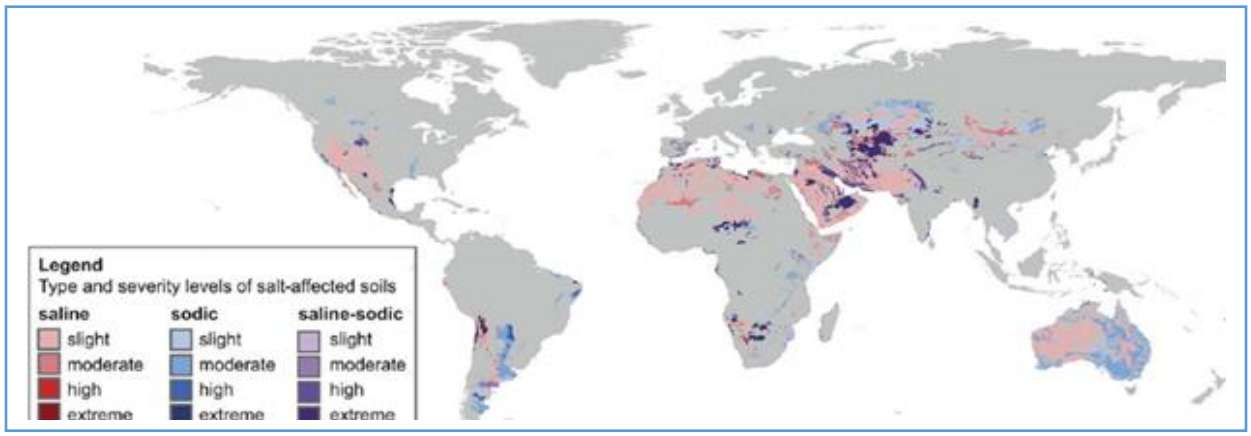
APPENDICES

Appendix I. Variation in Salt-Affected Areas in the World, in million hectares (M ha)

Regions	**Total area**	**Saline soils**	**%**	**Sodic soils**	**Percent**
Africa	1899.1	38.7	2.0	33.5	1.8
Asia and the pacific and australia	3107.2	195.1	6.3	248.6	8.0
Europe	2010.8	6.7	0.3	72.7	3.6
Latin America	2038.6	60.5	3.0	50.9	2.5
Near East	1801.9	91.5	5.1	14.1	0.8
North America	1923.7	4.6	0.2	14.5	0.8
Total	12781.3	397.1	3.1%	434.3	3.4%

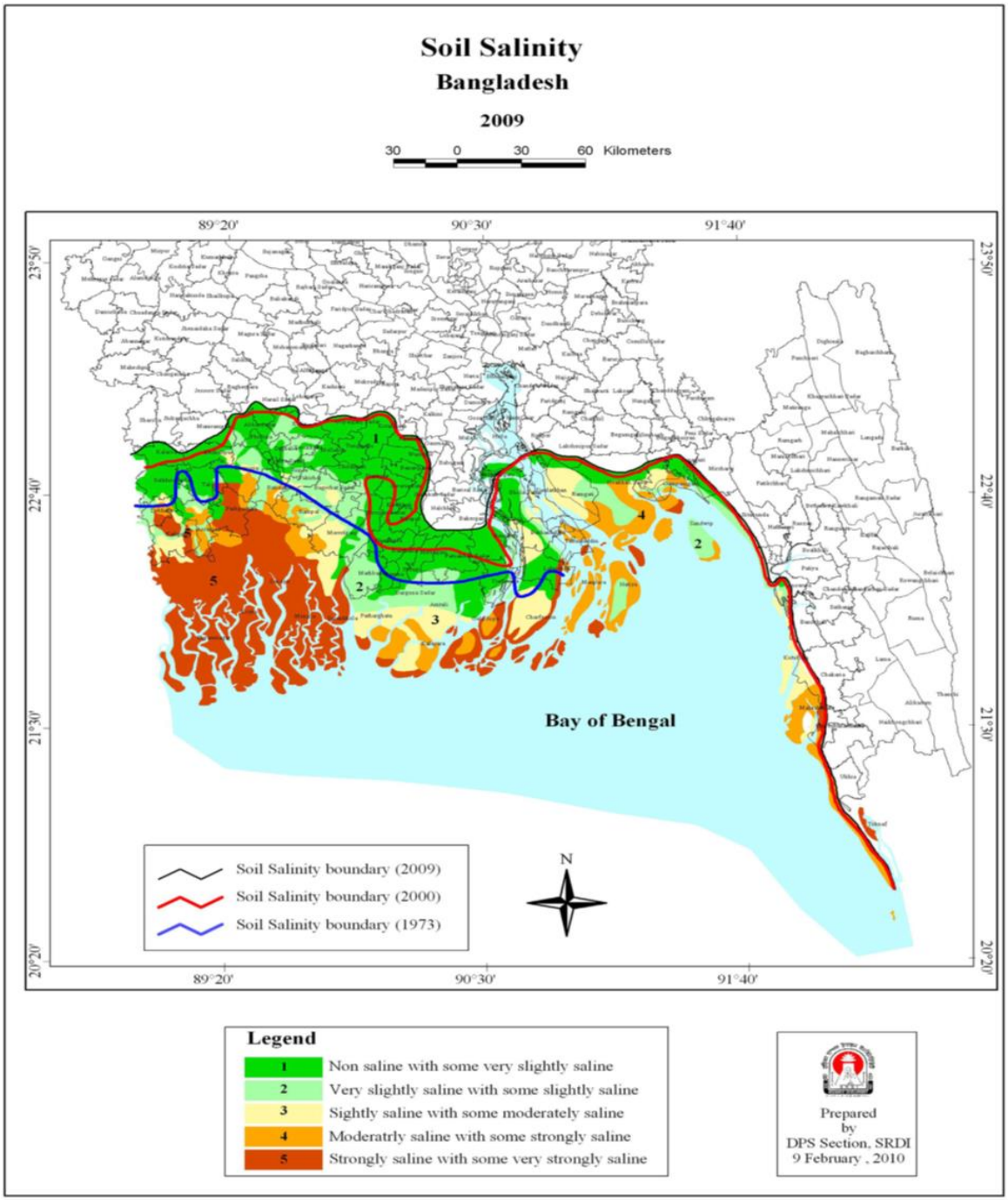
Source: FAO Land and Plant Nutrition Service (2008)

Appendix II. Global extents and distributions of salt affected soils



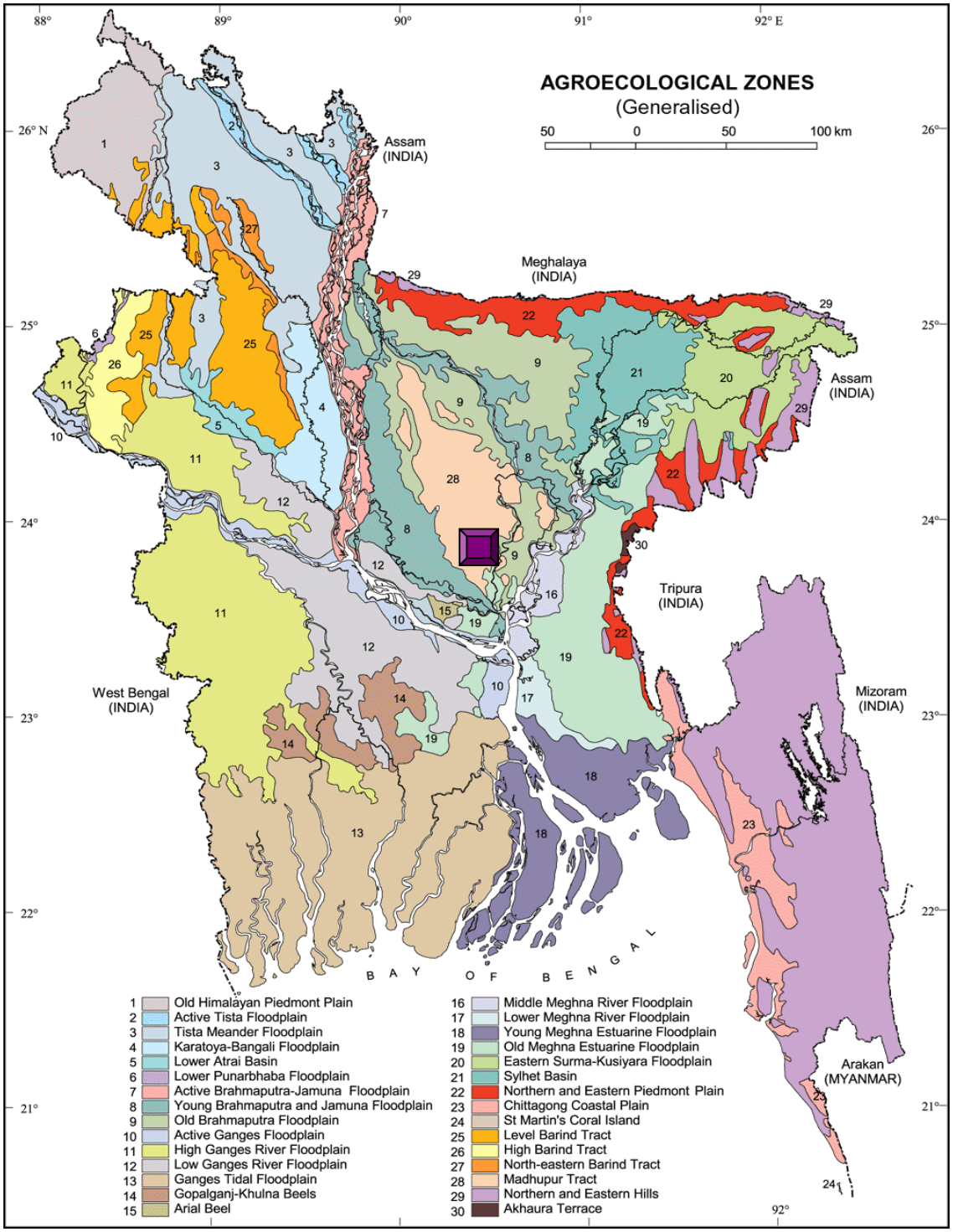
Source: Wicke *et al.*, (2011)

Appendix III. Soil Salinity map, 2009



Source: BARC (2009)

Appendix IV. Experimental location on the map of Agro-Ecological Zones of Bangladesh



The experimental site under study

Appendix V. Morphological Characteristics of the Experimental Field

Morphology	**Characteristics**
Location	SAU Farm, Dhaka
Agroecological zone	Madhupur Tract (AEZ- 28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur clay
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level
Soil series	Tejgaon

(SAU Farm, Dhaka)

Appendix VI. Physical and Chemical properties of the initial soil sample

Characteristics	**Value**
Particle size analysis	
% Sand (2.0-0.02 mm)	22.53
% Silt (0.02-0.002 mm)	56.72
% Clay (<0.002 mm)	20.75
Textural class	Silt Loam
pH (1: 2.5 soil- water)	5.6
Bulk Density (g/cc)	1.45
Particle Density (g/cc)	2.52
Organic carbon (%)	0.47
Organic matter (%)	0.81
Total N (%)	0.05
Available P (ppm)	18.1
Available K (meq/100g soil)	0.10
Available S (ppm)	2.006

(SAU Farm, Dhaka)

Appendix VII. Maximum and minimum monthly temperature (°C), relative humidity and rainfall during November, 2018 to April, 2019 at the farm of SAU

Name of the Months	Average air temperature (°C)		Relative Humidity (%)	Rainfall (mm)
	Maximum	Minimum		
November, 2018	31	18	63	1.9
December, 2018	28	16	61	3.5
January. 2019	27	13	57	12.3
February, 2019	34	15	57	8.1
March, 2019	34	16	57	73.4
April, 2019	35	20	66	178.5

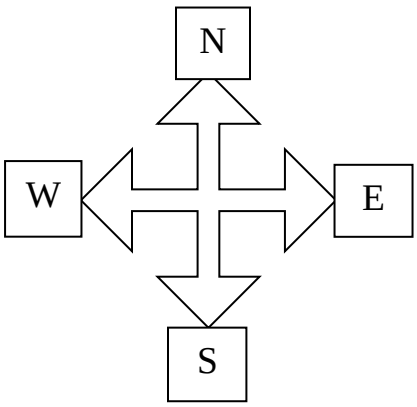
(Weather station, Sher-e-Bangla Agricultural University, Dhaka-1207)

Appendix VIII. Layout of the experiment

Pot size: 30 cm ×35 cm

Pot to pot distance: 0.15 m

Block to block distance: 0.30 m



$R_1 N_0 S_2$ ⇦ 0.30 m ⇨	$R_2 N_2 S_0$	$R_3 N_2 S_0$
⇧ 0.15 m ⇩		
$R_1 N_2 S_1$	$R_2 N_1 S_0$	$R_3 N_0 S_2$
$R_1 N_2 S_0$	$R_2 N_0 S_0$	$R_3 N_2 S_1$
$R_1 N_0 S_1$	$R_2 N_2 S_1$	$R_3 N_0 S_0$
$R_1 N_2 S_2$	$R_2 N_1 S_2$	$R_3 N_2 S_2$
$R_1 N_1 S_2$	$R_2 N_0 S_2$	$R_3 N_1 S_2$
$R_1 N_1 S_0$	$R_2 N_1 S_1$	$R_3 N_1 S_0$
$R_1 N_0 S_0$	$R_2 N_2 S_2$	$R_3 N_0 S_1$
$R_1 N_1 S_1$	$R_2 N_0 S_1$	$R_3 N_1 S_1$

Appendix IX. Analysis of variance of the data on plant height as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of plant height at different days after transplanting (DAT)			
		30	60	90	At Harvest
Replication	2	0.03	3.02	110.81	266.65
Salt Concentrations	2	778.49**	867.01**	858.56**	1023.60**
Sulfur Levels	2	108.22**	118.09**	283.92**	384.37**
Salt Concentrations X Sulfur Levels	4	16.86*	19.17NS	8.85*	21.29*
Error	16	4.48	8.91	2.69	6.00

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix X. Analysis of variance of the data on number of tillers plant^{-1} as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of number of tillers plant^{-1} at different days after transplanting (DAT)			
		30	60	90	120
Replication	2	1.59	4.48	1.44	9.17
Salt Concentrations	2	59.37**	101.59**	133.78**	81.47**
Sulfur Levels	2	54.70**	49.37**	44.33**	29.29**
Salt Concentrations X Sulfur Levels	4	1.82*	6.48*	1.44*	1.65*
Error	16	1.26	1.52	0.44	0.52

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XI. Analysis of variance of the data on leaf area, membrane stability index (MSI%) and relative water content (RWC%) as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of		
		Leaf Area	Membrane stability index (MSI%)	Relative water content (RWC%)
Replication	2	45.81	0.07	161.00
Salt Concentrations	2	1316.06**	1309.41**	999.85**
Sulfur Levels	2	155.65**	457.36**	132.26**
Salt Concentrations X Sulfur Levels	4	4.88NS	8.08*	3.31NS
Error	16	19.41	2.69	14.49

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XII. Analysis of variance of the data on chlorophyll content (SPAD value) as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of chlorophyll content (SPAD value) at different days after transplanting (DAT)	
		60	90
Replication	2	0.55	0.04
Salt Concentrations	2	85.84**	168.19**
Sulfur Levels	2	54.64**	182.52**
Salt Concentrations X Sulfur Levels	4	22.12**	29.13**
Error	16	2.66	4.06

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XIII. Analysis of variance of the data on dry weights of root, shoot, leaf and total dry matter (TDM) as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of dry weights of			
		Root	Shoot	Leaf	TDM
Replication	2	0.02	0.43	2.66	3.98
Salt Concentrations	2	17.09**	35.67**	73.00**	340.04**
Sulfur Levels	2	5.25**	27.59**	37.09**	183.88**
Salt Concentrations X Sulfur Levels	4	0.54**	2.15**	3.30**	9.33**
Error	16	0.08	0.36	0.53	1.12

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XIV. Analysis of variance of the data on Na and K content in shoots and roots as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of			
		Na Content (µmol/g DW) Shoot	Na Content (µmol/g DW) Root	K Content (µmol/g DW) Shoot	K Content (µmol/g DW) Root
Replication	2	367	23	462.2	406.1
Salt Concentrations	2	352931**	153588**	68884.2**	65758.1**
Sulfur Levels	2	43622**	41270**	29262.8**	22584.8**
Salt Concentrations X Sulfur Levels	4	12486**	12228**	6180.0**	3634.4**
Error	16	22	14	19.1	13.6

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XV. Analysis of variance of the data on days to flowering, panicle length and spikelet fertility% as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of		
		Days to Flowering	Panicle Length	Spikelet Fertility%
Replication	2	0.33	1.13	2.61
Salt Concentrations	2	203.44**	75.46**	190.22**
Sulfur Levels	2	24.78**	34.89**	469.78**
Salt Concentrations X Sulfur Levels	4	9.06**	0.62NS	23.97**
Error	16	1.42	0.89	1.69

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XVI. Analysis of variance of the data on number of effective and non-effective tillers plant^{-1} as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of number of	
		Effective Tillers Plant^{-1}	Non-Effective Tillers Plant^{-1}
Replication	2	8.93	0.01
Salt Concentrations	2	90.82**	0.55**
Sulfur Levels	2	59.37**	5.86**
Salt Concentrations X Sulfur Levels	4	1.59*	0.12**
Error	16	0.47	0.02

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

NS, indicates Non significant

Appendix XVII. Analysis of variance of the data on no. of filled and unfilled spikelets panicle^{-1} as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of number of	
		Filled Spikelets Panicle^{-1}	Unfilled Spikelets Panicle^{-1}
Replication	2	6.61	3.17
Salt Concentrations	2	6013.97**	68.59**
Sulfur Levels	2	1880.37**	453.73**
Salt Concentrations X Sulfur Levels	4	19.12**	13.26**
Error	16	3.45	2.63

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XVIII. Analysis of variance of the data on 1000 grain weight, grain yield plant^{-1}, straw yield plant^{-1}, biological yield plant^{-1}, relative grain yield% as influenced by combined effect of salt concentrations and sulfur levels of rice

Source of Variation	df	Mean square of				
		1000 Grain Weight	Grain Yield/Plant	Straw Yield/Plant	Biological Yield/ Plant	Relative Grain Yield %
Replication	2	0.04	8.03	4.22	23.75	1.42
Salt Concentrations	2	45.73**	270.46**	207.81**	950.44**	3795.20**
Sulfur Levels	2	17.98**	78.32**	127.35**	403.72**	1099.88**
Salt Concentrations X Sulfur Levels	4	0.22*	1.13*	9.19**	7.49**	15.85**
Error	16	0.05	0.34	0.88	1.09	1.64

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

PLATES

Plate I. Seedbed Preparation

Plate II. Uprooting of seeding from seedbed

Plate III. Transplanting of seedling in the experimental pot

Plate IV. Measurement of sulfur for application in the experimental pot

Plate V. Measurement of salinity for application in the experimental pot

Plate VI. Yellowing of leaves due to salt stress

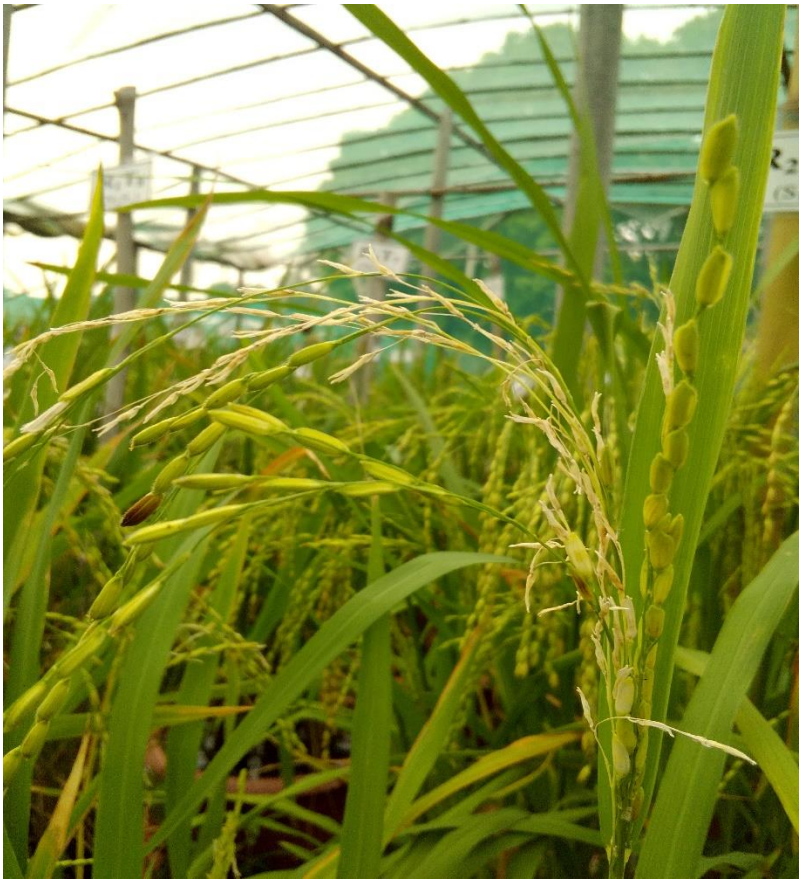


Plate VII. Spikelet degeneration of rice due to salt stress

Plate VIII. Collection of Data (Plant Height)

Plate IX. Collection of Data (SPAD reading)

Plate X. Boiling of the plant samples in water bath for MSI% measurement

Plate XI. Measuring weights of leaf laminas for RWC% calculation

Plate XII. Collection of Data (Leaf area)

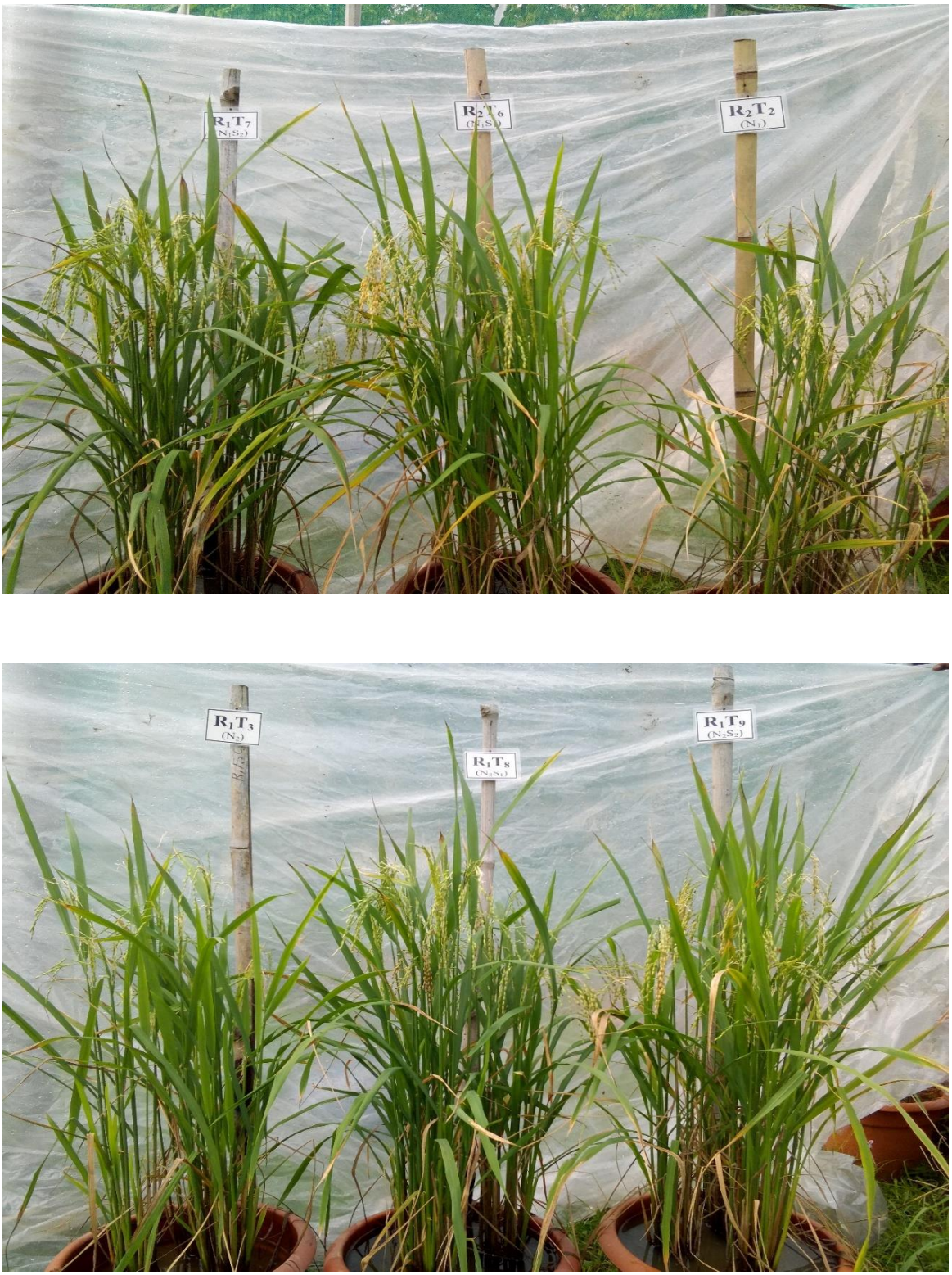


Plate XIII. Different treatment effects

Plate XIV. Collection of Data (No. of filled and unfilled spilelets planicle^{-1})

Plate XV. Oven drying of plant samples