MITIGATION OF SALT-INDUCED DAMAGES IN WHEAT BY EXOGENOUS APPLICATION WITH ASCORBIC ACID, SILICON AND GIBBERELLIN

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CERTIFICATE

This is to certify that the dissertation entitled, "MITIGATION OF SALT INDUCE DEMAGES IN WHEAT BY EXOGENOUS APPLICATION WITH ASCORBIC ACID, SILICON AND GIBBERELLIN" submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in the DEPARTMENT OF AGRONOMY, embodies the result of a piece of bona fide research work carried out by SHAHIDUL ISLAM, Registration No. 27549/00715 under my supervision and guidance. No part of the dissertation has been submitted for any other degree or diploma.

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

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Dated:
Place: Dhaka, Bangladesh

These research works are dedicated

to all of my beloved teachers

who thought and encourage

me to the path of

knowledge

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ABSTRACT

Considering the salinity issue three studies were conducted to find out the effect of salt stress on morph-physiological and biochemical changes of wheat (BARI Gom-26) as well as mitigation of the adverse effect through exogenous application of Ascorbic Acid (AsA), Silicon (Si) and Gibberellic Acid (GA3). The performances of secondary seeds were also evaluated. The studies were conducted at the net house and plant physiology laboratory of Agronomy department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. In field experiment, four levels of salt stress (0, 50, 80, 120 mM NaCl) were applied on growing media of wheat seedling at 20 days after sowing and grown up to harvest where in laboratory experiment salinity were applied 3 days after sowing grown upto 10 days. In both experiments AsA (2 mM ascorbic acid), Si (200 µM SiO_2), GA3 (100 µM gibberallic acid) were applied as foliar spraying (several times with interval) in another set of respective salt stress treatments. In field study salt stress decreased plant growth, biomass, water status and yield attributes by altering ionic balance, hampering osmotic status and reducing chlorophyll (chl) content. In the laboratory experiment, salt stress increased reactive oxygen species (ROS) generation and lipid peroxidation which contributes to alteration of osmotic status and chl content as well as growth and biomass of the plant. However, foliar application of AsA, Si and GA3 on salt affected plant decreased ROS generation, lipid peroxidation and proline production; increased water status and chlorophyll pigments which contribute in improved growth and yield of wheat seedling in contrast to respective stress. Moreover, the findings of the third experiment confirmed that AsA, Si and GA3 enhanced the performance of secondary seeds originated from the first experiment. Considering the results of all experiments GA3 performed better than the AsA and Si in mitigating salt stress.

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LIST OF ABBRIVIATIONS

Abbreviation	Full name
%	Percentage
°c	Degree Celsius
$_{1}O^{2}$	Singlet Oxygen
ABA	Abscisic acid
AEZ	Agro-Ecological Zone
Agric.	Agriculture
Agril.	Agricultural
Agron.	Agronomy
ANOVA	Analysis of Variance
Appl.	Applied
APX	Ascorbate peroxidase
AsA	Ascorbic acid/Ascorbate
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Biol.	Biology
CAT	Catalase
Chl	Chlorophyll
cm	Centimeter
CRD	Completely Randomized Design
CV	Coefficient of variance
DAE	Department of Agricultural Extension
DAS	Date After Sowing
DHAR	Dehydroascorbate reductase
DW	Dry weight
Ecol.	Ecology
Environ.	Environmental
et al.	And others (at elli)
Etc.	Etcetera
Exptl.	Experimental
FAO	Food and Agriculture Organization

FW	Fresh weight
g	Gram
GA3	Gibberellic Acid
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced Glutathione
H_2O_2	Hydrogen peroxide
ha	Hactare
HI	Harvest index
J.	Journal
kg	Kilogram
LSD	Least Significant Difference
m	Meter
M2	Square meter
MDA	Malondealdehyde
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
MG	Methylglyoxal
mm	Millimeter
mM	Milimolar
MOP	Murate of Potash
NAA	1-Naphthaleneacetic acid
NAPDH	Nicotinamide adenine dinucleotide phosphate
Nat.	Natural
No.	Number
Nutr.	Nutrition
O_2 •−	Superoxide radical
OH•-	Hydroxyl radical
PEG	Poly Ethylene Glycol
pH	Hydrogen ion conc.
Physiol.	Physiological
POD	Peroxidase
POX	Peroxidases Pro Proline

Res.	Research
ROS	Reactive oxygen species
RWC	Relative water content
Sci.	Science
SE	Standard Error
Soc.	Society
SOD	Superoxide dismutase
SOS1	Salt overly sensitive 1, Na^+ /H^+ antiporter
SPAD	Soil Plant Analysis Development
t ha^{-1}	Ton per hectare
TSP	Triple Super Phosphate
viz.	Namely
μM	Micromolar

CHAPTER 1

INTRODUCTION

Wheat is one of the most critical food crops in the world, grown in a large area and with large production among cereal crops. The main cultivated species of wheat is *Triticum aestivum* L. It is hexaploid and known as "common" or "bread" wheat (Shewry and Hey, 2015). In Bangladesh total production of wheat was 1100000 metric tons and the area covers 350000 hectares (BBS, 2019). The global wheat production in 2017 covers more land area than any other crops and it is about 238.56 million hectares and yield 765.49 million metric tons which are higher than rice (500.8 million tons) (FAO, 2018a). Between the world cereal production wheat is now held in the second position after maize. China, India, Russia, USA and Canada are the top 5 wheat producing countries (FAO, 2018b). Wheat can grow in diversified topography with the prospect of adjusting to acute weather conditions.

As a sessile organism, plants are affected by various kinds of environmental stresses (salinity, drought, heat, cold, flooding, heavy metals, ozone, UV radiation, etc) which influence plant growth, yield and productivity that challenge food security of ever extended population all over the world. (Cramer *et al*., 2011; Hasanuzzaman *et al*., 2012a). Due to abiotic stresses such as salinity, drought, heat, chilling and other factors; the estimated potential yield losses are 17%, 20%, 40%, 15% and 8% respectively (Ashraf and Harris, 2005). Unfortunately, the damaging effects of these stresses on crop yield are increasing due to anthropogenic contributions thus threatening global food security (Savvides *et al*., 2016). Among abiotic stresses, salt stress is one of the critical determinants limiting crop production (Wassmann *et al*., 2009). Now, about 20% of the world total agricultural areas are affected by salinity (FAO, 2015). It is expected that, by the middle of the twenty-first century, 50% of the world's arable area will be influenced by salinity (Machado and Serralheiro, 2017). All cultivable land and supplied irrigation water hold a various amount of salt. The quantity and category of salt's presence rely on the makeup of both the irrigation water and soil. The soils do not review saline conditions unless the salt gathering in the surrounding root zone is high enough to restrain optimum growth and yield (FAO, 2015). Of the environmental stresses, salinity is one of the most brutal environmental factors, as most crops are sensitive to salt stress (Hasanuzzaman *et al*.,

2012). Salinity is one of the most alarming environmental factors that affected 20 % of irrigated land and 2 % of dryland agriculture area (Munns and Tester, 2008). Besides, the problem of salinity is increasing day by day due to climate change and bad agricultural practices. However, salt-induced damages start from seed germination and exist till the maturity of the plant (Peng *et al.*, 2008). Salt stress conditions usually reduce plant growth and development through osmotic imbalance, ion toxicity, and/or their interactions (Munns, 2002). Plant hormones are signal molecules generate within the plant, and found in fewer concentrations, and are an essential component of the signal cascade incorporate in the induction of plant stress responses. Intensive endeavours have been made to reduce the unfavourable impact of salinity by the practice of plant growth regulators. Plant growth regulators are often used to breaking seed dormancy, and can remarkably upgrade seed germination in several species, especially by the activation of embryo growth, mobilization of reserves, and incapacitation of the endosperm layer (Pallaoro *et al.*, 2016). Gibberellic acids as plant hormones play an essential role in various plant growth and maturing procedure, including seed germination, stem elongation, and leaf enlargement (Khan and Chaudhry, 2006).

Saline conditions crucially influence various plant development phases; germination and early seedling are acknowledged as more delicate growth stages in most plant species (Ibrahim *et al.*, 2016). These circumstances may incite effective diminishing the germination rate and percentage. Higher salinity induced both ionic and osmotic stresses in the plant that causes growth inhibition, yield reduction and even death of the plant (Mahajan and Tuteja, 2005; Ahmad and Sharma, 2008; Hasanuzzaman *et al.*, 2013; Nahar *et al.*, 2016). The immediate response of plants to higher salinity is osmotic stress that reduces cell expansion, cell division, and stomatal closure consequently inhibits leaf area, photosynthesis and growth of the plant.

In the later stage, plants experience ionic stress due to the accumulation of Na^+ and Cl^- at the toxic level. Higher accumulation Na^+ causes premature senescence of adult leaves by disrupting protein synthesis and interfering with enzyme activity (Shabala *et al.*, 2006; Munns and Tester, 2008; Wu and Wang, 2012; Rahman *et al.*, 2016a). Salt-induced osmotic stress, ionic toxicity and a lower rate of photosynthesis increase the production of reactive oxygen species (ROS). A higher composition of

ROS interrupts the antioxidant defense system and hence causes oxidative stress (Hasanuzzaman *et al*., 2013; Mishra *et al*., 2013).

Enhancement of ionic and osmotic homeostasis and improvement of ROS detoxification are some salt-stress tolerance mechanisms in plants (Wu and Wang, 2012; Wutipraditkul *et al*., 2015; Rahman *et al*., 2016). Usually, plants produce Proline or other compatible solutes to maintain osmotic homeostasis by maintaining the water balance by stabilizing protein and enzyme complexes (Iqbal *et al*., 2015; Reddy *et al*., 2015; Nahar *et al*., 2016). Besides this, plants have well equipped antioxidative system composed of non-enzymatic and enzymatic antioxidants for scavenging ROS. Non-enzymatic antioxidants include glutathione (GSH), ascorbate (AsA), carotenoids, tocopherols, and flavonoids which are crucial for the detoxification of ROS (Gill and Tuteja 2010). The antioxidant enzyme includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), and glutathione *S*-transferase (GST) located in different sites of cell and works together to detoxify ROS (Gill and Tuteja 2010; You and Chan 2015). The equilibrium between ROS and antioxidative capacity determine the destiny of the plant under oxidative stress condition (Hasanuzzaman *et al*., 2012).

Regulation of antioxidant defense to detoxify overproduced ROS, improvement of ionic and osmotic homeostasis and reduction of Na^+ influx by applying exogenous phytoprotectants are main approach to minimize salt-induced damage in plants (Hasanuzzaman *et al*., 2011a; Wu and Wang, 2012; Nahar *et al*., 2015; Ozfidan-Konakci *et al*., 2015). Mitigation of abiotic stress including salinity by using antioxidants is one of the common approaches for plant biologists in recent times. The antioxidants including ascorbic acid (AsA), play a significant role in the mitigation of additional cellular reactive oxygen species function caused by salinity stresses (Hasanuzzaman *et al*., 2012; Venkatesh and Park, 2014).

Reduced Ascorbic acid (AsA) is the non-enzymatic antioxidant component that plays a vital role in the AsA-GSH cycle. As an efficient electron donor, AsA directly reacts with ROS (Sharma and Dubey, 2005, Hasanuzzaman *et al*., 2011a). Enhanced synthesis of AsA plays a crucial role in conferring stress tolerance by detoxifying ROS and maintaining other antioxidants GSH, α-tocopherol etc (De Tullio *et al*.,

2004). Some studies also confirmed that exogenous application of AsA upregulates antioxidant defense system which confers stress tolerance against different abiotic stresses (Bybordi, 2012 and Alam *et al*., 2014).

The effect of plant elements in mitigating the effect of abiotic stress in plants including salinity has become a matter of interest in recent decades. Among them, Silicon (Si) is the second most abundant element in the soil. Although it is a non-essential element, it has some beneficial role for plant growth and development. The beneficial role of Si plays a role against various abiotic stresses (Liang *et al*., 2006; Zeng *et al*., 2011; Srivastava *et al*., 2015). Silicon reduces Na^+ uptake acting as a mechanical barrier. Accumulation of Si by plant reduces transpiration through deposition in the cell wall of leaves which decreases Na^+ uptake and transpiration. Also, Si application improves salt stress tolerance by improving the water status of the plant (Wang *et al*., 2015). Moreover, Si mitigates salt-induced oxidative stress by detoxifying ROS by regulating the antioxidant defense system (Zhu and Gong, 2014).

Plant hormones are essential endogenous molecules that are involved in the regulation of plant development and tolerance towards various stresses including salinity (Ryu and Yong-Gu, 2015). Of the plant hormones, gibberellic acid (GA3) helps enhance plant growth and development under abiotic stress conditions (Shaddad *et al*., 2013; Tabatabei, 2013). The foliar applications of protectants are efficient in mitigating the damage generates by salinity (Nounjan *et al*., 2012; Badran *et al*., 2015; Hassan *et al*., 2015). Plant species possess domestic mechanisms to cope with stress-induced unfavourable effects; however, these procedures are often not enough (Shahbaz *et al*., 2017). The exogenous application of gibberellic acid (GA3) developed sufferance under abiotic stress by induction and developing of the endogenous levels of salicylic acid (Alonso- Ramírez *et al*., 2009). Moreover, Seed priming with gibberellic acid (GA3) alleviate the drastic effect of salinity and increase grain weight and grain quality by improving photosynthetic pigments, leaf area and plant growth (Shaddad *et al*., 2013). Foliar applications of GA3 also confer salt stress tolerance by increasing germination percentage, plant growth and up regulating antioxidant enzyme (Tabatabei, 2013). A different study showed that gibberellic acid (GA3) has a significant outcome on various of the biological

processes that happen in plants, before-mentioned as better germination percentage in various plants under natural circumstance (Kandil *et al*., 2014; Al Sahil, 2016).

Although many studies revealed the mechanisms, by which AsA, Si and GA3 alleviate salt stress through ionic and osmotic homeostasis, regulating antioxidant defense and another biochemical process, it was necessary to further study to understand the mechanisms how they alleviate salt-induced damages in wheat.

The present study was therefore conceived with the following aim and objectives:

1.1 Objectives

Considering the above strategies, this study was initited to understand the role of exogenous application of AsA, Si and GA3 on antioxidant defense system, yield and nutrient homeostasis of wheat under salt stress.

- To investigate the effect of salt stress on growth and yield of wheat and it's mitigation by AsA, Si and GA3 against salinity,
- To assess the physiological and biochemical changes of wheat due to exogenous AsA, Si and GA3 under salt stress, and
- To evaluate the influence of AsA, Si and GA3 on growth performance of seedling of secondary seeds under salt stress.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Crops such as wheat, corn, rice, barley and sorghum supply 90% of the world's mandatory food and 68% of which is provided by the main cereal. Wheat (*Triticum aestivum* L.) is a grass widely cultivated for its seed, is one of the world's major cereal crops, and a staple crop for about 35% of the human population (Khan *et al*., 2010 and James, 2014). The anthropology record suggests that within the regions of the geographic region as productive lunulae around 9600 BCE wheat was initially cultivated. Botanically, the wheat kernel is additionally a form of fruit known as a caryopsis. It is one of the cereal crops which are a member of the grass family. The most common uses of cereal crops are food, feed or coarse grains (Bushuk and Rasper, 1994).

Wheat is a dominant medium of carbohydrates (Shewry and Hey, 2015). That is a superior source of protein and more calories in human nourishment, having a daily protein requirement fulfilled of about 71% carbohydrate, 13% protein and 1.5% fat which is comparatively high compared to other vital cereals and calories for one third of the people globally (Arzani and Ashraf, 2017; Enghiad *et al*., 2017; FAO, 2018b and Yu and Tian, 2018) but relatively low in protein quality for supplying essential amino acids (USDA, 2016). When eaten as the whole grain and a source of multiple nutrients and dietary fiber, vitamin B and minerals (Kochak-Zadeh *et al.*, 2013).

Globally, Cereals are grown on about two-thirds of all planted lands. Human food since ancient time's cereal crops are very prime crops due to their simple mode of development, storage and mobilization as well as their nutritional value (Molina *et al*., 2015). Because of this adaptability, cereal crops are also able to grow in a variety of climates. For instance, wheat can be grown in regions of high or low temperature, drylands where irrigation is involved, and in areas with high rainfall (Molina *et al*., 2015).

2.2 Wheat in Bangladesh

Wheat is the second most vital staple food crop after rice in Bangladesh. In Bangladesh total production of wheat was about 11 lac metric tons and area cover 3.5 lac hectares (BBS, 2019). However, over the last decades, the wheat sector in Bangladesh has seen several important changes in terms of fabrication. Grain production capacity has declined in recent years due to severe atmospheric sequences and inherent disasters.

Mexican varieties 'Sonora 64' and 'Kalyansona', successfully inaugurated in collaboration with the International Maize and Wheat Improvement Centre (CIMMYT) which is the primary stage of wheat cultivation in Bangladesh. In 1972, the release of 'Sonalika' created a true breakthrough in wheat production and was adopted in 80 % of the wheat area by the early 1980s (WRC, 2009). Wheat Research Centre (WRC) and Bangladesh Agricultural Research Institute (BARI), declared in 1983 four more high-yielding varieties 'Ananda', 'Kanchan', 'Barkat' and 'Akbar'. 'Aghrani' in 1987 and 'Protiva' in 1993, high-yielding varieties were proposed by the Bangladesh National Seed Board. Breeding efforts to evolve high-yielding varieties continued till now and developed several high-yielding varieties. BARI Gom-19 (Sourav) and BARI Gom-20 (Gourab) released in 1998, BARI Gom-21 (Shatabdi) released in 2000, BARI Gom-22 (Sufi), BARI Gom-23 (Bijoy) and BARI Gom-24 (Prodip) released in 2005 (Pandit *et al*., 2011); and BARI Gom-25, BARI Gom-26 released in 2010 (BARI 2012), BARI Gom-27 and BARI Gom-28 released in 2012, BARI Gom-29 and BARI Gom-30 released in 2014, BARI Gom-31 and BARI Gom-32 and BARI Gom-33 released in 2017 and widely cultivated in Bangladesh.

In 1971, a series of disastrous harvests led to widespread food shortages in Bangladesh. Massive imports of grains, oils and dairy products became a regular feature of the economy. From March to December 1974, Bangladesh suffers a dreadful food shortage as the price of rice hiked sharply in the world market (OECD-FAO, 2009) and production declined (Alamgir, 1980). Rice prices rise sharply from 1971 to 1975 worldwide, as a result of food shortages in Bangladesh was serious (OECD, 2008; FAO, 2008). Production of Rice shrink (Index Mundi, 2012) and disruptions agricultural activities throughout the War of Liberation in 1971 and the speedy growth of natural disasters, like floods, droughts, cyclones (Alamgir, 1980;

Hugo, 2006; Pandit, *et al*., 2011). At that point, it was realized that rice alone couldn't meet the food needs of the country (Banglapedia, 2006). Wheat was so chosen as another winter food crop. Mexican varieties (Sonora 64 and Penjamo 62) were trial earliest within the north Bengol in 1965 (BARI, 2010). Their glorious performance inspired scientists to introduce wheat additional usually to the present a part of the country. From 1971, Bangladesh had become extremely passionate about wheat imports whereas dietary preferences were ever-changing such wheat was turning into an extremely fascinating food supplement to rice.

2.2.1 Importance of wheat

Wheat is a primitive agricultural crop in the world (Zohary and Hopf, 2000). It was the first cultivated crop in the world followed by rice and maize (Feldman, 1995). Wheat is commonly called the king of grains in field crops (Kotal *et al*., 2010). It is the most predominant food for 40% of the world's population, particularly for people living in the Western and Northern parts of Asia, Europe and North America. It ranks first in global grain production and makes up more than 20% of the total food calories in human nutrition (Peng *et al*., 2011; GRDC, 2012). The demand for wheat cultivation is growing faster than any other cereal crop and wheat grain production must increase an annual rate of 2% to meet out human demand by 2050 (Bhalla, 2006; Weigand, 2011). On average wheat supplies 0.33 kilocalorie of energy also it contains beneficial vitamins and nutrients which are good for the human meals (FAO, 2014; FAO, 2017). Wheat demand is increasing day by day due to its gluten protein which is responsible for many bakery products.

The global wheat production in 2017 covers more land area than any other crops and it is about 238.56 million hectares and yield 765.49 million metric tons (FAO, 2018a). At present, wheat production is decreasing day by day due to various kinds of natural disasters, pest and disease, contesting with other Rabi crops, etc. There are many reports about various types of biotic and abiotic stresses that are the main cause of wheat yield reduction. According to CIMMYT and ICARDA (2011), about 20-30% yield reduction occurs due to an increase of 2-30°C temperature. Disease like leaf rust may cause 10-30% yield loss depending on plant susceptibility (Singh *et al*., 2015). Wheat yield may be reduced totally due to severely wheat blast disease (Islam *et al*., 2016). The salinity itself may cause more than 50% yield reduction as it

has a detrimental effect on plant growth and development (Farooq *et al*., 2009). As a glycophytic plant, wheat is antagonistically pretentious by salt induce stress (Zhu, 2003). Wheat yield losses up to 45% due to salinity stress (Qureshi and Barrett-Lennard, 1998). Wheat cultivation under both irrigated and rain-fed conditions is affected by the salinity status of soil (Mujeeb-Kazi and De Leon, 2002). About 8-10% of spring bread wheat cultivated area in the world is already salt affected and it is predicted to increase in the future.

2.3 Salinity is an issue

Salinity is the most severe environmental stress affecting crop growth and productivity globally. It has been estimated that 25% of the whole of the cultivated world's land is affected by salinity and 33% of it is irrigated land (Haidarizadeh and Zarei, 2009). Ben-Mahmoud (2001), Moud and Maghsoudi (2008) and Sattar *et al*. (2010), estimated that 19% of the total 2.8 billion ha of agricultural land is affected by salinity in the world. Unlukara *et al*. (2010) reported that about 40,000 ha of the agricultural area become unavailable for agricultural production every year because of the increase in soil salinity. Saboora *et al*. (2006) mentioned that most of the salt affected soils are associated with cotton, rice, wheat, and sugarcane and rapeseed cultivation. Salinity can occur naturally (primary salinity) in soils from long-term chemical and physical weathering of parent materials. It is also occurs as a result of anthropogenic activities (secondary salinity) including overexploitation of irrigation schemes by low quality irrigation water to crop field. Total salt-contaminated soil around the world is about 1125 million ha here 76 milion hectare are created by manmade salinization (Wicke *et al*., 2011 and Sanower, 2019). Hasanuzzaman *et al*. (2014), stated that a salt affected soil around the world continues in such method, 50% of tillable lands are going to be lost by 2050.

2.3.1 Salinity of Bangladesh

Bangladesh could be a deltaic country with a total space of 147, 570 km^2. The most important part (80%) of the country consists of sediments deposited by the stream Ganges River, Tista, Jamuna, Meghna and their tributaries. Terraces with the associate altitude of 20 to 30 m covered almost 8% of the country, whereas hilly areas with an associate altitude of 10 to 1000 m occur within the south-eastern and

north-eastern portion. The coastal region covers virtually 29000 km^2 or regarding 20% of the country. In Bangladesh, salinity affected land were 83.3 million hectares in 1973, which increased up to 102 million hectares in 2000 and the area gradually has risen to 105.6 million hectares in 2009 and continues to hike. Last 35 years, salinity has escalated to 26% nationwide. Coastal areas of Bangladesh covered an area of 274.22 and 351.69 million hectares moderately and strongly saline soil, respectively (SRDI, 2010).

The coastal areas of Bangladesh covered 30% of the productive lands of the country. Regarding 53% of the coastal land measures are stricken by salinity. Agricultural land use in these areas is incredibly poor, which is far less than the country's average cropping intensity. Salinity causes an unfavourable setting and hydrological state of affairs that forbid the standard crop production throughout the year. The factors that contribute considerably to the event of saline soil unit, prevalence flooding throughout the wet season (June-October), direct inundation by saline water, and upward or lateral movement of saline water throughout the time of year (November-May). The severity of salinity drawback in the Bangladesh will increase with the desiccation of the soil. It affects crops depending on the degree of salinity at the very important stages of growth, which reduces yield and in severe cases total yield is lost. Soil reaction values (pH) in the coastal region vary from 6.0 to 8.4. The organic matter content of the soils is additionally pretty low (1.0-1.5 %). Micronutrients, like metal and metal square measure widespread (Sattar *et al*., 2010).

Throughout the wet monsoon, the severity of the salt injury is reduced because of dilution of the salt at intervals the root-zone of the standing crop. The dominant crop robe within the saline areas is a native transplanted Aman rice crop with low yields. The cropping patterns followed within the coastal square measures are chiefly Fallow Fallow-Transplanted Aman rice. Salinity drawback received little attention within the past. It’s become imperative to explore the possibilities of the skyrocketing potential of this (saline) lands for hyperbolic production of crops (SRDI, 2010).

In Eurasia, salt-affected soils occur in most countries and occupy a calculable space of 242 million hectares, fixing danger food security and therefore the action of 2030 (Zhang *et al*., 2015).

2.3.2 Soil salinity

Environmental distresses pessimistic influence plant growth, enlargement and overall crop production. Salinity, drought and nutrient imbalances are the prime environmental stresses (Ghassemi *et al*., 1995). It's been reported that but 10% of the world's tillable lands are unbound from these environmental difficulty, with drought and salinity being the foremost extensive (Goudarzi and Pakniyat, 2008a). Salt induce stress remains one among the oldest and most consequential environmental things, that detrimentally influences and considerably obstructs the expansion and productivity of crops significantly in arid and semi arid region (Qayyum *et al.,* 2007; Goudarzi and Pakniyat, 2008a; Mostafazadeh-Fard *et al.,* 2009; Dkhil and Denden, 2010; Abari *et al.,* 2011; Bhutta, 2011) wherever precipitation isn't sufficient and water provides also are scarce as compared to water required for crop production (Unlukara *et al*., 2010). Soil salinity is outlined because the increase within the accumulation of salts likes common salt, soda ash, and sulfate (Alamgir *et al.,* 2007). There are variety of alternative definitions, Ashraf and Foolad (2005), outlined soil salinity because the existence of an excessive content of absorbable salts, that impede or influence the expansion of crops. A saline soil may be conjointly outlined as a soil that consists of associate in nursing satisfactory quantity of dissolvable salts which may hamper the expansion of crops (James *et al*., 1982).

According to Munns and Tester (2008), soil can be classified as saline when the electrical conductivity (EC) equals or exceeds 4 dS m^{-1} and this amount of salinity can decrease crop fabrication vastly. Pessarakli (1994) observed that soil salinity can be divided into five types. Firstly, saline soil that occurs due to the effect of electrolytes of sodium salts. This soil can be formed in desert and semi-desert regions. Secondly, alkaline soil which is produced due to the effect of electrolytes of alkaline hydrolysis. This kind of soil is found in all climatic regions. Thirdly, soil which is salt-affected by $CaSO_4$ or $CaCl_2$. It can be formed in arid and semi-arid regions (North America, North Africa, the Middle and Far East, and Australia). Fourthly, magnesium induced soil salinity occurs in desert and semi-desert regions. It can also be formed in semi-humid regions. Finally, acid sulphate soil which is formed as a result of $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$ accumulation. This soil can occur throughout the world in regions close to the seacoast and in tidal marsh areas.

2.4 Causes of Salinity

Soil salinity is in addition a complex development that might have a natural origin caused by raise of water level or marine intrusion, and an associate thropogenetic origin owing to agricultural augmentation, use of poor quality irrigation water and uncontrolled application of mineral fertilizers. This process are often accentuated by global climate change and causes a discount in crop yields, affecting small farmers and rural communities more acutely, who have fewer means for its minimization or adaptation. Many factors can affect the formation of, or increase in, soil salinity and these are directly include the weathering of minerals from parent rocks (Munns and Tester, 2008; Ozturk *et al*., 2009), and accumulate of oceanic salts carried by wind and rain (Ozturk *et al*., 2009). Also soils may become saline as a result of human activities such as irrigation practices, which increase salt accumulation in arid and semi-arid regions (Noori *et al*., 2006). Furthermore, in tropical and subtropical regions, deforestation processes are considered as the main source of salinity (Chan, 2001; Hachicha and Abdelgawed, 2003). Moreover, chemical fertilizers can also contribute to producing saline soil when they are used excessively (Chhabra, 1996). Many other factors can cause salinity including dust deposited on the soil, herbicides, insecticides, fungicides, and solid wastes can cause salt surface deposition (Munns and Tester, 2008).

2.4.1 Weathering Reactions

Salts can be formed as a result of the weathering process. The chemical weathering of rock minerals in the revealed layer of the earth's crust significantly supply of all soluble salts. Weathering processes, including hydrolysis, hydration, oxidation, carbonation and many other processes decompose minerals in rocks. These processes discharge dissoluble ions that join together to form salts. The type of ions released depends on the kind of rocks which are exposed to weathering processes but are mainly sodium chloride, calcium chloride and magnesium chloride (Munns and Tester, 2008). The geochemical processes that are involved in weathering reactions depend on the efficient elimination of the weathering products from the reaction location and the reaction of weathering processes can form salts that are not related to the rocks.

2.4.2 Surface Deposition

Many sources can contribute to soluble salts, mainly rainwater, irrigation water, dust deposited on the soil, herbicides, insecticides, fungicides, and solid wastes. Rainwater carries from 6 to 50 mg kg^{-1} of NaCl which decreases as the distance from the coast increases (Munns and Tester, 2008). However, Munns (2009); Ozturk *et al.* (2009), yearly estimated that rainwater distributes 10 kg ha^{-1} of sodium chloride for every 100 mm of rainfall. Cultivated lands increase in line with the need to produce more food, which leads to more irrigation practices. The increase in irrigation practices drives the increase in salt accumulation in soil. The use of bad quality or slightly saline irrigation water can cause an increase in soil salinity over time. Lakhdar *et al.* (2008) reported that irrigation with poor water is one of the main causes of increasing salt accumulation and reducing crop yield. Deef (2007), and Unlukara *et al.* (2010), declared that 50% or more of irrigated areas around the world are exposed to the effects of salinization and waterlogging. Moreover, Curtis *et al.* (2002), mentioned that irrigation water might carry from 1 to 4 kg m^{-3} of soluble salts yearly presenting 1-60 t ha^{-1} annually of soluble salts. According to the FAO (2009), secondary salinity, which is caused by human activities, has affected over 30 million ha of the estimated 1,500 million ha of agricultural land. However, Patel *et al.* (2010), reported that over 40 million ha of irrigated areas are affected by salinity, which is approximately one-third of the whole irrigated area in the world. However, Sattar *et al.* (2010), reported that about 50% of whole irrigated lands in the world are affected by salinity.

2.4.3 Deforestation

It has been estimated that 9 million km^2 of the globe's arid lands have been turned into manmade deserts over the past half century (Tavili *et al.*, 2011). Salinity has also occurred in 10 tropical and subtropical regions due to deforestation processes (Bhutta, 2011). It is believed that it is due to the migration of ions in the soils. For instance, trees use groundwater as a source of water for their growth; when the trees are felled, the soil water table is increased due to the filtration of rainwater and irrigation water (Chan, 2001). Thus, dissolved salts from raw rock material are raised with the water to the depth close to the surface of the soil. Consequently, as a result of the evaporation process, salts are lifted to the surface of the soil causing salinity

(Chan, 2001). For instance, in India wild regions of former forests become extremely saline in a few years after the removal of the trees (Pessarakli, 1994).

2.4.4 Contamination with Chemicals

Although the quantity of chemical fertilizers, which are used in agriculture, is low compared with the amount of salt in some soils, they have also been considered year after year as a major source of salinity which occurs due to intensive agricultural production, especially in greenhouses where chemical fertilizers are often heavily used. Sewage sludge and industrial emissions can increase the accumulation of some ions causing saline soil which leads to a reduction in the productivity of soil (Pessarakli, 1994; Chhabra, 1996; Bond, 1998).

2.5 Major constrains imposed by salinity stress

The mechanisms of growth inhibition as affected by salinity include the osmotic or water deficit effect and specific ion excess affect (Blumwald *et al*., 2000; Flowers and Flowers, 2005; Salama *et al*., 2011). Saline condition effects crop by causing inferior germination and seedling formation. It has been reported that during germination and early seedling establishment, plants are more sensitive to salinity (Farooq, 2009; Dhanapackiam and Ilyas, 2010). The osmotic effect is the decrease of osmotic potential due to the high accumulation of ions in the solution of the growth medium, which reduces the ability of a plant to take up water and leads to decrease growth. The ion specific effect is described as the increase of toxic ions (e.g Na^+ and Cl^-) in the plant tissue with a decrease in beneficial ions (e.g K^+ and Ca_2^+), thus decreasing plant growth (Munns, 2002; Munns *et al*., 2006; Karimi *et al*., 2009; Khayatnezhad *et al*., 2010; Salama *et al*., 2011). Sayar *et al*. (2010a, 2010b), and Abari *et al*. (2011), reported that salt affected soils contain enough soluble salts to restrict the growth of, and cause damage to plants through a series of interacting factors such as osmotic potential and ion toxicity. The latter may not be anticipated to possess a straight impact as plants have a reserve of nutrients that they will mobilize (Akram *et al*., 2007). Furthermore, it has been reported that the germination of seeds may be affected by soil salinity either by creating osmotic potential external to the seeds, which inhibits water uptake or by the toxic effects of Na^+ and Cl^- ions on germinating seeds (Janmohammadi *et al*., 2008; Mohammadi, 2009; Patel *et al*., 2010). Distinguishing between these two types of stress is important to understand

the physiological mechanisms for the salinity tolerance of plants (Munns and Tester, 2008). Moreover, Physiological and biochemical processes can be impacted by both osmotic and ionic stresses (Alamgir *et al*., 2007; Azooz, 2009). In the root zone excess soluble salts shown adverse effects on plant development and yield through nutritional disproportion, osmotic effects and particular ion toxicities (Munns, 2005; Tahir *et al*., 2006). Basant *et al*. (2018), found that salt stress conditions (0, 4 and 8 dSm^{-1}) have a significant decrease in plant height, leaf area, number of effective tillers per plant, number of spikes per plant.

2.5.1 Osmotic stress

The osmotic stress helps to water uptake of plants can be limited by salinity due to a reduction in the osmotic potential of the growth medium (Dixit and Chen, 2010). The osmotic pressure of soil increases by the increase of soluble salts which affect the ability of plants to absorb sufficient amounts of water (Epstein, 1980). Tavili *et al*. (2011) observed that the accumulation of salt concentration in the soil results in a reduction in moisture potential, which impacts moisture accessible. Water shortage or osmotic effects are probably the main physiological mechanisms for growth reduction as salinity stress reduces the soil water potential. When salts are accumulated around the root zone, the osmotic pressure will be increased to the threshold level and plants will be affected straight and leaf and shoot development ratio drastically decline (Epstein and Bloom, 2005). The emergence rate of new leaves will be slower than usual and the development of lateral buds will also be slow or will stay dormant. These effects are a result of the osmotic impact of the accumulation of ions in the rooting zone. Subsequently, the reduced rate of photosynthesis (caused by stomata closure) increases the formation of ROS, and increases the activity of enzymes that detoxify these species.

In the osmotic effect, immediately shrink cell expansion in root tips and juvenile leaves, and causes stomatal closure (Munns and Tester, 2008). Normally the most rapid response by plants to osmotic stress, which is presented due to salinity or increased drought, is to reduce the consumption of water by closing stomata or decreasing the leaf surface area. However, these mechanisms may affect the exchange of gases and the ability of the leaf to reduce the temperature of the plant caused by transpiration processes. Also long periods of osmotic stress lead to the extension of the roots to attain deeper soil moisture and transfer it to the plant

(Epstein and Bloom, 2005). The number of tillers in the whole leaf area is heavily influenced by the increase of salinity in cereal crops (Munns and Tester, 2008). The reduction in water uptake by the plant from the soil is due to the decrease in leaf production, which leads to the retention of moisture in the soil, preventing salt uptake. In addition, the evapotranspiration process can also be affected by the increase of salinity (Chhabra, 1996). This effect is due to the decrease in the availability of water as a result of the decrease in osmotic potential, reduction in leaf area and higher maintenance of water in the plant to reduce the absorbed salts (Chhabra, 1996).

2.5.2 Ionic Toxicity

Na^+ appears for the most species, to be the vital toxic ion as it could reach a toxic level premature than Cl^- does (Munns and Tester, 2008). When the concentration of salts in the old leaves rise to toxic levels this causes the death of the old leaves. Moud and Maghsoudi (2008) reported that when the rate of transpiration is high, the salt will be concentrated in the leaf causing it to die. Moreover, Neumann (1997) reported that when ions are accumulated in the transpiring leaves (old leaves), leaf senescence and necrosis are accelerated. Consequently, the provision of carbohydrates and hormones are reduced. Munns and Tester (2008) mentioned that in low and moderate concentrations of salinity, the ionic effect has less effect on the growth than the osmotic effect. Ion toxicity may affect the cell membrane. As the regulation of the exchange of materials between the cell and the surrounded environment is an important function of the cell membrane, the accumulation of salts leads to the destruction of the membrane structure and the replacement of Ca_2^+ by Na^+ at the binding sites (Jacoby, 1994). This effect on membrane structure produces enhanced membrane permeability and ion leakage from the cell (Bewley and Black, 1994). Limitation of Na^+ uptake, segregation of Na^+ into vacuoles active Na^+ exclusion back to the soil solution and to maintain cellular homeostasis, control of xylem Na^+ loading and its extraction from xylem are the main implementation for salt tolerance at the cellular level (Munns and Tester, 2008; Shabala and Cuin, 2008; Maathuis, 2014). A high accumulation of ions in the growth medium or in the plant itself may cause toxicity to the plant. Therefore, the normal growth of plants will be affected (Chhabra, 1996). It has been reported that Ca^{2+} decreases the effect of salinity on membrane integrity (Easterwood, 2002; Iqbal, 2005; Afzal *et al*., 2008)

by maintaining the selectivity of K^+ : Na^+ ratio (Royo and Abio, 2003; Gobinathan *et al*., 2009).

Active transport of Na^+ out of cytoplasm across the plasma membrane is mostly mediated by the Na^+ /H^+ antiporters (Barrett-Lennard and Shabala, 2013; Maathuis, 2014). The salt overly sensitive (SOS1) gene can affect the long-distance Na^+ transport by controlling Na^+ concentrations in xylem sap by unloading Na^+ from or loading Na^+ into xylem vessels depending on the severity of salinity stress (Guo *et al*., 2009). Transporters NHXs mediate both Na^+ /H^+ and K^+ /H^+ exchange and therefore affect both salinity tolerance and K^+ nutrition (Leidi *et al*., 2010). The high-affinity K^+ transporters (HKTs) were first isolated from wheat (Schachtman and Schroeder, 1994). High-affinity K^+ transporters can also play an important role in Na^+ translocation to the shoots and Na^+ accumulation in leaves by contributing to Na^+ unloading from the arising xylem sap and favouring Na^+ recirculation from leaves surface to roots (Flower and Yeo, 1986; Mian *et al*., 2011). The up-regulation of the high-affinity K^+ transport activity is highly correlated with a decrease in leaf Na^+ content (Plett *et al*., 2010).

2.5.3 Cytosolic K^+ homeostasis

Nowadays, the third major obstruction of salt induces stress, namely its distraction to cytosolic K^+ homeostasis was uncover (Shabala and Cuin, 2008). As a crucial macronutrient K^+ is essential for a varying cellular activity like osmotic regulation, maintenance of membrane potential, enzyme activity, synthesis of protein and starch, respiration and photosynthesis (Schachtman and Schroeder, 1994). Ion transport across the plasma and intra-organelle membranes K^+ is essential as a counter ion for the charge balance (Anschutz *et al*., 2014; Dreyer and Uozumi, 2011). High cytosolic K^+ levels are also essential to suppress the activity of caspase-like proteases and endonucleases, thus reduce the cell risk in transition to PCD (Bortner *et al*., 1997). A strong positive correlation between the ability of plant roots to retain K^+ and salinity tolerance was revealed in a wide range of crop species in barley (Chen *et al*., 2007), wheat (Cuin *et al*., 2008). In wheat, these K^+ retention capabilities give out to 60% of genetic variance in salinity stress tolerance (Anschutz *et al*., 201; Cuin *et al*., 2012). Since excessive Na^+ ions repress various important cellular processes many of which are directly correlated with K^+ transport and essential functions of K^+, it is well recognized that K^+ alleviates the toxic effects of Na^+, and that a high K^+ / Na^+ ratio in

shoots, especially in leaves, is important in glycophytes for enhanced salinity tolerance (Hauser and Horie, 2010). It was also shown that the supply of K^+ fertilizers has a beneficial impact on plant performance under salinity stress (Siringam *et al*., 2013; Umar *et al*., 2011). Massive K^+ leak from cytosol has been observed under saline conditions. It is suggested that outward-rectifying depolarization activated K^+ channels (GORK in Arabidopsis) are the main pathway for the salinity induced K^+ efflux from the cytosol (Shabala and Cuin, 2008). An important factor contributing to a massive K^+ leak is ROS. In salt-stressed plants ROS levels are known to be much higher (Jacoby *et al*., 2011). Several types of K^+ permeable channels, including non-selective ion channels (NSCC) (Demidchik, 2014) and GORK (Demidchik *et al*., 2010) are activated by ROS, providing an additional (independent of membrane depolarization) pathway for K^+ leakage from the cytosol and even the vacuoles (Demidchik, 2014). If the K^+ leakage process takes too long, the vascular K^+ pool is depleted and the cell collapses. Restricting the Na accumulation or avert the K^+ loss from the cell can be maintained by the optimal Na^+ /K^+ ratio (Shabala and Cuin, 2008), and Na^+ /K^+ ratio has been considered as a coherent indicative index for salt induce stress tolerance.

2.5.4 Pernicious effects of ROS

Plant's have the ability to repair ROS-influence damage to essential cellular structures has also been contemplated as a lead attribute of salt stress tolerance principally in halophytes, which are converted to elevated saline soils (Maksimovic *et al*., 2013; You and Chan, 2015). A comparison of photosynthetic response between the halophyte *Thellungiella salsuginea* and the glycophyte *Arabidopsis thalina* during salt stress revealed that electron transport through PSII and the activity of plastid terminal oxidase protein (diverting up to 30% of total PSII electron flow to O_2) is substantially increased whereas they are inhibited in Arabidopsis (Stepien and Johnson, 2009), suggesting that alternative electron sinks have the potential to decrease salt-induced ROS production in halophytes (Bose *et al*., 2014). Additionally, the production of ROS is critically dependent on K^+ accessibility and Increases in the severity of K^+ deficiency were also associated with enhanced activity of enzymes involved in the detoxification of H_2O_2 and utilization of H_2O_2 in oxidative processes, and K^+ deficiency also caused an increase in NADPH-dependent $O_2{\bullet}^-$ generation in root cells (Cakmak, 2005). Thus, increasing the K^+ nutritional

supply can reduce the detrimental build-up of ROS either by enhancing photosynthetic electron transport or by inhibiting the membrane-bound NADPH oxidases and lead to enhanced salinity tolerance (Shabala and Pottosin, 2014).

2.6 Physiological mechanisms of adaptation to salinity

Crop research for salinity tolerance has become increasingly important because of the need to increase the productivity of crops in saline areas (Strogonov, 1964; Epstein, 1980). Due to the increase in population, it is very important to increase the production of wheat to overcome the serious difficulties in sustaining a wheat food supply (Curtis *et al*., 2002). Better wheat production can be attained in two methods: (1) increasing the area of wheat cultivated lands including saline and acid soils, (2) increasing the production of wheat per unit area sown (Curtis *et al*., 2002; Dixit and Chen, 2010). The salt tolerance of plants is defined as the capability of plants to grow in a saline environment (Parida and Das, 2004). Chhabra (1996) reported that it is difficult to fix a limit of salinity where the plant will fail to grow. Plant salinity tolerance is a complex phenomenon process that varies between species and different varieties (Azooz, 2009). This difference intolerance is based on a number of factors including plant physiology and growth stage (Chhabra, 1996; Nilsen, 2000; Orcutt and Azooz, 2009). Crops vary in their salinity tolerance. According to Munns and Tester (2008) rice (*Oryza sativa*) is very sensitive to salinity. On the other hand, the salinity tolerance of barley (*Hordeum vulgare*) is high. Wheat (*Triticum aestivum* L.) is contemplated to be quite tolerant.

Three different strategies or physiological salt tolerances are used by plants in order to adjust to soil salinity, namely, tolerance to osmotic stress, the exclusion of Na^+ and Cl^-, and plant tissue tolerance to accumulated Na^+ and Cl^- (Munns and Tester, 2008).

2.6.1 Osmoregulation

Many plants can use inorganic salts to increase their own osmotic pressure to an equal level with the soil osmotic pressure in order to extract water (Chhabra, 1996). This process is called osmotic acclimatization. Ashraf and Harris (2005), and Flowers and Colmer (2008), reported that organic (compatible) solutes can be used as osmotic adjusters. Moreover, plants also can use inorganic salts such as Na^+ , Cl^- , K^+ and Ca^{2+} to adjust their own osmotic potential (Ashraf, 2004). In new leaves and root tips, osmotic stress affects the plant by decreasing cell expansion and

encouraging stomatal closure. With greater leaf growth and stomatal conductance, the response to osmotic stress would be decreased but in plants that have enough available water, the leaf area would be increased due to the synthesis of sufficient carbohydrates especially in irrigated lands where water is guaranteed (Munns and Tester, 2008).

2.6.2 Exclusion of Na^+, K^+ and Cl^-

Plants are able to avoid absorbing salts which are not needed for growth, using their roots. Roots can avoid absorbing Na^+ to prevent accumulation at toxic levels in the leaves. This selective avoidance of ions is achieved via the cell membrane (Chhabra, 1996). To avoid salt concentrating in the shoots, roots must exclude 98% of the soluble salts, permitting just 2% to be moved through the xylem to the shoots (Munns, 2009). Cereals differ in terms of salt exclusion. In 50 mM NaCl, Janz cultivar can embargo 99% of resolvable salts while bread wheat can exclude 98%. Although, durum wheat, barley and rice exclude 94% of resolvable salts (Munns, 2005). The adaptation to Na^+ toxicity is not the only thing that is required for salt tolerance, the acquisition of K^+ is also required for salt tolerance, but because of the chemical similarity of K^+ and Na^+ ions, the high accumulation of Na^+ in the root zone influences the absorption of K^+ (Rodríguez-Navarro, 2000; Karimi *et al*., 2009). It is suggested that the transport systems of K^+, which involve good selectivity of K^+ over Na^+ can be considered a significant salt tolerance determinant (Rodríguez-Navarro, 2000). Bagcl *et al*. (2007) reported that the key trait contributing to salt tolerance is the discrimination of K^+ over Na^+. Also Aslam *et al*. (2003) reported that Na^+ ions that entered into the shoots are accumulated in the old leaves. On the other hand, the transportation of K^+ ions is continued and these K^+ ions are accumulated in the younger leaves. Any deficiency in excluding Na^+ leads to toxicity. This effect can occur in days or weeks depending upon the kind of plant (Munns and Tester, 2008). Tissue tolerance requires disassociation of Na^+ and Cl^- at the cellular and intracellular level to limit Na^+ accumulation and avoid toxicity in the cytoplasm. Plants should be able to compartmentalize the salt in vacuoles, thus the cytoplasm will be protected from ion toxicity, and prevent dehydration by avoiding a build-up of salts in the cell wall (Flowers and Yeo, 1986; Garthwait *et al*., 2005; Munns, 2009). Otherwise, the salt will be concentrated in the cells and cause death to the old leaves (Munns, 2009).

2.7 The effect of salinity on wheat

Like other crops, the production of wheat can be affected by both soil and water salinity. Salinity in the root area has negative impacts on the growth of wheat (Mc William, 1986). The influence of salinity can be extremely harmful for wheat. Even though wheat is grownup beneath each irrigated and rain-fed condition, each kinds of agriculture square measure vulnerable by salinization (Qayyum *et al*., 2007). Curtis *et al*. (2002) reported that the yield of wheat was decreased up to 50% by an increase in salinity to 13 dS m^{-1}. It has been reported that the low yield of wheat under salinity stress is often attributed to low seed germination, emergence and poor establishment of seedlings (Abro *et al*., 2009; Parida and Das, 2004). Seed germination and seedling establishment are the most sensitive phases to salt stress in many crops and are critical stages of the crop life cycle (Atia *et al*., 2006; Dkhil and Denden, 2010).

Akbari *et al*. (2007) reported that rapid seed germination and seedling establishment are important factors for crop yield in saline environments in arid and semi-arid regions. Many studies of seed germination under salt stress have shown that seeds of most crop species obtain their greatest germination in distilled water, and germination and seedling stages are decreased with an increase in salt level (Gulzar *et al*., 2003; Akbari *et al*., 2007). Kaya *et al*. (2009), studied the effect of salinity on germination and seedling establishment, and their results revealed that germination and emergence of wheat seeds were negatively affected by NaCl and the mean germination time was delayed. Saboora *et al*. (2006) studied the effect of salinity on nine wheat cultivars and the results showed that the increase of salt concentration had a negative effect on germination %, germination rate, and shoot and root dry weight. They also reported that, in the first phases of growth, wheat is highly sensitive to saline environments. The growth of seedlings can be affected by salt accumulation by reducing the speed of mobilization of the reserve 24 nutrients. Soil salinity affects the emergence of wheat and reduces by 50% with 8.8 dS m^{-1} soil salinity. However, Monasterio *et al*. (2002) reported that soil salinity affects wheat yield when electrical conductivity is greater than 6 dS m^{-1}. Generally the germination percentage, rate and growth of wheat are also affected by salinity. Furthermore, Fallah (2008), investigated the effects of salt stress on four wheat cultivars, and the results indicated that germination percentage, germination rate, the length of shoot and root, and dry

weight of shoots and roots were diminished with the increase of NaCl in all cultivars. Rahman *et al*. (2008) also reported that the presence of high concentrations of NaCl in the soil reduces the weight and the length of shoots and roots in wheat. Haidarizadeh and Zarei (2009) studied the effect of different concentrations of sodium chloride on seedling establishment in wheat and the results showed that the growth of shoots and roots significantly decreased with an increase in NaCl concentration. Also they reported that the total number, leaf weight, and leaf length were affected by the increase of NaCl. Moreover, it has been reported that leaf cell expansion and leaf area can be decreased by accumulation of salts in the plant (Ahmad *et al*., 2009). The length of the stem can be also affected due to the increase of Na^+ in the tissues (Naseem *et al*., 2001). It has also been reported that salt accumulated in the soil leads to a reduction in the quantity of leaves in the main shoot and reduces the quantity of spikelets in the main spike (Naseem *et al*., 2001; Curtis *et al*., 2002). The viability of tillers is also affected by salinity. The number of primary and secondary tillers is reduced with the increase in salt concentration to 7.5 dS m^{-1} in the soil (Curtis *et al*., 2002). Grieve *et al*. (1994), highlight that all phenological phases in wheat are hastened by salt accumulation in soil. The decreasing quantity of culms is the main aspect of growth that is affected by salinity (Maas *et al*., 1994).

2.7.1 Effect of salinity on germination

Germination of seed is one of the most basic and required stages in the plant's survival cycle. Salt creates the osmotic potential of germination media which obstructs the imbibitions of water, produces disequilibrium in the usual activities of enzymes and protein metabolism and declines the food storage of seed (Hasanuzzaman *et al*., 2013). Seeds required a longer time for germination under saline conditions (Afzal *et al*., 2008; Ghiyasi *et al*., 2008). Kaya *et al.* (2009) found that the effect of salinity stress on germination and seedling establishment and their results disclosed that germination and emergence of wheat were pessimistically affected by NaCl and the mean germination time was delayed. In the salinity stress, decreased germination rate and germination index, increased mean germination time at 12.5 ds m^{-1} (Akbarimoghaddam *et al*., 2011; Kochak-Zadeh *et al*., 2013) and decreasing germination percentage (Fuller *et al*., 2012).

2.7.2 Effect of salinity on morphological traits

Wheat responses to salinity stresses are complex and depend upon several factors such as duration of salinity, group of salts and developmental stage of the plant at exposure (Hamada and Al-Hakimi, 2001; Cramer, 2002; Saqib, 2002).

2.7.2.1 Growth

Fallah (2008), investigated the effects of salt stress on four wheat cultivars, and the results indicated that germination percentage, germination rate, the length of shoot and root, and dry weight of shoots and roots were diminished with the increase of NaCl in all cultivars. Akhtar *et al*. (2015), found that Salt induces stress significantly decreased all growth parameters of wheat like plant survival rate, shoot length, shoot dry weight, number of tillers, number of spikes, spike length, number of spikelet per spike and grain yield. Salt stress restricted growth and yield with maximum and has been attributed to reduced water availability and Na^+ toxicity to the plants. The length of the stem can be also affected due to escalating of Na^+ in the tissues and salt accumulated in the soil leads to a reduction of leaves in the primary shoot that shrinks the capacity of spikelets in the principal spike (Naseem *et al.,* 2001; Curtis *et al*., 2002).

2.7.2.2 Leaf

Increasing salinity result thickness of the epidermis, thickness of mesophyll tissue, diameter and length of palisade tissue, diameter of spongy parenchyma cell, and a diameter of the vascular bundle were increased (Makbul *et al*., 2011; Xu *et al*., 2014). In wheat salt stress conditions indicated a significant reduction in leaf-area (Kumar *et al*., 2017). Huge salts accumulation in photosynthetic tissues causes a decline in leaf-area and leaf senescence (Rauf *et al*., 2010). Delicate salinity stress leads quickly to development inhibition of leaves and stems whereas roots might still extend (Nonami and Boyer, 1990; Spollen *et al*., 1993; Lacerda *et al*., 2003).

2.7.2.3 Tiller

Salt induced stress decreased the number of tillers per plant with increasing NaCl salt and salinity stress at tiller emergence can inhibit their development and can cause their death at last stages (Nicolas *et al*., 1994). At 250 mM NaCl stress, maximum secondary tillers were dried and several numbers of primary tillers were greatly

consolidated (El-Hendawy *et al*. 2009; Shahzad *et al*., 2012). The amount of primary and secondary tillers is a decline with the rising salt concentration to 7.5 dS m^{-1} in the soil and tillers efficacy is also inhibit (Curtis *et al*., 2002; Mass *et al*., 1994). Grieve *et al*. (1994), observed that all phonological stages in wheat are accelerated by a salt deposit in soil.

2.7.2.4 Shoot and root dry weight

In wheat, root morphological changes under various environmental conditions have straight influences to the plant inhabitant form, above ground parts and its biomass composition (Zhang *et al*., 2002; Liu *et al*., 2006). Increased portioning of assimilates towards the leaf at the consumption of the stem due to increased NaCl and Cd stress (Sifola and Postiglione, 2002). Shoot dry weight reduced by almost two times in NaCl treated plants (Lin and Kao, 2001). Massive and fast augmentation of the young cells developed by meristematic division's results in plant growth. Cell expansion in roots and leaves can be inhibited by salinity (Hussain *et al*., 2004). The detrimental effects of NaCl on the dry weight of the shoot may be due to the direct effect on photosynthesis (Sayad, 2003).

2.7.2.5 Root length and volume

Rahman *et al.* (2008) reported that the presence of high concentrations of NaCl in the soil reduces the weight and the length of shoots and roots in wheat. Lin and Kao, (2001) reported that $NaCl^-$ induced cell wall thickening is a possible mechanism of NaCl abstracting root enlargement of wheat seedlings. Munns (2002) reported that toxicity of salinity have detrimental roots responds through changes in term of root volume and root average diameter. Hassan *et al*. (2008) reported that the root of rice retained higher Cd concentration than the stem, which resulted in reduced root weight. Mohammad *et al*. (2010) find that increasing concentration of NaCl had negatively affected root like inhibited total root length, root average diameter and total root volume. NaCl was functional in stimulating the deposition of ammonium in roots and that ammonium preceded retardation of root growth (Jbir *et al*., 2001).

2.7.3 Grain yield

Ghulam *et al*. (2013) reported that grain yield and yield components were decreased significantly by saline conditions as compared to non saline conditions and the

maximum reduction was tillers per plant 62.5% followed by grain yield per plant 57.65% and plant height 24.4%. Reduction in the total grain yield and yield contributing factors due to salinity (Saqib *et al*., 2004; Ghogdi *et al*., 2012). Generally, salt stress acutely affects various developmental variables and yield of wheat like other grain. Yield reduction of wheat may range from a tiny loss to severe crop defeat depending upon the extremity of the salinity complication (Chang and Sipio, 1991). In accordance with Kamkar *et al*. (2004), the salinity induced source limitation generally reduces yield by an extreme reduction in grain number and grain yield (Khan *et al*., 2010b). Due to higher concentrations of salts in the leaves metabolic processes like photosynthesis and protein assimilations are negatively affected and result in the reduced grain weight (Katerji *et al*., 2005). Monasterio *et al.* (2002) receded that salinity affects the yield of wheat when electrical conductivity is above 6 dS m^{-1}. In wheat, salinity stress reduced yield by more than 60% in their completely different growth phase by minimizing the multiplication and yield causative attributes (Farooq *et al*., 2009; Hakim *et al*., 2014).

2.7.4 Effect of salinity on physiological attributes of wheat

Stress responses of wheat plants have been complemented by the growing shreds of evidence for stress-induced physiological, biochemical and epigenetic changes (Kumar *et al*., 2017). Physiological disorders caused by salinity stress on plants have been substantially studied (Flowers, 2004).

2.7.4.1 Osmotic status, chl contents, ROS generation and membrane damage

Sairam *et al*. (2002) investigated that long-term salinity treatments (5.4 and 10.6 dS m^{-1}, 60 d) caused significant increase in H_2O_2 and lipid peroxidation in wheat seedlings, which were higher in salt-sensitive cultivar than salt-tolerant cultivar. Salinity also impacts the physiological and molecular functioning of the photosynthetic components like chlorophyll, PSII and carotenoids, which are degraded, thereby decreasing the photosynthetic efficiency of the plants (Demetriou *et al*., 2007). Increased lipid peroxidation and H_2O_2 levels with increased salinity stress in *T. aestivum* were observed in the study of Hasanuzzaman *et al*. (2011a). They exposed wheat seedlings to 300 mM NaCl and found 60 and 73% increase of H_2O_2 and MDA contents. Salt stress also decreased ascorbic acid (AsA) content by 52%.

Mishra *et al*. (2013) studied the differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive indica rice (*Oryza sativa* L.) seedlings and found salinity disrupts the antioxidant defense system through over production of ROS which consequently results in oxidative stress. Rao *et al*. (2013a), observed dose dependent increase in lipid peroxidation in wheat exposed to salt (2, 4, 8, and 16 EC) and these effects were variable among the cultivars. They found increased MDA content in cultivars, ZARDANA (55.9%), ROHTAS-90 (42.26%), SAUGHAT-90 (51%), and SHAHEEN-94 (52%), and hence they were designated as salt sensitive, whereas PUNJAB-85 (33%), BHAKAR 2002 (35%), PIRSBAK-05 (31%), and AUQAB (28%) showed decreased levels of lipid peroxidation and were categorized as salt tolerant (Rao *et al*. 2013b).

Saeidi-Sar *et al*. (2013) conducted an experiment with common bean and found NaCl treatment increased H_2O_2 content and lipid peroxidation indicated by accumulation of malondialdehyde (MDA) which contributed in cellular damage as well as reduction of growth of plant. Nahar *et al*. (2015) investigated that salt stress (200 mM NaCl) significantly increased the malondialdehyde (MDA), methylglyoxal (MG), H_2O_2, and Pro content, $O_2^{\bullet -}$ generation rate, and lipoxygenase (LOX) activity of as well as decreased the growth and development of mung bean.

Rahman *et al*. (2016a) carried out an experiment to investigate the effect of salt stress in rice seedlings. Hydroponically grown 13-day-old rice (*Oryza sativa* L. cv. BRRI dhan47) seedlings were exposed to 200 mM NaCl Salt stress caused oxidative stress in seedlings through lipid peroxidation, loss of plasma membrane integrity, higher reactive oxygen species (ROS) production and methylglyoxal (MG) formation. Salt stress also increases Pro content.

Rahman *et al*. (2016b) also observed salinity effect in another experiment. They hydroponically exposed 12-day-old rice (*Oryza sativa* L. cv. BRRI dhan 47) seedlings to150 mM NaCl and found salt stress resulted in disruption of ion homeostasis by Na^+ influx and K^- efflux. Higher accumulation of Na and water imbalance under salinity caused osmotic stress, chlorosis, and growth inhibition. Salt-induced ionic toxicity and osmotic stress consequently resulted in oxidative stress by disrupting the antioxidant defense and glyoxalase systems through overproduction of ROS and MG, respectively. According to Zou *et al*. (2016), *T. aestivum* leaves showed 35% increase in MDA content upon 100 mM NaCl

treatment for 5 d which further increased by 68% after 10 d of treatments.

Daoud *et al*. (2018) recorded that salt stress markedly increased the H_2O_2 content of wheat leaves at the four growth stages and hampered the normal morpho-physiological processes of plant. Mahmud *et al*. (2020) checked the effect of salt stress of Indian mustard and found that salt stress increased Na content and decreased K content in shoot and root. It disrupted the antioxidant defense system by producing reactive oxygen species (ROS; H_2O_2 and $O_2^{\bullet-}$), MG content and causing oxidative stress. It also reduced the growth and photosynthetic pigments of seedlings but increased Pro content.

2.7.4.2 Photosynthesis

Salinity may affect plant growth partially by declining the photosynthesis rate and a notable reduction in photosynthesis has been corresponding with a decrease in total chlorophyll content and malformation in chlorophyll ultra-structures (Platten *et al*., 2006; Meng *et al*., 2011; Lee *et al*., 2013; Waters *et al*., 2013). Chlorophyll effects content is directly correlated with growth and development of the plant and maintenance of chlorophyll content in salt-tolerant crops as wheat and legume species (Munns *et al*., 2006; Zhang *et al*., 2005 Baek *et al*., 2011; Arzani and Ashraf, 2016). The inhibitory consequence of salt on chlorophylls could be due to the repression of individual enzymes accountable for the assimilation of chlorophyll (Horie *et al*., 2009; Wu *et al*., 2014; Kumar *et al*., 2017). Salinity stress causes leaf senescence and metabolic damage by photosynthetic collapse prime to lower plant productivity (Kumar *et al*., 2015; Singh *et al*., 2015).

Salt stress significantly increased leaf Abscisic Acid (ABA) concentration (Akhtar *et al*., 2015) and that regulates gs, which directly influences photosynthesis (Liu *et al*., 2006), decreased leaf Chlorophyll content index (CCI) and total leaf N content were reduced and significant positive correlation was found between leaf Na^+ concentration and [ABA] leaf cell growth rate is decline under saline conditions, cells become tiny in size resulting in increased cell density (Karimi *et al*., 2005; Adolf *et al*., 2012). Salt stress caused a reduction in the chlorophyll content index is probably due to decreased uptake of N (van Hoorn *et al.,* 2001) and increased chlorophyllase activity (Reddy and Vora, 1986). Accumulation of ABA within the leaves has often according in plants exhibit to elevated salinity (Shahbaz and

Ashraf, 2013; Amjad *et al*., 2014) and consistent with this, a strong positive graphical affinity between [ABA] leaf and leaf Na^+ concentration (Shahbaz *et al*., 2017). Increased [ABA] leaf is regulation plant responses to associate degree extent of abiotic stresses besides salinity and drought (Keskin *et al*., 2010). It acts as a protracted distance signal molecule that meets the physiological responses between the roots and shoots (Wilkinson and Davies, 2002). Keskin *et al*. (2010) reported that under saline conditions the strength of the ABA signal is negatively correlated with stomatal conductance and thus conserving water. Increasing leaf succulence and size of pavement cells decreased stomatal density under salt stress condition (Shabala *et al*., 2012; Akhtar *et al*., 2015)

2.7.4.3 Accumulation of Ion

A detrimental effect of salinity is the sodium ion (Na^+) accumulation in plant tissues and elevated concentration of Na^+ discourage uptake of vital macronutrients from the soil like potassium (K^+) and calcium (Ca_2^+) (Demidchik *et al*., 2010; Hamamoto *et al*., 2015). Raised Na^+ concentration in the cytosol can cause a degradation of chlorophyll and avert the ordinary functioning of huge proteins and enzymes resulting from the contesting by Na^+ for K^+ binding sites (Dreyer and Uozumi, 2011, Anschutz *et al*., 2014; Cherel *et al*., 2014). The accumulated huge amount of Na^+ and Cl^- ions interrupt the uptake of fundamental ions which irritate the plant growth system. Salt-sensitive cultivars tend to uptake more Na^+ and this uptake rate increases with increasing concentration of salt (Mandhania *et al*., 2006). Lower accumulation of NO_3^- and PO_4^{3-} ions were recorded by Wahid *et al*. (2007). Higher uptake of Na^+ and Cl^-, and reduced uptake of K^+ and Ca_2^+ by salt induced stressed wheat seedlings (Afzal *et al*., 2013). Jamal *et al*. (2011) reported increased uptake of Na^+ and K^+ ions and decreased K^+ /Na^+ ratio in wheat shoots when exposed to 120 mM of NaCl. Asgari *et al*. (2012) and Afzal *et al*. (2013) recorded that under saline condition (15–16 dS m^{-1}) significant decrease of K^+ uptake. Higher accumulation of Na^+ and Cl^- and lower uptake of K^+, Ca_2^+ and Zn_2^+ ions under medium salinity (Guo *et al*., 2015).

2.7.4.4 Water relation

Arzani and Ashraf (2016) found that salt stress affects metabolic processes in wheat through impairment of water potential of cells, intake of crucial mineral nutrients,

membrane integrity and function, and ion toxicity. Excessive salt concentrations stimulate osmotic stress to plants, result in low water potential. Relative water content (RWC) declined by 3.5% in the salt-sensitive and 6.7% tolerant cultivars compared to their controls applying of 100 mM NaCl after 6 d manifestation (Mandhania *et al*., 2006). Lowering of osmotic potential with increasing salt concentrations they also reported. The proportion of water volume reduces in the root but increases in shoot and spike of cultivar of wheat named Banysoif 1 (Tammam *et al*., 2008). Lv *et al*. (2016) found that minor relative water content (RWC) in leaves of *T. monococcum* seedlings revealed to salt stress of 320 mM NaCl. Arfan *et al*. (2007) found that reduced water use efficiency (WUE) of sensitive and tolerant cultivars under salinity stress. Leaf water potential also decreased under salt stress of 150 mM NaCl (Wahid *et al*., 2007) and 16 dS m^{-1} (Poustini *et al*., 2007).

2.8 Mitigation of salinity stress by exogenous application of ascorbic acid

Ascorbic acid (Vitamin C) or Ascorbate (AsA) was discovered in the 1930's by Hungarian scientist Albert Szent-Györgyi chemical (Squires, 2011). The chemical formula of ascorbic acid is C6H8O6 (figure 3), Melting point 190–192 °C, Boiling point 552.7 °C and Molar mass 176.12 g·mol^{-1}. Ascorbic acid exhibit vital multiple roles in plants development, segregation and metabolic process and with higher amount of AsA content of plant expose superior protection against oxidative stress (Hasanuzzaman, *et al*., 2012b).

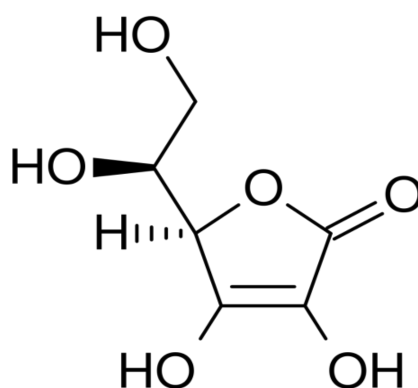

Figure 1. Ascorbic acid (reduced form)

Ascorbic acid ubiquitously gifts in plants and has been rumored to play an important role in assuaging the adverse effects of salt on plant growth and metabolism in several crop plants. (Hamada, 1998; Gest *et al*., 2013; Ozgur *et al*., 2013). It's an

abundant small molecule in plants. It’s a serious substance within the network of antioxidants that include glutathione, a-tocopherol, ascorbate, and a series of antioxidant enzymes. It’s also been exhibit to play numerous characters in plant growth, like in biological processes, cell membrane expansion, and other developmental processes (Pignocchi and Foyer, 2003; Buettner and Schafer, 2004). It’s also relying to detoxify O_2 and OH (Conklin, 2001).

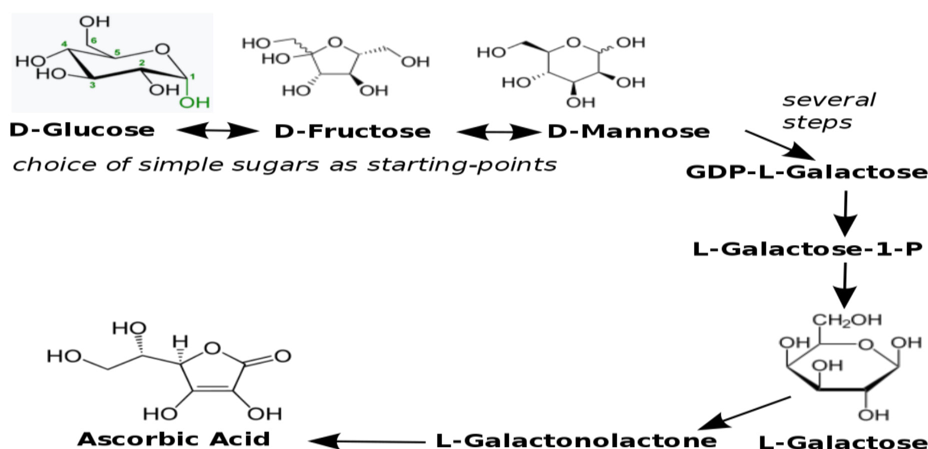

Figure 2. Ascorbic acid biosynthesis in plants (Dewick, 2009)

Generally, the consequences of the water-soluble vitamin in mitigating the adverse effects of salt stress are ascribed to the activation of a number of the enzymatic reactions (Venkatesh and Park, 2014; Hossain *et al*., 2017). Positive effects of antioxidants in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and also the photosynthetic apparatus from oxidative damage (Zhang, 2013).

Ascorbic acid functions as a significant reaction buffer, a compound for enzymes control phytohormones, chemical action, regeneration of antioxidants, and rising photosynthetic pigments. Ascorbic acid additionally controls the biological process, fruit growth, seed germination and plant growth (Younis *et al*., 2009; Bybordi, 2012; Gallie, 2013; Zhang, 2013; Akram *et al*., 2017). Moreover, AsA isn't solely essential for fruit ripening in fruit senescence (Green and Prof, 2005) however additionally plays a key role in check the cells from aerophilous harm by removing the ROS throughout organic phenomenon and non living stresses (Hemavathi *et al.,* 2010; Khan *et al.,* 2011; Venkatesh and Park, 2014; Akram *et al.,* 2017). Plants area unit able to circumvent ROS to stimulate aerophilous stress through the inhibitor system.

The inhibitor system restricts and alleviates aerophilous harm, and will increase the environmental stress resistance that always correlates with a well-organized inhibitor system (Conklin and Barth, 2004; Suzuki *et al.,* 2012). The activities of inhibitor enzymes like SOD (SOD), oxidase (POD), and enzyme (CAT) act as a key indicator in the inhibitor unconscious process (Gill and Tuteja, 2010; Khan *et al.,* 2016).

2.8.1 Germination

Germination is subjected by a proper ratio ABA/GA3 and during this procedure of ABA decline in favour of GA3 which prolongs germination up to the seedling stage (Kucera *et al*., 2005; Finkelstein *et al*., 2008). Exogenous application of AsA can partially recover seed germination from an ABA treatment (Nenghui and Jianhua, 2012). Consequently, AsA can control ABA/GA3 biosynthesis during seed germination and developed seeds are capable to germinate under favourable conditions. The water imbibitions of seed revive metabolism and the development of seminal tissues. Increasing of the ROS and antioxidants together with AsA, thioredoxin (Trx) or peroxiredoxin (Prx) (De Gara *et al*., 1997). Oxidative imbalances can reduce the potential for proper seed germination (Chen *et al*., 2014) and so a compact arrangement is required to equal ROS fabrication and scavenging. AsA contents enhance in the primary stages of germination and this is correlated with a rising in the enzyme l-galactono-1, 4-lactone dehydrogenase (GLDH), the last enzyme in AsA synthesis.

2.8.2 Photosynthesis

Ascorbic acid is connected in regulating photosynthetic ability by controlling stomatal movement (Chen and Gallie, 2004). Ascorbate is furthermore a serious co-factor of few enzymes or protein complexes that are connected within the regulation of photosynthesis (Davey *et al*., 2000). Habib *et al*. (2008, 2009), observed that exogenous application of AsA enhanced the stomatal conductance thereby favoring higher assimilation of CO_2 and increased endogenous level of AsA and CAT which had a protective outcome on growth and photosynthetic strength of wheat against salt induced oxidative stress. Application of AsA enhanced chlorophyll a, chlorophyll b contents under several abiotic stress conditions like salinity (Khan *et al*., 2006; Athar *et al*., 2009; Azzedine *et al*., 2011; Agami 2014; Billah *et al*., 2017), and osmotic stress (Alam *et al*., 2014). The progress of chlorophyll content and

composition fetched on by AsA application could also enhance photochemical efficiency (Dolatabadian *et al*., 2009).

2.8.3 Ion Homeostasis

Habib *et al*. (2008, 2009), observed that AsA raised K^+ aggregation in the leaves and that of Na^+ in the roots. Dilutes K^+ and Ca^+, and obstruct the harmful effects of salt pressure on wheat development by improving the photosynthetic efficiency of wheat plants while maintaining ion homeostasis. Exogenous application of AsA assist maintain plant ion homeostasis, by minimizing Na^+, rising K^+, and thus decreasing the Na^+/K^+ ratio (Al-Hakimi and Hamada, 2001; Athar *et al*., 2008, 2009; Farouk, 2011). The mechanism of AsA mediated ion homeostasis remains unspecified, but it is possibly due to the improvement of cell membrane equilibrium and/or plasma membrane Na^+-H^+ antiporter SOS1 (Zhu, 2003). Exogenous application of AsA elevated the depletion of both Mg_2^+ and Ca_2^+ contents under salt stress (Al-Hakimi and Hamada, 2001; Athar *et al*., 2008, 2009; Farouk, 2011).

2.8.4 Plant growth and yield

Externally applied AsA has shown that wheat boost large-scale growth (Hamada and al-Hakimi, 2001; Al-Hakimi, 2001), and tomatoes (Shalta and Newman, 2001). AsA-induced increase in growth beneath non-saline may area unit because of stimulating organic process and cell enlargement (Arrigoni, 1994). Shah *et al*. (2019) demonstrated that AsA application significantly promotes Germination, chlorophyll content, tillers per unit area, number of grains per spike, and 1000-grain weight, yield, productivity and biomass. The varied non-enzymatic antioxidants, like tocopherols, carotenoids, and phenols, vitamin C happens ubiquitously in plants and has been rumoured to play an important role in assuaging the adverse effects of salt on plant growth and metabolism in several crop plants (Hamada, 1998). Shalata and Neumann (2001) found that AsA applied through the growing medium counteracted the salt-induced reduction in growth and AsA was less effective in up the enlargement of salinity stressed wheat plant. Farouk (2011) found that exogenous application of ascorbic acid at plants can carry over the leaf aging by peroxide/phenolic/ascorbate system which is complicated in scavenging the ROS generate during leaf aging.

Generally, its concentration is superior in leaves than that in other plant parts and it's

5–10 times far away that of glutathione (GSH) (Smirnoff, 2005). Moreover, experimental studies on whole different plants have shown that exogenous application of Ascorbic acid might decrease salt induced adverse effects and finishes up during a huge increment of growth and yield. Ascorbic acid is concerned with rising strain tolerance and causes a substantial increase in plant growth and yield (Gul *et al.,* 2015). Batool *et al*. (2012) reported that abnormal result of salt stress on growth and chemistry attributes of pot-grown sugarcane plants were significantly improved by externally applied of water-soluble vitamin C and could overcome the damaging effects of salinity by increasing the endogenous levels of substance enzymes and organic compound, that successively was reflected in improved growth.

2.8.5 Antioxidant mechanism

Ascorbic acid is one in every of the foremost important antioxidants abundantly occurring in plants (Smirnoff, 2000 and Veljovic-Jovanovic *et al*., 2001). The role of AsA as an antioxidant has been shown by Muller-Moul ¨ e.´ *et al*. (2003, 2004), who demonstrated that AsA deficient mutants of Arabidopsis (vtc mutants) were more sensitive to ozone, dioxide, or UV-B light (). Similarly, vtc2 mutants of Arabidopsis experienced severe high light-induced oxidative stress along with increased lipid peroxidation and photo-inhibition when vtc2 mutants were transferred from low to high light (Muller-Moul ¨ e. *et al*., 2003, 2004). According to Ameer *et al*. (2006), Ascorbic acid was exogenously applied as a foliar spray with varying levels (0, 50, a hundred mg L^{-1}) in agriculture. Salt stress severely reduced the growth of S-24 (salt-tolerant) and MH-97 (moderately salt-sensitive) wheat cultivars. Foliar spray with AsA improved the expansion of non-stressed plants however didn't alleviate the adverse effects of salt stress on plants. Antioxidant protected the chemical action mechanism from the damaging effects of salt stress, it didn't improve the growth of both wheat cultivars beneath saline conditions. Ascorbic acid not exclusively acts as an associate degree substance but the cellular levels of AsA unit connected with the activation of sophisticated defense mechanisms (Conklin, 2001). It’s to boot been accustomed counteract the adverse effects of salt stress in many crop plants (Khan *et al*., 2010a). It’s projected functions in whole plant metabolism (Debolt *et al*., 2007). Billah *et al*. (2017) noted that AsA is involved in antioxidant defense directly by serving as a substrate of antioxidant enzymes to reduce H_2O_2 or indirectly by affecting other antioxidant pathways. Abiotic stress attributed upon plants causes

additional production of ROS, while AsA is engaged in the depletion of H_2O_2 by directly extinguish ROS in a nonenzymatic procedure, or through APX enzymatic scavenging in the water-water cycle and ascorbate-glutathione (AsA-GSH) cycle, escort ROS level to a sub-lethal state (Wang *et al*., 2017).

Habib *et al*. (2008, 2009) observed that Ascorbic acid is one of the most important and abundant water-soluble antioxidants for plants. Crop plants naturally accumulate of osmoprotectants and different organic compounds are incredibly low and this deficiency will be overcome by their exogenous application (Makela *et al*., 1998). Exogenous application of antioxidants has recently gained ground as an awfully promising means that of alleviating the unfavourable effects of salt on plant development and metabolism (Kefeli, 1981; Janda *et al*., 1999; Shalata and Neumann, 2001). The effects of vitamin C in mitigating the adverse effects of salt stress are ascribed to the activation of several protein reactions (Kefeli, 1981). The positive reaction of vitamin C in overcoming the unfavourable effects of salt stress was attributed to the stabilization and a safe gourd of synthetic action pigments and also the equipment from aerophilous trauma (Choudhury *et al*., 1993; Hamada, 1998). Exogenous application of osmoprotectants, antioxidants, or plant growth regulators is considered to be an alternative short-term solution to induce salt tolerance in some of the important crop cultivars (Barth *et al*., 2004; Pavet *et al*., 2005; Raza *et al*., 2006; Waseem *et al*., 2006; Younis, *et al*., 2012).

Ascorbic acid is taken into thought one in every of the foremost effective growth regulators against abiotic stresses (Conklin, 2001). Ascorbic acid synchronizes sort of cellular processes, like biological process, cell separation, and senescence (Venkatesh and Park, 2014). Moreover, AsA protects lipids and proteins and ameliorate patience against varied abiotic stresses, induces growth of the plant, action, transpiration, aerobic differentiation defense ability, and activity pigments (Khan *et al*., 2010c; Naz *et al*., 2016; Akram *et al*., 2017).

2.9 Mitigation of salinity stress by exogenous application of Silicon

Silicon is a chemical element with atomic number 14, melting point 1414 °C and boiling points 3265 °C. Glass bearing silica was manufactured by the Egyptians since at least 1500 BC by the ancient Phoenicians (Silicon, 2019). Silicon was given its present name in 1817 by Scottish chemist Thomas Thomson (Voronkov, 2007). In

1823, Swedish scientist Jöns Jacob Berzelius discovered amorphous silicon (Thomas, 1817 and Weeks, 1932).

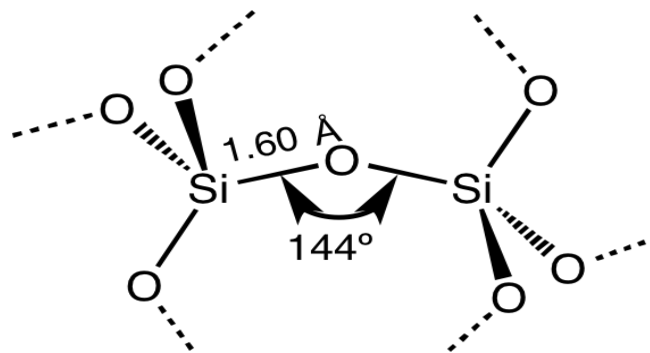

Figure 3. Structural motif of silicon dioxide

Silicon dioxide commonly known as silica, chemical formula SiO_2 usually found in nature as quartz and in different living creature (Iler, 1979) and Silicon (Si) is one of the most complicated and sufficient materials (Fernández *et al*., 2015). Silicon (Si) is that the second most plenteous mineral part within the soil after oxygen (Epstein, 1999; Liang *et al*., 2007) and is also a significant structural element of the cell walls in few monocotyledonous varieties (Inanaga and Okasaka, 1996). Silicon (Si) is the remunerative component for plants and that have been reported to strengthen resistance to salinity stresses (Hasanuzzaman *et al*., 2010; Hasanuzzaman and Fujita, 2011b; Tahir *et al*., 2012). Although Si could be a major constituent of plants up to now its essentialness has not been fully established, however numerous studies have incontestable that Si application considerably augmented plant growth under stress condition together with the organic phenomenon and abiotic stresses as salt stress (Rodrigues *et al*., 2003; Ma, 2004; Mukkram *et al*., 2010). Elevation of the unfavorable effects of salinity in huge plants together with *Oryza sativa* (Lekklar and Chaidee, 2011), common wheat (*Triticum aestivum*) by exogenous application of Si (Tuna *et al*., 2008; Tahir *et al*., 2012), *Brassica napus* (Hashemi *et al*., 2010), *Lycopersicon esculentum* (Romero-Aranda *et al*., 2006) and *Zea mays* (Moussa, 2006). Hashemi *et al*. (2010), found that exogenous Si elevated the disadvantageous effects of salinity on the development through lowering tissue Na^+ content, protecting the membrane integrity and enlarge ROS scavenging capability.

2.9.1 Si on growth

Salt susceptibility of wheat is documented and famous like other glycophytes (Erdei and Trivide, 1989; Trivedi *et al*., 1991; Zhu *et al*., 2004). Wheat plants show toxic responses to salt stress (Sharma *et al*., 2005). Wheat has also been designated as a Si accumulator (Mayland *et al*., 1991). Si application significantly increased the Si-content in flag leaves of wheat under saline and non-saline conditions and Silicon was deposited within the roots (Gong *et al*., 2003). Silicon deposits in plants at a rate equivalent to those of calcium, magnesium and phosphorous (Epstein, 1999). Si is beneficial for the growth of many plants under multiple abiotic and biotic stresses (Liang *et al*., 2003 and Ma, 2004). Si has a crucial significance for better plant development under salinity (Tahir *et al*., 2006). In the presence of Si in plants maintained the photosynthetic activity to increase dry matter production (Agurie *et al*., 1992).

Adatia and Besford (1986) found that exogenously application of Si increased the growth of a number of monocot and dicot species under saline conditions. Exogenous application of nutrients as Si has many more beneficial effects on plant growth and crop yields under stressful environments (Epstein, 2001 and Ma *et al.,* 2001). Ahmad *et al*. (1992) found that root growth remains unaffected in wheat by Si application. Gong *et al*. (2006) announced that attaching Si in solution culture did not change the development of glycophytes root showing a tiny effect on root length and root dry weight (15%). Similar findings have been reported by Moussa (2006), Al-aghabary *et al*. (2004); Yeo *et al*. (1999); Takahashi *et al*. (1990). The possible mechanisms responsible for better crop growth in the presence of Si under stressful circumstances might be the prohibition of loss of water from aerial parts of plant by holding the water status conserve by the plant (Takahashi *et al*., 1990). Ali *et al*. (2011) found that Si supplementation into the root medium improved the K^+ and K^+ : Na^+ ratio, leaf water potential and stomatal conductance, but reduced the Na^+ in *T. aestivum* . These plants also showed a concomitant increase in number of tillers, number of grains per spike, grain and straw yield and that element is beneficial in profoundly affecting physiological phenomena and improving wheat growth under salt stress.

2.9.2 Si on Ion transportation

Si obstructs the Na^+ transportation to gaseous parts of plants by its outcome on the transpiration movement (Yeo *et al.*, 1999) or by preparing with Na^+ (Ahmad *et al.*, 1992). Anser *et al.* (2012) reported that Silicon inclusion into the growth medium is of assistance for wheat growth by maintaining plant water status, better $K^+ : Na^+$, low electrolyte leakage and elevated plant protection system unfavorably affected by salt stress. Reduced growth under salt stress is the consequence of the high osmotic potential and Na^+ concentrations to toxic levels (Hasegawa *et al.*, 2000). Si application decrease Na^+ deposition in the roots of barley (Liang, *et al.*, 1999) and sugarcane (Ashraf *et al.*, 2010) or in the leaves of rice (Gong *et al.*, 2006), sorghum (Yin *et al.*, 2013) and alfalfa (Wang *et al.*, 2004) as well as in both roots and leaves of wheat (Tuna *et al.*, 2008). This effect is likely to be mediated by a Si-induced increase in H^+ATPase and/or Na^+ /H^+ antiporter activities under salt stress (Liang *et al.*, 2007; Zhu and Gong, 2014). The Si induced physical barrier in the roots is another mechanism by which Si mediates salt tolerance in plants. In tomato (Romero-Aranda *et al.*, 2006) and tobacco (Hajiboland *et al.*, 2017) incorporation of Si had no remarkable consequence on Na^+ concentration in leaves or roots. In rice, Si was observed to be deposited on the exodermis and endodermis of roots, which dramatically decreased apoplastic transport and transpirational bypass flow and, thereby Na^+ accumulation (Gong *et al.*, 2006). The different levels of contributions are made by the mechanisms supplying osmotic and ion homeostasis under mild and extreme salinity (Hasegawa *et al.*, 2000). Liang *et al.* (1999) observed that the salt tolerance due to Si application is attributed to selective uptake and transport of K^+ and Na^+ by plants.

2.9.3 Ameliorative effects of Si

Various mechanisms have been claimed to be involved in such ameliorative effects of Si under salt stress as activation of antioxidative defence and improvement of plants ability to uptake moisture and obtainable nutrients from the soil (Liang *et al.*, 2007; Currie and Perry, 2007; Hajiboland, 2012; Zhu *et al.*, 2020). Under salt stress, Si reportedly reduces Na^+ uptake but increases its $K^+ : Na^+$ ratio, mitigating the ion toxicity outcome in various plant species such as rice (Yeo *et al.*, 1999) and barley (Liang *et al.*, 1999). Probable mechanism for Si-treated amelioration of salinity stress comes from the Si-treated Mn_2^+ (Rogalla and Römheld, 2002) and Al_3^+ (Wang *et al.*,

2004) toxicities are the impact of capture these metals to the cell wall. Mn_2^+ is bound more strongly to the cell wall In the presence of Si to decrease Mn_2^+ concentration in the symplast (Rogalla and Römheld, 2002). Detoxification of Al_3^+ in the apoplast of the root apex and its reduced mobility are achieved by forming hydroxyl aluminum silicates (Wang *et al*., 2004). Saqib *et al*. (2008) exhibited those Si-arbitrate salt stress enhancement rely not only on minimizing Na^+ uptake and shoot-root conduction and diminish cell sap Na^+ concentration. Hajiboland *et al*., (2015) observed that effect of Si supplementation (1 and 4 mM) on wheat (*Triticum aestivum* cv. Homa) plants grown hydroponically under salinity stress (50 and 150 mM NaCl) plant biomass was found to decrease at both salinity levels and diminished leaf concentration of Na^+ in the cell sap but developed the cell wall-bound fraction, indicating a Na^+ detoxification motion negotiate by Si. Saqib *et al*. (2008) stated that indicates a role for cell wall Na^+ binding in the Si-arbitrate development amelioration in salt-stressed wheat. Wheat having a high strength for Si uptake and accumulation and the effects of Si concentrations on the salt stress alleviation and growth (Broadley *et al*., 2012). Tahir *et al*. (2012) reported that application of Si increased shoot and root dry weight and plant water contents and decreased Shoot Na^+ and $Na^+ : K^+$ ratio under stress conditions. Salt-stressed wheat by Si application was improved plant water contents in shoots, chlorophyll content, decreased Na^+ and increased K^+ concentrations in shoots (Tahir *et al*., 2012).

2.9.4 Si on mitigate salt stress

In wheat, Si application can tolerate salt stress effect (Ahmad *et al*., 1992). The effects of Si were mainly observed during the whole growing season under field conditions and the reproductive organs were the most responsive parts to Si addition (Tahir *et al*., 2006; Ali *et al*., 2012; Ahmed *et al*., 2013). Si-induced anatomical interruption in roots is a prime mechanism by negotiate salt tolerance in accumulator plants (Zhu and Gong, 2014). Some possible mechanisms through which Si may increase salinity tolerance in plants (Liang *et al*., 2003) include: increased plant water status (Romero-Aranda *et al*., 2006), stimulation of ROS (Zhu *et al*., 2004); immobilization of toxic Na^+ ion (Liang *et al*., 2003); reduced Na^+ uptake in plants and enhanced K^+ uptake (Yeo *et al*., 1999; Liang *et al*., 2005) and higher $K^+ : Na^+$ selectivity (Hasegawa *et al*., 2000). Wheat is a moderately salt resistance crop and a Si accumulating species (Broadley *et al*., 2012). Relief of salt stress has been

detected as a result of Si approach in hydroponics (Saqib *et al*., 2008 and Tuna *et al*., 2008) and its approach to soil (Tahir *et al*., 2006; Ali *et al*., 2012). It has been demonstrated that Si decreases Na^+ concentrations in the leaves and roots of wheat, leading to its enhanced salt resistance (Tahir *et al*., 2006; Saqib *et al*., 2008; Tuna *et al*., 2008; Ali *et al*., 2012). Ali *et al*. (2009) found that Si application may alleviate the salinity induced damages. Application of Si as 0, 50, 100, 150 and 200 mg L^{-1} as calcium silicate, an experiment was conducted on two contrasting wheat genotypes (salt sensitive; Auqab-2000 and salt tolerant; SARC-5) in 10 dS m^{-1} and 2 dS m^{-1} solutions. A silicon addition into the solution ameliorates wheat growth and minimizes Na^+ and enhanced K^+ uptake (Liang *et al*., 2005).

2.10 Mitigation of salinity stress by exogenous application of gibberellic acid

Gibberellic acid is well known as Gibberellin A3, GA, and GA3. It is a hormone that founds in both plants and fungi (Silva *et al*., 2013; Camara, 2015). The chemical formula of GA3 is $C_{19}H_{22}O_6$ (Figure 1). Authentic Gibberellic acid is white to pale yellow color and solid in nature (Silva *et al*., 2013). Melting point 233-235^{0}C and solubility in water is 5 g L^{-1} at 20^{0}C and molar mass is 346.37 g mol^{-1}.

The first invasion into the perception of GA3 was evolutions from the bakanae, or "foolish seedling" disease in rice. The foolish seedling disease causes a strong prolongation of rice stems and leaves and finally causes them to topple over (Stowe and Yamaki, 1957). In 1926, Japanese scientist Eiichi Kurosawa identified that foolish seedling disease was caused by the fungus *Gibberella fujikuroi* (Stowe and Yamaki, 1957).

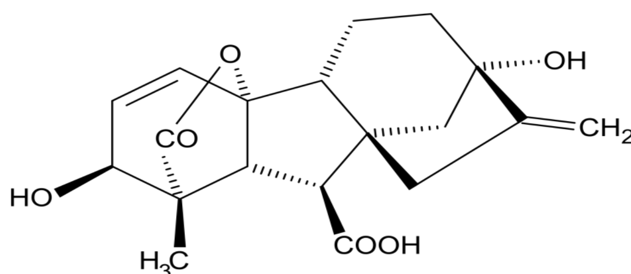

Figure 4. Chemical structure of gibberellic acid

Gibberellic acid (GA3) are plant hormones that regulate various developmental processes,including stem elongation, germination, dormancy, flowering, flower devel opment, and leaf and fruit senescence and grain development (Hedden and Sponsel,

2015). GA3 is one of the longest-known classes of plant hormone and thought that the selective breeding of crop strains that were insufficient in GA3 synthesis was one of the crucial drivers of the "green revolution" in the 1960s (Hedden and Phillips, 2000), a revolution that is credited to have saved over a billion lives worldwide (Spielmeyer *et al*., 2002; Dewick, 2009).

2.10.1 Seed germination

Wheat Germination has been elevated by the application of Gibberellic acid (GA3), growth and grain yield was reduced with growing salinity levels, but advanced comparatively by seed treated by GA3 (Kumar and Singh, 1996). Gibberellic acid (GA) helps germination by breaking the seed dormancy. Seed dormancy or germination relies on a few parameters like light, temperature, moisture, and few hormone and enzymes. Gibberellic acid and abscisic acid (ABA) is one of the most dominant growth regulating hormones. GA3 reviving the seed germination whereas, ABA is engaged in the inception and preservation of dormancy (Debeaujon and Koornneef, 2000; Gupta and Chakrabarty, 2013). GA3 plays a vital role in germination in two ways like firstly it raises the growth potential of en embryo and then it inspires the operation of hydrolytic enzymes (Ogawa *et al*., 2003; Kucera *et al*., 2005). Another thing is GA3 biosynthesize and shows its response in an embryo and the aleurone layer and helps to develop shoot cell division or elongation by upregulating the expression of the α-amylase gene. This α-amylase gene is synthesized in the aleuronic layer (Gubler *et al*., 2002).

2.10.2 Stem elongations

It is stated that GA plays an important role in stem elongation and it is proved by the physiologist (Ross *et al*., 1997). It cell division and expansion in response to light or dark that allows plant to internodes elongation (Alabadí *et al*., 2008; Feng *et al*., 2008). The GA3 biosynthetic pathway is complex (Gallego-Bartolomé *et al*., 2011) and it is painful to understand the actual site of GA biosynthesis in plants. Very little is known about this and still we have to understand the actual signal transduction pathway that is associated with the stem elongation of plants (Gupta and Chakrabarty, 2013). Sakamoto *et al*. (2001), stated that GA3 biosynthesis has a concern with cell fate determination. A protein named NTH15 is present at the corpus region of the shoot apical meristem and when its activity is under controlled

GA3 starts its function to stimulate cell division and enlargement. It was reported that in Arabidopsis GA3 synthesis occurred in growing tissue (Silverstone *et al*., 1997). Li and He (2013) stated that GA3 releases DELLA protein which helps the plant to cell elongation. Shaddad *et al*. (2013) found that exogenous application of GA3 at 150 mg L^{-1} improved leaf area, photosynthetic pigment, carbohydrates, protein, amino acid and Pro content, grain weight of wheat under different salt stress condition.

2.10.3 GA3 in the flowering

GA plays a role in the floral improvement and it can determine the male flower or female flower. The development of the floral part or flower inducing mostly depends on its concentration (Gupta and Chakrabarty, 2013). Goto and Pharis (1999) said that in Arabidopsis plant GA3 requires higher concentration to develop stamens than any other floral part. Griffiths *et al*. (2006) explained that GA3 is required for flower initiation and flower fertility. If GA3 is deficient in tomato or Arabidopsis stamen development becomes abnormal (Chhun *et al*., 2007; Hu *et al*., 2008) and in extreme scarcity situation female flower remains in a sterile condition. GA3 deficiency may increase the non-viable pollen (Nester and Zeevaart, 1988), undeveloped floral parts like sepals, petals, and pistils (Goto and Pharis, 1999) and sometimes flower abortion (Chhun *et al*., 2007) In the case of rice same result was also reported. GA3 plays a role in pollen germination and pollen tube development and it is mediated in early anther development (Chhun *et al*., 2007). GA3 helps in sex expression in plants but it may vary from crops to crops and with concentration. In cucumber repeated use of GA3 induce male flower whereas in minor concentration it propagates female flower in bitter gourd and improves fruit quality (Banarjee and Basu, 1992). Gupta and Chakrabarty (2013) reported that GA3 influences flower in castor bean, corn.

2.10.4 GA3 on yield

Iqbal and Ashraf (2010) described that increased grain yield in *Triticum aestivum* was a trait of the GA3 priming-introduced conjugation of ions uptake and differentiation within shoots and roots and hormones homeostasis under saline conditions. GA3 has a role in increasing yield as we know it helps to initiate flowering (Achard *et al*., 2006; Gupta and Chakrabarty, 2013). Abdel and Al-Rawi (2012), showed that 200 mg per litter GA3 application enhances the total yield and

yield parameters of lentil. Experiments were conducted with three different verities of lentil and GA3 gave superior yield and seed yield per plant hiked 7.85% and harvest index was 14.94% higher than control, 1000 seed weight was not remarkably changed. GA3 has a positive effect on chickpea. It helps to increase the branch number that is associated with a yield increase (Iqbal *et al*., 2001; Hasanuzzaman *et al*., 2007). Emongor (2007) observed a similar result by exogenous application of GA3 on cowpea and GA3 increased the nodulation number, leaf area index, 1000 seed weight, total yield and harvest index. Takahashi and Kobayashi (1991) described that GA plays a positive role on rice. In the dwarf variety, it is not visible but in the case of normal genotypes, GA3 increased growth and yield. GA3 has a dominant role in growth and yield and It helps to promote growth, flowering and yield, and it is proved in the case of mustard (Khan *et al*., 2002), mungbean (Mohammed, 2007), Potato (Sharma *et al*., 1998). Uddain *et al*. (2009) found that a higher number of fruit clusters, fruits plant^{-1} and individual fruit weight and 11.24% higher yield than no application of plant growth regulators due to the application of GA3.

2.10.5 GA3 on stress mitigation

Under the saline condition, plant growth regulators improved nutrient uptake, physiology, and metabolic activities of plants (Hedden and Sponsel, 2015). Sang-Mo *et al*. (2014) stated that in drought conditions GA3 mitigated the adverse effect of drought and improved plant growth in soybean. Cohen *et al*. (2009) supported this and said that in maize gibberellins producing *Azospirillum lipoferum* alleviates the drought stress. In the stress condition Gibberellic acid works as a protectant and can scavenge the ROS. GA3 helps a plant to endure in saline conditions (Hedden and Thomas, 2012). It is assumed that GA3 helps plants in stress conditions by raising the nutrient elevation (Lopes *et al*., 2013). GA3 increases the nitrogen use efficiency in stress condition that helps plant to adjust with the adverse condition (Iqbal *et al*., 2001). Application of GA3 remarkably expand leaf area, dry matter, photosynthesis rate, leaf Chlorophyll content and stomatal conductance differentiate to salt alone (Shah, 2007). Singh *et al*. (2005) gave the same opinion an also said that GA3 increased the chlorophyll content and mineral nutrients uptake that also helped in stress mitigation. In wheat, under saline stress condition GA3 helped plant by modulation of ions uptake, root-shoot partitioning and hormones homeostasis (Afzal

et al., 2005; Iqbal and Ashraf, 2013). In the case of rice plant growth regulators like NAA-Na, GA3 or 6-BA improve the photosynthetic ability, and decrease the leaf senescence and helps to increase the seed-setting in different environmental conditions (Li *et al*., 2010). Pan *et al*. (2013) experimented and showed that different growth hormones like gibberellic acid (GA3), paclobutrazol (PBZ), 6-Benzylaminopurine (6-BA) played a role as the aid of an Antioxidant enzyme that in deleting ROS. He showed that after GA3 applying SOD, POD activities were increased and MDA content decrease. Achard *et al*. (2008) stated that GA3 helps in regulating the ROS level.

CHAPTER 3

MATERIALS AND METHODS

This chapter deals with a brief description of the materials used and techniques employed during the course of studies that have been given below.

3.1 Experimental Site

This experiment was conducted at the net house, Sher-e-Bangla Agricultural University, Dhaka, under the Agro-ecological zone of Modhupur Tract (AEZ-28) and Shallow Red Brown Terrace Soil during the November, 2017 to March, 2018.

3.1.1 Geographical location

The land area is situated at 23°41′N Latitude and 90°22′E Longitude at an altitude of 8.6 meter above sea level. The experimental location is shown in the AEZ Map of Bangladesh in Appendix I.

3.1.2 Soil and its characteristics

The soil belongs to the "Madhupur tract" (AEZ-28) having General soil type, Shallow Red Brown Terrace Soils under Tejgaon Series. Top soils were silty loam in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. The physicochemical properties of the soil are presented in Appendix II.

3.1.3 Climatic conditions

The climate of the experimental area is under the sub-tropical climate with high temperatures and high humidity and heavy rainfall. Occasional stormy winds in kharif season (April-September) and scanty rainfall affiliated with moderately low temperature during the Rabi season (October-March) is visible. The weather information regarding temperature, rainfall, relative humidity and sunshine hours prevailed at the experimental site during the cropping season have been presented in Appendix III.

3.2 Source of Seed

Seeds of BARI Gom–26 were collected from Wheat Research Center, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3 Experiment-1: GROWTH AND YIELD OF WHEAT AS AFFECTED BY EXOGENOUS APPLICATION OF ASCORBIC ACID, SILICON AND GIBBRALLIC ACID UNDER SALT STRESS CONDITION

3.3.1 Experiment location

The experiment was carried out at the net house of Agronomy field, Sher-e-Bangla Agricultural University, Dhaka-1207. Details of the experiments conducted are given below.

3.3.2 Experiment period

The present studies were accomplished by conducting experiments from November 2017 to March 2018.

3.3.3 Plant materials

Wheat research centre, Bangladesh Agriculture Research Institute (BARI) was released BARI Gom-26 in 2010. This variety was developed by hybridization between three exotic wheat varieties. It was developed by crossing BAW-1064 genetic line. Characteristics of BARI Gom-26: Plant height: 92-96 cm. Duration: 100-105 days. Grain no. spike^{-1}: 45-50. 1000-seed weight: 48-52 g. Seed: white, shiny and big in size. Yield: 4.0-5.5 t ha^{-1}.

This variety is high yielding, short duration, and resistant to high temperature, tolerant to leaf spot disease and resistant to rust disease. The variety is susceptible to wheat blast disease and this variety is resistant to UG 99 race of stem rust disease.

3.3.4 Fertilizers dose

Fertilizer and manure	Doses (kg ha^{-1})
Cowdung	7500
Urea	220
TSP	145
MoP	100
Zypsum	110
Boric Acid	6

Source: Krishi Projukti Hatboi, BARI, Joydebpur, Gazipur, 2019

3.3.5 Treatments

T_0	Control
T_1	AsA (2 mM ascorbic acid)
T_2	Si (200 µM SiO_2)
T_3	GA3 (100 µM gibberalic acid)
T_4	50 mM NaCl
T_5	50 mM NaCl + 2 mM AsA
T_6	50 mM NaCl + 200 µM SiO_2
T_7	50 mM NaCl + 100 µM GA3
T_8	80 mM NaCl
T_9	80 mM NaCl + 2 mM AsA
T_{10}	80 mM NaCl + 200 µM SiO_2
T_{11}	80 mM NaCl + 100 µM GA3
T_{12}	120 mM NaCl
T_{13}	120 mM NaCl + 2 mM AsA
T_{14}	120 mM NaCl + 200 µM SiO_2
T_{15}	120 mM NaCl + 100 µM GA3

3.3.6 Pot preparation

Plastic pot with a diameter of 14 inches and a depth of 18 inches is used for the experiment. Soils for pot were collected from the agronomic field and weeds and stubbles were removed. After mixing cow dung and all the chemical fertilizers in the soil together, 16 kg of the soil is filled in the pot and kept for 7 days.

3.3.7 Germination test

Before sowing, a germination test was carried out in the Plant Physiology Laboratory, Department of Agronomy and the percentage of germination was found to be over 95.

3.3.8 Seed sowing

Seeds were sown on 24 November 2017 by hand and it was in line sowing method. 40 nos. wheat seeds were sown in each pot. After accomplishing sowing, a fine layer of soil was spread over the seeds. Light watering through sprinkling was assured to

avoid the washing off of the seeds and then pots were covered with clear plastic sheets.

3.3.9 Intercultural operations

Seed germination was started after 3 days of sowing. After seed germination, various kinds of intercultural operations were done like thinning, weeding and irrigation.

3.3.9.1 Thinning

After germination, pulls up some seedlings to maintain 30 nos. population in each pot. Thinning out was done at two times. One is after 10 days of germination and the second one was after 20 days of germination.

3.3.9.2 Weeding

There were so many weeds found in the pot like kakpaya ghash (*Dactyloctenium aegyptium* L.), Shama (*Echinochloa crussgalli*), Durba (*Cynodon dactylon*), Mutha (*Cyperus rotundus* L.), Shaknatey (*Amaranthus viridis*), and so on. Weeding was done in four times. First one was 15 DAS (day after sowing) with the thinning operation, the second one was at 30 DAS, the third one was at 45 DAS and the last one at 60 DAS.

3.3.10 Irrigation and application of salt stress

Irrigation was applied very carefully and provide as salt stress coz. Treatments were irrigation related. Each pot was applied 500 ml 50 mM NaCl solution to T_4, T_5, T_6, T_7 80 mM NaCl solution to T_8, T_9, T_{10}, T_{11} and 120 mM NaCl solution to T_{12}, T_{13}, T_{14}, T_{15} in every time. Salt solution was provided twice a week from 20 DAS.

3.3.11 Application of ascorbic acid

Ascorbic acid (2 mM) exogenously applied to T_1, T_5, T_9 and T_{13}. 10 ml Ascorbic acid solution per pot dose was spraying very carefully to ensure the foliar application from 20 DAS. Ascorbic acid was applied after salt stress irrigation.

3.3.12 Application of silicon

Silicon (200 µm) exogenously applied to T_2, T_6, T_{10} and T_{14}. 10 ml silicon solution per pot dose was spraying very carefully to ensure the foliar application from 20 DAS.

Silicon was applied after salt stress irrigation.

3.3.13 Application of gibberalic acid

Gibberalic acid (100 µm) exogenously applied to $T_{3,}$ $T_{7,}$ T_{11} and $T_{15.}$ 10 ml Gibberalic acid solution per pot dose was spraying very carefully to ensure the foliar application from 20 DAS. GA3 was applied after salt stress irrigation.

3.3.14 Harvesting and threshing

The maturity of wheat was determined when 90% of the plants became golden yellow color. Harvesting was done on 10 March 2018. All population was carefully harvested and separated from each pot. They were properly tagged and brought to the threshing floor for recording data. The harvested plants were sun-dried on the threshing floor. After sun drying, the biological yield (Grain + Straw) for the net harvested areas was recorded. Threshing was done manually, seeds and straw were sun-dried, cleaned and weighed for calculation of seed yield (ton ha^{-1}) containing moisture of 12%.

3.3.15 Data collection

Grain yield and straw yield was collected from untouched three replications. Then this yield was converted to ton/ha. Growth parameters were collected from another three replications. Ten plants were marked to identify the same plants for data collection. Destructive all morphological and physiological data were collected from the other three replications which is middle in position.

3.3.16 Growth character

3.3.16.1 Plant height

Plant height was measured at 15 days interval starting from 35 days after sowing (35, 50, 65, 80 DAS) and continued up to harvest. The height of the plant was determined by measuring the distance from the soil surface to the tip of the leaf before heading, and to the tip of spike after heading. The collected plant heights were finally averaged. The mean was computed for ten plants in all treatments of each replication and expressed in cm.

3.3.16.2 Tillers plant^{-1}

Tillers plant^{-1} was measured at 35, 50, 65, 80 DAS and continued up to harvest time and data were finally averaged. The mean was computed for ten plants in all treatments of each replication and expressed in no.

3.3.16.3 Leaf number plant^{-1}

The leaves of each plant were counted during the data collection procedure at 35, 50, 65, 80 DAS. Leaf number was counted for ten plants then it was calculated as average number leaves plant^{-1}.

3.3.16.4 Leaf area

The leaf area of each plant was counted during the data collection procedure at 35 and 50 DAS. Leaf area was counted for ten plants then it was calculated as average number leaf area plant^{-1}.

3.3.16.5 Relative water content

Leaf relative water content (RWC) was measured in accordance with Barrs and Weatherly (1962). RWC was measured at at 35, 50, 65 and 80 DAS. Leaf laminas were weighed (fresh weight, FW) then placed immediately between two layers of filter paper and submerged in distilled water in a Petri dish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight (DW) was measured after 48 h oven drying at 70 °C. Leaf RWC was determined by using the following formula:

$$RWC\ (\%) = (FW - DW) / (TW - DW) \times 100$$

3.3.16.6 Fresh and dry weight

Five sample plants in each plot were selected on 35, 50, 65, 80 DAS and at harvest time. They were first air dried for one day, then oven dried at 70°C till a constant weight was attained. The dry weight of the sample plants was weighed and averaged.

3.3.16.7 SPAD value

SPAD value was measured with the help of SPAD meter. The top, middle, and bottom of each leaf blade were measured with this instrument. Then it was averaged and counted as chlorophyll content.

3.3.17 Yield attributes and yield

3.3.17.1 Spike length

Spike length was counted from ten plants from the basal node of the rachis to the apex of each spike and then averaged. It was measured at harvesting time.

3.3.17.2 No. of spike plant^{-1}

The number of spikelets were counted from 10 spikes and averaged to determine the number of spike plant^{-1}.

3.3.17.3 Grains spike^{-1}

The total number of filled grains spike^{-1} was counted. Average data were recorded randomly from ten spike bearing plants in each pot during the time of harvest by the following formula:

$$\text{No. of grains spike}^{-1} = \frac{\text{Total no. of grains}}{\text{Number of spike}} \times 100$$

3.3.17.4 1000-grain weight

After dried Spikes were thrashed then 1000-seed grains were cleaned and counted. Then these seeds were measured with a digital electric balance.

3.3.17.5 Grain yield

All plants from each pot were harvested for recording yield data. After threshing, proper drying (12% moisture level) and cleaning, the yield of each sample pot were weighed and values will be converted to t ha^{-1}.

3.3.17.6 Straw yield

All plants from each plot were harvested and straw weight was determined after threshing and drying and finally converted them into t ha^{-1}.

3.3.17.7 Biological yield

Grain yield and straw yield together were considered as biological yield. The biological yields were calculated with the following formula:

$$\text{Biological yield} = \text{Grain yield} + \text{Straw yield}$$

3.3.17.8 Harvest index

The harvest index will be calculated from the grain and straw yield of wheat for each pot and expressed in percentage.

$$HI\ (\%) = \frac{\text{Economic yield (grain weight)}}{\text{Biological yield (Total dry weight)}} \times 100$$

3.3.17.9 Determination of Na^+, K^+, Ca^{2+} and Mg^{2+} contents

The samples were selected to give a range in total K, Na, Ca, and Mg content and to represent a variety of plant parts and tissues. All grain and straw samples were oven-dried (forced-draft type oven at 65°C for 3 d) and ground (Wiley mill) to pass a 40-mesh sieve. About 0.1 g of oven-dried plant material was transferred into a 70-mL porcelain evaporating dish and ashed. Plant sample was treated with three drops of alcoholic sulfuric acid (5% H_2SO_4 in ethanol) to saturate the sample, ignited to burn the sample, and the porcelain dish was placed in a cold muffle furnace and heated to 550°C for 3 h as described by Johnson and Ulrich (1959). A 25-mL aliquot of 5 mM HCl was added to the ignited residue and heated 90°C on a steam plate and solution was transferred into a 100-mL volumetric flask. This heating and transfer was repeated for a total of four times. The solutions were combined in the 100 mL volumetric flask, and the volume was adjusted to 100 mL with 5 mM HCl. Before analysis, the plant digest was centrifuged at 12 000 X g for 10 min to remove any particulates. The ion chromatograph (1C) used was a Dionex model 10 instrument (Dionex, Sunnyvale, Calif.). Separate precolumns and separator columns were employed for determination of alkali (K and Na) and alkaline earth (Ca and Mg) metals. A 3 X 150-mm precolumn, 6 X 250-mm separator column, and a 9 X 100-mm suppressor column were used for determination of K and Na. A 4 X 50-mm precolumn, 3 X 250-mm separator column, and a 9 X 100-mm suppressor column were used for determination of Ca and Mg. Other components of the Dionex model 101C include eluent and suppressor-column regenerating reagent reservoirs, injector valve, 100 µL sample loop, two Milton Roy pumps, conductivity cell, and a valving system to direct the flow through various parts of the instrument described by Mulik *et al*. (1978). The eluent for determination of K and Na was 5 mM HCl at a flow rate of 180 mL h^{-1} and a pump pressure of 2.8 MPa (400 psi). The eluent used for determination of Ca and Mg was 2.5 mM HCl + 2.5 mM m-phenylenediamine

dihydrochloride [$C_6H_4(NH_2)2.2HCl$] (m-PDA) at a flow rate of 115 mL h^{-1} and a pump pressure of 3.4 MPa (500 psi). The K, Na, Ca, and Mg standards were prepared by using KCl, NaCl, $CaCO_3$, and Mg ribbon, respectively. The results obtained by the ion chromatograph (IC) method were compared with those by flame photometry (IL Model 443) and by atomic absorption spectrophotometry (Perkin-Elmer Model 272) described by Basta and Tabatabai (1985).

3.4 Expriment-2: ALLEVIATION OF SHORT TERM SALT STRESS EFFECT ON WHEAT SEEDLINGS BY FOLIAR SPRAY WITH ASCORBIC ACID, SILICON AND GIBBERELLIC ACID

3.4.1 Experiment location

The experiment was conducted at Plant Physiology Laboratory, Sher-e-Bangla Agricultural University during the period from October 2018 to December, 2018.

3.4.2 Plant matter, growth and stress treatment

Uniform sized Wheat (*Triticum aestivum* L. cv. BARI Gom-26) seeds were surface sterilized with 70% ethanol for 8–10 min for sterilization. Seeds were cleaned a few times with sterilized distilled water. The seed was sown in petri plates (9 cm) lined with 6 layers of filter paper moistened with 10 ml of distilled water for germination and kept for 2 days in dark condition. Each petri dish contained 30 germinated seedlings and those were grown under controlled conditions (light, 350 µmol photon m^{-1} s^{-2}; temperature, 25±2°C; relative humidity, 65–70%). 5,000-fold diluted Hyponex solution (Hyponex, Japan) was applied as nutrient every day according to necessity. Control seedlings were grown in Hyponex solution only. Foliar spray with AsA, Si and GA3 were started daily after germination of seed and continuous until 7days. Salt stress was induced with different concentrations of salt (0, 50, 80 and 120 mM NaCl) on 3d-old seedlings. Different physiological and biochemical parameters such as plant height, fresh weight, dry weight, relative water content (RWC), chlorophyll, lipid peroxidation (malondialdehyde), ROS (H_2O_2, O_2•−) Proline (Pro) content, were observed after 3 days of treatment. After seven days of salt treatment, leaves were harvested and used for studying various morphological and physiological parameters.

3.4.3 Treatments

T_0	Control
T_1	AsA (2 mM ascorbic acid)
T_2	Si (200 µM SiO_2)
T_3	GA3 (100 µM gibberalic acid)
T_4	50 mM NaCl
T_5	50 mM NaCl + 2 mM AsA
T_6	50 mM NaCl + 200 µM SiO_2
T_7	50 mM NaCl + 100 µM GA3
T_8	80 mM NaCl
T_9	80 mM NaCl + 2 mM AsA
T_{10}	80 mM NaCl + 200 µM SiO_2
T_{11}	80 mM NaCl + 100 µM GA3
T_{12}	120 mM NaCl
T_{13}	120 mM NaCl + 2 mM AsA
T_{14}	120 mM NaCl + 200 µM SiO_2
T_{15}	120 mM NaCl + 100 µM GA3

3.4.4 Data collection

3.4.4.1 Relative water content

Leaf relative water content was measured in accordance with Barrs and Weatherly (1962). Leaf laminas were weighed (fresh weight, FW) then placed immediately between two layers of filter paper and submerged in distilled water in a Petri dish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight (DW) was measured after 48 h oven drying at 70 °C. Leaf RWC was determined by using the following formula:

$$RWC (\%) = (FW-DW) / (TW-DW) \times 100$$

3.4.4.2 Shoot length

Seedlings were taken randomly and shoot length was measured in centimeters with the scale.

3.4.4.3 Root length

Seedlings were taken randomly and root length was measured in centimeters with the scale.

3.4.4.4 Root shoot ratio

Length basis root-shoot ratio was calculated by following the method of Khandakar (1980) to estimate root efficiency to support production.

$$\text{Root-shoot ratio (\%)} = \frac{\text{Root length}}{\text{Shoot length}} \times 100$$

3.4.4.5 Fresh and dry weight

For fresh weight and dry weight measurement, 10 seedlings from each treatment were selected. These selected seedlings were uprooted carefully, weighed in a digital balance; data were reported and deliberated as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 80^0 C for 48 h.

3.4.4.6 Measurement of chlorophyll content

Chlorophyll content was measured as a fresh leaf sample of 0.25g was taken from randomly selected seedlings to measure the Chlorophyll content. The samples were homogenized with 10 ml of acetone (80% v/v) using pre-cooled pestle and mortar and the homogenate was centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants was measured with a UV visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b* respectively. The amount of Chlorophyll contents was calculated using the equations suggested by Arnon (1949).

3.4.4.7 Determination of hydrogen peroxide content

H_2O_2 was examined according to the method narrated by Yu *et al.* (2003b). H_2O_2 was withdrawn by homogenizing 0.5 g of leaf samples with 3 ml of 50 mM potassium phosphate (K-P) buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 11,500× g for 15 minutes and 3ml of supernatant was mixed with 1 ml of 0.1% $TiCl^{4-}$ in 20% H_2SO_4 (v/v) and kept at room temperature for 10 min. Subsequently, the mixture was again centrifuged at 11,500× g for 12 min. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content using the extinction coefficient 0.28 μM^{-1} cm^{-1} and expressed as nmolg^{-1} fresh weight.

3.4.4.8 Measurement of lipid peroxidation

To estimate lipid peroxidation, malondialdehyde (MDA) content was measured according to Heath and Packer (1968), with a slight modification by Hasanuzzaman *et al*. (2012b). 0.5 gm leaf samples were set down in 5% (w/v) trichloroacetic acid (TCA) and the homogenates were centrifuged at 11,500×g for 15 min. The supernatant was then mixed with thiobarbituric acid (TBA) and heated at 95°C for 30 min in a water bath and the cooled and centrifuged again at 11,500×g for 10 min. Absorbance was read at 532 nm after cooling the supernatant. The volume of MDA was counted by using the eradication coefficient 155 mM^{-1} cm^{-1} and reveled as n mol of MDA g^{-1} FW.

3.4.4.9 Measurement of proline content

Free Proline in leaf tissues was identified by following the protocol of Bates *et al*. (1973). 0.25 g fresh leaf tissue was homogenized well in 5 ml of 3% sulfo-salicylic acid on ice-cooled morted on ice and the homogenate was centrifuged at 11,500×g for 15 min. 2 ml of the supernatent was than mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid. The mixture was settled at 100°C in a water bath for 1 hour, then shifted in to test tube and kept in ice to be cooled, after a while when it was cooled, 2 ml of toluene was added and mixed thoroughly by vortex mixture. After sometimes by transferring the upper aqueous layer the optical consistency of the chromophore bearing toluene was read spectrophotometrically at 520 nm exercising toluene as a blank. The amount of Proline was computed from the standard curve using laboratory grad Pro.

3.5 Expriment-3: GERMINATION AND GROWTH PERFORMANCE OF SEEDLINGS OF ASCORBIC ACID, SILICON AND GIBBERELLIC ACID INDUCED SECONDARY WHEAT SEEDS UNDER SALT STRESS.

3.5.1 Experiment location

The experiment was conducted at Plant Physiology Laboratory, Sher-e-Bangla Agricultural University during the period from October 2018 to December, 2018.

3.5.2 Plant matter, growth and treatment

Wheat (*Triticum aestivum* L. cv. BARI Gom-26) seeds were collected from experiment no. 1. These secondary BARI Gom-26 Seeds were surface sterilized with 70% ethanol for 8–10 min for sterilization. Seeds were cleaned a few times with sterilized distilled water. 50 nos. seeds were sown in hydroponix plates with 250 ml of distilled water. Data was colleted from 10 days old seedling.

Data collection

3.5.3 Relative water content

Leaf relative water content was measured in accordance with Barrs and Weatherly (1962). Leaf laminas were weighed (fresh weight, FW) then placed immediately between two layers of filter paper and submerged in distilled water in a Petri dish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight (DW) was measured after 48 h oven drying at 70 °C. Leaf RWC was determined by using the following formula:

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

3.5.4 Germination %

Count out 50 seeds and soak them in water for an hour then spread on a layer of Moisten filter paper in the petri dish. Put on the lid and place the dish in the dark and record the number of seeds that germinate daily. A germination test was carried out in the Plan Physiology Laboratory, department of agronomy. Germination % was determined by using the following formula:

$$\text{Germination \%} = (\text{Seeds germinated / Total seed}) \times 100$$

3.5.5 Shoot length

Seedlings were taken randomly and shoot length was measured in centimeters with the scale.

3.5.6 Root length

Seedlings were taken randomly and root length was measured in centimeters with the scale.

3.5.7 Root shoot ratio

Length basis root-shoot ratio was calculated by following the method of Khandakar (1980) to estimate root efficiency to support production.

$$\text{Root-shoot ratio (\%)} = \frac{\text{Root length}}{\text{Shoot length}} \times 100$$

3.5.8 Vigor index

Seedling vigor index was calculated by using by the formula of Abdul-Baki and Anderson (1973).

$$\text{Vigor index (VI)} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

3.5.9 Survibility %

$$\text{Survibility \%} = \frac{\text{No. of servable seedling}}{\text{Total no. of seed}} \times 100$$

3.5.10 Fresh and dry weight

For fresh weight and dry weight measurement, 10 seedlings from each treatment were selected. These selected seedlings were uprooted carefully, weighed in a digital balance; data were reported and deliberated as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 80^0 C for 48 h.

3.5.11 Chlorophyll

Chlorophyll (chl) content was measured as a fresh leaf sample of 0.25g was taken from randomly selected seedlings to measure the chlorophyll content. The samples were homogenized with 10 ml of acetone (80% v/v) using pre-cooled pestle and mortar and the homogenate was centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants was measured with a UV visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b* respectively. The amount of chlorophyll contents was calculated using the equations suggested by Arnon (1949).

3.6 Statistical analysis

The data obtained for different parameters, mean values of each parameter were calculated from three replications and subjected to analysis of variance (ANOVA)

applying computer based CoStat v.6.400 (CoStat, 2008) software. The average of three replications ($n = 3$) was used to determine mean (± SD) for each treatment and mean differences were compared using Fisher's LSD test where a variation of $p \leq 0.05$ due to Fisher's LSD test was considered significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Experiment 1. GROWTH AND YIELD OF WHEAT AS AFFECTED BY EXOGENOUS APPLICATION OF ASCORBIC ACID, SILICON AND GIBBERELLIC ACID UNDER SALT STRESS CONDITION

4.1.1 Plant height

Sharp decreases in plant height was observed in response to salt stress, compared to the untreated control at 50, 65, 80 DAS and at harvest for wheat (Figure 5). However, AsA, Si and GA3 supplementation with salt treatment increased plant height under salinity stress conditions at 50, 65, 80 DAS and at harvest for wheat (Figure 5). Plant height was found about 65.1cm, 71.3 cm, 76.4 cm and 74.8 cm at 50, 65, 80 DAS, and during harvest respectively under control conditions. Plant height was observed about 61.5 cm, 65.8 cm, 69.5 cm and 68.7 cm under 50 mM NaCl; 58.4 cm, 63.2 cm, 66.1 cm and 65.2 cm under 80 mM NaCl and 56.3 cm, 61.2 cm, 63.3 cm and 62.8 cm under 120 mM NaCl at 50, 65, 80 DAS, and during harvest respectively. However, exogenous application of AsA increased plant height 2%, 4%, 3% and 2% under 50 mM NaCl; 5%, 3%, 1% and 3% under 80 mM NaCl and 5%, 2%, 4% and 3% under 120 mM NaCl at 50, 65, 80 DAS, and during harvest respectively. Supplementation of Si increased plant height 6%, 6%, 5% and 6% under 50 mM NaCl; 8%, 5%, 6% and 5% under 80 mM NaCl and 8%, 6%, 5% and 6% under 120 mM NaCl at 50, 65, 80 DAS, and during harvest respectively. Addition of GA3 also increased plant height 9%, 10%, 8% and 7% under 50 mM NaCl; 2%, 9%, 10% and 9% under 80 mM NaCl and 10%, 8%, 9% and 8% under 150 mM NaCl at 50, 65, 80 DAS, and during harvest respectively. Salinity stress limit plant growth due to osmotic stress, ionic toxicity, and a reduced ability to take up essential plant minerals (Munns *et al.*, 2006). In case of severe salinity stress, plant root cells may lose water instead of absorbing it due to the hyperosmotic pressure of the soil solution. Water deficits affect a cascade of physical, signaling, gene expression, biochemical, and physiological pathways and processes, resulting in decreased cell elongation, cell expansion, wilting, and, ultimately, decreased plant height (Apse and Blumwald, 2002; Munns and Tester, 2008). However, supplementation of osmoprotectants and synthetic plant growth regulator like AsA,

Si and GA3 increased adaptation of wheat cultivars to salinity to some extent. This may be achieved through osmoregulation which in turn increased osmotic adjustment by increasing water flow and water status by using the organic solutes (Shaddad *et al*., 2013; Islam and Mehraj, 2014).

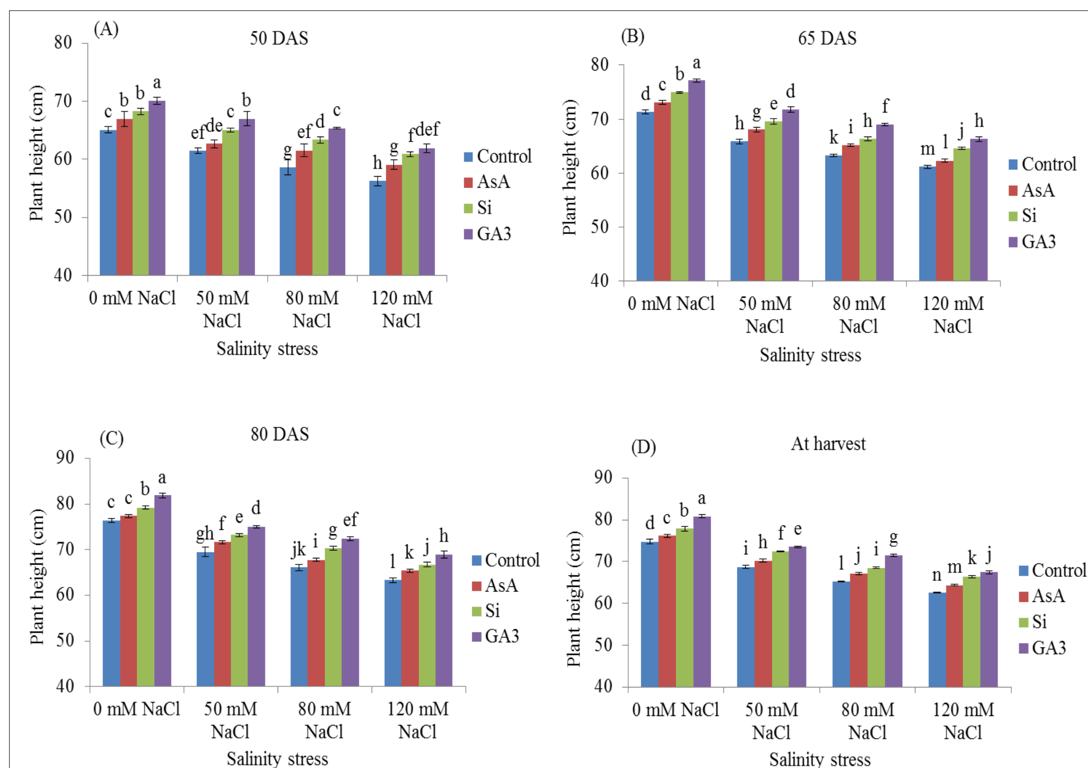

Figure 1. Effect of different salinity level, and AsA, Si and GA3 on plant height at (A) 50 days after sowing (DAS), (B) 65 DAS, (C) 80 DAS and (D) at harvest of wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.2 Tillers plant^{-1}

Different salinity treatments affected tiller production significantly throughout the growing period. Salinity treatment reduced tiller number compared to control (Figure 6). The salt-stressed seedlings had significantly lower tiller number plant^{-1} (3, 10, and 16% at 35 DAS; 7, 24 and 30% at 50 DAS; 7, 8 and 9% at 65 DAS and 7, 8 and 19% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Supplementation of AsA significantly increased tiller number plant^{-1} (7, 8 and 4% at 35 DAS; 7, 22 and 19% at 50 DAS; 27, 13 and 1% at 65 DAS and 7, 2 and 1% at 80 DAS at AsA treated 50, 80 and 120 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without AsA treatment (Figure 6). On the contrary, Si with saline treatments increased tiller number (18, 16 and 33% at 35 DAS; 11, 31 and

34% at 50 DAS; 40, 27 and 7% at 65 DAS and 13, 7 and 15% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without Si treatment. Furthermore, exogenous application of GA3 with saline treatments increased tiller number (21, 20 and 38% at 35 DAS; 21, 35 and 43% at 50 DAS; 40, 27 and 13% at 65 DAS and 20, 13 and 15% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without GA3 treatment. In this study, tiller number plant^{-1} decreased with increasing salinity. Maas *et al*. (1994) suggested that salinity stress at the root zone significantly decreased the number of primary and secondary tillers in wheat cultivars. This may indicate that tiller number and their behaviour under salinity can be used as simple and non-destructive measurement to evaluate wheat genotypes in breeding programs. Goudarzi and Pakniyat (2008a) found that salinity stress during tiller emergence can inhibit their formation and can cause their abortion at later stages. When salinity levels are greater than 50 mM NaCl, the number of primary tillers was greatly reduced and most of the secondary tillers of wheat genotypes were eliminated (Zeeshan *et al*., 2020). It has been suggested that high-tillering capacity of wheat had greater grain yield on poor soil. Therefore, increasing the salinity tolerance in wheat may require an increase in the capacity of tillering (El-Bassiouny and Bekheta, 2005).

However, in this study exogenous application of AsA, Si and GA3 increased tiller number of wheat under salinity stress conditions to some extent. Exogenous applications of non-enzymatic antioxidants AsA have been reported to markedly improve the inhibitory effects of salinity stress on plant growth and metabolism (Malik and Ashraf, 2012). Some studies also confirmed that, exogenous application of AsA upregulate antioxidant defence system which confers stress tolerance against different abiotic stresses (Bybordi, 2012; Alam *et al*., 2014). AsA as antioxidant plays an important role on the impact on cell growth and division, differentiation and metabolism in plants. AsA improves the adverse effect of salinity stress by increasing nutrient uptake, total chlorophyll content, protein synthesis, transpiration, photosynthesis and plant growth (Athar *et al*., 2009; Xu e*t al*., 2015). On the other hand, Si plays role against various abiotic stress (Liang *et al*., 2005; Zeng *et al*., 2011; Srivastava *et al*., 2015). Silicon reduces Na^+ uptake acting as mechanical barrier. Accumulation of Si by plant reduces transpiration through deposition in the

cell wall of leaves which decreases Na^{+} uptake and transpiration. In addition, Si application improves salt stress tolerance by improving water status of plant (Wang *et al*., 2015). Moreover, Si mitigates salt-induced oxidative stress by detoxifying ROS through regulating antioxidant defense system (Zhu and Gong, 2014). Furthermore, plant hormones gibberellic acid (GA3) is helpful in enhancing plant growth and development under abiotic stress condition (Shaddad *et al*., 2013). The exogenous application of gibberellic acid (GA3) improved tolerance under abiotic stress by induction and increasing of the endogenous levels of salicylic acid (Alonso-Ramírez *et al*., 2009). Foliar applications of GA3 also confer salt stress tolerance by increasing germination percentage, plant growth and upregulating antioxidant enzyme (Tabatabei, 2013).

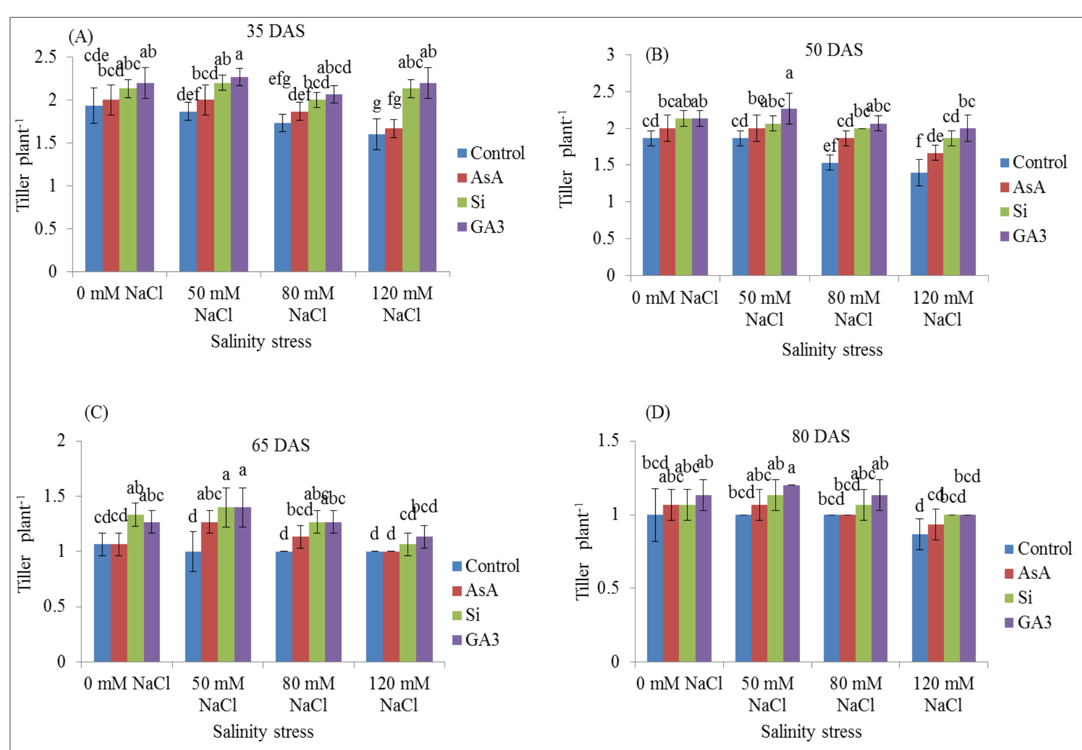

Figure 2. Effect of different salinity level, and AsA, Si and GA3 on number of tillers plant^{-1} at (A) 35 days after sowing (DAS), (B) 50 DAS, (C) 65 DAS and (D) 80 DAS of wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.3 Number of leaf plant^{-1}

Different salinity treatments affected number of leaf significantly throughout the growing period. Salinity treatment reduced number of leaf compared to control (Figure 7). The salt-stressed seedlings had significantly lower number of leaf plant^{-1}

(5, 4, and 12% at 35 DAS; 7, 9 and 23% at 50 DAS; 13, 28 and 43% at 65 DAS and 15, 22 and 43% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Supplementation of AsA significantly increased number of leaf plant^{-1} (7, 6 and 16% at 35 DAS; 6, 5 and 4% at 50 DAS; 17, 21 and 26% at 65 DAS and 1, 9 and 24% at 80 DAS at AsA treated 50, 80 and 120 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without AsA treatment (Figure 7). On the contrary, Si with saline treatments increased number of leaf plant^{-1} (26, 20 and 38% at 35 DAS; 22, 6 and 13% at 50 DAS; 26, 30 and 41% at 65 DAS and 17, 17 and 37% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without Si treatment. Furthermore, exogenous application of GA3 with saline treatments increased number of leaf plant^{-1} (24, 17 and 31% at 35 DAS; 25, 9 and 9% at 50 DAS; 22, 21 and 33% at 65 DAS and 1, 9 and 24% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without GA3 treatment. In our study, leaf number at vegetative stage decreased with increasing salinity. Similar decrease in leaf number due to salinity stress was reported earlier (El-Hendawy *et al*., 2005). Reduced leaf number, a marked disruption in photosynthetic attributes along with reduced chlorophyll contents resulting from salt stress were observed in the wheat cultivars. These could be happened due to either single or combined effects of reduced stomatal conductance, inhibition of metabolic phenomena, and increased ROS generation which can increase oxygen-induced cellular damage. The reductions in stomatal conductance, photosynthesis, and leaf chlorophyll contents due to salinity stress ultimately reduced the plant growth and leaf number of plant (Zeeshan *et al*., 2020).

However, in the present study, exogenous application of AsA, Si and GA3 increased number of leaf plant^{-1} of wheat under salinity stress conditions to some extent. In previous studies, it was confirmed that, exogenous application of AsA upregulate antioxidant defence system, increase cell growth and division, differentiation and metabolism, increase nutrient uptake, total chlorophyll content, protein synthesis, transpiration, photosynthesis and plant growth (Athar *et al*., 2009; Xu *et al*., 2015; Malik and Ashraf, 2012). On the other hand, Si plays a significant role to reduce the toxic effect by decreasing Na^+ uptake and transpiration and mitigates salt-induced oxidative stress by detoxifying ROS through regulating antioxidant defense system

(Zhu and Gong, 2014; Srivastava *et al*., 2015). Furthermore, supplementation of gibberellic acid (GA_3) improved salinity tolerance by enhancing plant growth and development under abiotic stress condition (Shaddad *et al*., 2013; Tabatabei, 2013).

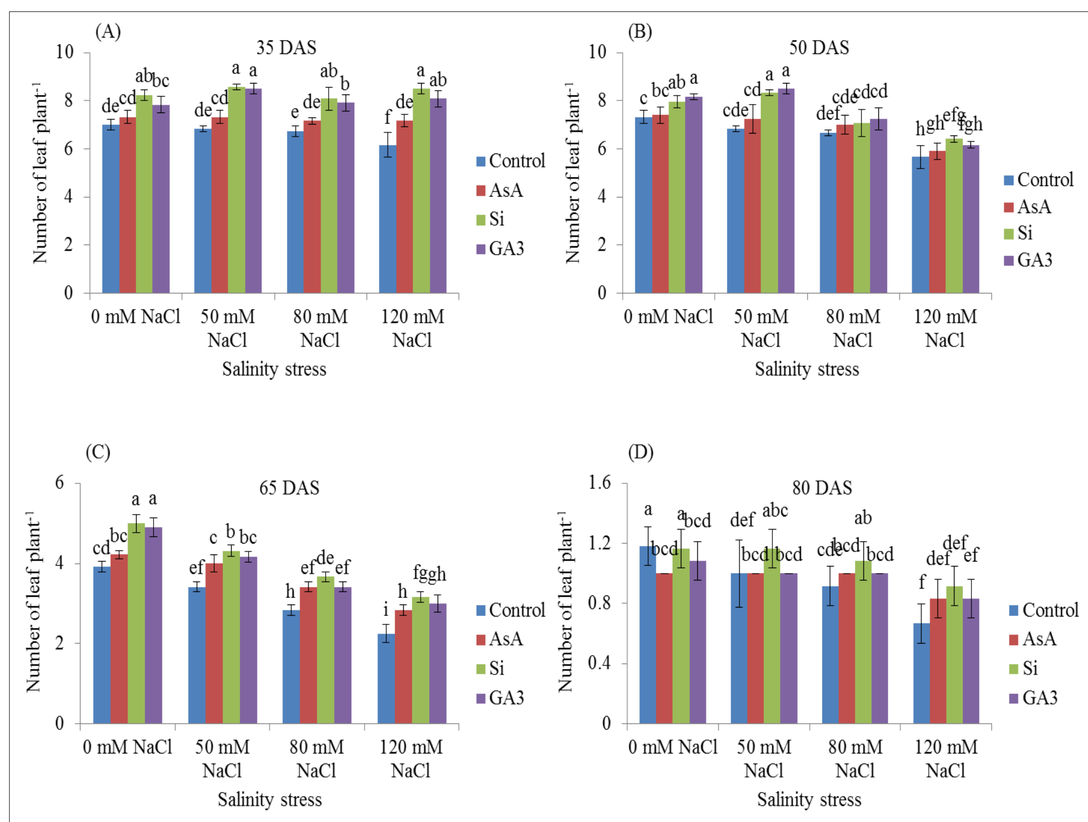

Figure 3. Effect of different salinity level, and AsA, Si and GA3 on number of leaf plant^{-1} at (A) 35 days after sowing (DAS), (B) 50 DAS, (C) 65 DAS and (D) 80 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.4 Leaf area

Different salinity treatments affected leaf area significantly throughout the growing period. Salinity treatment reduced leaf area compared to control (Figure 8). The salt-stressed seedlings had significantly lower leaf area (12, 28, and 37% at 35 DAS and 7, 20 and 28% at 50 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Supplementation of AsA significantly increased leaf area (3, 9 and 8% at 35 DAS and 6, 15 and 17% at 50 DAS at AsA treated 50, 80 and 120 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without AsA treatment (Figure 8). On the contrary, Si with saline treatments increased leaf area (11, 26 and 31% at 35 DAS and 20, 33 and 33% at 50 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without Si

treatment. Furthermore, exogenous application of GA3 with saline treatments increased leaf area (2, 21 and 23% at 35 DAS and 6, 27 and 20% at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without GA3 treatment. Generally leaf area depends on both cell division and cell elongation. It was found that leaf area, which is governed by cell division, was shown to be affected by salinity stress in different crops (Läuchli and Grattan, 2007; El-Hendawy *et al*., 2005).

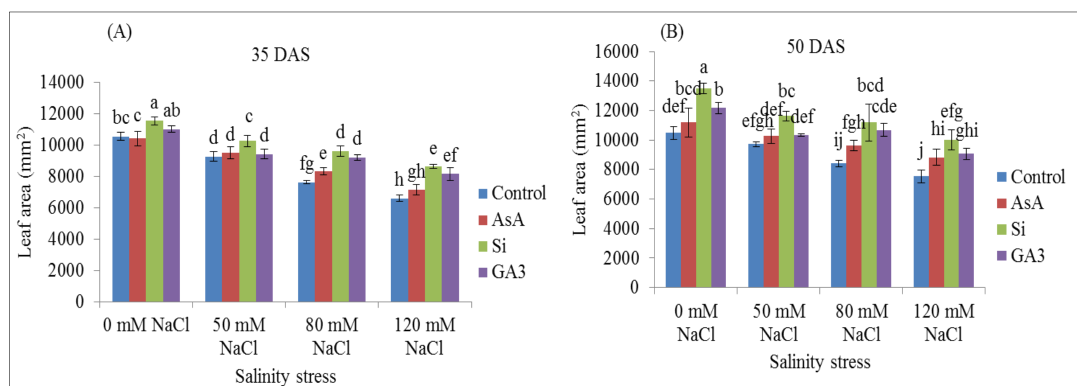

Figure 4. Effect of different salinity level, and AsA, Si and GA3 on leaf area at (A) 35 days after sowing (DAS) and (B) 50 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

However, synthetic plant growth regulators treatments (that is GA3) increased adaptation of wheat cultivars to salinity to some extent. These may be achieved through osmoregulation which in turn increased conservation and water use efficiency using the organic solutes (saccharides and proteins), which in turn increased the leaf photosynthetic area (Shaddad *et al*., 2013; Ashraf and Harris, 2004; Iqbal and Ashraf, 2013). Exogenous application of Si reduced up taking Na^+ ion in root and leaf and reduces the detrimental effect of Na^+ toxicity (Tahir *et al*., 2010; Ali *et al*., 2008). Supplementation of AsA upregulate antioxidant defence system against ROS, increase protein synthesis, increase nutrient uptake, ultimately increase cell growth and division and plant growth (Malik and Ashraf, 2012).

4.1.5 Plant biomass

4.1.5.1 Fresh weight plant^{-1}

Fresh weight plant^{-1} significantly affected by different salinity treatments throughout the growing period. Salinity treatment reduced fresh weight plant^{-1} compared to

control plant (Figure 9).

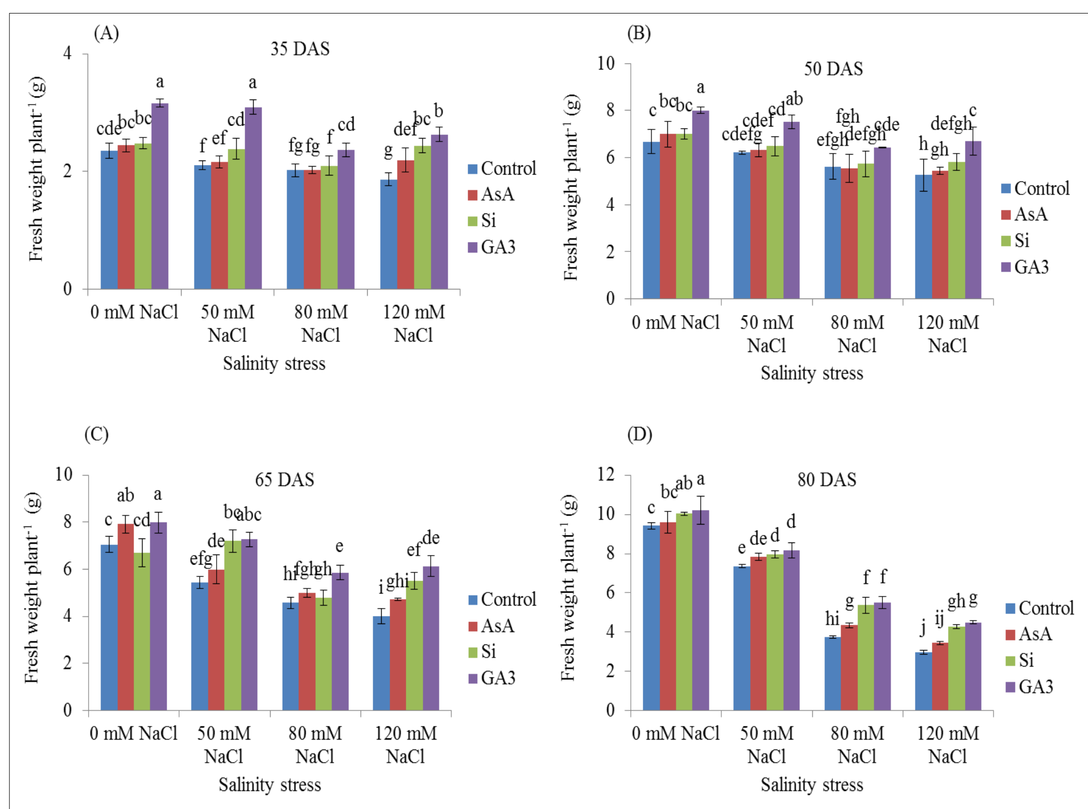

Figure 5. Effect of different salinity level, and AsA, Si and GA3 on fresh weight plant^{-1} at (A) 35 days after sowing (DAS), (B) 50 DAS, (C) 65 DAS and (D) 80 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

The salt-stressed plant had significantly lower fresh weight plant^{-1} (10, 14, and 20% at 35 DAS; 7, 16 and 21% at 50 DAS; 23, 35 and 43% at 65 DAS and 22, 60 and 69% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions, respectively. Application of AsA significantly increased fresh weight plant^{-1} (2, 1 and 18% at 35 DAS; 2, 1 and 4% at 50 DAS; 10, 9 and 18% at 65 DAS and 6, 16 and16% at 80 DAS at AsA treated 50, 80 and 120 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without AsA treatment (Figure 9). On the contrary, Si with saline treatments increased fresh weight plant^{-1} (8, 4 and 31% at 35 DAS; 4, 2 and 11% at 50 DAS; 32, 5 and 38% at 65 DAS and 8, 44 and 45% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without Si treatment. Furthermore, exogenous application of GA3 with saline treatments increased fresh weight plant^{-1} (40, 17 and 41% at 35 DAS; 21, 14 and 27% at 50 DAS; 33, 28 and 53% at 65 DAS and 11, 47 and 52% at 80 DAS at

50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without GA3 treatment.

4.1.5.2 Dry weight plant^{-1}

Dry weight plant^{-1} significantly affected by different salinity treatments throughout the growing period. Salinity treatment reduced dry weight plant^{-1} compared to control plant (Figure 10). The salt-stressed plant had significantly lower dry weight plant^{-1} (19, 23, and 29% at 35 DAS; 10, 13 and 14% at 50 DAS; 24, 34 and 37% at 65 DAS and 18, 43 and 51% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions, respectively. Application of AsA significantly increased dry weight plant^{-1} (3, 5 and 3% at 35 DAS; 2, 34 and 11% at 50 DAS; 13, 7 and 18% at 65 DAS and 8, 5 and 6% at 80 DAS at AsA treated 50, 80 and 120 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without AsA treatment (Figure 10). On the contrary, Si with saline treatments increased dry weight plant^{-1} (10, 11 and 11% at 35 DAS; 14, 4 and 16% at 50 DAS; 22, 12 and 41% at 65 DAS and 15, 18 and 18% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without Si treatment. Furthermore, exogenous application of GA3 with saline treatments increased dry weight plant^{-1} (24, 26 and 24% at 35 DAS; 14, 5 and 21% at 50 DAS; 42, 44 and 83% at 65 DAS and 19, 23 and 24% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress without GA3 treatment.

In our present study, biomass production (fresh and dry weight plant^{-1}) reduced due to the detrimental effect of salinity stress. It was revealed that reduction of plant biomass directly related to the reduction of the number of tiller and leaf number and leaf area (Huh *et al*., 2002). In this study the number of tiller, number of leaf and leaf area also reduced with the increasing salinity stress. The reduction in total biomass of the wheat genotypes probably occurred due to the extra energy utilization for osmotic accumulation, which is much more ATP-consuming for osmotic adjustment (Wyn Jones and Gorham, 1993). Moreover, reduction of plant biomass, a significant disturbance in photosynthetic parameters along with reduced chlorophyll contents and chlorophyll fluorescence, reduction of stomatal conductance, photosynthesis rates, inhibition of metabolic phenomena, and increased ROS generation which can increase oxygen-induced cellular damage (Neill *et al*., 2002). Usually, plants close

their stomata upon the onset of stressful conditions to save water, consequently reducing stomatal conductance and photosynthesis (Netondo *et al*., 2004).

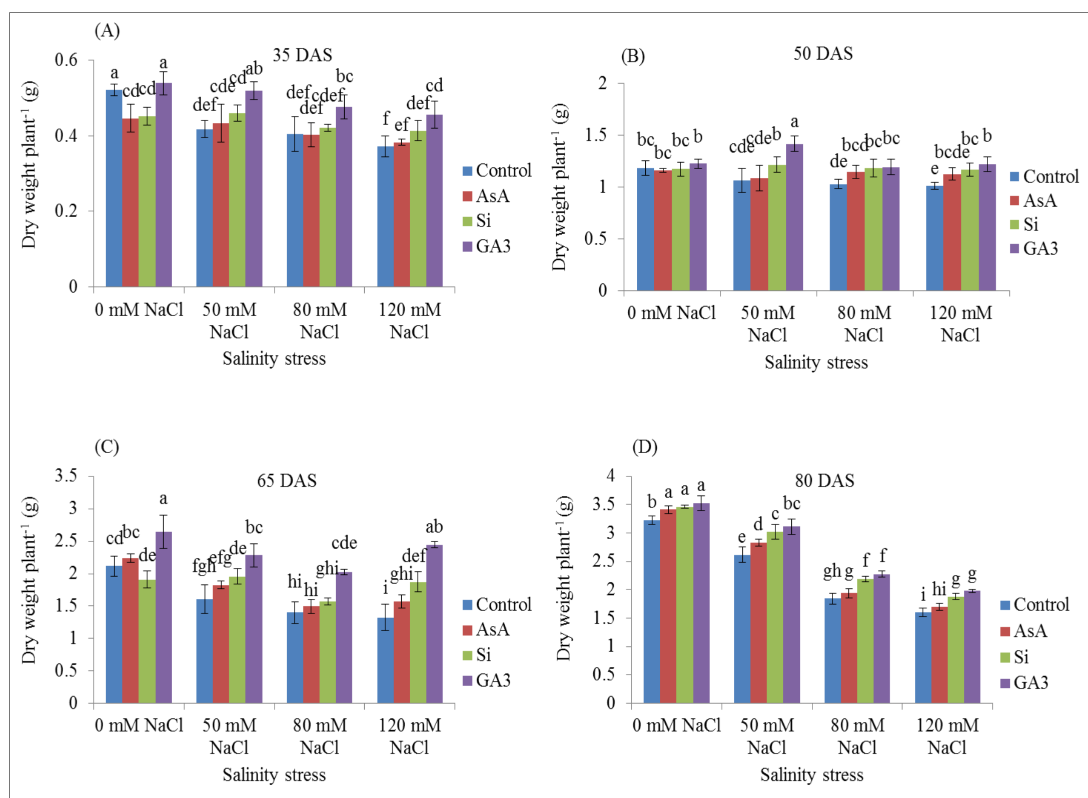

Figure 6. Effect of different salinity level, and AsA, Si and GA3 on dry weight plant^{-1} at (A) 35 days after sowing (DAS), (B) 50 DAS, (C) 65 DAS and (D) 80 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

The effect of salinity might be a secondary influence, arbitrated by the lower partial pressure of CO_2 in the green parts of the plant due to the stomata closure on the photosynthesis-related enzyme activities (Lawlor and Cornic, 2002; Meloni *et al*., 2003). However, exogenous application of GA3, AsA and Si increased total biomass of wheat in the present study. Gibberellic acid (GA3) is plant hormones that are associated with various plant growth and development processes found in previous studies. It has been stated that GA3 play a central role in tolerance to salinity stress by improving the activity of antioxidant enzymes and preventing lipid peroxidation, increased the accumulation of proline and potassium and increased the different physiological parameters, ultimately produced total biomass (Miceli *et al*., 2019; Maggio *et al*., 2010; Alsudays *et al*., 2020). On the other hand, application of scorbic acid (ASA) helps to uptake nutrients (N, P and K), protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities,

minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes (Farahat *et al*., 2013; Shao *et al*., 2008). Furthermore, it was showed that Si supplementation ameliorated the adverse effects of NaCl on plants growth, biomass, and oxidative stress by increasing the content of proline and cytokinin (Zhu *et al*., 2020). Si supplement could induce salt tolerance in plants by improving photosynthesis, membrane integrity, and detoxification of toxic radicals and elevating its antioxidant capacity under salinity stress (Robatjazi *et al*., 2020).

4.1.6 Relative water content (RWC)

Upon exposure to salt, stress leaf RWC decreased significantly in wheat when compared to their controls (Figure 11). At 50 mM, 80 mM and 120 mM of NaCl it was decreased by 6%, 8% and 10% at 35 DAS, 7%, 10% and 11% at 50 DAS and 9%, 12% and 19% at 65 DAS, respectively over control (Figure 11). The application of AsA, Si and GA3, in combination with salt stress significantly increased and effectively maintained the RWC compared to addition of salt only. But, in case of 50 mM NaCl, AsA, Si and GA3 treatment could not increase RWC of wheat plant. AsA could increase RWC by 3% and 5% at 35 DAS, 2% and 3% at 50 DAS and 3% and 4% at 65 DAS in seedlings exposed to 80 and 120 mM NaCl, respectively, while Si could increase the RWC by 13 and 34%. On the contrary, GA3 application with saline treatment increased RWC throughout the growing period. Since salt stress causes osmotic stress, the reduction in RWC is a common phenomenon in plants growth under salinity stress conditions and hence RWC is considered as an important parameter for evaluating plants for tolerance to salinity stress (Boyer *et al*., 2008). In this study, salinity stress led to a significant decrease of RWC in wheat with increasing NaCl concentration (Figure 11). In previous studies, the RWC of the plant decrease under salinity stress conditions in a diverse group of plants (Polash *et al*., 2018; El-Bassiouny and Bekheta, 2005; Keyvan, 2010). Decrease in RWC was due to loss of turgor that results in physiological drought for cell extension processes (Shamsi and Kobraee, 2013). However, when salt treated wheat plants were supplemented with AsA, Si and GA3, plant showed increased RWC which was due to the retention in water in their tissue (Figure 11). The enhanced water content in plants due to exogenous application was also observed by other researchers (Hasanuzzaman *et al*., 2013).

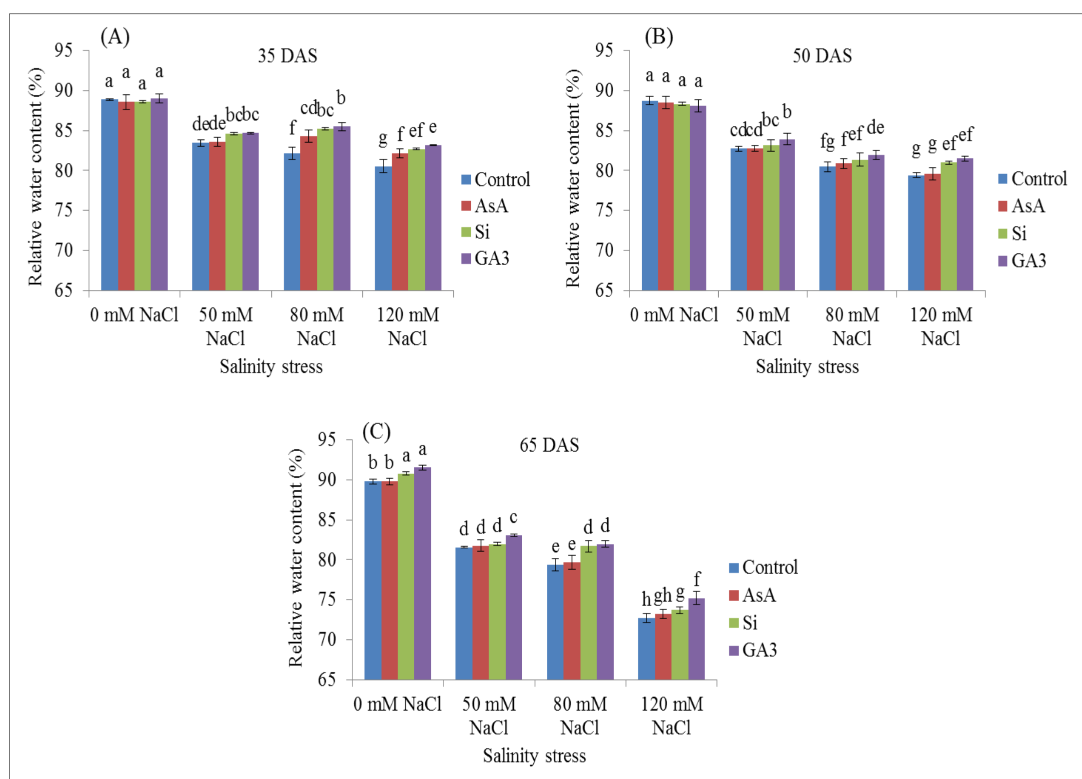

Figure 7. Effect of different salinity level, and AsA, Si and GA3 on relative water content (RWC) at (A) 35 days after sowing (DAS), (B) 50 DAS and 65 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.7 SPAD value

Different salinity treatments affected SPAD value significantly throughout the growing period. Salinity treatment reduced total SPAD value compared to its respective control. SPAD value decreased over control by 7%, 12%, 15% at 50, 80 and 120 mM NaCl; 11%, 14%, 20% at 50, 80 and 120 mM NaCl; 7%, 12%, 17% at 50, 80 and 120 mM NaCl and 37%, 56%, 77% at 50, 100 and 150 mM NaCl during 35 DAS, 50 DAS, 65 DAS and 80 DAS, respectively (Figure 12). On the contrary, AsA, Si and GA3 with saline treatments increased SPAD value throughout the growing period. In our experiment salinity caused reduction in SPAD value in wheat. Salinity stress often causes alteration in photosynthetic pigment biosynthesis (Cuin *et al*., 2010; Maxwell and Johnson, 2000). Kiani-Pouya and Rasouli (2014), reported that the chlorophyll content decreased significantly under severe salinity stress in wheat. However, exogenous application of AsA, Si and GA3 in salt treated plant increased chl content (Tahir *et al*., 2012; Tuna *et al*., 2008).

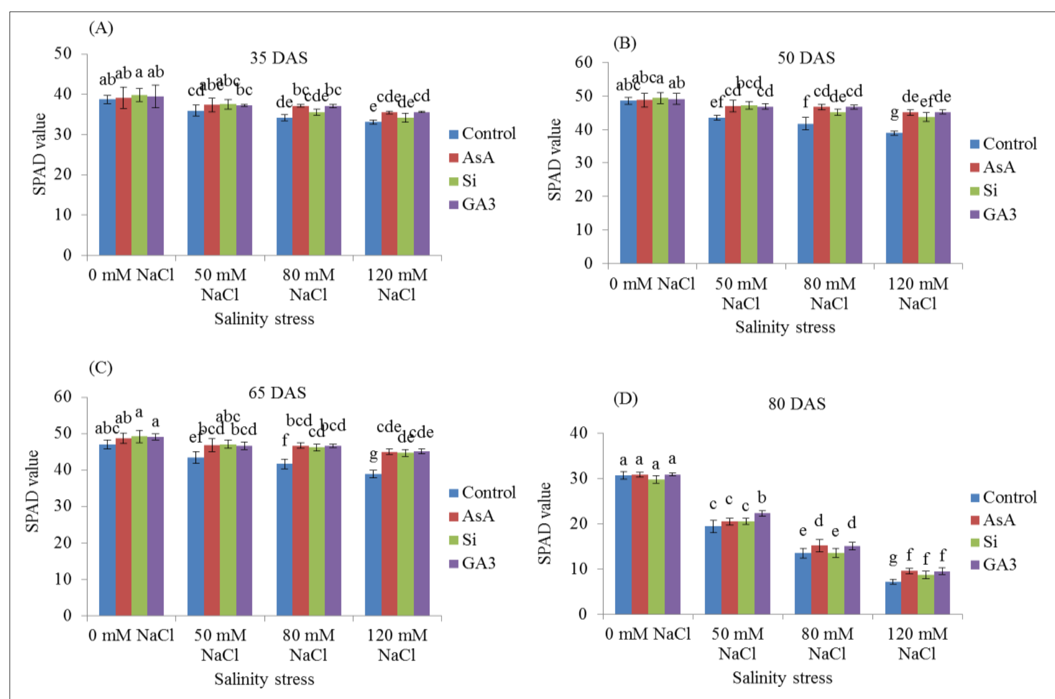

Figure 8. Effect of different salinity level, and AsA, Si and GA3 on SPAD value at (A) 35 days after sowing (DAS), (B) 50 DAS, (C) 65 DAS and (D) 80 DAS on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at *p* ≤ 0.05 applying LSD test.

4.1.8 Yield contributing characters and yield

4.1.8.1 Spike length plant^{-1}

Length of spike was also affected by salinity stress, but it had no statistically significance with each other according to Figure 13. Exogenous AsA and Si supplementation caused increased the length of spike under salinity stress conditions, but it had also no statistically significance with each other. In contrary, exogenous GA3 supplementation caused increased the length of spike by 6%, 6% and 9% under 50, 80 and 120 mM NaCl conditions, respectively.

4.1.8.2 Effective spike pot^{-1}

Salinity caused a considerable reduction of effective spike pot^{-1} compared to control (Figure 14). Exposure to salt stress resulted in significant decreases in effective spike pot^{-1}: 5, 8 and 11% at 50, 80 and 120 mM salinity stressed conditions, respectively when compared to unstressed control plant (Figure 14). On the contrary, the highest effective spike per pot was found in Si and GA3 treated plant under control conditions.

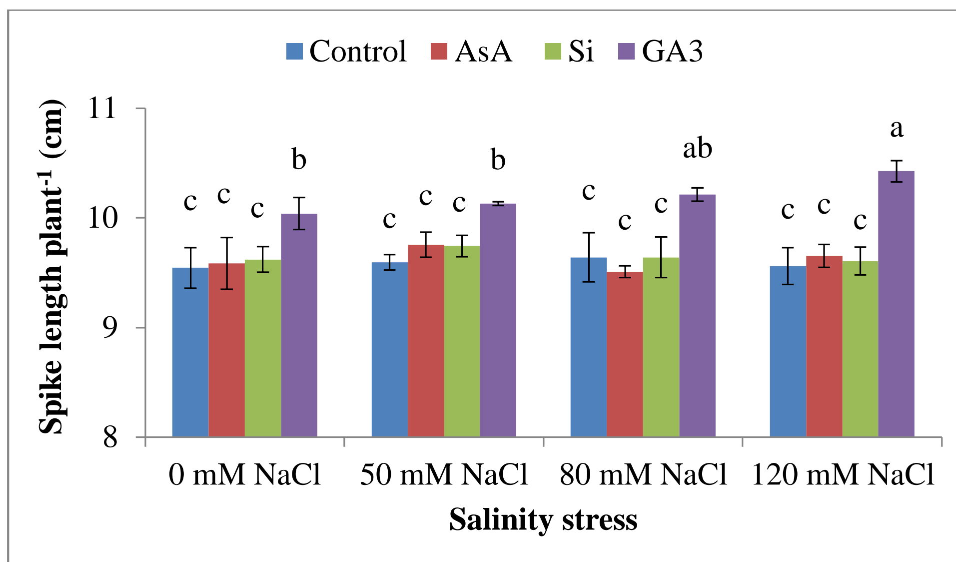

Figure 9. Effect of different salinity level, and AsA, Si and GA3 on spike length plant^{-1} on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

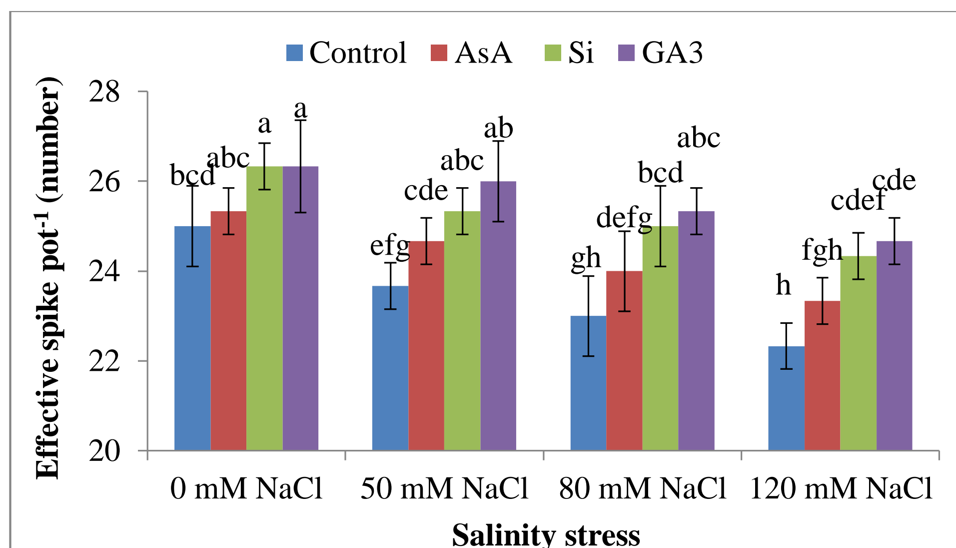

Figure 10. Effect of different salinity level, and AsA, Si and GA3 on effective spike pot^{-1} on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

AsA, Si and GA3 application increased effective spike per pot compared to its respective salt stress conditions. AsA treated plant increased effective spike pot^{-1} compare it's salt stress treatment (5, 4 and 5% at 50, 80 and 120 mM stressed condition, respectively). Si treated plant increased effective spike pot^{-1} compare it's salt stress treatment (7, 9 and 9% at 50, 80 and 120 mM stressed condition,

respectively). Furthermore, in case of GA3 supplementation the effective spike pot^{-1} increased by 10, 10 and 11% at 50, 80 and 120 mM stressed condition, respectively compare its salt stress treatment.

4.1.8.3 Number of grains spike^{-1}

For different salinity stress treatments, significant variation was observed for number of grains spike^{-1} (Figure 15). The number of grains per spike decreased harshly in case of salt stressed treatment compared to AsA, Si and GA3 treated stressed plant. Number of grains spike^{-1} was also decreased in the same way which was 3, 11 and 37% at 50, 80 and 120 mM salinity stressed conditions, respectively when compared to unstressed control plant. However, exogenous application of AsA and GA3 increased the number of grains spike^{-1} significantly under saline and non-saline conditions.

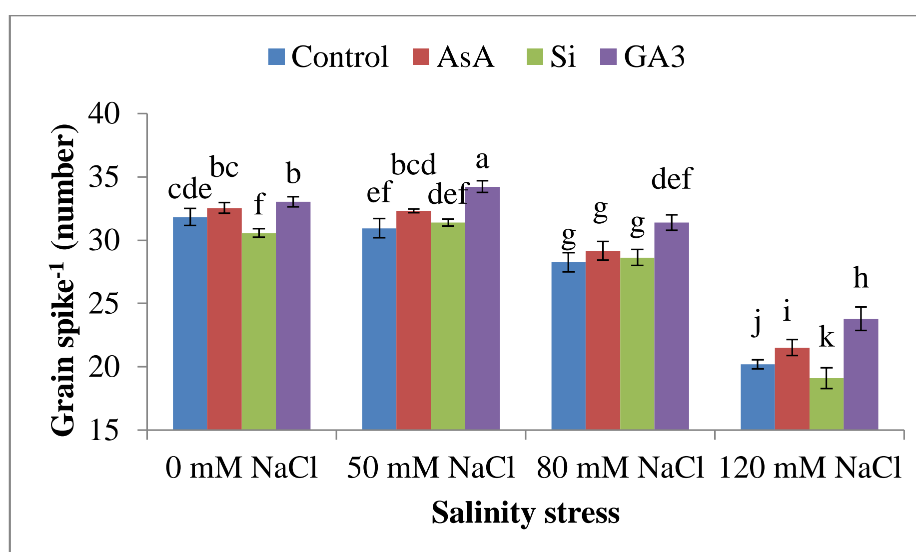

Figure 11. Effect of different salinity level, and AsA, Si and GA3 on number of grains spike^{-1} on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

AsA treated plant increased the number of grains spike^{-1} compare it's salt stress treatment (5, 4 and 7% at 50, 80 and 120 mM stressed condition, respectively). Furthermore, in case of GA3 supplementation the effective spike pot^{-1} increased by 11, 11 and 17% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment. On the contrary, exogenous Si supplementation caused increased the number of grains spike^{-1} under salinity stress conditions at 50 and 80 mM NaCl, but it had no statistically significance with each other.

4.1.8.4 1000-grain weight

As shown in Figure 16, 1000-grain weight of wheat plants decreased under salinity stress. Distinctly decreases in 1000-grain weight were observed (17, 28 and 52% at 50, 80 and 120 mM, respectively) in response to salt stress. The highest 1000-grain weight (42.03 g) was found with the application of GA3 under control condition and the lowest (18.54 g) was in 120 mM NaCl conditions. However, foliar application of AsA, Si and GA3 increased 1000-grain weight of wheat compared to its respective salt stress conditions. AsA treated plant increased 1000-grain weight of wheat compare it's salt stress treatment by 13, 6 and 6% at 50, 80 and 120 mM stressed condition, respectively. Supplementation of Si on plant increased 1000 grain weight of wheat compare it's salt stress treatment by 19, 11 and 16% at 50, 80 and 120 mM stressed condition, respectively. Furthermore, in case of GA3 supplementation on wheat plant, the 1000-grain weight increased by 25, 18 and 12% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

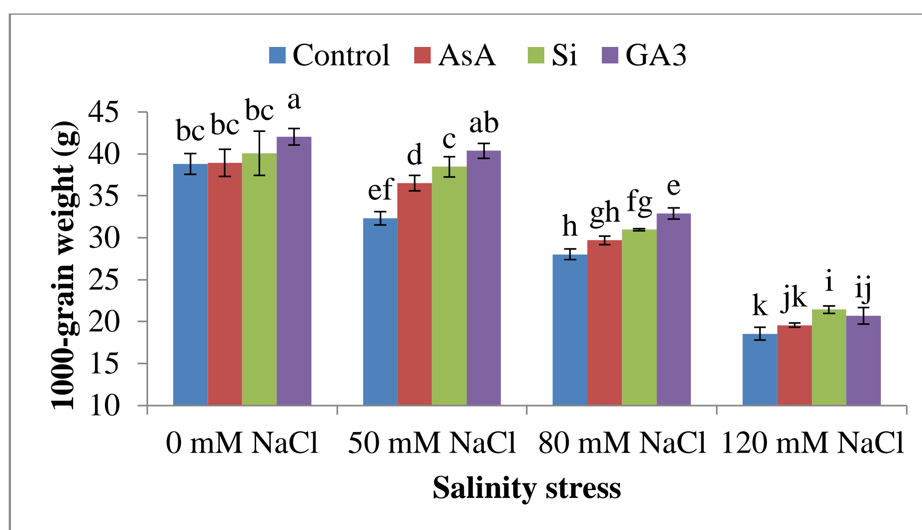

Figure 12. Effect of different salinity level, and AsA, Si and GA3 on 1000-grain weight on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.8.5 Seed yield pot^{-1}

Salinity caused a significant reduction in grain yield pot^{-1} of wheat plants compared to those in non-saline conditions (Figure 17). About 23, 41, 41 and 73% grain yield pot^{-1} decreased at 50, 80 and 120 mM NaCl, respectively. The highest grain yield pot^{-1} (36.60 g) was found in only GA3 treated plant. For all that, the lowest grain

yield pot (8.35 g) was found in 120 mM saline conditions. However, exogenous application of AsA, Si and GA3 increased grain yield per pot of wheat compared to its respective salt stress conditions. AsA treated plant increased grain yield pot^{-1} of wheat compare it's salt stress treatment by 23, 14 and 18% at 50, 80 and 120 mM stressed condition, respectively. Supplementation of Si on plant increased grain yield per pot of wheat compare it's salt stress treatment by 39, 22 and 19% at 50, 80 and 120 mM stressed condition, respectively. In case of GA3 supplementation on wheat plant, the grain yield pot^{-1} was the highest in all the concentration of salinity stress. Furthermore, GA3 supplementation on wheat plant, the grain yield pot^{-1} increased by 52, 44 and 45% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

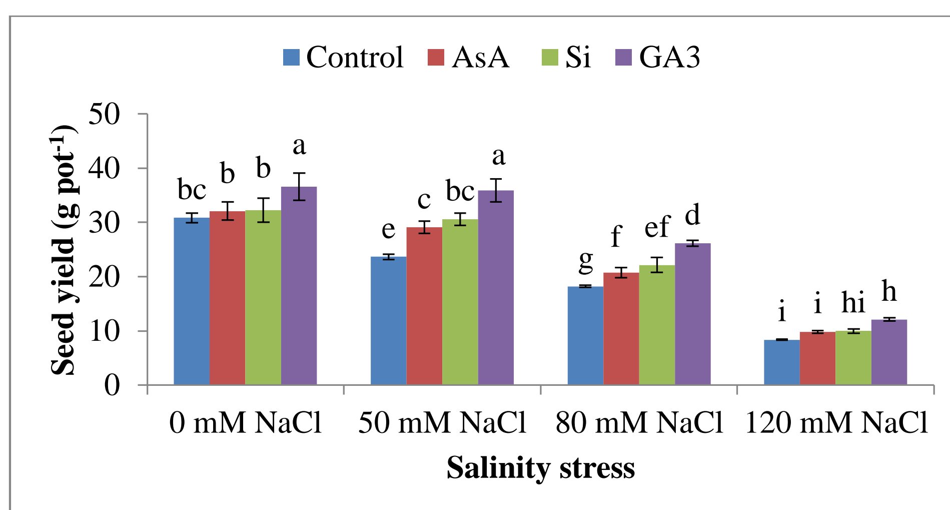

Figure 13. Effect of different salinity level, and AsA, Si and GA3 on grain yield pot^{-1} on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.8.6 Seed yield

Salinity caused a significant reduction in grain yield ton ha^{-1} of wheat plants compared to those in control conditions (Figure 18). About 23, 41, 41 and 73% grain yield pot^{-1} decreased at 50, 80 and 120 mM NaCl, respectively. However, exogenous application of AsA, Si and GA3 increased grain yield ton ha^{-1} of wheat compared to its respective salt stress conditions. AsA treated plant increased grain yield ton ha^{-1} of wheat compare it's salt stress treatment by 23, 14 and 18% at 50, 80 and 120 mM stressed condition, respectively. Supplementation of Si on plant increased grain yield ton ha^{-1} of wheat compare it's salt stress treatment by 39, 22 and 19% at 50, 80 and

120 mM stressed condition, respectively. In case of GA3 supplementation on wheat plant, the grain yield ton per ha was the highest in all the concentration of salinity stress. Furthermore, GA3 supplementation on wheat plant, the grain yield ton ha^{-1} increased by 52, 44 and 45% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

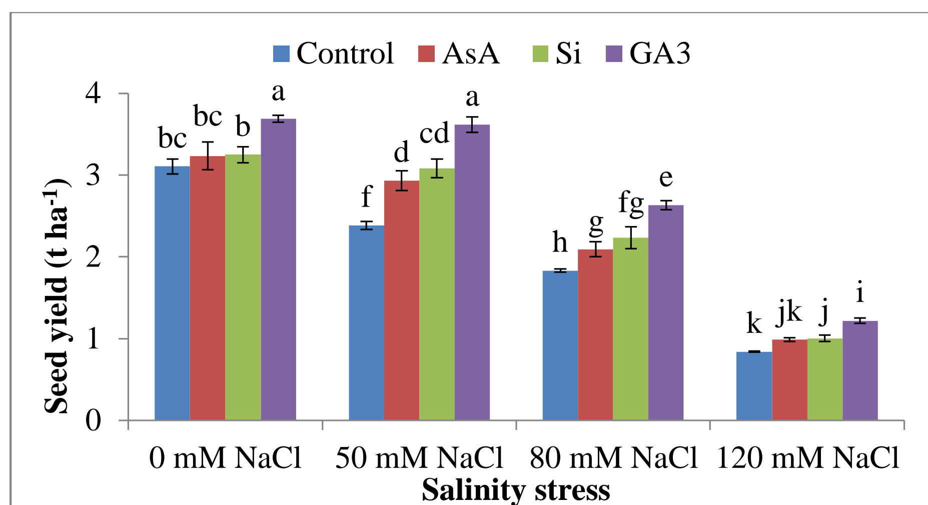

Figure 14. Effect of different salinity level, and AsA, Si and GA3 on grain yield (t ha^{-1}) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.8.7 Straw yield pot^{-1}

Straw yield (g pot^{-1}) was noticeably decreased in wheat varieties under salt stressed condition. Straw yield was decreased by 4, 10 and 28% at 50, 80 and 120 mM respectively, compared to respective control (Figure 19). The highest straw yield was observed in only Si treated plant (53.47 g pot^{-1}) when grown under 50 mM NaCl conditions. AsA treated plant grown under 50 mM NaCl conditions produced 52.40 g pot^{-1} straw yield which is statistically similar with the highest result (Figure 19). The lowest straw yield (32.50 g pot^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. However, exogenous application with AsA, Si and GA3 mitigated the salt effect of wheat. AsA treated plant increased straw yield (g pot^{-1}) of wheat compare it's salt stress treatment by 20, 4 and 9% at 50, 80 and 120 mM stressed condition, respectively. In case of Si supplementation on wheat plant, the straw yield (g pot^{-1}) was the highest in all the concentration of salinity stress. Supplementation of Si on plant increased straw yield (g pot^{-1}) of wheat compare it's salt stress treatment by 23, 8 and 14% at 50, 80 and 120 mM stressed condition,

respectively. Furthermore, GA3 supplementation on wheat plant, the straw yield (g pot^{-1}) increased by 14, 2 and 3% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

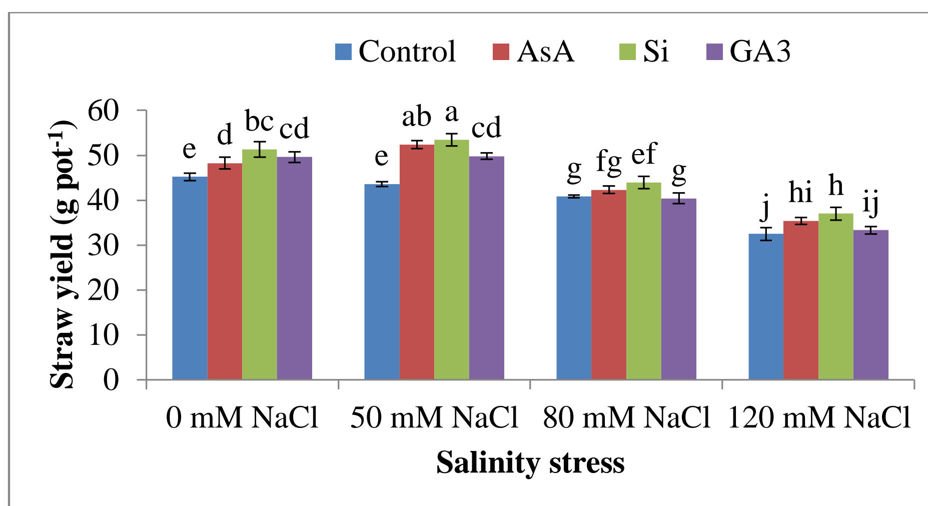

Figure 15. Effect of different salinity level, and AsA, Si and GA3 on straw yield pot^{-1} on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.8.8 Straw yield

Straw yield (t ha^{-1}) was noticeably decreased in wheat varieties under salt stressed condition. Straw yield was decreased by 4, 10 and 28% at 50, 80 and 120 mM respectively, compared to respective control (Figure 20). The highest straw yield was observed in only Si treated plant (5.29 t ha^{-1}) when grown under 50 mM NaCl conditions. AsA treated plant grown under 50 mM NaCl conditions produced (5.28 t ha^{-1}) straw yield which is statistically similar with the highest result (Figure 20). The lowest straw yield (3.27 t ha^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. However, exogenous application with AsA, Si and GA3 mitigated the salt effect of wheat. AsA treated plant increased straw yield (t ha^{-1}) of wheat compare it's salt stress treatment by 20, 4 and 9% at 50, 80 and 120 mM stressed condition, respectively. In case of Si supplementation on wheat plant, the straw yield (t ha^{-1}) was the highest in all the concentration of salinity stress. Supplementation of Si on plant increased straw yield (t ha^{-1}) of wheat compare it's salt stress treatment by 23, 8 and 14% at 50, 80 and 120 mM stressed condition, respectively. Furthermore, GA3 supplementation on wheat plant, the straw yield (t ha^{-1}) increased by 14, 2 and 3% at 50, 80 and 120 mM stressed condition,

respectively compare it's salt stress treatment.

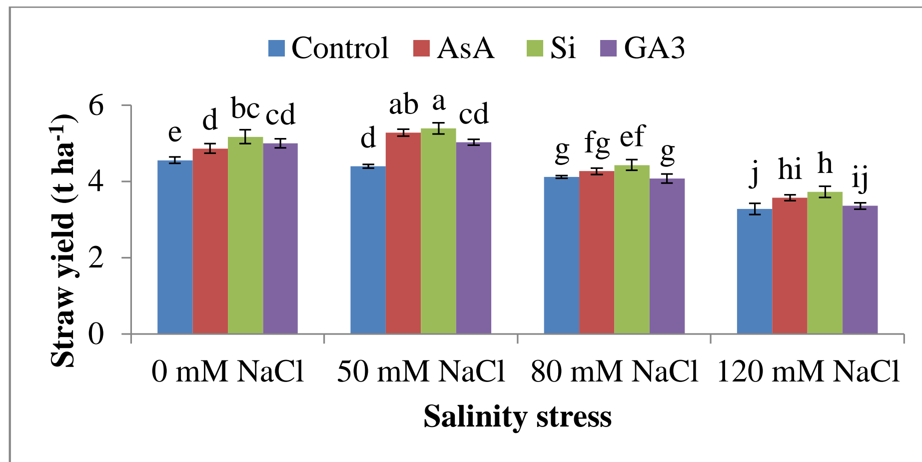

Figure 16. Effect of different salinity level, and AsA, Si and GA3 on straw yield (t ha^{-1}) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.8.9 Biological yield pot^{-1}

Biological yield (g pot^{-1}) was noticeably decreased in wheat varieties under salt stressed condition. Biological yield was decreased by 12, 22 and 46% at 50, 80 and 120 mM respectively, compared to respective control (Figure 21).

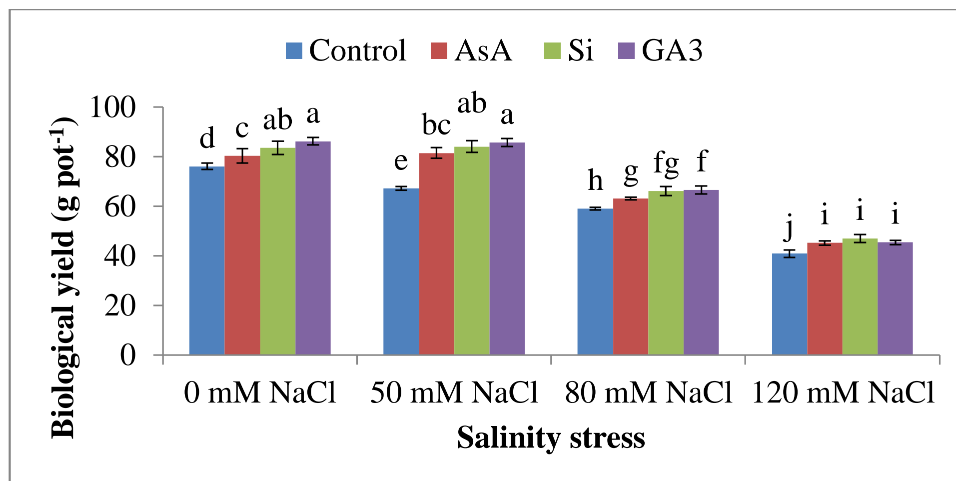

Figure 17. Effect of different salinity level, and AsA, Si and GA3 on biological yield (g pot^{-1}) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

The highest biological yield was observed in only GA3 treated plant (86.22 g pot^{-1}) when grown under control conditions. GA3 treated plant when grown under 50 mM

NaCl conditions produced 85.77 g pot^{-1} biological yield which is statistically similar with the highest result (Figure 21). The lowest biological yield (40.85 g pot^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. However, exogenous application with AsA, Si and GA3 mitigated the salt effect of wheat with increasing biological yield compare to it's salt stress conditions. AsA treated plant increased biological yield (g pot^{-1}) of wheat compare it's salt stress treatment by 21, 7and 11% at 50, 80 and 120 mM stressed condition, respectively. Supplementation of Si on plant increased biological yield (g pot^{-1}) of wheat compare it's salt stress treatment by 25, 13 and 15% at 50, 80 and 120 mM stressed condition, respectively. In case of GA3 supplementation on wheat plant, the biological yield (g pot^{-1}) was the highest at 50 and 80 mM NaCl concentration of salinity stress compare to AsA and Si application, but it was not at 120 mM NaCl conditions. Furthermore, GA3 supplementation on wheat plant, the biological yield (g pot^{-1}) increased by 22, 13 and 12% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

4.1.8.10 Biological yield

Biological yield (t ha^{-1}) was noticeably decreased in wheat varieties under salt stressed condition. Biological yield was decreased by 12, 22 and 46% at 50, 80 and 120 mM respectively, compared to respective control (Figure 22). The highest biological yield was observed in only GA3 treated plant (8.68 t ha^{-1}) when grown under control conditions. GA3 treated plant when grown under 50 mM NaCl conditions produced 8.64 t ha^{-1} biological yield which is statistically similar with the highest result (Figure 22). The lowest biological yield (4.11 t ha^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. However, exogenous application with AsA, Si and GA3 mitigated the salt effect of wheat with increasing biological yield compare to it's salt stress conditions. AsA treated plant increased biological yield (t ha^{-1}) of wheat compare it's salt stress treatment by 21, 7and 11% at 50, 80 and 120 mM stressed condition, respectively. Supplementation of Si on plant increased biological yield (t ha^{-1}) of wheat compare it's salt stress treatment by 25, 13 and 15% at 50, 80 and 120 mM stressed condition, respectively. In case of GA3 supplementation on wheat plant, the biological yield (t ha^{-1}) was the highest at 50 and 80 mM NaCl concentration of salinity stress compare to AsA and Si application, but it was not at 120 mM NaCl conditions. Furthermore, GA3

supplementation on wheat plant, the biological yield (t ha^{-1}) increased by 22, 13 and 12% at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment.

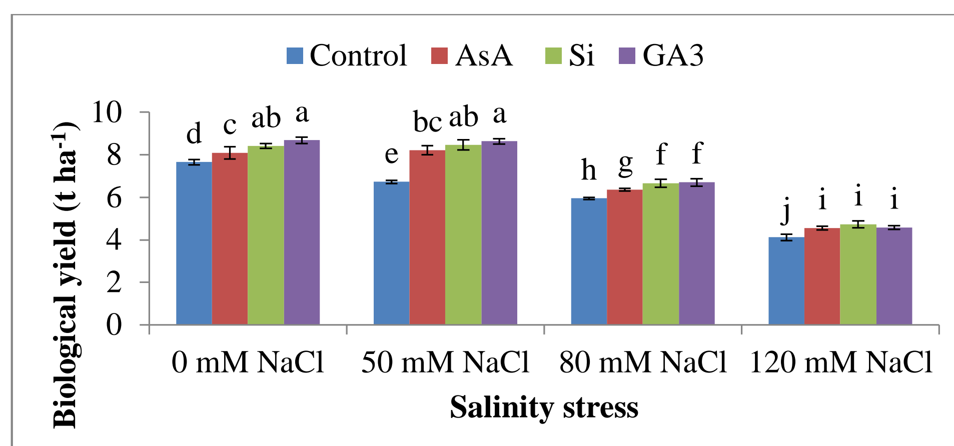

Figure 18. Effect of different salinity level, and AsA, Si and GA3 on biological yield (t ha^{-1}) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.1.9 Harvest index

For different salinity treatments with or without AsA, Si and GA3 spraying, significant variation was observed for harvest index (Figure 23). The highest harvest index was observed in only GA3 treated plant (42.46%) when grown under control conditions. GA3 treated plant when grown under 50 mM NaCl conditions produced 41.90% harvest index which is statistically similar with the highest result. The lowest harvest index (20.46%) was observed in only when plant grown under 120 mM NaCl conditions. Meaningful decreases in harvest index were observed (13, 24 and 50% at 50, 80 and 120 mM NaCl stresses, respectively) in response to salt stress, compared to the respective control and untreated control (Figure 23). However, AsA treated stressed plant produced harvest index (35.70, 32.90 and 21.71% at 50, 80 and 120 mM salt stressed condition, respectively) compared to salt stressed condition. Si treated stressed plant produced harvest index (36.38, 33.51 and 21.22% at 50, 80 and 120 mM salt stressed condition, respectively) compared to salt stressed condition. Furthermore, GA3 treated stressed plant produced harvest index (41.90, 39.29 and 26.66% at 50, 80 and 120 mM salt stressed condition, respectively) compared to salt stressed condition. Improving the grain yield of wheat is always the main target in plant breeding. The final yield of wheat is determined by the number of spikes per

plant and yield components, such as effective tiller, spikelet number, grain number and 1000-grain weight. The number of spikes is highly correlated with the the number of effective tillers. The effect of salinity on tiller number and spikelet number, which both initiate during early growth stages, has a greater influence on final grain yield than on yield components in the later stages.

The effect of salinity on tiller number and number grains spike^{-1}, which both initiate during early growth stages, has a greater influence on final grain yield than on yield components in the later stages (El-Hendawy *et al.*, 2005). The decrease in grain yield might be caused by the salinity, which induced reduction of photosynthetic capacity leading to less starch synthesis and accumulation in the grain (Turki *et al.*, 2012; Maha *et al.*, 2017). Similar results were found in previous literature in different crops and observed that yield reduced during the vegetative stage by affecting yield contributing parameters such as tiller number, grain in spike, grain number and grain weight in cereals such as rice (Zeng and Shannon, 2000) or wheat (Maas *et al.*, 1994). The harvest index has been shown to be affected by salinity (Gholizadeh *et al.*, 2014).

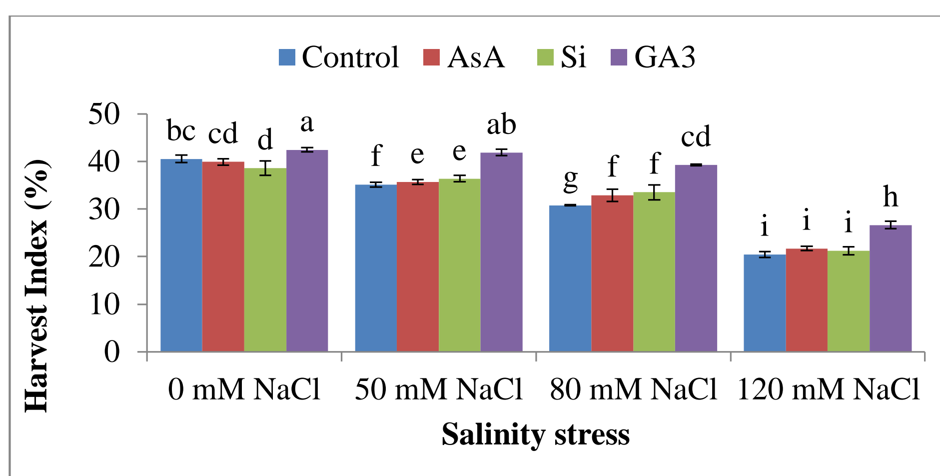

Figure 19. Effect of different salinity level, and AsA, Si and GA3 on harvest index (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

A plant capable of maintaining harvest index under salinity stress conditions will often have a higher yield. The reason for the maintenance of HI under salinity for changes in harvest index may include a lower shoot biomass reduction, maintenance of tiller number (Zeng and Shannon, 2000) or earlier flowering (Saade *et al.*, 2016).

Besides harvest index, other parameters, such as yield and yield components including grain weight, spikelets spike^{-1}, spike length, fertility rates in the spikes and 1000-grain weight, have also been shown to be affected by salinity stress (Gholizadeh *et al.*, 2014; Mishra *et al.*, 2014).

However, exogenous application of AsA, Si and GA3 significantly increased all the yield contributing attributes and yield. Similar result was found in previous studies (Tahir *et al*., 2010; Alsudays *et al*., 2020). Si may be increased tolerance of plants against salinity stress which is a major yield limiting factor in arid and semiarid areas. Application of Si to plant significantly increased dry matter production and grain yield in wheat under salinity stress conditions (Al-aghabary *et al*., 2004). Tahir *et al*. (2010), also observed that Si application significantly increased dry matter production and yield contributing parameters, ultimately increased yield. It was found that gibberellic acid (GA3) alleviated the adverse effects of salt stress on the growth, ion accumulation and photosynthetic capacity, finally increased the yield of wheat cultivars (Ashraf *et al*., 2002). On the other hand, a enlightened increase in plant height, number of tiller and spikes, flag leaf area, blades area plant^{-1}, spike length, grain index, grain and straw yield plant^{-1} was found by increasing ascorbic acid level up to 400 mg L on wheat under salinity stress conditions (Amin *et al*., 2008).

4.1.10 Plant ionic relations in shoot

Wheat showed a high variability in the plant sap total Na^+ content ranging between 0.56 % in control and 2.92% in GA3 treatment under 120 mM NaCl conditions (Figure 24). Meaningful increase in total Na^+ content were observed (183, 262 and 389% at 50, 80 and 120 mM NaCl stresses, respectively in response to salt stress, compared to the untreated control (Figure 24). However, exogenous application of AsA, Si, GA3 at 50, 80 and 120 mM salt stressed conditions was not decreased compared to control condition.

K^+ content in the plant sap ranged between 1.00% in Si treated control plant and 1.88% in AsA treated 120 mM NaCl conditions (Figure 25). In salt-grown plant, total K^+ content in shoot significantly decrease by 18, 28 and 44% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant (Figure 25). However, exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions

was not significantly differed total K^+ content in wheat shoot compared to 50 mM salt stressed conditions. Similar trend was found in case of 80 mM and 120 mM NaCl conditions.

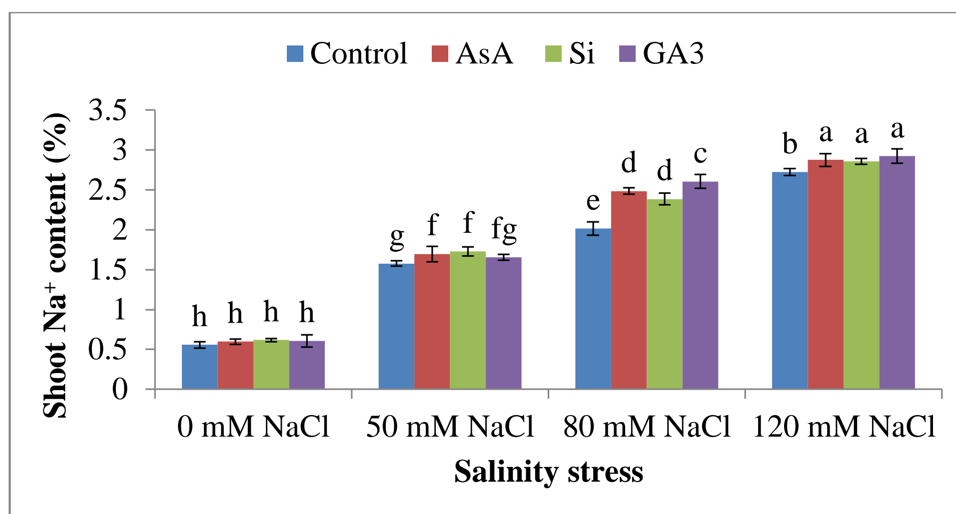

Figure 20. Effect of different salinity level, and AsA, Si and GA3 on shoot Na^+ content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

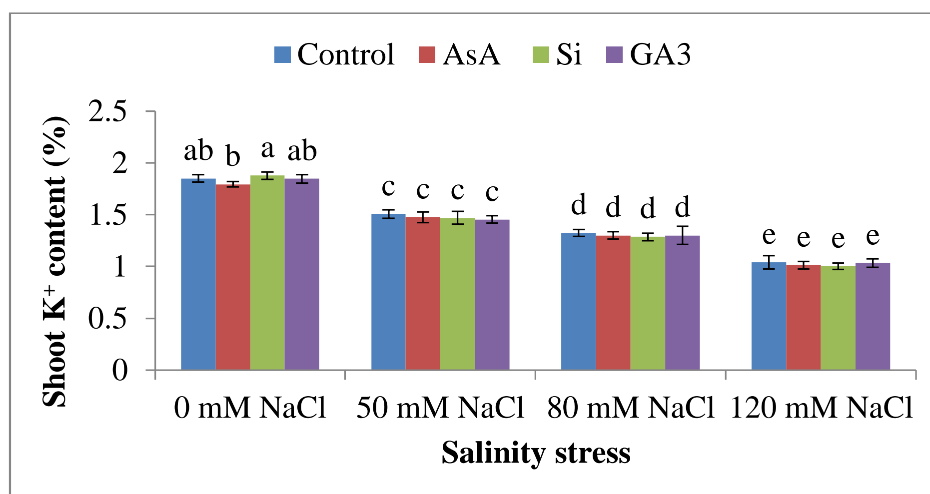

Figure 21. Effect of different salinity level, and AsA, Si and GA3 on shoot K^+ content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Ca^{2+} content in the plant sap ranged between 1.54 % in 120 mM NaCl conditions and 2.38 % in AsA treated control plant (Figure 26). In salt-grown plant, total Ca^{2+} content in shoot significantly decrease by 20, 25 and 34% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant (Figure 26).

Supplementations of AsA, Si and GA3 significantly increased Ca^{2+} content in shoot under 50 mM NaCl stress condition. Similar trend was observed in case of 80 mM NaCl stress conditions. However, exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed Ca^{2+} content in wheat shoot compared to 120 mM salt stressed conditions.

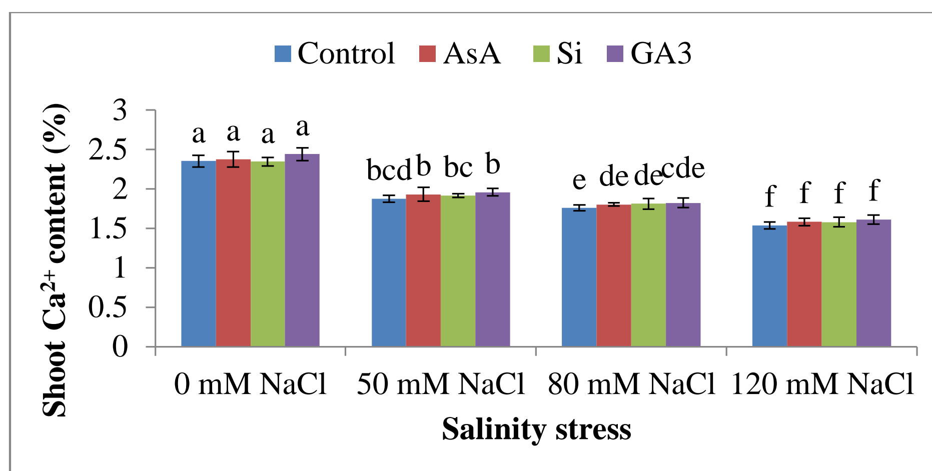

Figure 22. Effect of different salinity level, and AsA, Si and GA3 on shoot Ca^{2+} content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

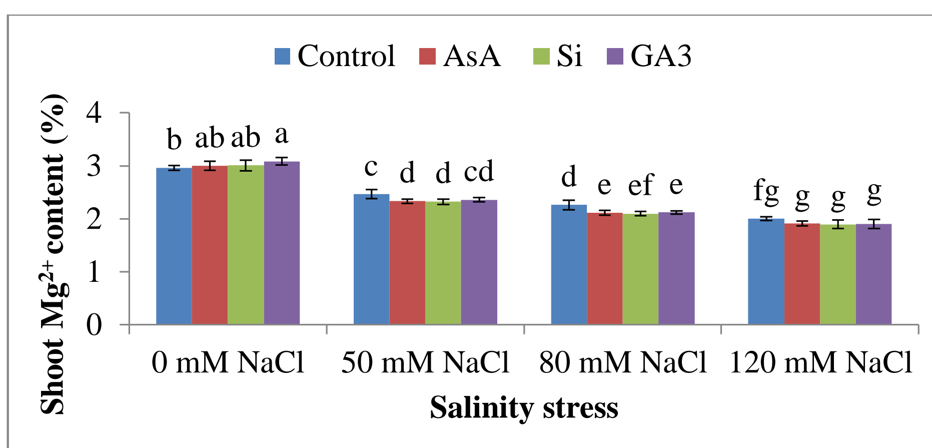

Figure 23. Effect of different salinity level, and AsA, Si and GA3 on shoot Mg^{2+} content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Mg^{2+} content in the plant sap ranged between 1.90 % in Si treated 120 mM NaCl stressed plant and 3.08 ppm in GA3 treated control plant (Figure 27). In salt-grown plant, total Mg^{2+} content in shoot significantly decrease by 17, 24 and 32% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant

(Figure 27). Supplementations of AsA, Si and GA3 significantly decreased Mg^{2+} content in shoot under 50 mM NaCl stress condition. Similar trend was observed in case of 80 mM NaCl stress conditions. However, exogenous application of AsA, Si, GA3 at 120 mM salt stressed conditions was not significantly differed Ca^{2+} content in wheat shoot compared to 120 mM salt stressed conditions.

4.1.11 Plant ionic relations in grain

Na^+ content in the grain of wheat ranging between 0.12 % in control and 0.25 % in AsA, Si and GA3 treatment plant under 120 mM NaCl conditions (Figure 28). Meaningful increase in total Na^+ content in the grain were observed (33, 50 and 100% at 50, 80 and 120 mM NaCl stresses, respectively in response to salt stress, compared to the untreated control (Figure 28). However, exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed Na^+ content in wheat grain compared to 50 mM salt stressed conditions. Similar trend was found in case of 80 mM and 120 mM NaCl conditions.

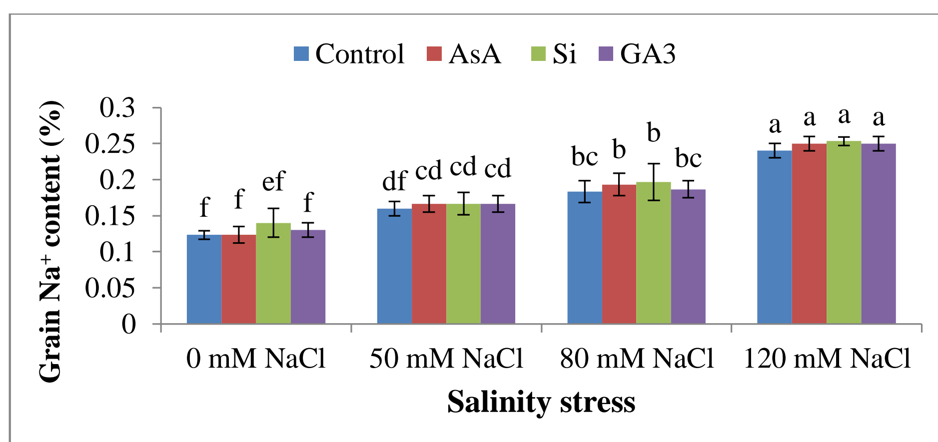

Figure 24. Effect of different salinity level, and AsA, Si and GA3 on grain Na^+ content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

K^+ content in the wheat grain ranged between 0.55 % in 120 mM NaCl conditions and 0.76 % in Si treated control conditions (Figure 29). In salt-grown plant, total K^+ content in grain significantly decrease by 22, 24 and 25% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant (Figure 29). However, exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed K^+ content in wheat grain compared to 50 mM salt

stressed conditions. Similar trend was found in case of 80 mM and 120 mM NaCl salinity stress conditions.

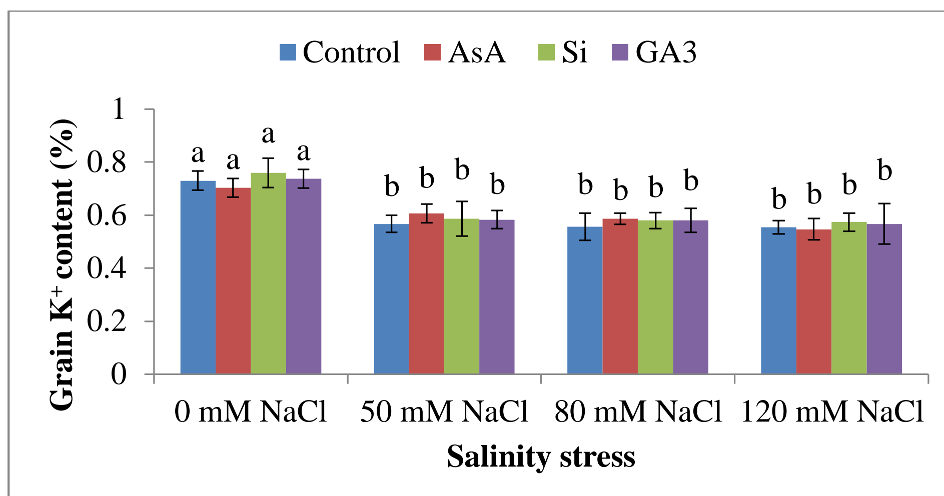

Figure 25. Effect of different salinity level, and AsA, Si and GA3 on grain K^+ content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

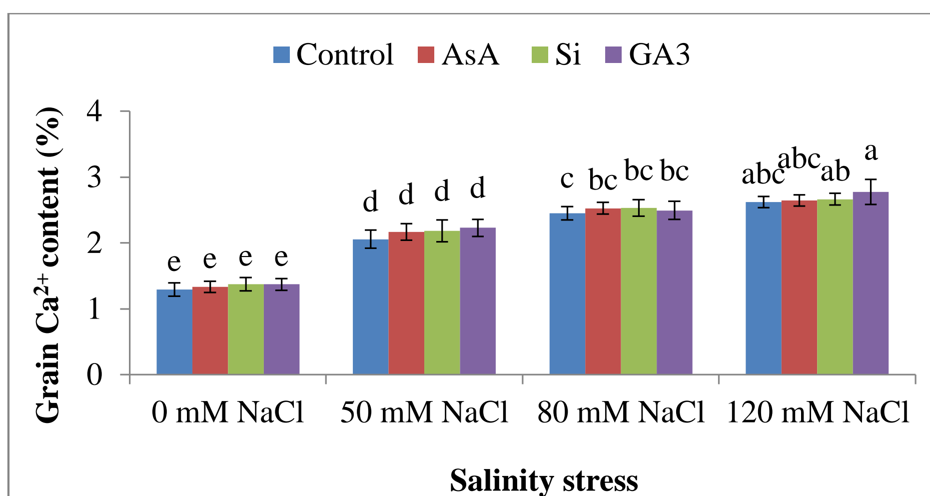

Figure 26. Effect of different salinity level, and AsA, Si and GA3 on grain Ca^{2+} content (%) on wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Ca^{2+} content in the wheat grain ranged between 1.29 % in control plant and 2.77 % in 120 mM NaCl stress conditions (Figure 30). In salt-grown plant, total Ca^{2+} content in grain significantly increased by 60, 90 and 103% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant (Figure 30). No

significant difference was observed with supplementations of AsA, Si and GA3 at 50 mM NaCl stress conditions for Ca^{2+} content in the grain. However, exogenous application of AsA, Si, GA3 at 80 and 120 mM salt stressed conditions was significantly differed Ca^{2+} content in wheat grain compared to 80 and 120 mM salt stressed conditions.

Mg^{2+} content in the wheat grain ranged between 0.74 % in control plant and 1.60 % in GA3 treated control plant (Figure 31). In salt-grown plant, total Mg^{2+} content in grain significantly increased by 17, 24 and 32% at 58, 95 and 104 mM NaCl stresses, respectively, compared to the untreated control plant (Figure 31). Supplementations of AsA, Si and GA3 significantly increased Mg^{2+} content in grain under 50 mM NaCl stress condition. Similar trend was observed in case of 80 and 120 mM NaCl stress conditions.

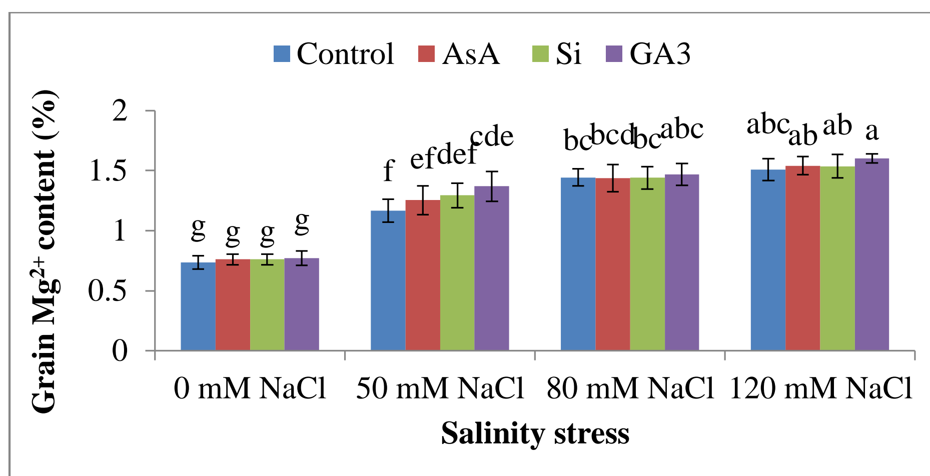

Figure 27. Effect of different salinity level, and AsA, Si and GA3 on grain Mg^{2+} content (%) of wheat. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Salinity stress is known to enhance the uptake and accumulation of toxic Na^+ ions in different crop species, including wheat with increasing salinity level. The contents of K^+ were reduced due to imposition of salinity in wheat plants. The accumulation of toxic ions (Na^+) in plants results in reduced uptake of K^+ under saline conditions and consequently, low levels of K^+ are transported to leaves (Tuna *et al.*, 2013). Salinity causes an excessive influx of Na^+ in roots and leaves, which ultimately leads to K^+ efflux, thus causing K^+ deficiency in plants (Demidchik *et al.*, 2014). However, application of AsA, Si and GA3-treated plants accumulated more Na^+ in both shoot

and grain than the untreated salinity stressed plants. Similar result was reported by Tahir *et al*. (2010). However, these results do not agree with other authors (Noreen *et al*., 2020) who reported that AsA, Si and GA3 reduced the accumulation of Na^+ in shoot of wheat under saline conditions. However, the contradiction other studies could be due to the difference in plant growth stage at which AsA, Si and GA3 was applied.

4.2 Experiment 2. ALLEVIATION OF SHORT TERM SALT STRESS EFFECT ON WHEAT SEEDLINGS BY FOLIAR SPRAY WITH ASCORBIC ACID, SILICON AND GIBBERELLIC ACID

4.2.1 Plant growth biomass parameter

4.2.1.1 Root and shoot length and their ratio

Salt toxicity severely arrest the growth and development of wheat seedlings, including shoot and root length, fresh weight (FW) and dry weight (DW) (Table 1). In contrast to control seedlings, shoot length was decreased by 4%, 18% and 47% due to 50, 80 and 120 mM NaCl concentration, respectively. Whereas root length decreased by 20%, 36% and 56% under 50, 80 and 120 mM NaCl concentration, respectively. Exogenous application (foliar spraying) of AsA, Si and GA3 improve the growth performance of all types of salt affected seedlings where GA3 performed better (Table 1).

Almost similar trend was observed from root/shoot ratio. Salinity stress limit plant growth by reducing overall root and shoot length due to osmotic stress, ionic toxicity, and a reduced ability to take up essential plant minerals (Munns *et al*., 2006; Rahman *et al*., 2016a,b). Salinity create water deficits condition which affect cell elongation, cell expansion, wilting, and, ultimately, decreased plant height (Apse and Blumwald, 2002; Munns and Tester, 2008; Mahmud *et al*., 2020). Shabani *et al*. (2013), also reported that salinity-induced osmotic stress inhibits growth of rapeseed by reducing water uptake capacity and photosynthesis efficiency. Similar salt-induced growth inhibition has been reported in previous studies in different plant (Tuncturk *et al*., 2008; Munns, 2011; Rahman *et al*., 2016a,b; Mahmud *et al*., 2020).

In present study, osmoprotectants and synthetic plant growth regulator like AsA, Si and GA3 increased plant height slightly or significantly under different level of salt stress. This may be achieved through osmoregulation which in turn increased osmotic adjustment by increasing water flow and water status by using the organic solutes (Shaddad *et al*., 2013; Iqbal and Ashraf, 2013; Saeidi-Sar *et al*., 2013; Islam and Mehraj, 2014).

Table 1. Effect of AsA, Si and GA3 on shoot length, root length, root/shoot ratio, fresh weight and dry weight of wheat seedlings under salt stress.

Treatments	Shoot length (cm)	Root length (cm)	Root / Shoot ratio	Fresh weight (gm $plant^{-1}$)	Dry weight (gm $plant^{-1}$)
Control	18.40±0.29 d	13.47±0.19 b	0.732±0.021 a	0.252±0.004 b	0.026±0.0015 a
AsA	18.87±0.36 c	13.75±0.10 ab	0.729±0.010 ab	0.251±0.003 b	0.026±0.0010 b
Si	19.25±0.25 b	13.92±0.31 ab	0.723±0.015 ab	0.270±0.002 a	0.028±0.0012 a
GA3	19.54±0.13 a	14.17±0.20 a	0.725±0.015 ab	0.253±0.004 b	0.026±0.0010 b
Salt 50	17.72±0.09 e	10.77±0.50 de	0.608±0.030 de	0.222±0.002 d	0.022±0.0006 d
Salt 50 + AsA	18.13±0.13 d	11.21±0.58 d	0.618±0.028 cde	0.236±0.008 c	0.023±0.0006 cd
Salt 50 + Si	18.37±0.12 d	11.72±0.08 c	0.638±0.003 c	0.245±0.009 bc	0.025±0.0010 bc
Salt 50 + GA3	18.75±0.10 c	11.92±0.18 c	0.636±0.006 cd	0.240±0.004 c	0.023±0.0010 d
Salt 80	15.17±0.18 h	8.68±0.08 g	0.572±0.012 f	0.184±0.005 fg	0.018±0.0006 f
Salt 80 + AsA	15.80±0.0 g	9.51±0.21 f	0.602±0.010 e	0.194±0.003 ef	0.019±0.0006 f
Salt 80 + Si	16.07±0.10 g	9.79±0.15 f	0.610±0.009 cde	0.198±0.005 e	0.022±0.0006 e
Salt 80+ GA3	16.46±0.08 f	10.39±0.40 e	0.631±0.022 cde	0.185±0.006 fg	0.020±0.0006 e
Salt 120	9.80±0.13 l	5.97±0.11 j	0.609±0.020 cde	0.154±0.007 i	0.014±0.0006 h
Salt 120 + AsA	10.23±0.08 k	7.25±0.35 i	0.708±0.029 ab	0.171±0.012 h	0.015±0.0006 g
Salt 120 + Si	10.97±0.13 j	7.68±0.20 hi	0.701±0.010 b	0.180±0.012 gh	0.017±0.0015 g
Salt 120+ GA3	11.53±0.20 i	8.07±0.20 h	0.700±0.015 b	0.175±0.006 gh	0.016±0.0006 g
$LSD_{0.05}$	**0.1402**	**0.2283**	**1.4532**	**0.005281**	**0.3639**
CV (%)	**1.08**	**2.66**	**2.70**	**3.04**	**4.35**

AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM Gibberellic acid, respectively. Means (±SD) were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.2.1.2 Fresh and dry weight

Fresh and dry weights of wheat seedling were studied to evaluate the salt-induced negative alteration in plant as well as performance of AsA, Si and GA3 against NaCl toxicity. Salt severely affected the growth and biomass of plant where FW and DW notably decreased in dose-dependent fashion (Table 1). Compared to control treatment, fresh weight declined by 12%, 27% and 39% due to exposure of wheat seedling to 50, 80 and 120 mM NaCl concentrations, respectively. Dry weight of wheat seedling followed similar trend as usual. Dry weight decreased by 15%, 30% and 46% under 50, 80 and 120 mM NaCl concentration, respectively. Exogenous application (foliar spraying) of AsA, Si and GA3 improve the fresh and dry weight of all types of salt affected seedlings notably or slightly. However interestingly for biomass improvement Si performed better than AsA and GA3 (Table 1). In current study, salinity significantly decreased the biomass production (fresh and dry weight plant^{-1}) of wheat seedling. It is proportionally related with the shoot and root length of plant. In this study, plant height (shoot + root length) also reduced with the increasing salinity stress. Rahman *et al*. (2016a,b), confirmed that salt-affected rice seedlings showed growth inhibition in terms of plant height, seedling fresh and dry weight. The reduction in total biomass of the wheat genotypes probably occurred due to the extra energy utilization for osmotic accumulation, a noteworthy disorder in photosynthetic parameters, reduction of stomatal conductance, photosynthesis rates, increased ROS generation, lipid peroxidation as well as cellular damage (Wyn Jones and Gorham, 1993; Neill *et al*., 2002; Mahmud *et al*., 2020).

On the other hand, foliar spraying of GA3, AsA and Si increased total biomass of wheat in the present study. Gibberellic Acid (GA3) is plant hormones that are associated with various plant growth and development processes found in previous studies. It has been stated that gibberellins (GA3) play a central role in tolerance to salinity stress by improving the activity of antioxidant enzymes and preventing lipid peroxidation, increased the accumulation of proline and potassium and increased the different physiological parameters, ultimately produced total biomass (Miceli *et al*., 2019; Maggio *et al*., 2010; Alsudays *et al*., 2020). On the other hand, application of ASA, and GA3 helps to continue normal metabolic processes against H_2O_2 generation and reduce membrane peroxidation and reduce cellular damages (Farahat *et al*., 2013; Shao *et al*., 2008). Addition of Si to salt affected plant restores

photosynthesis and improves membrane integrity by improving antioxidant defense system which regulates plant growth and biomass (Daoud *et al*. 2018; Robatjazi *et al*., 2020).

4.2.2 Water status and osmotic adjustment

4.2.2.1 Relative water content

Salt stress negatively affected the water status of wheat seedlings. A notable reduction in leaf relative water content (RWC) due to salt stress confirmed the water deficit condition in wheat seedling. In current study salt stress decreased leaf RWC in dose dependant manner (Figure 32). In contrast to control, 50 mM, 80 mM and 120 mM NaCl decreased leaf RWC by 2, 6 and 9%, respectively. But exogenous foliar application of AsA, Si and GA3 increased leaf RWC considerably whereas among the performance of three applied chemical no significant differences were observed (Figure 32). So, in present experiment, salt stress resulted in osmotic stress indicated by leaf relative water content, which decreased with the increasing duration of stress.

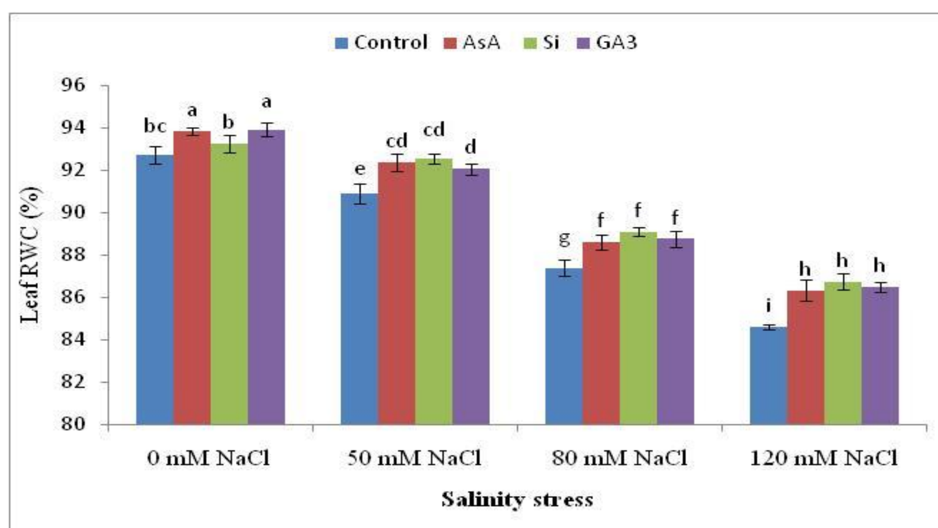

Figure 28. Effect of AsA, Si and GA3 on relative water content of wheat seedlings under salt stress. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Salt stress causes both ionic toxicity and osmotic stress which creates physiological drought due to exosmosis and interruption of water uptake (Munns, 2011). Wheat seedlings exposed to salt showed lower RWC and higher Pro accumulation, which

indicated a salt-induced water imbalance and osmotic stress. This result corroborates previous studies (Hasanuzzaman *et al*. 2014a; Nahar *et al*. 2015; Ozfidan-Konakci *et al*., 2015; Mahmud *et al*., 2020), which reported salinity increased osmotic potential and decreased water retention capacity with higher Pro for osmotic adjustment. However application of AsA, Si and GA3 restored water loss (indicated from leaf relative water content) in our experiment which may due to improve turgor of cell. The enhanced leaf relative water content in plants due to exogenous application of different chemicals was also observed by other researchers (Hasanuzzaman *et al*., 2013).

4.2.2.2 Proline content

Proline content of wheat seedling followed reverse tendency as leaf RWC in current study. It increased by 44%, 100% and 235% under 50, 80 and 120 mM NaCl concentration, respectively (Figure 33). Proline accumulation is a universal stress response. Salt application increased the activity of the proline biosynthetic enzyme, i.e., pyrroline-5 carboxylate reductase, and decreased the activity of peroxidase enzyme in crop species (Anjum *et al*. 2005). An extreme augmentation of Pro content owing to salt stress indicated the water shortage situation of wheat seedling (Mahmud *et al*., 2020).

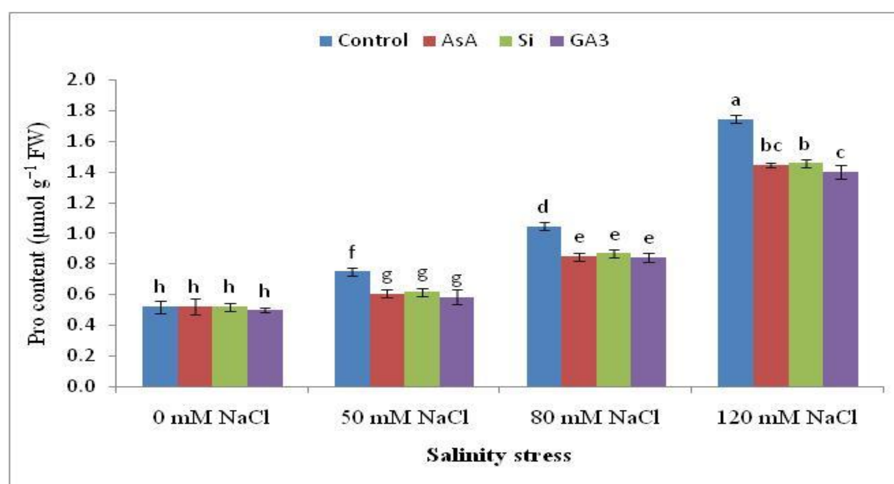

Figure 29. Effect of AsA, Si and GA3 on proline content of wheat seedlings under salt stress. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 μM silicon di-oxide and 100 μM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

However at certain level it has been documented that Pro helps to mitigate stresses through different mechanisms, including the detoxification of toxic ions, protection

of membrane integrity and stabilization of proteins/enzymes, and protects cell from ROS damage (Iqbal *et al*., 2014). Proline content increased with increasing salinity level was also reported by Datta *et al.* (2009). They confirmed salt stress on five varieties of wheat (*Triticum aestivum* L.) cultivars enhanced Pro concentration in cellular level by dose dependant manner from 0 to 100 mM salinity under laboratory condition. Similar salt-induced Pro accumulation was also recorded in salt-affected rice seedlings (Hasanuzzaman *et al*., 2014). Exogenous foliar application of AsA, Si and GA3 decreased the content of Pro which indicated the stress released condition. Under 120 mM NaCl stress the rate of decline Pro was higher by GA3 than the others. Under 120 mM NaCl the reduction of Pro was 17% by AsA and 16% by Si but 20% by GA3 in contrast to respective stress. However in case of 50 mM and 80 mM stress their performance was statistically similar (Figure 33). Application of such chemical (AsA) was recorded to ameliorate salt stress by reducing Pro content also recorded in wheat (Azzedine *et al*. 2011) and barley (Agami, 2014) seedling under salt stress condition. Moreover, GA3 play a vital role in tolerance to salinity stress by enhancing the action of antioxidant enzymes, preventing lipid peroxidation and regulating the Pro content (Miceli *et al*., 2019; Maggio *et al*., 2010; Alsudays *et al*., 2020).

4.2.3 Photosynthetic pigments

Salt stress severely affected the photosynthesis progression as it lessened concentration of essential photosynthetic pigments, including chl *a* and chl *b*. In contrast to control seedlings, chl *a* content decreased by 19%, 28% and 50% due to 50, 80 and 120 mM NaCl concentration, respectively (Figure 34). While level of chl *b* decreased by 27%, 50% and 56% under 50, 80 and 120 mM NaCl concentration, respectively. Therefore, in comparison with control seedlings chl (*a*+*b*) content reduced by 22%, 36% and 52% under 50, 80 and 120 mM NaCl concentration, respectively. Foliar spraying with AsA, Si and GA3 reinstates the chl *a* and chl *b* content. Here, Si performed better in increasing chl *a* and chl *b* content of wheat leaf compared to almost all of the respective treatments (Figure 34). Salinity-induced ionic toxicity and osmotic stress decreased chlorophyll content by increasing the activity of chlorophyllase and overproduction of ROS (Saha *et al*. 2010; Hasanuzzaman *et al*. 2014a, b) or both. In current study, salt stress decreased chl a, chl b and chl (a+b) contents, which were remarkably recovered by AsA, Si and GA3

supplementation. This restoration of photosynthetic pigments might be due to lower production of ROS, lower Na uptake, and upregulation of Mn uptake by Mn supplementation. Similar finding related to positive function of AsA, Si and GA3 against different abiotic stress were obtained from different studies (Saeidi-Sar *et al*., 2013; Daoud *et al*., 2018). In another experiment it is found that seed priming with GA3 alleviates the drastic effect of salinity and increases grain weight and grain quality by improving photosynthetic pigments, leaf area and plant growth (Shaddad *et al*., 2013).

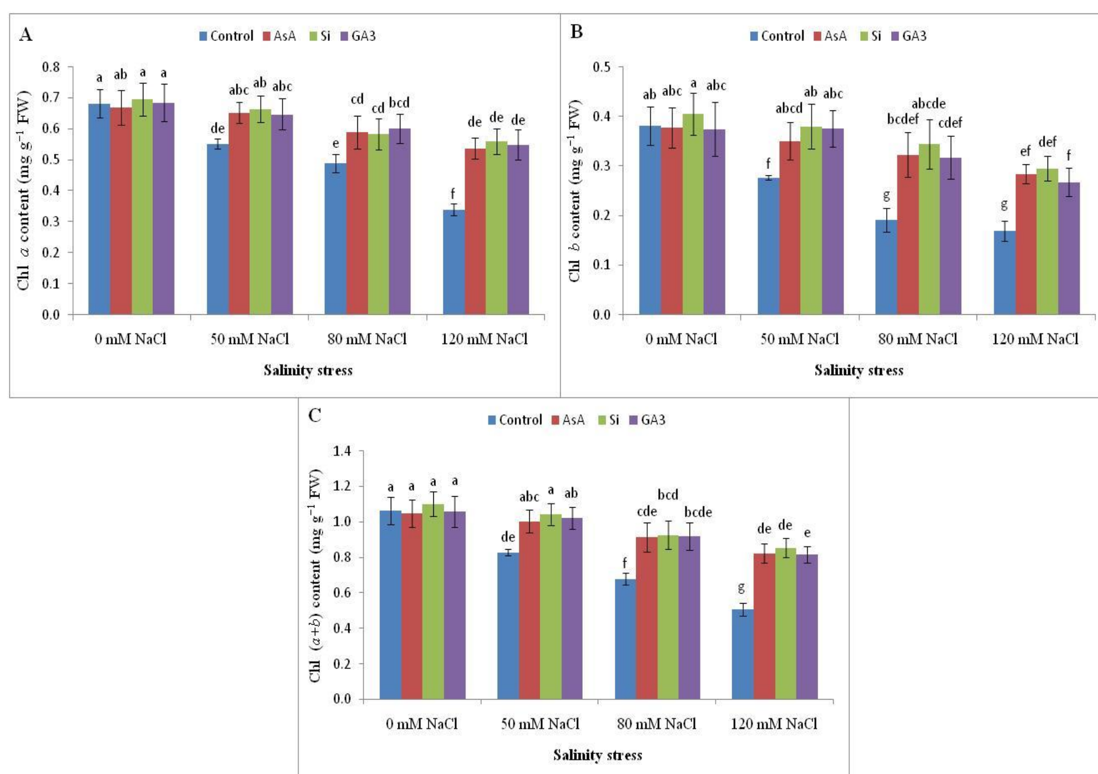

Figure 30. Effect of AsA, Si and GA3 on chl a, chl b and chl (a+b) content of wheat seedlings under salt stress. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon dioxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.4 Reactive oxygen species (ROS) generation and membrane damage

4.2.4.1 Hydrogen peroxide (H_2O_2) Content

The level of H_2O_2 increased in dose dependant manner of salt stress. In comparison with control treatment content of H_2O_2 increased by 19, 50 and 85% under 50, 80 and 120 mM NaCl concentration, respectively (Figure 35). Exogenous application of

AsA, Si and GA3 as foliar spraying reduced the generation of H_2O_2 under all level of stress. There performance was statistically similar (though the reduction capacity is bit higher for GA3) under all treatments except GA3 under 120 mM NaCl stress where GA3 significantly lowered H_2O_2 content than the AsA and Si (Figure 35).

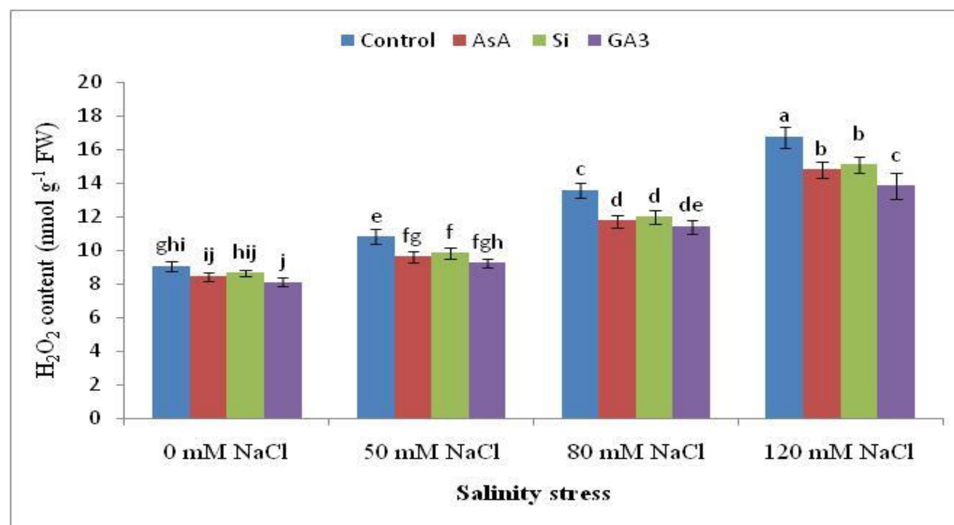

Figure 31. Effect of AsA, Si and GA3 on H_2O_2 content of wheat seedlings under salt stress. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

The most common biological and physiological effects in plants experiencing salt stress are reduced water potential, ion imbalance and toxicity, diminished CO_2 assimilation and enhanced generation of ROS including H_2O_2 leading to oxidative stress. Excess of ROS triggers phytotoxic reactions such as lipid peroxidation, inactivating enzymes, protein degradation and denaturing DNA molecules (Gill and Tuteja, 2010; Hasanuzzaman *et al*., 2012a). Salt induced higher production of ROS also recorded in rice (Rahman *et al*., 2016a,b), wheat (Wahid *et al*. 2007, Hasanuzzaman *et al*., 2011b), rapeseed (Hasanuzzaman *et al*., 2014b; Mahmud *et al*., 2020), mung bean (Nahar *et al*., 2015). Like present study exogenous application of different plant hormone, organic acid, trace element or many chemicals are being used in different research to mitigate salinity stress (Hasanuzzaman *et al*., 2011a; 2014a,b; Rahman *et al*., 2016a,b; Mahmud *et al*., 2020). Ascorbic acid, Si and GA3 contribute in enhancing the component of antioxidant defense system which helps directly or indirectly to scavenge ROS from cellular level and reduced salt toxicity. GA3 are externally used for alleviating various kinds of abiotic stresses including salinity. Foliar application of GA3 confers salt stress tolerance by increasing

germination percentage, plant growth and upregulating antioxidant enzyme (Tabatabei, 2013). Silicon inhibits the production of H_2O_2 and reduced and develops tolerance of wheat (*Triticum aestivum* L.) against salt stress at different growth stages (Daoud *et al*., 2018). Azzedine *et al*. (2011) observed improvement of salt tolerance in durum wheat by ascorbic acid application. They found addition of ascorbic acid to wheat plant decrease the level of ROS (H_2O_2) and improve growth in contrast to salt affected plant.

4.2.4.2 Malondialdehyde (MDA) content

Oxidative stress of salt-affected wheat seedlings was recognized from increased lipid peroxidation. All the levels of salinity stress increased lipid peroxidation or MDA level drastically. 50, 80 and 120 mM NaCl stress in wheat seedling enhanced salinity level by 29, 61 and 97%, respectively (Figure 36). Use of AsA, Si and GA3 decreased MDA content significantly under all level of stress. However the MDA reduction capacity of GA3 is slightly better than the other chemicals which might helped wheat seedling to perform better than the AsA and Si (Figure 36).

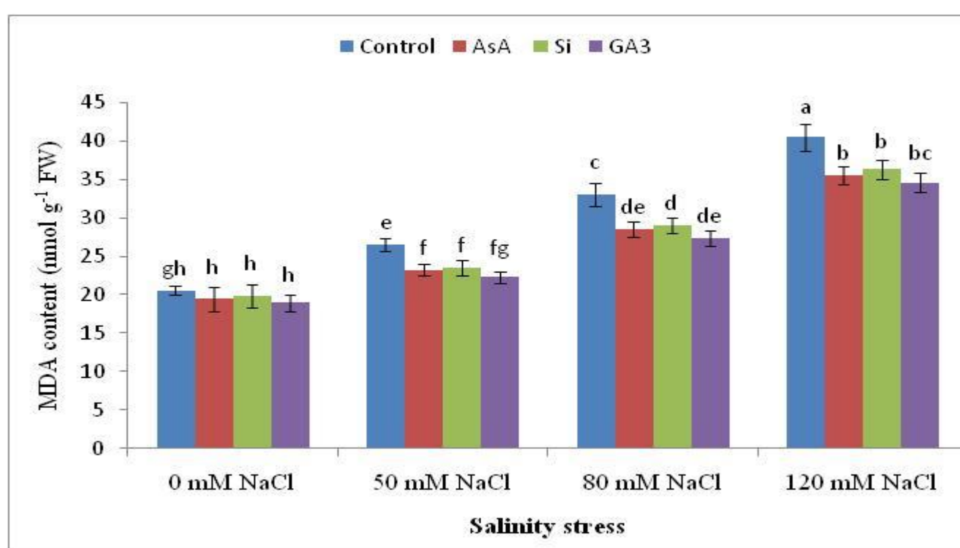

Figure 32. Effect of AsA, Si and GA3 on MDA content of wheat seedlings under salt stress. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

In response to increasing salinity, many metabolic pathways are inhibited and proteins are catabolized (Mahmud *et al*., 2020). The increased ROS generation can alter normal cellular metabolism through increased oxygen-induced cellular damage

(MDA content), oxidative damage to DNA, proteins and membrane lipids, which led to the membrane lipid peroxidation (Khan and Khan, 2017). Salinity disrupts the antioxidant defense system, increase the generation of ROS as well as increase the concentration of MDA in plant cell which contribute in lipid peroxidation resulted cellular damage (Rahman *et al*., 2016a,b). Incoherent growth and inappropriate uptake of water and nutrients eventually result in deterioration of cell membrane properties of plants. Lipid peroxidation, accumulation of H_2O_2, and increased membrane permeability are some common phenomenon of wheat seedlings under salt stress. Mandhania *et al*. (2006) reported higher damage of cellular membranes of salt-sensitive cultivar due to higher H_2O_2 accumulation and lipid peroxidation which enhanced the electrolyte leakage compared to the tolerant one. Lipid peroxidation (MDA content) increased by 68% under NaCl treatment of 100 mM for 10 d compared to control (Zou *et al*., 2016).

In many studies similar enhancement of MDA was observed in different plant due to salt stress which enhance cellular oxidative damage and hamper the growth of plant (Wahid *et al*., 2007; Hasanuzzaman *et al*., 2011; Hasanuzzaman *et al*., 2014b; Nahar *et al*., 2015; Rahman *et al*., 2016a,b; Mahmud *et al*., 2020). So, inhibition of ROS generation can reduce the lipid peroxidation in cell under stress condition which contributes in plant stress tolerance. Ascorbic acid, Si and GA3 considerably decrease the production of ROS under abiotic stress condition and lessened the lipid peroxidation as well as cellular damage (Azzedine *et al*., 2011; Saeidi-Sar *et al*., 2013; Tabatabei, 2013; Daoud *et al*., 2018).

4.3 Experiment 3. GERMINATION AND GROWTH PERFORMANCE OF SEEDLINGS OF ASCORBIC ACID, SILICON AND GIBBERELLIC ACID INDUCED SECONDARY WHEAT SEEDS UNDER SALT STRESS

4.3.1 Germination percentage

In this study, germination percentages significantly increased in secondary seed with exogenous application of GA3 under minimal or no salt stresses (0 mM= S_1, 50 mM= S_2) from salt stress mitigation experiment (Figure 37). Neither higher salt stress 80 mM= S_3 or 120 mM= S_4 showed significant variations with the application of GA3 in secondary seeds. Germination percentages significantly increased 1.33% and 1.66% in S_1 and S_2, respectively from the secondary seeds of previously exogenous application of GA3 during plant growth stages in salinity stress. However, results showed that the percentage of germination decrease collaterally with the increase of salt stresses throughout the treatments in secondary seeds.

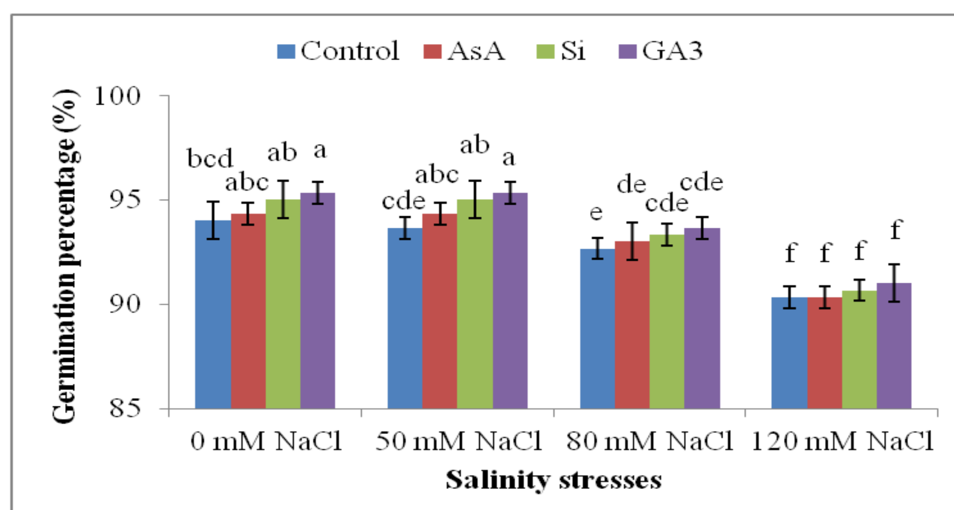

Figure 33. Germination percentage of salt-stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.2 Relative water content

Relative water content of salt stressed secondary seedlings showed insignificant variations in S3 with different mitigating agents (Ascorbic acid (2 mM), Silicon (200 µM) and Gibberellic Acid (100 µM)), while GA3 showed significant results in S_1 and AsA in S_2, respectively (Figure 38). Highest increased of relative water content

was observed in S_2 by AsA under previously salt stressed secondary seedlings. Relative water contents in previously treated seeds were decreased with the increased level of salt stresses.

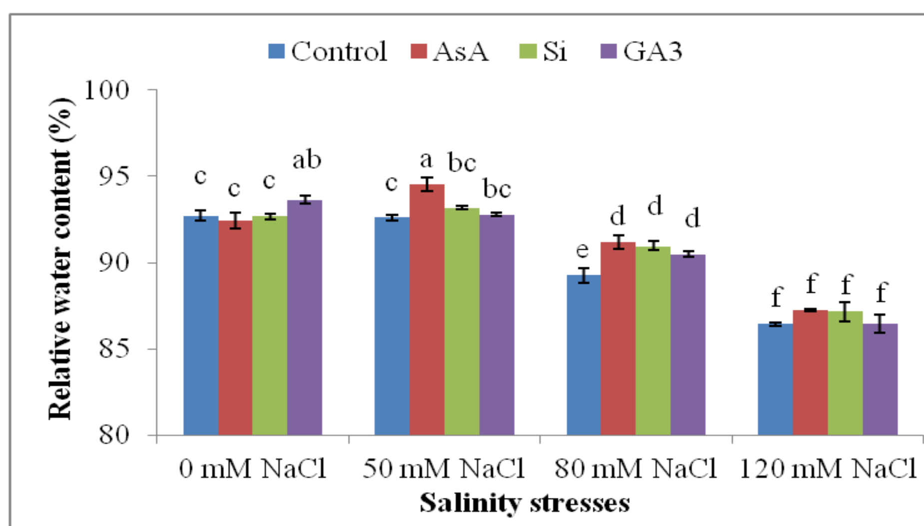

Figure 34. Relative water content of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.3 Growth Parameter

4.3.3.1 Shoot length (cm)

The exogenous application of GA3 significantly increased (4.0%, 7.4%, 9.5% and 10.6%, respectively) the shoot length in previously salt stressed (0 mM= S_1, 50 mM= S_2, 80 mM= S_3 and 120 mM= S_4) secondary seeds (Figure 39). Shoot lengths were higher in lower or minimal salt stressed seedlings. The exogenous application of Ascorbic acid (2 mM), Silicon (200 µM) and Gibberellic Acid (100 µM) improved the shoot length in salt stressed secondary seeds. In addition, shoot lengths were decreased respectively in previously increased doses of salt treated seeds.

4.3.3.2 Root length (cm)

Root length of salt stressed secondary seeds were significantly increased in exogenous application of Silicon (200mM) and Gibberellic Acid (100mM) under different salt stresses (0 mM= S_1, 50 mM= S_2, 80 mM= S_3 and 120 mM= S_4). Highest results (9.6%, 19%, 13% and 8.5% increase under S_1, S_2, S_3 and S_4) were found from the GA3 (100 µM) application under all the salt stresses (Figure 40).

Results showed the similar decrease in root length as shoot length under salt stresses in previous experiment.

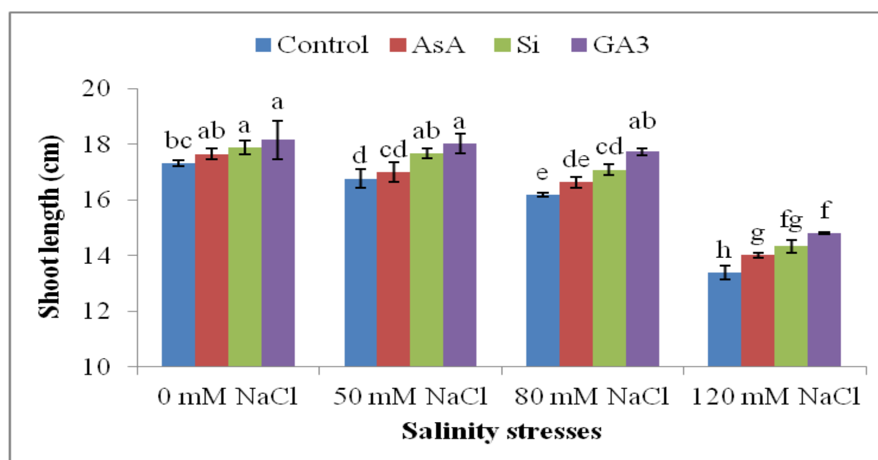

Figure 35. Shoot length of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

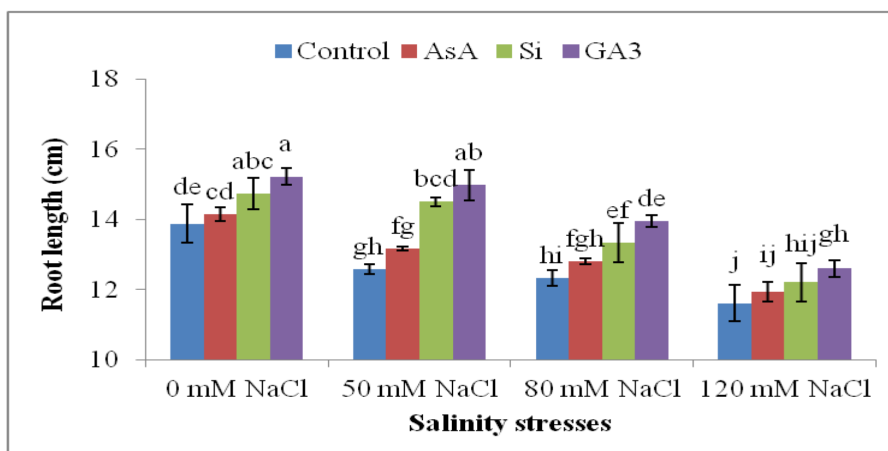

Figure 36. Root length of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.3.3 Root-Shoot ratio

The salt stressed secondary seeds showed significant increase (10.8%) in root-shoot ratio in exogenous application of GA3 (100 µM) under S2 (50 Mm NaCl) slat stressed secondary seeds, while other doses of mitigating agents (AsA 2 mM), Silicon (200 µM) and GA3 (100 µM)) were found insignificant under different salt stressed seeds (Figure 41). The root-shoot ratio increased simultaneously with

increased salt stresses in every previously treated seeds.

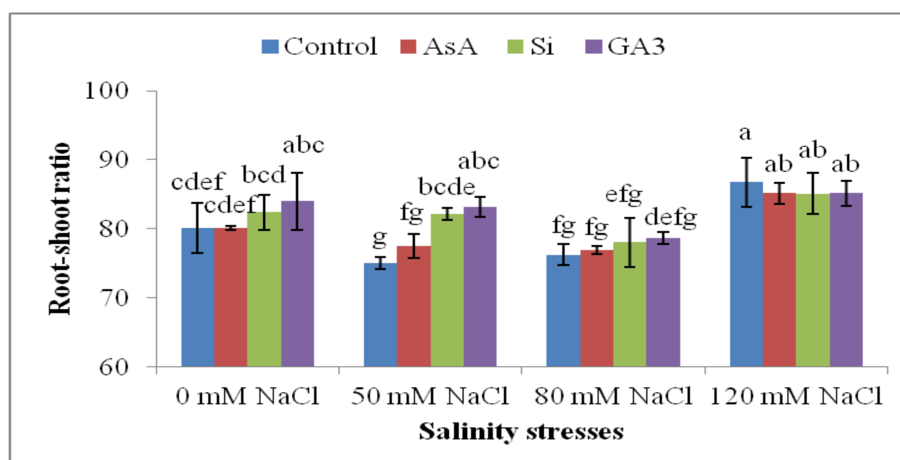

Figure 37. Root-shoot ratio of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.3.4 Fresh weight seedling^{-1}

Fresh weight of salt stressed secondary seedlings from different salt stresses showed insignificant variations in S_1 and S_2 with different mitigating agents (Ascorbic acid (2 mM), Silicon (200 µM) and Gibberellic Acid (100 µM)), while Silicon and GA3 showed significant results in S_3 and $_{S4}$, respectively. The highest increases in fresh weights were observed by silicon (3.84%) in S_3 and GA3 (12.5%) under S_4 salt stressed secondary seedlings (Figure 42). Fresh weights in different in previously treated plants seeds were decreased with the increased salt stresses.

4.3.3.5 Dry weight seedling^{-1}

Dry weight of salt stressed secondary seedlings from previously salt treated plants seeds showed significant increase by Silicon (200 µM) and Gibberellic Acid (100 µM). Exogenous application of salt stress mitigating agent Silicon (200 µM) increased the seedling dry weight by 9.52% under S_2 and S_3 salt stressed conditions, while Gibberellic Acid (100 µM) increased dry weight of salt stressed secondary seedlings by 12.5% under S_4= 120 mM salt stress (Figure 43). Dry weights in different treatments were also decreased with the increased salt stresses as like as fresh weights of seedlings.

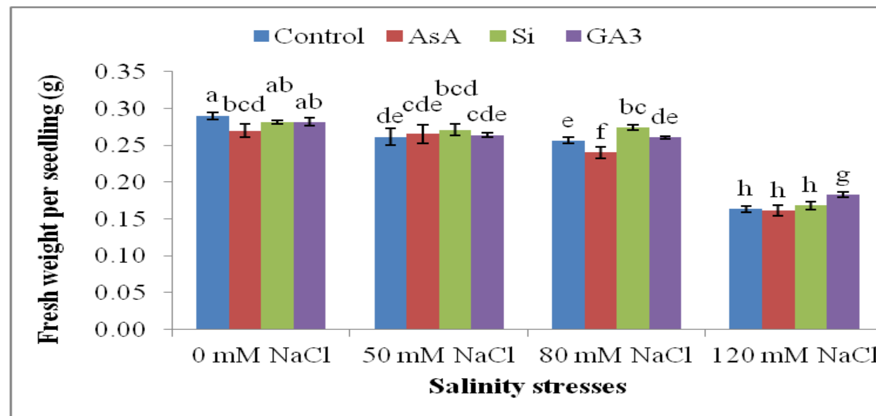

Figure 38. Fresh weight per seedling of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

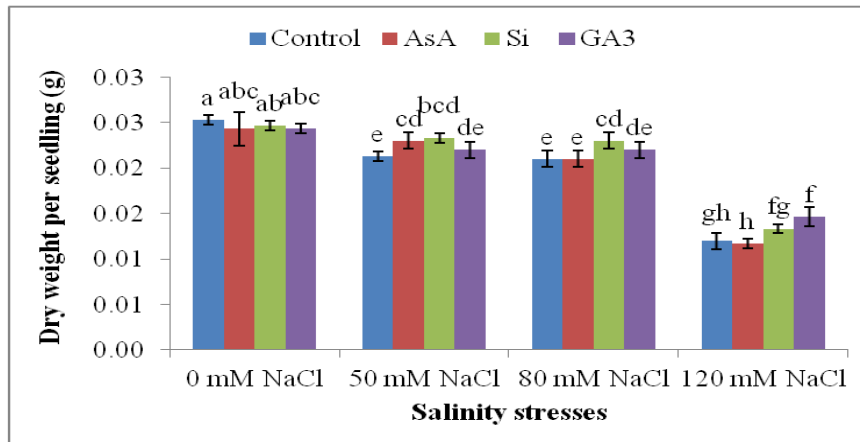

Figure 39. Dry weight per seedling of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.4 Vigor index

Salt stress mitigating agents (Ascorbic acid (2 mM), Silicon (200 µM) and Gibberellic Acid (100 µM)) treated slat stressed secondary seed showed significant increase in seedling vigor index. Ascorbic acid treated plants seeds increased 2.27%, 3.54%, 3.61%, and 3.83%, respectively; Silicon treated plants seeds increased 5.63%, 11.17%, 7.41% and 6.55%, respectively and GA3 treated plants seeds increased 8.49%, 14.45%, 12.26% and 10.43% respectively in S_1, S_2, S_3 and S_4 salt stresses (Figure 44). The highest vigor indexes were found from the Gibberellic Acid (100 µM) treated plants seeds in every salt stresses.

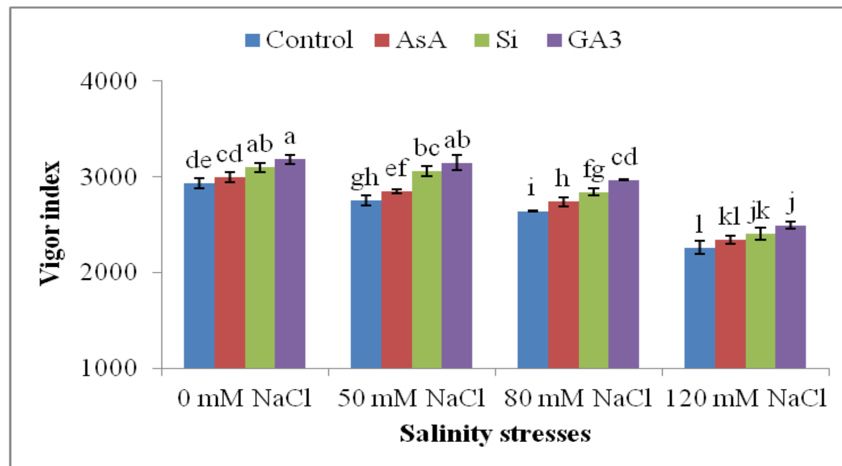

Figure 40. Vigor index of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at *p* ≤ 0.05 applying LSD test.

4.3.5 Survibility percentage

There were no significant variations in survibility percentages with mitigating agents (Ascorbic acid (2 mM), Silicon (200 µM) and Gibberellic Acid (100 µM)) in different slat stressed secondary seeds except for S_4 treatment. Survibility percentages increased 3.74% and 4.68% by Ascorbic acid (2 mM) and Gibberellic Acid (100 µM), respectively (Figure 45). However, survibility percentages decreased with increased salt stresses in salt stressed secondary seeds.

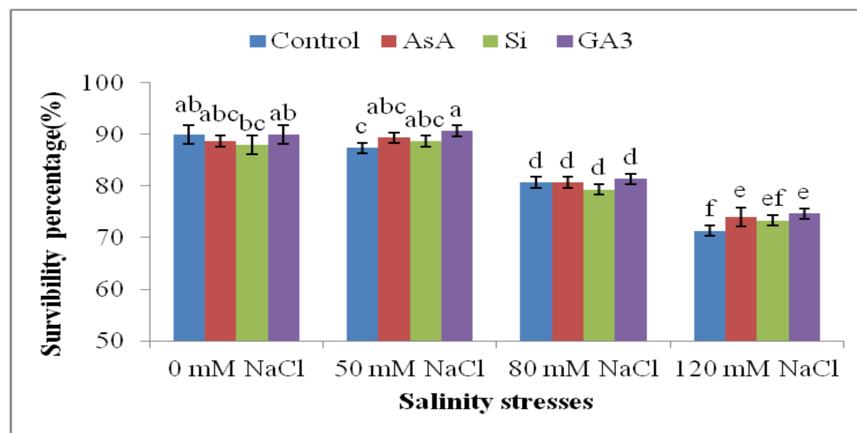

Figure 41. Survibility percentage of salt stressed secondary seeds from different salinity level comprising salt stress mitigating agents. AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM silicon di-oxide and 100 µM gibberellic acid, respectively. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at *p* ≤ 0.05 applying LSD test.

4.3.6 Chlorophyll content

In this experiment, chlorophyll (*a*, *b* and *a+b*) of salt stressed secondary seedlings did not show any significant variation with the application of salt stress mitigating agents (AsA (2 mM), Si (200 µM) and GA3 (100 µM)) under previously four different salt stress conditions including control treatment (0 mM= S_1, 50 mM= S_2, 80 mM= S_3 and 120 mM= S_4) (Table 2). However, the application of salt stress mitigation agent Silicon (200 µM) in previous experiment showed simultaneous increase in chlorophyll (*a*, *b* and a+b) under different salt treatments (S_1, S_2, S_3 and S_4) in secondary seeds. The highest increase in chlorophyll-*a* (2.23%, 2.20%, 2.08% and 2.5% in S_1, S_2, S_3 and S_4, respectively), chlorophyll-*b* (7.74%, 4.68%, 7.68% and 9.02% in S_1, S_2, S_3 and S_4, respectively) and Chlorophyll a+b (4.18%, 3.11%, 4.10% and 4.76% in S_1, S_2, S_3 and S_4, respectively) were found in salt stressed secondary seedlings from previously treated plants by Si (Table 2). In addition, the lowest Chlorophyll *a*, *b* and a+b were found from the highest salt stress S4 (120 mM).

In our present experiment, germination percentages increased in secondary seeds which were collected from the treatments including the application of mitigating agents (AsA, Si, and GA3) under salinity stress. These results revealed that secondary seeds from saline conditions with exogenous application of AsA, Si, and GA3 increased seed performances. Usually, salt stresses reduce photosynthetic parameters, chlorophyll content and stomatal opening, and hinder the normal growth of plant, while affecting the proper seed development (Neill *et al*., 2002; Netondo *et al*., 2004).

However, the application of exogenous AsA, Si, and GA3 improved the seed development by improving the normal growth of total biomass of the plant in saline stress. AsA helps to uptake nutrients (N, P and K), protects metabolic processes and enzyme activities, minimize the damage caused by oxidative processes, and stabilize membranes in salt stress (Farahat *et al*., 2013; Shao *et al*., 2008). On the other hand, Si induces salt tolerance in plants by improving photosynthesis, membrane integrity, and detoxification of toxic radicals and elevating its antioxidant capacity under salinity stress (Robatjazi *et al*., 2020).

Table 2. Chlorophyll (*a*, *b* and *a+b*) of salt stressed secondary seeds of different salinity level comprising of salt stress mitigating agents.

Treatment	chl *a* (nmol/g DW)	chl *b* (nmol/g DW)	chl (a+b) (nmol/g DW)
Control	0.67 ± 0.02 ab	0.38 ± 0.02 ab	1.06 ± 0.01 ab
AsA	0.66 ± 0.01 abc	0.39 ± 0.03 ab	1.05 ± 0.02 abc
Si	0.69 ± 0.02 a	0.41 ± 0.03 a	1.10 ± 0.02 ab
GA3	0.67 ± 0.01 ab	0.38 ± 0.04 ab	1.05 ± 0.03 ab
50 mM NaCl	0.68 ± 0.01 a	0.40 ± 0.02 ab	1.08 ± 0.01 ab
50 mM NaCl + AsA	0.67 ± 0.01 ab	0.40 ± 0.25 ab	1.07 ± 0.03 ab
50 mM NaCl + Si	0.70 ± 0.02 a	0.41 ± 0.04 a	1.11 ± 0.02 a
50 mM NaCl + GA3	0.68 ± 0.01 a	0.39 ± 0.04 ab	1.07 ± 0.04 ab
80 mM NaCl	0.67 ± 0.05 ab	0.37 ± 0.01 bc	1.04 ± 0.05 abc
80 mM NaCl + AsA	0.66 ± 0.05 abc	0.37 ± 0.01 bc	1.03 ± 0.06 bc
80 mM NaCl + Si	0.69 ± 0.05 a	0.40 ± 0.02 ab	1.08 ± 0.05 ab
80 mM NaCl+ GA3	0.67 ± 0.05 ab	0.36 ± 0.03 bcd	1.03 ± 0.06 bc
120 mM NaCl	0.60 ± 0.04 cd	0.33 ± 0.01 cd	0.93 ± 0.05 d
120 mM NaCl + AsA	0.58 ± 0.05 d	0.33 ± 0.01 cd	0.92 ± 0.07 d
120 mM NaCl + Si	0.61 ± 0.04 bcd	0.36 ± 0.01 bcd	0.97 ± 0.05 cd
120 mM NaCl + GA3	0.60 ± 0.04 cd	0.32 ± 0.02 d	0.92 ± 0.07 d
$LSD_{0.05}$	**0.0299**	**0.0205**	**0.0370**
CV (%)	**5.57**	**6.70**	**4.38**

AsA, Si and GA3 indicate 2 mM ascorbic acid, 200 µM SiO_2 and 100 µM gibberellic acid, respectively. Means (±SD) were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

Foliar application of AsA, Si and GA3 increased 1000-grain weight and seed yield of wheat under salinity stress (Chapter 1, Figure 16 and 17), which also increased the

germination percentages with healthier seed in this experiment as well. GA3 play a vital role in tolerance to salinity stress by improving the activity of antioxidant enzymes and preventing lipid peroxidation, increased the accumulation of proline and potassium, and increased the different physiological parameters, ultimately produced total biomass (Miceli *et al*., 2019; Alsudays *et al*., 2020). Thus, the application of GA3 improved the plant growth and seed development, while secondary seed showed the highest performances in seed germination.

CHAPTER 5

SUMMARY AND CONCLUSION

Summary

Three experiments were conducted to find out the effect of salt stress on morph-physiological and biochemical changes of wheat as well as mitigation by using AsA, Si and GA3. The collective effect of salt stress along with AsA, Si and GA3 on growth, physiology, biochemistry and yield was also recorded. All data were analyzed through CoStat v. 2008 software package.

The first experiment was conducted at net house of Agronomy field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. In this experiment seeds were sown in 24 November, 2017 and harvested at 5 March, 2018. Here effect of salt stress along with AsA, Si and GA3 on ionic status, physiological attributes, growth parameters and yield characteristics of wheat were observed. To maintain and achieve respective salinity each pot was irrigated by NaCl solution for several times during vegetative period after 20 DAS. Ascorbic acid (2 mM) exogenously applied to T_1, T_5, T_9 and T_{13}, Silicon (200 μm) exogenously applied to T_2, T_6, T_{10} and T_{14} and Gibberalic acid (100 μm) exogenously applied to T_3, T_7, T_{11} and T_{15} respectively start at 20 DAS after salt stress irrigation and continued throughout the vegetative period with seven days interval. Growth parameters were collected after a definite period of time and the yield data was collected after harvest.

Plant height was observed about 61.5 cm, 65.8 cm, 69.5 cm and 68.7 cm under 50 mM NaCl; 58.4 cm, 63.2 cm, 66.1 cm and 65.2 cm under 80 mM NaCl and 56.3 cm, 61.2 cm, 63.3 cm and 62.8 cm under 120 mM NaCl at 50, 65, 80 DAS, and during harvest respectively. However, compared to respective stress, exogenous application of AsA, Si and GA3 in stressed plant increased plant height. But, the performance of GA3 in improving plant height by counteract salt stress was considerably higher than the AsA and Si. The salt-stressed seedlings had significantly lower tiller number plant^{-1} (3, 10, and 16% at 35 DAS; 7, 24 and 30% at 50 DAS; 7, 8 and 9% at 65 DAS and 7, 8 and 19% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Application of GA3 with saline treatments considerably increased tiller number compared to the seedlings subjected to salt stress without GA3 treatment. NaCl

treated seedlings had significantly lower number of leaf plant^{-1} (5, 4, and 12% at 35 DAS; 7, 9 and 23% at 50 DAS; 13, 28 and 43% at 65 DAS and 15, 22 and 43% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Foliar application of Si with saline treatments increased number of leaf plant^{-1} (26, 20 and 38% at 35 DAS; 22, 6 and 13% at 50 DAS; 26, 30 and 41% at 65 DAS and 17, 17 and 37% at 80 DAS at 50, 80 and 120 mM NaCl, respectively), compared to the seedlings subjected to salt stress with AsA and GA3 treatment. The salt-stressed wheat seedlings had significantly lower leaf area (12, 28, and 37% at 35 DAS and 7, 20 and 28% at 50 DAS at 50, 80 and 120 mM NaCl salinity stress conditions. Here Si showed increased leaf area 11, 26 and 31% at 35 DAS and 20, 33 and 33% at 50 DAS at 50, 80 and 120 mM NaCl, respectively. But, the performance of Si in improving leaf area by counteract salt stress was considerably higher than the AsA and GA3. The salt-stressed plant had significantly lower fresh weight plant^{-1} (10, 14, and 20% at 35 DAS; 7, 16 and 21% at 50 DAS; 23, 35 and 43% at 65 DAS and 22, 60 and 69% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions, respectively. But, the performance of GA3 in improving fresh weight plant^{-1} by counteract salt stress was considerably higher than the AsA and Si. Similarly significantly lower dry weight plant^{-1} (19, 23, and 29% at 35 DAS; 10, 13 and 14% at 50 DAS; 24, 34 and 37% at 65 DAS and 18, 43 and 51% at 80 DAS at 50, 80 and 120 mM NaCl salinity stress conditions, respectively. Performance of GA3 improving dry weight plant^{-1} is higher than the AsA and Si. In contrast to control, 50 mM, 80 mM and 120 mM of NaCl it was decreased leaf RWC by 6%, 8% and 10% at 35 DAS, 7%, 10% and 11% at 50 DAS and 9%, 12% and 19% at 65 DAS, respectively. But, in case of 50 mM NaCl, AsA, Si and GA3 treatment could not increase relative water content of wheat plant.

SPAD value decreased over control by 7%, 12%, 15% at 50, 80 and 120 mM NaCl; 11%, 14%, 20% at 50, 80 and 120 mM NaCl; 7%, 12%, 17% at 50, 80 and 120 mM NaCl and 37%, 56%, 77% at 50, 80 and 120 mM NaCl during 35 DAS, 50 DAS, 65 DAS and 80 DAS, respectively. GA3 supplementation caused increased the length of spike by 6%, 6% and 9% under 50, 80 and 120 mM NaCl conditions, respectively. AsA and Si supplementation caused increased the length of spike under salinity stress conditions, but it had also no statistically significance with each other. Exposure to salt stress resulted in significant decreases in effective spike per pot: 5, 8 and 11% at 50, 80 and 120 mM salinity stressed conditions, respectively when

compared to unstressed control plant. AsA (5, 4 and 5%), Si (7, 9 and 9%) and GA3 (10, 10 and 11%) treated plant increased effective spike per pot compare it's salt stress treatment at 50, 80 and 120 mM stressed condition, respectively. Exogenous application of AsA and GA3 increased the number of grains per spike significantly under saline and non-saline conditions. AsA and GA3 treated plant increased the number of grains per spike compare it's salt stress treatment (5, 4 and 7%) and (11, 11 and 17%) at 50, 80 and 120 mM stressed condition, respectively compare it's salt stress treatment. Noticeably decreases in 1000-grain weight were observed (17, 28 and 52% at 50, 80 and 120 mM, respectively) in response to salt stress. The highest 1000-grain weight (42.03 g) was found with the application of GA3 under control condition and the lowest (18.54 g) was in 120 mM NaCl conditions. About 23, 41, 41 and 73% grain yield pot^{-1} a significant reduction at 50, 80 and 120 mM NaCl, respectively. The highest grain yield per pot (36.60 g) was found in only GA3 treated plant and the lowest grain yield pot (8.35 g) was found in 120 mM saline conditions. Similarly the highest grain yield ton per ha (3.69 t ha^{-1}) was found in only GA3 treated plant and the lowest grain yield ton per ha (0.84 t ha^{-1}) was found in 120 mM saline conditions. Straw yield was notably decreased by 4, 10 and 28% at 50, 80 and 120 mM respectively, compared to respective control. The highest straw yield was observed in only Si treated plant (53.47 g pot^{-1}) when grown under 50 mM NaCl conditions. The lowest straw yield (32.50 g pot^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. Similarly the highest straw yield was observed in only Si treated plant (5.29 t ha^{-1}) when grown under 50 mM NaCl conditions. AsA treated plant grown under 50 mM NaCl conditions produced (5.28 t ha^{-1}) straw yield which is statistically similar with the highest result. The lowest straw yield (3.27 t ha^{-1}) was observed in only when plant grown under 120 mM NaCl conditions.

Biological yield was decreased by 12, 22 and 46% at 50, 80 and 120 mM respectively, compared to respective control. The highest biological yield was observed in only GA3 treated plant (8.68 t ha^{-1}) when grown under control conditions and the lowest biological yield (4.11 t ha^{-1}) was observed in only when plant grown under 120 mM NaCl conditions. Significant variation was observed for harvest index. The highest harvest index was observed in only GA3 treated plant (42.46%) when grown under control conditions and meaningful decreases in harvest

index were observed (13, 24 and 50% at 50, 80 and 120 mM NaCl stresses, respectively) in response to salt stress, compared to the respective control and untreated control

Consequential increase in total Na^+ content in shoot were observed (183, 262 and 389% at 50, 80 and 120 mM NaCl stresses, respectively in response to salt stress, compared to the untreated control and application of AsA, Si, GA3 at 50, 80 and 120 mM salt stressed conditions was not decreased compared to control condition. Total K^+ content in shoot significantly decrease by 18, 28 and 44% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant. But exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed total K^+ content in wheat shoot compared to 50 mM and 80 mM salt stressed conditions. Total Ca^{2+} content in shoot significantly decrease by 20, 25 and 34% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant. Supplementations of AsA, Si and GA3 significantly increased Ca^{2+} content in shoot under 50 mM NaCl stress condition. Similar trend was observed in case of 80 mM NaCl stress conditions. In salt-grown plant, total Mg^{2+} content in shoot significantly decrease by 17, 24 and 32% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant. Supplementations of AsA, Si and GA3 significantly decreased Mg^{2+} content in shoot under 50 mM and 80 mM NaCl stress condition.

Meaningful increase in total Na^+ content in the grain were observed (33, 50 and 100% at 50, 80 and 120 mM NaCl stresses, respectively in response to salt stress, compared to the untreated control. However, exogenous application of AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed Na^+ content in wheat grain compared to 50 mM, 80 mM and 120 mM salt stressed conditions. Total K^+ content in grain significantly decrease by 22, 24 and 25% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant. AsA, Si, GA3 at 50 mM salt stressed conditions was not significantly differed K^+ content in wheat grain compared to 50 mM and 80mM salt stressed conditions. Total Ca^{2+} content in grain significantly increased by 60, 90 and 103% at 50, 80 and 120 mM NaCl stresses, respectively, compared to the untreated control plant. Exogenous application of AsA, Si, GA3 at 80 and 120 mM salt stressed conditions was significantly differed Ca^{2+} content in wheat grain compared to 80 and 120 mM salt

stressed conditions. Total Mg^{2+} content in grain significantly increased by 17, 24 and 32% at 58, 95 and 104 mM NaCl stresses, respectively, compared to the untreated control plant. AsA, Si and GA3 significantly increased Mg^{2+} content in grain under 50 Mm, 80 and 120 mM NaCl stress condition.

The second experiment was conducted at Plant Physiology Laboratory of Agronomy department, Sher-e-Bangla Agricultural University, Bangladesh during the period from October, 2018 to December, 2018. In this experiment CRD design was followed with three replications. The lab experiment was conducted to observe the changes of growth and physiological parameters of wheat seedlings under different levels of salt treatments (0, 50, 80, 120mM NaCl). Moreover the effect of AsA (2 mM), Si (200 mM) and GA3 (100 mM) as foliar spraying was demonstrated under salt stress. Plant root and shoot length, fresh and dry weight, leaf relative water content, Pro content, chl content, H_2O_2 level and MDA content were investigated under the salt (sole) and combined (salt in combination with AsA, Si and GA3) treatments. Data were taken from 10 days old wheat seedlings (7 days after treatment). Compared to control shoot length decreased by 4%, 18% and 47% due to 50, 80 and 120 mM NaCl concentration, respectively. Whereas root length decreased by 20%, 36% and 56% under 50, 80 and 120 mM NaCl concentration, respectively. Fresh weight declined by 12%, 27% and 39% due to exposure of wheat seedling to 50, 80 and 120 mM NaCl concentrations, respectively, in contrast to control treatments. Similarly dry weight decreased by 15%, 30% and 46% under 50, 80 and 120 mM NaCl concentration, respectively. In contrast to control, 50 mM, 80 mM and 120 mM NaCl decreased leaf RWC by 2, 6 and 9%, whereas respectively. Under 120 mM NaCl stress the rate of decline Pro was higher by GA3 than the others. Under 120 mM NaCl the reduction of Pro was 17% by AsA and 16% by Si but 20% by GA3 in contrast to respective stress. In contrast to control seedlings, chl *a* content decreased by 19%, 28% and 50% due to 50, 80 and 120 mM NaCl concentration, respectively. While level of chl *b* decreased by 27%, 50% and 56% under 50, 80 and 120 mM NaCl concentration, respectively. Therefore, in comparison with control seedlings chl (*a*+*b*) content reduced by 22%, 36% and 52% under 50, 80 and 120 mM NaCl concentration, respectively. In comparison with control treatment content of H_2O_2 increased by 19, 50 and 85% under 50, 80 and 120 mM NaCl concentration, respectively. 50, 80 and 120 mM NaCl stress in wheat seedling enhanced salinity

level by 29, 61 and 97%, respectively. But, foliar spraying of AsA, Si and GA3 significantly decreased MDA level, H_2O_2 content and Pro content which considerably enhance the chl content, leaf relative water content as well as plant root and shoot length and fresh and dry weight of plant under stress condition.

The third experiment was conducted at Laboratory of Agronomy department, Sher-e-Bangla Agricultural University, Bangladesh during the period from October, 2018 to December, 2018. CRD design was followed for the experiment with three replications. The lab experiment was conducted to observe the changes of growth and physiological parameters of wheat seedlings obtained from experiment 1. Secondary seeds were grown for 10 days to take different physiological and growth parameters. Secondary seeds obtained from 80 and 120 mM NaCl showed significantly lower germination rate compared to control. However, germination percentage of secondary seeds did not differ significantly except GA3 at 0 mM NaCl and GA3 and Si at 50 mM NaCl treatments. Relative water content decreased notably of wheat seedlings grown from secondary seeds of first experiment under 80 and 120 mM NaCl stress. Interestingly AsA treated secondary seeds showed slight or considerable higher RWC than the other treatments. The exogenous application of GA3 significantly increased (4.0%, 7.4%, 9.5% and 10.6%, respectively) the shoot length in previously salt stressed (0, 50, 80 and 120 mM NaCl, respectively) secondary seeds. Similar results were also obtained from seedlings of Si treated seeds (except 120 mM NaCl). Like shoot length, root length also indicated same trend for seedlings of secondary seeds. The salt stressed secondary seeds showed significant increase (10.8%) in root-shoot ratio in exogenous application of GA3 under 50 mM salt stressed secondary seeds, while other mitigating agents were found insignificant under different salt stressed seeds. Highest increases in fresh weights were observed by silicon (3.84%) in S_3 and GA3 (12.5%) under 120 mM NaCl stressed secondary seedlings. Dry weight of salt stressed secondary seedlings from previously salt treated plants seeds showed significant increase by Si and GA3. Dry weights in other different treatments were also decreased with the increased salt stresses as like as fresh weights of seedlings. Vigor index of secondary salt affected seeds showed significant reduction in dose dependant manner. Ascorbic acid treated plants seeds increased 2.27%, 3.54%, 3.61%, and 3.83%, respectively; Si treated plants seeds increased 5.63%, 11.17%, 7.41% and 6.55%, respectively and GA3 treated plants

seeds increased 8.49%, 14.45%, 12.26% and 10.43% respectively in 0, 50, 80 and 120 mM NaCl concentration salt stresses. Survibility percentages decreased with increased salt stresses in salt stressed secondary seeds. There were no significant variations in survibility percentages with mitigating agents. Secondary seeds obtained from 80 mM NaCl stress decreased chl b content and 120 mM NaCl stress declined chl *a*, *b* and *a+b* content. Chlorophyll (*a*, *b* and *a+b*) of salt stressed secondary seedlings did not show any significant variation with the application of AsA, Si and GA3.

Conclusion

The findings of the first study revealed that salt stress decreased plant growth (plant height, tiller plant^{-1}, leaf no plant^{-1}, leaf area) biomass (fresh weight, dry weight and relative water content) yield attributes (spike length, number of spikes plant^{-1}, grains spike^{-1}, 1000-grain weight, grain yield, straw yield, biological yield and harvest index) by altering ionic balance (total Na^+, Ca^{2+}, K^+ and Mg^{2+} of both grain and straw) hampering osmotic status and reducing chl content which ultimately reduced the yield of wheat. Conversely foliar spraying of AsA, Si and GA3 on salt affected plant improves the growth and yield of wheat seedling in contrast to respective stress. Considering the most of the growth, physiological and yield attributes performance of GA3 is ahead than the other two chemicals.

The results of this second study indicate that salt stress increased ROS generation and lipid peroxidation which contribute in alteration of osmotic status and chl content as well as growth and biomass of plant. However exogenous application of AsA, Si and GA3 as foliar spraying was effective in alleviating the inhibitory effects of salt stress on the biomass production of wheat by increasing chl content, improving water status and reducing ROS generation and MDA content. In aspect of plant protection against salt stress GA3 perform slightly (in some cases significantly) better than the AsA and Si.

The application of salt stress mitigating agents like AsA, Si and GA3 all together improve the plant growth and development as well as enhance the secondary seed performances. In third study on secondary seeds and seedlings performances from the previously exogenous applied salt stress mitigating agents in wheat plant's seeds showed significant variations in germination percentage, shoot length, root length, fresh and dry weight seedling^{-1} and vigor indexes. However, the performances of

secondary seed and seedlings in this study elucidates that exogenous application of salt stress mitigating agents may significantly reduce the stress impacts in wheat plants under different salt stresses as well as in secondary seeds during their germination and seedling growth. In this study, secondary seeds from GA3 application in salt stressed plants significantly have increased the seed germination and seedling growth parameters, while silicon mostly improved the fresh weight and chlorophyll (*a*, *b* and *a*+*b*) and AsA showed better relative water contents with other parameters in secondary seeds. Preceding research hardly explain the secondary seeds performances, thus further research is necessary on this topic. The results of this study have evaluated the secondary seeds performances which plants treated by salt stress mitigating agents during salt stress conditions, and these findings will provide knowledge for further research and development of sustainable agriculture in wheat cultivation in saline areas as well as for other crops.

RECOMMENDATION

This study showed the mitigating effects of AsA, Si and GA3 on salt stress damage on the process of germination, seedling growth, vegetative growth, reproductive growth and yield of wheat grown in petri-dishes and pots under controlled environmental conditions. For this reason, it is recommended that further research be done under natural field conditions (farm trials) to confirm this tendency. The underlying biochemical and molecular mechanism of salt tolerance due to exogenous application of AsA, Si and GA3 need to be elucidated. The experiment with the higher concentration of AsA, Si and GA3 along with their different combinations under the various higher salt stress to be done for synergestic effects. In addition, preceding research hardly explains the performances of seeds from using AsA, Si and GA3 on salt stress wheat, thus further research is necessary on this topic.

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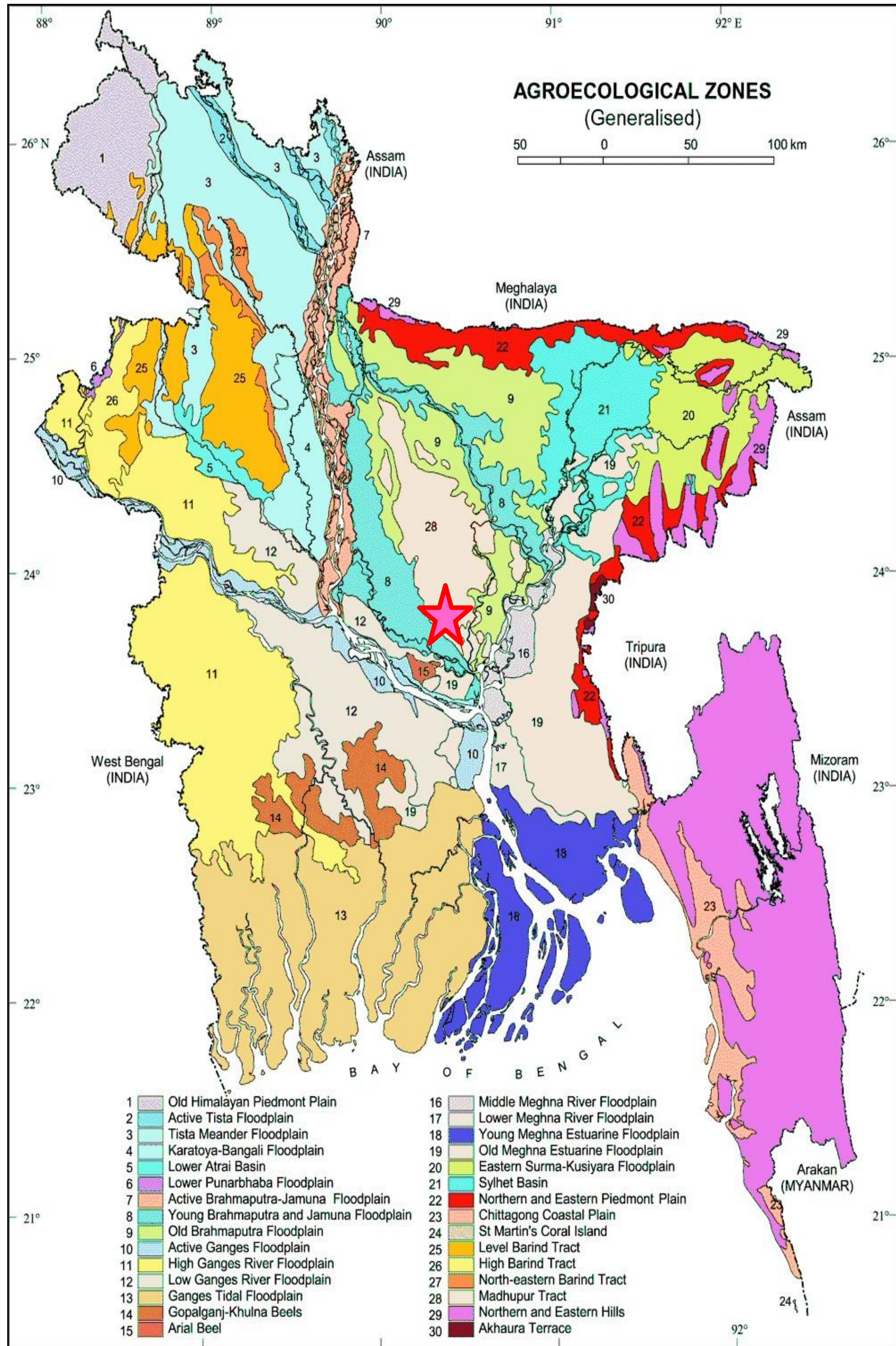
APPENDICES

Appendix I. Map showing the geographical location of experiments.



Appendix II. Soil morphological, physical and chemical characteristics of experimental field as analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka.

Morphological properties of the soil

Morphological features	**Characteristics**
Location	Agronomy field , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow Red Brown Terrace Soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Drainage	Well drained
Flood level	Above flood level

Physical properties of the soil

Particle size analysis	**Results**
Organic matter	1.38%
Sand (%) (0.0-0.02 mm)	21.75
Silt (1%) (0.02-0.002 mm)	66.60
Clay (%) (<0.002 mm)	11.65
Soil textural class	Silty loam
Consistency	Granular and friable when dry

Source: Soil Resources Development Institute (SRDI), Dhaka.

Chemical properties of the soil (0 - 15 cm depth)

Soil characters	**Value**
pH (1: 2.5 soil-water)	5.9 – 6.2
CEC (cmol kg^{-1})	17.25
Potassium	0.17 meq/100 g soil
Calcium	3.80 meq/100 g soil
Magnesium	1.20 meq/100 g soil
Total nitrogen	0.062%
Phosphorus	21.25µg/g soil
Sulphur	20.35 µg/g soil
Boron	0.52 µg/g soil
Copper	2.77 µg/g soil
Iron	275.3 µg/g soil
Manganese	171 µg/g soil
Zinc	3.19 µg/g soil

Appendix III. Monthly record of weather data of the experimental site during the period from November 2017 to March 2018

	November, 2017	December, 2017	January, 2018	February, 2018	March, 2018
*Maximum temperature (^{0}C)	30	27	25	30	34
*Minimum temperature (^{0}C)	21	19	15	19	22
*Average Temperature (^{0}C)	25	22	19	24	28
Average rainfall (mm)(monthly total)	5.25	16.61	0	1.21	17.62
*Average wind speed (kmph)	7.2	6.4	7.9	6.8	7.8
*Average air pressure (mb)	1011.3	1013.8	1012.7	1012.6	1008.9
*Relativehumidity (%)	58	59	47	44	46
*Average Cloud (%)	10	11	2	9	12
*Average UV index	6	6	5	7	7
Sunshine hour (hr) (monthly total)	206	210	232.5	207	324

* Monthly average

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

Experiment: 1

Appendix IV. Analysis of variance of the data for plant height at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.605	0.3026	1.1282	0.3370
Salt stress	3	54.271	18.0903	67.4382	$1.944e^{-13}$ **
Protectent	3	82.808	27.6028	102.8993	$6.949e^{-16}$ **
Salt × Protectent	9	2.354	0.2616	0.9750	0.4796
Error	30	8.048	0.2683		

*Significant at 5% level ** Significant at 1% level

Appendix V. Analysis of variance of the data for plant height at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.17	0.084	0.0965	0.9083
Salt stress	3	412.81	137.604	158.5009	$< 2.2e^{-16}$ **
Protectent	3	219.13	73.042	84.1343	$1.052e^{-14}$ **
Salt × Protectent	9	6.13	0.681	0.7841	0.6326
Error	30	26.04	0.868		

*Significant at 5% level ** Significant at 1% level

Appendix VI. Analysis of variance of the data for plant height at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	1.49	0.743	6.0750	0.006093
Salt stress	3	744.00	247.999	2027.6908	$< 2.2e^{-16}$ **
Protectent	3	208.29	69.431	567.6857	$< 2.2e^{-16}$ **
Salt × Protectent	9	1.80	0.200	1.6374	0.149436
Error	30	3.67	0.122		

*Significant at 5% level ** Significant at 1% level

Appendix VII. Analysis of variance of the data for plant height at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.32	0.16	0.4824	0.6220
Salt stress	3	1047.88	349.29	1044.6700	$<2e^{-16}$ **
Protectent	3	217.01	72.34	216.3404	$<2e^{-16}$ **
Salt × Protectent	9	3.75	0.42	1.2449	0.3062
Error	30	10.03	0.33		

*Significant at 5% level ** Significant at 1% level

Appendix VIII. Analysis of variance of the data for plant height at Harvest

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.19	0.10	0.6990	0.5050037
Salt stress	3	985.46	328.49	2391.0790	$< 2.2e^{-16}$ **
Protectent	3	204.24	68.08	495.5509	$< 2.2e^{-16}$ **
Salt × Protectent	9	5.98	0.66	4.8382	0.0004894**
Error	30	4.12	0.14		

*Significant at 5% level ** Significant at 1% level

Appendix IX. Analysis of variance of the data for tiller plant^{-1} at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.00167	0.00083	0.0348	0.965835
Salt stress	3	0.33667	0.11222	4.6868	0.008429 **
Protectent	3	1.29000	0.43000	17.9582	$7.324e^{-07}$ **
Salt × Protectent	9	0.21000	0.02333	0.9745	0.480046
Error	30	0.71833	0.02394		

*Significant at 5% level ** Significant at 1% level

Appendix X. Analysis of variance of the data for tiller plant^{-1} at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.06167	0.03083	1.4644	0.2473
Salt stress	3	0.80917	0.26972	12.8100	1.459e^{-05} **
Protectent	3	1.36250	0.45417	21.5699	1.218e^{-07} **
Salt × Protectent	9	0.15417	0.01713	0.8135	0.6079
Error	30	0.63167	0.02106		

*Significant at 5% level ** Significant at 1% level

Appendix XI. Analysis of variance of the data for tiller plant^{-1} at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.03167	0.015835	1.0000	0.37981
Salt stress	3	0.28667	0.095556	6.0351	0.00242 **
Protectent	3	0.54000	0.180000	11.3684	3.793e^{-05} **
Salt × Protectent	9	0.13333	0.014815	0.9357	0.50950
Error	30	0.47500	0.015833		

*Significant at 5% level ** Significant at 1% level

Appendix XII. Analysis of variance of the data for tiller plant^{-1} at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.011667	0.005833	0.5676	0.572872
Salt stress	3	0.150000	0.050000	4.8649	0.007109 **
Protectent	3	0.150000	0.050000	4.8649	0.007109 **
Salt × Protectent	9	0.016667	0.001852	0.1802	0.994746
Error	30	0.308333	0.010278		

*Significant at 5% level ** Significant at 1% level

Appendix XIII. Analysis of variance of the data for leaf plant^{-1} at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.0937	0.0469	0.4341	0.65186
Salt stress	3	0.8906	0.2969	2.7492	0.06008
Protectent	3	21.0885	7.0295	65.0965	$3.075e^{-13}$ **
Salt × Protectent	9	1.6406	0.1823	1.6881	0.13579
Error	30	3.2396	0.1080		

*Significant at 5% level ** Significant at 1% level

Appendix XIV. Analysis of variance of the data for leaf plant^{-1} at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.3582	0.1791	1.3328	0.2789
Salt stress	3	22.9110	7.6370	56.8262	$1.754e^{-12}$ **
Protectent	3	6.7777	2.2592	16.8107	$1.358e^{-06}$ **
Salt × Protectent	9	2.1810	0.2423	1.8032	0.1091
Error	30	4.0318	0.1344		

*Significant at 5% level ** Significant at 1% level

Appendix XV. Analysis of variance of the data for leaf plant^{-1} at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.1001	0.0501	1.5272	0.2336
Salt stress	3	19.7260	6.5753	200.6245	$< 2.2e^{-16}$ **
Protectent	3	5.9731	1.9910	60.7495	$7.489e^{-13}$ **
Salt × Protectent	9	0.4726	0.0525	1.6020	0.1597
Error	30	0.9832	0.0328		

*Significant at 5% level ** Significant at 1% level

Appendix XVI. Analysis of variance of the data for leaf plant^{-1} at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.00000	0.000000	0.0000	1.0000
Salt stress	3	0.45833	0.152778	13.7500	8.073e^{-06} **
Protectent	3	0.59375	0.197917	17.8125	7.910e^{-07} **
Salt × Protectent	9	0.17708	0.019676	1.7708	0.1161
Error	30	0.33333	0.011111		

*Significant at 5% level ** Significant at 1% level

Appendix XVII. Analysis of variance of the data for leaf area at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	8963	4481	0.0376	0.96312
Salt stress	3	68480925	22826975	191.6440	< 2.2e^{-16} **
Protectent	3	15926872	5308975	44.5714	3.595e^{-11} **
Salt × Protectent	9	3178024	353114	2.9646	0.01206 *
Error	30	3573341	119111		

*Significant at 5% level ** Significant at 1% level

Appendix XVIII. Analysis of variance of the data for leaf area at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	317458	158729	0.4025	0.6722
Salt stress	3	54991679	18330560	46.4846	2.152e^{-11} **
Protectent	3	40971995	13657332	34.6337	7.161e^{-10} **
Salt × Protectent	9	3346823	371869	0.9430	0.5038
Error	30	11830078	394336		

*Significant at 5% level ** Significant at 1% level

Appendix XIX. Analysis of variance of the data for fresh weight plant^{-1} at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.1991	0.09954	7.3181	0.002579
Salt stress	3	1.5388	0.51294	37.7112	$2.656e^{-10}$ **
Protectent	3	3.6478	1.21593	89.3947	$4.668e^{-15}$ **
Salt × Protectent	9	0.6743	0.07492	5.5079	0.000176 **
Error	30	0.4081	0.01360		

*Significant at 5% level ** Significant at 1% level

Appendix XX. Analysis of variance of the data for fresh weight plant^{-1} at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.1047	0.0524	0.2201	0.8037
Salt stress	3	15.8725	5.2908	22.2341	$8.948e^{-08}$ **
Protectent	3	10.8380	3.6127	15.1819	$3.417e^{-06}$ **
Salt × Protectent	9	0.4821	0.0536	0.2251	0.9883
Error	30	7.1388	0.2380		

*Significant at 5% level ** Significant at 1% level

Appendix XXI. Analysis of variance of the data for fresh weight plant^{-1} at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.005	0.0024	0.0125	0.987534
Salt stress	3	47.604	15.8679	81.8384	$1.522e^{-14}$ **
Protectent	3	14.348	4.7826	24.6662	$3.048e^{-08}$ **
Salt × Protectent	9	7.116	0.7906	4.0776	0.001689 **
Error	30	5.817	0.1939		

*Significant at 5% level ** Significant at 1% level

Appendix XXII. Analysis of variance of the data for fresh weight plant^{-1} at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.106	0.053	0.4810	0.62282
Salt stress	3	277.380	92.460	842.9458	< 2.2e^{-16} **
Protectent	3	11.486	3.829	34.9065	6.541e^{-10} **
Salt × Protectent	9	1.963	0.218	1.9886	0.07654
Error	30	3.291	0.110		

*Significant at 5% level ** Significant at 1% level

Appendix XXIII. Analysis of variance of the data for dry weight plant^{-1} at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.001588	0.0007938	0.6853	0.511637
Salt stress	3	0.021723	0.0072412	6.2519	0.001997 **
Protectent	3	0.070456	0.0234854	20.2776	2.258e^{-07} **
Salt × Protectent	9	0.007869	0.0008743	0.7549	0.657224
Error	30	0.034746	0.0011582		

*Significant at 5% level ** Significant at 1% level

Appendix XXIV. Analysis of variance of the data for dry weight plant^{-1} at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.030004	0.015002	2.5601	0.09407
Salt stress	3	0.040342	0.013447	2.2948	0.09798
Protectent	3	0.244292	0.081431	13.8963	7.377e^{-06} **
Salt × Protectent	9	0.122558	0.013618	2.3239	0.04027 *
Error	30	0.175796	0.005860		

*Significant at 5% level ** Significant at 1% level

Appendix XXV. Analysis of variance of the data for dry weight plant^{-1} at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.0213	0.01065	0.3950	0.6771
Salt stress	3	2.3139	0.77129	28.6029	$6.186e^{-09}$ **
Protectent	3	3.6323	1.21077	44.9009	$3.287e^{-11}$ **
Salt × Protectent	9	0.7328	0.08142	3.0196	0.0109 *
Error	30	0.8090	0.02697		

*Significant at 5% level ** Significant at 1% level

Appendix XXVI. Analysis of variance of the data for dry weight plant^{-1} at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.0157	0.0079	0.8161	0.4517
Salt stress	3	20.0069	6.6690	693.1682	$< 2.2e^{-16}$ **
Protectent	3	1.1521	0.3840	39.9153	$1.357e^{-10}$ **
Salt × Protectent	9	0.0705	0.0083	0.8666	0.5643
Error	30	0.2886	0.0096		

*Significant at 5% level ** Significant at 1% level

Appendix XXVII. Analysis of variance of the data for Relative water content at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.480	0.240	0.7000	0.504496
Salt stress	3	280.037	93.346	272.5502	$< 2.2e^{-16}$ **
Protectent	3	23.520	7.840	22.8913	$6.638e^{-08}$ **
Salt × Protectent	9	13.259	1.473	4.3015	0.001162 **
Error	30	10.275	0.342		

*Significant at 5% level ** Significant at 1% level

Appendix XXVIII. Analysis of variance of the data for Relative water content at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.02	0.010	0.0224	0.977819
Salt stress	3	469.14	156.379	360.6540	$< 2.2e^{-16}$ **
Protectent	3	8.22	2.740	6.3182	0.001884 **
Salt × Protectent	9	8.35	0.927	2.1385	0.057418
Error	30	13.01	0.434		

*Significant at 5% level ** Significant at 1% level

Appendix XXIX. Analysis of variance of the data for Relative water content at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.85	0.43	1.2479	0.3016
Salt stress	3	1698.48	566.16	1660.5179	$< 2.2e^{-16}$ **
Protectent	3	32.06	10.69	31.3438	$2.234e^{-09}$ **
Salt × Protectent	9	4.48	0.50	1.4607	0.2077
Error	30	10.23	0.34		

*Significant at 5% level ** Significant at 1% level

Appendix XXX. Analysis of variance of the data for SPAD value at 35 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	1.939	0.970	0.4220	0.65956
Salt stress	3	141.183	47.061	20.4853	$2.041e^{-07}$ **
Protectent	3	27.439	9.146	3.9813	0.01683 *
Salt × Protectent	9	10.479	1.164	0.5068	0.85783
Error	30	68.919	2.297		

*Significant at 5% level ** Significant at 1% level

Appendix XXXI. Analysis of variance of the data for SPAD value at 50 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	10.823	5.412	3.2727	0.05180
Salt stress	3	204.866	68.289	41.2992	$9.029e^{-11}$ **
Protectent	3	115.114	38.371	23.2059	$5.765e^{-08}$ **
Salt × Protectent	9	40.735	4.526	2.7373	0.01841 *
Error	30	49.605	1.654		

*Significant at 5% level ** Significant at 1% level

Appendix XXXII. Analysis of variance of the data for SPAD value at 65 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	3.479	1.739	1.0135	0.3751
Salt stress	3	157.593	52.531	30.6098	$2.915e^{-09}$ **
Protectent	3	147.816	49.272	28.7108	$5.935e^{-09}$ **
Salt × Protectent	9	22.089	2.454	1.4301	0.2197
Error	30	51.485	1.716		

*Significant at 5% level ** Significant at 1% level

Appendix XXXIII. Analysis of variance of the data for SPAD value at 80 DAS

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	8.16	4.08	5.9830	0.006507
Salt stress	3	3153.00	1051.00	1540.6105	$< 2.2e^{-16}$ **
Protectent	3	22.79	7.60	11.1340	$4.456e^{-05}$ **
Salt × Protectent	9	11.10	1.23	1.8071	0.108316
Error	30	20.47	0.68		

*Significant at 5% level ** Significant at 1% level

Appendix XXXIV. Analysis of variance of the data for Spike length plant^{-1}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.05058	0.02529	1.0403	0.3657
Salt stress	3	0.10635	0.03545	1.4583	0.2457
Protectent	3	3.06705	1.02235	42.0554	$7.261e^{-11}$ **
Salt × Protectent	9	0.29020	0.03224	1.3264	0.2651
Error	30	0.72929	0.02431		

*Significant at 5% level ** Significant at 1% level

Appendix XXXV. Analysis of variance of the data for effective spike length pot^{-1}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.667	0.3333	0.5357	0.5907
Salt stress	3	28.167	9.3889	15.0893	$3.607e^{-06}$ **
Protectent	3	31.833	10.6111	17.0536	$1.189e^{-06}$ **
Salt × Protectent	9	1.333	0.1481	0.2381	0.9857
Error	30	18.667	0.6222		

*Significant at 5% level ** Significant at 1% level

Appendix XXXVI. Analysis of variance of the data for effective grain spike^{-1}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	2.46	1.23	3.0891	0.06027
Salt stress	3	967.55	322.52	809.3672	$< 2.2e^{-16}$ **
Protectent	3	73.36	24.45	61.3679	$6.576e^{-13}$ **
Salt × Protectent	9	10.40	1.16	2.8986	0.01363 *
Error	30	11.95	0.40		

*Significant at 5% level ** Significant at 1% level

Appendix XXXVII. Analysis of variance of the data for 1000 grain weight

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	11.00	5.50	4.6137	0.017905
Salt stress	3	2794.20	931.40	781.4015	$< 2.2e^{-16}$ **
Protectent	3	140.56	46.85	39.3074	$1.628e^{-10}$ **
Salt × Protectent	9	38.61	4.29	3.5994	0.003843 **
Error	30	35.76	1.19		

*Significant at 5% level ** Significant at 1% level

Appendix XXXVIII. Analysis of variance of the data for Seed yield (g pot^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	7.8	3.91	2.0839	0.142090
Salt stress	3	3752.9	1250.98	667.0048	$< 2.2e^{-16}$ **
Protectent	3	340.3	113.43	60.4819	$7.924e^{-13}$ **
Salt × Protectent	9	66.9	7.43	3.9608	0.002058 **
Error	30	56.3	1.88		

*Significant at 5% level ** Significant at 1% level

Appendix XXXIX. Analysis of variance of the data for Seed yield (t ha^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.010	0.0051	0.5261	0.5963
Salt stress	3	38.037	12.6790	1305.6925	$< 2.2e^{-16}$ **
Protectent	3	3.452	1.1507	118.4959	$< 2.2e^{-16}$ **
Salt × Protectent	9	0.685	0.0762	7.8420	$7.631e^{-06}$ **
Error	30	0.291	0.0097		

*Significant at 5% level ** Significant at 1% level

Appendix XL. Analysis of variance of the data for Straw yield (g pot^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	1.43	0.72	0.4412	0.6474
Salt stress	3	1975.75	658.58	405.6385	$< 2.2e^{-16}$ **
Protectent	3	140.01	46.76	28.7448	$5.858e^{-09}$ **
Salt × Protectent	9	26.26	2.92	1.7970	0.1104
Error	30	48.71	1.62		

*Significant at 5% level ** Significant at 1% level

Appendix XLI. Analysis of variance of the data for Straw yield (t ha^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.0142	0.0071	0.4302	0.6543
Salt stress	3	20.0442	6.6814	404.7196	$< 2.2e^{-16}$ **
Protectent	3	1.4237	0.4746	28.7465	$5.854e^{-09}$ **
Salt × Protectent	9	0.2668	0.0296	1.7955	0.1107
Error	30	0.4953	0.0165		

*Significant at 5% level ** Significant at 1% level

Appendix XLII. Analysis of variance of the data for Biological yield (g pot^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	12.4	6.2	1.8139	0.18044
Salt stress	3	10974.7	3658.2	1072.3579	$< 2.2e^{-16}$ **
Protectent	3	587.1	195.7	57.3683	$1.555e^{-12}$ **
Salt × Protectent	9	82.3	9.1	2.6817	0.02043 *
Error	30	102.3	3.4		

*Significant at 5% level ** Significant at 1% level

Appendix XLIII. Analysis of variance of the data for Biological yield (t ha^{-1})

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.039	0.020	0.6252	0.54198
Salt stress	3	111.248	37.083	1185.4060	$< 2.2e^{-16}$ **
Protectent	3	5.953	1.984	63.4343	$4.295e^{-13}$ **
Salt × Protectent	9	0.842	0.094	2.9909	0.01149 *
Error	30	0.938	0.031		

*Significant at 5% level ** Significant at 1% level

Appendix XLIV. Analysis of variance of the data for harvest index

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	2	0.40	0.20	0.2184	0.805
Salt stress	3	2144.43	714.81	784.0978	$< 2.2e^{-16}$ **
Protectent	3	290.09	96.70	106.0681	$4.593e^{-16}$ **
Salt × Protectent	9	54.14	6.02	6.5987	$3.764e^{-05}$ **
Error	30	27.35	0.91		

*Significant at 5% level ** Significant at 1% level

Appendix XLV. Analysis of variance of the data for Shoot Na^{+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replication	15	35.1936	2.34624	590.87	0.0000
Error	32	0.1271	0.00397		

Appendix XLVI. Analysis of variance of the data for Shoot K^{+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	4.25003	0.28334	133.47	0.0000
Error	32	0.06793	0.00212		

Appendix XLVII. Analysis of variance of the data for Shoot Ca^{2+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	4.15733	0.27716	76.28	0.0000
Error	32	0.11627	0.00363		

Appendix XLVIII. Analysis of variance of the data for Shoot Mg^{2+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	8.00771	0.53385	128.57	0.0000
Error	32	0.13287	0.00415		

Appendix XLIX. Analysis of variance of the data for Grain Na^{+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.09176	0.006117	34.55	0.0000
Error	32	0.00567	0.000177		

Appendix L. Analysis of variance of the data for Grain K^{+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.24070	0.01605	8.56	0.0000
Error	32	0.06000	0.00187		

Appendix LI. Analysis of variance of the data for Grain Ca^{2+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	12.7063	0.84709	60.74	0.0000
Error	32	0.4463	0.01395		

Appendix LII. Analysis of variance of the data for Grain Mg^{2+}

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	4.53685	0.30246	40.24	0.0000
Error	32	0.24053	0.00752		

EXPERIMENT: 2

Appendix LIII. Analysis of variance of the data for H_2O_2 content

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	325.512	21.7008	123.65	0.0000
Error	32	5.616	0.1755		

Appendix LIV. Analysis of variance of the data for Malondialdehyde (MDA) content

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	2111.15	140.743	102.05	0.0000
Error	32	44.13	1.379		

Appendix LV. Analysis of variance of the data for Shoot length

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	527.233	35.1489	1191.40	0.0000
Error	32	0.944	0.0295		

Appendix LVI. Analysis of variance of the data for root length

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	294.919	19.6612	251.50	0.0000
Error	32	2.502	0.0782		

Appendix LVII. Analysis of variance of the data for root shoot ratio

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	1392.79	92.8525	29.31	0.0000
Error	32	101.37	3.1677		

Appendix LVIII. Analysis of variance of the data for fresh weight

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.06064	0.0040426	96.64	0.0000
Error	32	0.00134	0.0000418		

Appendix LIX. Analysis of variance of the data for dry weight

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	237.639	15.8426	79.75	0.0000
Error	32	6.357	0.1987		

Appendix LX. Analysis of variance of the data for Relative water content

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	425.723	28.3815	235.44	0.0000
Error	32	3.857	0.1205		

Appendix LXI. Analysis of variance of the data for prolin content

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	7.42249	0.49483	512.50	0.0000
Error	32	0.03090	0.00097		

Appendix LXII. Analysis of variance of the data for Chl *a*

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.38288	0.02553	12.90	0.0000
Error	32	0.06331	0.00198		

Appendix LXIII. Analysis of variance of the data for Chl *b*

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.21425	0.01428	10.35	0.0000
Error	32	0.04417	0.00138		

Appendix LXIV. Analysis of variance of the data for Chl a+b

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	1.14386	0.07626	18.49	0.0000
Error	32	0.13194	0.00412		

EXPERIMENT: 3

Appendix LXV. Analysis of variance of the data for germination percent

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	141.667	9.44444	17.44	0.0000
Error	32	17.333	0.54167		

Appendix LXVI. Analysis of variance of the data for relative water content

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	406.817	27.1211	30.11	0.0000
Error	32	28.821	0.9007		

Appendix LXVII. Analysis of variance of the data for shoot length

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	107.987	7.19912	75.75	0.0000
Error	32	3.041	0.09504		

Appendix LXVIII. Analysis of variance of the data for root length

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	58.4663	3.89775	26.21	0.0000
Error	32	4.7596	0.14874		

Appendix LXIX. Analysis of variance of the data for root shoot ratio

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	609.400	40.6267	6.00	0.0000
Error	32	216.788	6.7746		

Appendix LXX. Analysis of variance of the data for vigor index

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	3865699	257713	86.63	0.0000
Error	32	95199	2975		

Appendix LXXI. Analysis of variance of the data for survibility %

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	2136.00	142.400	71.20	0.0000
Error	32	64.00	2.000		

Appendix LXXII. Analysis of variance of the data for fresh weight

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.09490	0.0063266	116.31	0.0000
Error	32	0.00174	0.0000543		

Appendix LXXIII. Analysis of variance of the data for dry weight

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.0009958	0.00006639	70.81	0.0000
Error	32	0.0000300	0.00000094		

Appendix LXXIV. Analysis of variance of the data for Chl *a*

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.05897	0.003931	2.94	0.0051
Error	32	0.04281	30.00133		

Appendix LXXV. Analysis of variance of the data for Chl *b*

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.03486	0.002324	3.67	0.0010
Error	32	0.02024	0.0006325		

Appendix LXXVI. Analysis of variance of the data for Chl *a*+ *b*

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	15	0.17551	0.01170	5.71	0.0000
Error	32	0.06554	0.00205		

PLATES

Plates 1. Different growth stage, data collection and various scenareo under experiment 1: GROWTH AND YIELD OF WHEAT AS AFFECTED BY EXOGENOUS APPLICATION OF ASCORBIC ACID, SILICON AND GIBBRALLIC ACID UNDER SALT STRESS CONDITION.

a. Pot preparation

b. Pot covered by polythene sheet

c. Polythene removed at 5 DAS

d. A view of 20 DAS Wheat seedling

e. A view of seedling after foliar spray

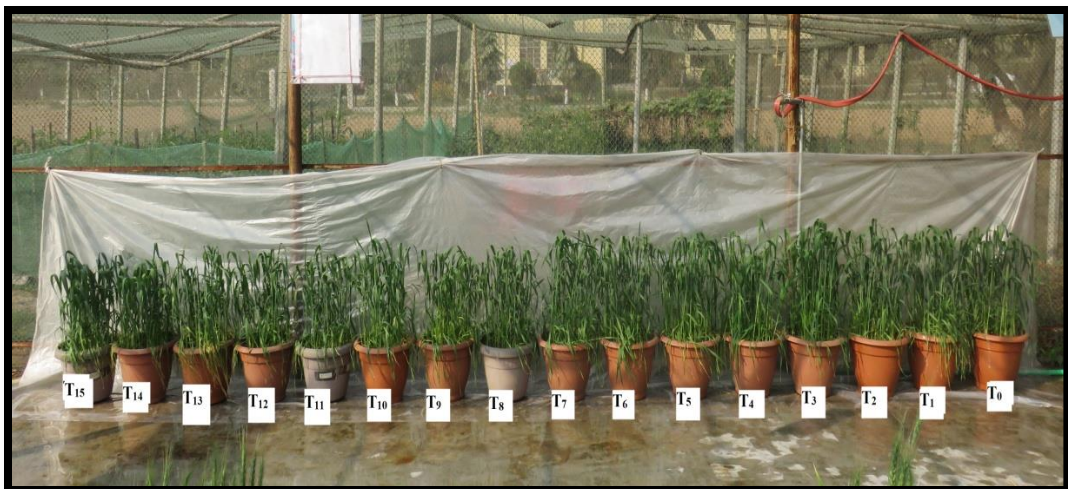
f. A view of Sample collection for biomass determination at 35 DAS

g. A view of leaf area measurement

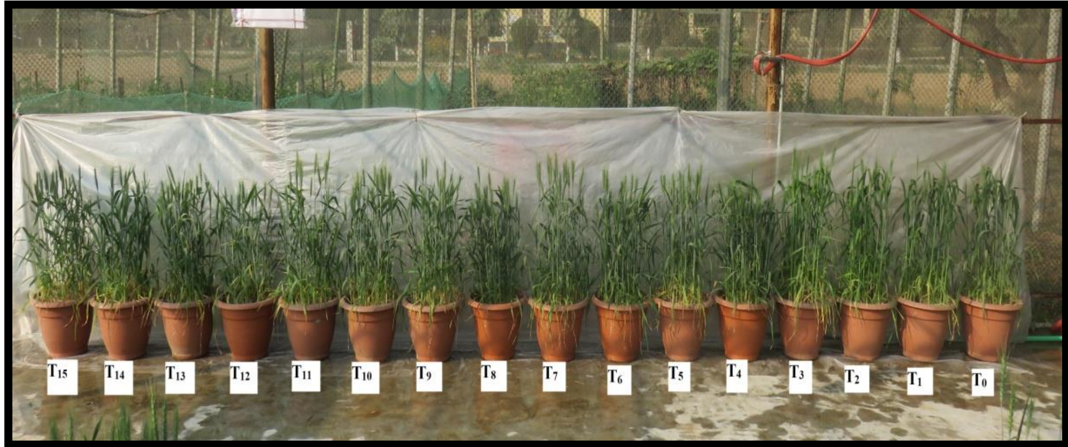
h. Data recorded of various growth character

i. Measurement of electric conductivity

j. A view of ion determination



k. A view of wheat plant at 35 DAS



l. A view of wheat plant at 50 DAS

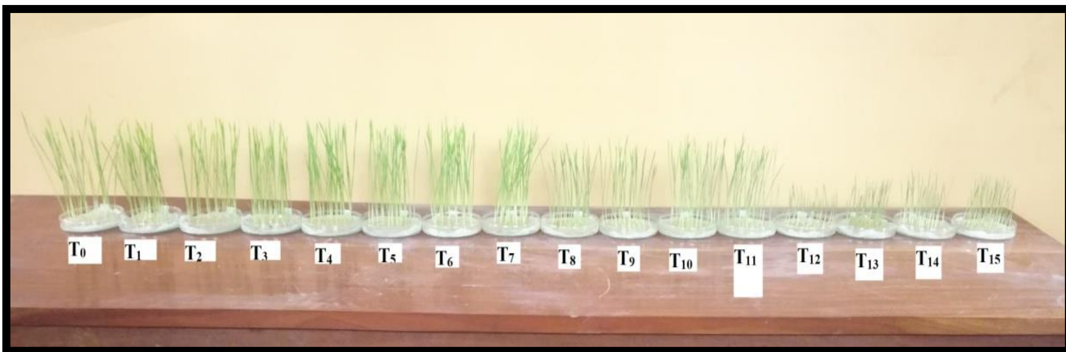
m. A view of wheat plant at 65 DAS

n. A view of wheat plant at Harvest

Plates 2. **Showing various procedures under experiment 2: ALLEVIATION OF SHORT TERM SALT STRESS EFFECT ON WHEAT SEEDLINGS BY FOLIAR SPRAY WITH ASCORBIC ACID, SILICON AND GIBBRILLIC ACID**

a. A view of seed sown in petri dish

b. Hyponex and NaCl treatment



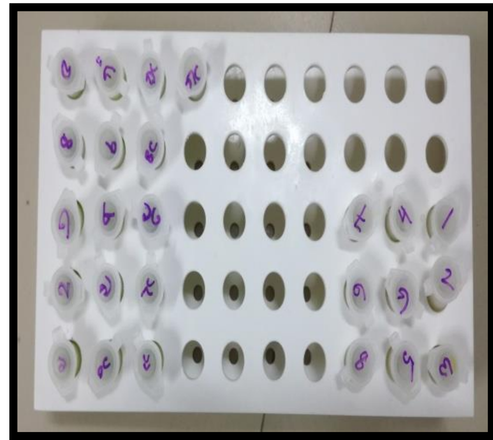
c. A view of wheat plant at 10 DAS

d. Electric conductivity and pH measurement

e. Wheat seedling collection for biomass determination at 10 DAS

f. Collecting sap from seedling

g. Chemical preparation



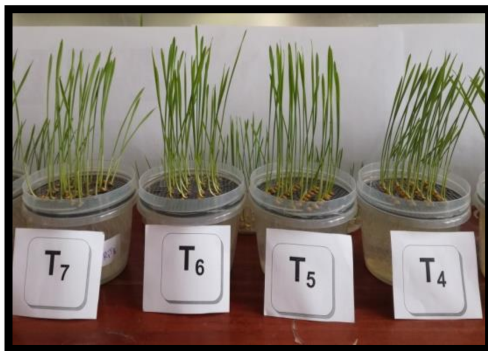
h. Centrifuging plant sap

i. Plant sap

Plates 3. Showing growth performance of secondary seed under experiment 3: GERMINATION AND GROWTH PERFORMANCE OF SEEDLINGS OF ASCORBIC ACID, SILICON AND GIBBERELLIC ACID INDUCED SECONDARY WHEAT SEEDS UNDER SALT STRESS

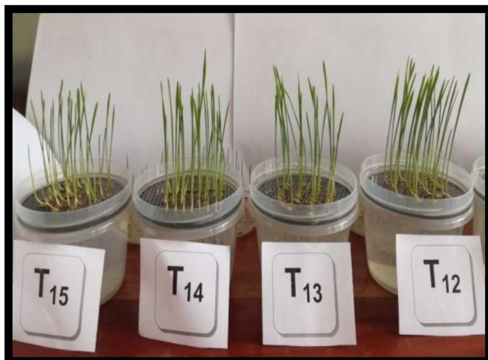
a. A view of secondary seed sown in hydroponix plates

b. Measuring growth data of secondary seed

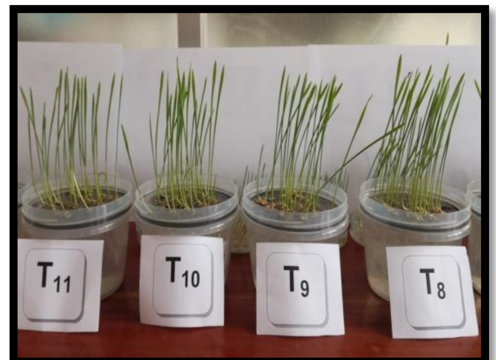


c. Growth of secondary seed

d. Growth of secondary seed



e. Growth of secondary seed



f. Growth of secondary seed