EFFECT OF MANNITOL ON MORPHOPHYSIOLOGY AND YIELD OF WHEAT PLANT UNDER SALINITY

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CERTIFICATE

This is to certify that thesis entitled, "Effect of Mannitol on Morphophysiology and Yield of Wheat Plant Under Salinity" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE (MS) in AGRICULTURAL BOTANY****, embodies the result of a piece of bona fide research work carried out by* ***MD. TANVIR AHMED,*** *bearing Registration No.* ***12-05044*** *under my supervision and guidance. No part of the thesis 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 been duly acknowledged.

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TO

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EFFECT OF MANNITOL ON MORPHOPHYSIOLOGY AND YIELD OF WHEAT PLANT UNDER SALINITY

ABSTRACT

The experiment was conducted in the net house of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, during the period of November, 2018-March, 2019 to find out the effect of mannitol on morphophysiology and yield of wheat plant under salinity. BARI Gom-25 was used as a plant material. The experiment consists of nine treatments viz. T_1 = Control, T_2 = Mannitol 15 mM (M_1), T_3= Mannitol 30 mM (M_2), T_4 = 4 dS m^{-1} NaCl (S_1), T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1), T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2), T_7 = 8 dS m^{-1} NaCl (S_2), T_8 = 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1) and T_9 = 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2). The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data on different growth, physiological and yield contributing parameters of BARI Gom-25 were recorded. Wheat plants exposed to maximum levels of salinity (8 dS m^{-1} NaCl) resulted in the lowest value of the studied parameters such as plant height (cm), tillers number $plant^{-1}$, leaf chlorophyll content (SPAD reading), filled grains $spike^{-1}$, unfilled grains $spike^{-1}$, 1000-seed weight (g), dry weight of plant at harvest (g) and grain yield $plant^{-1}$ (g). Plants in non-saline and control treatments showed the highest value of the studied parameters. Mannitol was applied in both saline and non-saline treated plants. Application of mannitol to salinized wheat plants resulted in the significant improvement of the studied parameters. But the effect of mannitol to non-salinized wheat plants was similar to the effect of control plants. Mannitol application @ 30mM (M_2) performed best over 15mM (M_1) for mitigating the damaging effect of salinity on different growth, physiological, yield contributing and yield parameters of wheat.

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ABBREVIATIONS AND ACRONYMS

%	=	Percentage
AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BAU	=	Bangladesh Agricultural University
Ca	=	Calcium
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAT	=	Days After Transplanting
dS m^{-1}	=	deciSiemens per metre
df	=	Degrees of freedom
e.g.	=	exempli gratia (L), for example
et al.,	=	And others
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
GM	=	Geometric mean
i.e.	=	id est (L), that is
K	=	Potassium
kg	=	Kilogram (s)
L	=	Litre
LSD	=	Least Significant Difference
M.S.	=	Master of Science
m^2	=	Meter squares
mg	=	Miligram
ml	=	Mili Litre
mM	=	Millimolar
NaOH	=	Sodium hydroxide
NaCl	=	Sodium Chloride
No.	=	Number
^oC	=	Degree Celceous
P	=	Phosphorus
ppm	=	Parts per million
SAU	=	Sher-e-Bangla Agricultural University
t ha^{-1}	=	Ton per hectare
USA	=	United States of America
var.	=	Variety
WHO	=	World Health Organization
µg	=	Microgram

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the family Gramineae is an important cereal crop in Bangladesh as well as in the world and ranks first globally and third in Bangladesh both in terms of production and acreage (FAO, 2014). It is a staple food crop for more than one third of the world population (Shirazi *et al*., 2001). Wheat is grown on more land area than any other food crop (220.4 million hectares, FAOSTAT, 2014). Global demand for wheat is increasing due to the unique viscoelastic and adhesive properties of gluten proteins, which facilitate the production of processed foods, whose consumption is increasing as a result of the worldwide industrialization process and the westernization of the diet (Day *et al.*, 2006). Globally, it is the leading source of vital protein in human food, having a protein content of about 13%, which is relatively high compared to other major cereals but relatively low in protein quality for supplying essential amino acids. Wheat is an important source of carbohydrates. When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber (Shewry and Hey, 2015). In Bangladesh, the area under wheat cultivation during 2017-2018 was about 8,67,884 acres producing 10,99,373 metric tons with an average yield of 3.130 metric tons per hectare (BBS, 2018). The present population of Bangladesh will progressively increase to 223 million by 2030 requiring 48.0 million tons of food grains (Karim *et al*., 1990). Owing to population pressure the cultivable area is decreasing in the country day-by day and this problem will gradually but soon be acute.

Salinity is one of the most serious abiotic stresses limiting the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop yield (Munns and Tester, 2008). Salinization affects many irrigated areas mainly due to the use of brackish water. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Munns and Tester, 2008). In Bangladesh, the salinity affected area was 83.3 million ha in 1973, 102 million ha in 2000 and in 2009 it has reached up to 105.5 million ha and the area is being expanded with times being reported by Soil Resource and Development Institute. The dramatic increasing of saline area is caused by rise of the sea

levels due to global warming. In Bangladesh, over 30% of the net cultivable areas lie in the coastal zone close to the Bay of Bengal of which approximately 53% are affected by varying degrees of salinity (Haque, 2006). Ali (2011) showed that the salt-affected areas in the coastal region of Bangladesh increased sharply, by 26.71%, to 950,780 hectares in 2009 from 750,350 hectares in 1973. High salinity affects plants in two main ways: high concentrations of salts in the soil disturb the capacity of roots to extract water and high concentrations of salts within the plant itself can be toxic, resulting in an inhibition of many physiological and biochemical processes such as nutrient uptake and assimilation (Hasegawa *et al.,* 2000). Most of the high yielding salt sensitive crop might not be suitable for cultivation in the existing cropping pattern. Wheat is cultivated over a wide range of environments, because of wide adaptation to diverse environmental conditions. It is a moderately salt-tolerant crop (Moud and Maghsoudi, 2008). Salinity reduces the growth of wheat plant by reducing the plants ability to absorb water from soil. Salinity affects wheat seedling growth by changing phytohormone levels (Shakirova *et al*., 2003). Furthermore, salinity induces reduction in photosynthetic rate and stomatal conductance in wheat. Adding more NaCl increases the action of superoxide dismutase and peroxidase and reduces the transpiration rate in *Triticum aestivum* (Sharma *et al*., 2005). Moreover, increased salinity induces a considerable reduction in height, number of fertile tillers and dry weight of shoots in wheat (Iqbal *et al*., 2005). It has been demonstrated that about 61% reduction of seed germination and 23-25% yield loss can be occurred when wheat seeds were cultivated under salt stress condition (AL-Musa *et al*., 2012). Exposing wheat to salt stress leads to decrease in cell growth which causes reduction in leaf area, biomass and yield because many physiological processes are affected by salinity (Asadi *et al*., 2007).

For mitigating salt-induced damages, in recent decades, the use of exogenous osmoprotectants or osmolytes has been found effective (Hasanuzzaman *et al*., 2014). Mannitol, an important osmolyte, is normally synthesized in large amount in many plant species (Mitoi *et al*., 2009). Mannitol is a six-carbon, non-cyclic sugar alcohol having different roles like coenzyme regulation, free-radicals scavenging and osmoregulation (Rahnama *et al*., 2011), thereby imparting abiotic stress tolerance. However, pretreatment with exogenous mannitol (100 mM) reversed the deleterious salt effects by increasing

antioxidant enzymes (such as SOD, POD, CAT, APX and GR) activities, appearance of SOD and POD isozyme activity bands and reducing lipid peroxidation (Seckin *et al*., 2009). Expression of *mtlD* gene encoding mannitol-1-phosphate dehydrogenase resulted in enhanced salt stress tolerance in wheat due to defensive roles of mannitol against salt stress (Abebe *et al*., 2003). The *mtlD* gene encoding mannitol-1-phosphate dehydrogenase transformation in *T. aestivum* cv. Giza conferred salt stress tolerance by inducing mannitol and reducing sugars in tissues of plant (Ramadan *et al*., 2013). External use of mannitol serve as osmo-protectants under stress conditions which maintain membrane structure and act as free radical scavengers preventing lipid peroxidation or as regulators of K^+ and Na^+ channels in stomata. The pre-sowing soaking treatment of seeds with mannitol positively affected the osmotic potential, shoot and root dry mass, Na^+/K^+ ratio and contents of photosynthetic pigments in wheat seedlings, under saline and non- saline conditions. (Parida and Das, 2005). Although mannitol plays an important role in osmotic adjustment, it acts as an antioxidant to scavenge of hydroxyl radicals (OH^-) (Srivastava *et al*., 2010). Wheat plant cannot produce mannitol. However, the effect of exogenously applied mannitol to the wheat plants has not been evaluated under salt stress. So, the present study was carried out to observe the effect of exogenously applied mannitol on morphophysiology and yield of wheat plant under salinity.

Considering the fact described above, the present work was undertaken to achieve the following objectives:

1. To study the physiology, yield contributing characters and yield of wheat plant under salt stress.
2. To observe the role of mannitol in mitigating salinity stress in wheat plant.
3. To determine the effective dose of mannitol for mitigating salt stress.

CHAPTER II

REVIEW OF LITERATURE

2.1 Wheat

Wheat (*Triticum* spp.) ranks first in the world's grain production. Wheat is consumed as staple food by more than 36% of world population. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman *et al*., 1995).

Wheat (*Triticum aestivum* L.) is the 1st ranking cereal crop globally and major staple food for more than one third of the world population rather than the main staple food for Asia (Shirazi *et al*., 2001).

Wheat ranks third in position among the cereals in Bangladesh next to rice. The winter season of Bangladesh is favorable for wheat cultivation. Wheat is gaining popularity as a staple crop of our country day by day. It plays a vital role in the national economy to reduce the deficit between the food production and food import.

Due to continuous food shortage, changing food habit and introduction of dwarf type high yielding wheat varieties, cultivation of wheat have become popular to the farmers of Bangladesh after the world wide successful campaign of green revolution in mid sixties.

In Bangladesh, the area under wheat cultivation during 2017-2018 was about 8,67,884 acres producing 10,99,373 metric tons with an average yield of 3.130 metric tons per hectare (BBS, 2018).

2.2 Salt stress

Among the abiotic stresses, salt stress is a major environmental threat to agriculture, and its adverse impacts are getting more serious problems in regions where saline water is used for irrigation (Turkan and Demiral, 2009).

Salt stress can be generally viewed as the toxicity to the plants due to development of salinity. The stress condition resulting from higher salt concentration which is sufficient to lower the water potential (0.5 to 10 bars) is termed salt stress. The soils in which the

electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeds 4 dSm^{-1} (approximately 40 mM NaCl) at 25 °C and has exchangeable sodium of 15% are identified as saline soils.

When the salinity in soil develops as a result of natural accumulation of salts for a longer time period, whether in the form of sea salt deposition by winds and water or due to salts release from erosion of rocks, is referred to as primary salinization. However, secondary soil salinization is the results of human activities such as faulty practices of irrigation in agriculture. There is excessive accumulation of salts ions in the soil such as sodium (Na^+), chlorine (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfate (S), and bicarbonate (HCO_3^-), resulting in buildup of salinity and affecting plant growth and development (Lewis, 1984).

It is estimated that at least 20% of total irrigated lands in the world is salt-affected (Pitman and Lauchli, 2002). Due to climate change the area affected by soil salinity in Bangladesh increased from about 0.83 million ha in 1973 to 1.02 million ha in 2000, and 1.05 million ha in 2009 (Anon., 2010). In most of the cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence causes many physiological disorders in plant, Cl^- is the most dangerous (Tavakkoli *et al*., 2010).

Salinity at higher levels causes both hyper ionic and hyper osmotic stress and can lead to plant demise. The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leading to plant death (Mahajan and Tuteja, 2005).

Molecular and biochemical studies of the salt stress responses of plants have demonstrated significant increases in reactive oxygen species (ROS) such as, singlet oxygen, superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Perez-Lopez *et al*., 2010).

Among the cereals production worldwide, 69% of the total wheat production is adversely affected by high salinity (Isayenkov, 2012). Salt stress results in a considerable decrease

in the fresh and dry weights of leaves, stems, tillers, fertile tillers and roots (Chartzoulakis and Klapaki, 2000). Salinity stress changes the physiological, morphological and biochemical responses of plants (Siringam *et al*., 2011). It causes significant changes in the SOD (Super oxide dismutase), antioxidant enzymes, growth regulators, lipid per-oxidation, total chlorophyll content and roots and shoots fresh weight of the plant. The different physiological processes which are adversely affected by salt stress are mineral distribution, membrane permeability, membrane instability due to calcium displacement by sodium (Gupta *et al*., 2002) and decrease in photosynthetic efficiency (Hasegawa *et al.*, 2000).

According to Carvajal *et al*. (1999) the direct effects of salts on plant growth may be divided into three broad categories: (i) a reduction in the osmotic potential of the soil solution that reduces plant available water, (ii) a deterioration in the physical structure of the soil such that water permeability and soil aeration are diminished and (iii) increase in the concentration of certain ions that have an inhibitory effect on plant metabolism (specific ion toxicity and mineral nutrient deficiencies).

On account of the shrinking agricultural land area for the sustainable crop production there is a need to utilize the areas under stress conditions for crop production and other uses through appropriate management techniques like adopting suitable agronomic practices involving tilling with sub-soiler, mulching, growing of salt tolerant crops, reclamation measures, judicious application of fertilizers, practicing INM (Integrated nutrient management) and irrigating with saline water in combination with two-three turns of fresh water particularly in regions under salt stress.

2.3 Effect of salt stress on germination

Seed germination is one of the most fundamental and vital phases in the growth cycle of a plant that determines the yield. Salinity confines the seed germination and vigor of a several crops species like rice (Xu *et al*., 2011), wheat (Akbarimoghaddam *et al*., 2011), Maize (Khodarahmpour *et al*., 2012), Mustard (Ulfat *et al*., 2007), Pulses (Jabeen *et al*., 2003) and Sunflower (Mutlu and Buzcuk, 2007).

Salinity impairs the imbibitions of seeds due to lower osmotic potential which alters the activity of enzymes associated with nucleic acid metabolism and protein metabolism leading hormonal imbalance and lessens the utilization of seed reserves thus reduces seed germination.

Kaveh *et al*. (2011) found a significant negative correlation between salinity and the rate and percentage of germination which resulted in delayed germination and reduced germination percentage in *Solanum lycopersicum*.

Bordi (2010) reported that the germination percentage in *Brassica napus* significantly reduced at 150 and 200 mM NaCl. Germination rate also decreased on increasing concentration of salinity levels.

In a recent study, Khodarahmpour *et al*. (2012) observed drastic reduction in germination rate, length of radical, plumule, seedling length and seed vigor in Maize seeds exposed to 240 mM NaCl.

Seed germination at 80 mM NaCl needs 50% more days whereas it requires almost 100% more days at 190 mM NaCl than control (Cuartero and Fernandez-Munoz, 1999).

Salt stress is believed to damage the ultra structure of cell, tissue and organs (Rasheed, 2009) that hinder the germination processes.

Seed germination of rice was not significantly affected up to 16.3 dsm^{-1} but was severely inhibited when salinity increased to 22 dsm^{-1}. The suppression of seed germination was might be due to increased osmotic pressure due to salt stress (Heenan *et al.*, 1988).

Even though there are exceptions, the majority of the research indicates that most annual crops are tolerant at germination but are sensitive during emergence and early vegetative development (Maas and Grattan, 1999).

Mauromicale and Licandro (2002) observed that at higher salt concentration percentage of germinated seeds will reduce, but in case of sugar beet which considered as salt tolerant crop is somewhat sensitive to salinity at germination (Lauchli and Epstein, 1990).

The salinity tolerant barley varieties showed much higher germination percentage and faster germination rate then sensitive one (Tajbakhsh *et al.,* 2006).

Belaqziz *et al.* (2009) reports that salinity affects germination by destroying the embryo or drastically decreasing the soil potential might be due to hampered water uptake.

2.4 Effect of salt stress on growth of plants

Salinity stress results in a clear stunting of plants. Salt stress also results in a considerable decrease in the fresh and dry weights of leaves, stems, and roots (Chartzoulakis and Klapaki, 2000).

The optimum growth of plants is obtained at 50% seawater and declines with further increases in salinity in *Rhizophora mucronata* (Aziz and Khan, 2001).

Fresh and dry weights of plants increase with an increase in salinity in *Salicornia rubra* while the optimal growth occurrs at 200mM NaCl and the growth declines with a further increase in salinity (Khan, 2001).

In *Raphanus sativus* (radish) total plant dry weight decreases at higher salinities and about 80% of the growth reduction at high salinity can be attributed to reduction of leaf area expansion and hence to reduction of light interception. The remaining 20% of the salinity effect on growth is most likely explained by a decrease in stomatal conductance. The small leaf area at high salinity is related to a reduced specific leaf area and increased tuber/shoot weight ratio and the latter can be attributed to tuber formation starting at a smaller plant size at high salinity (Marcelis and VanHooijdonk, 1999).

Kurban *et al*. (1999) have reported that in *Alhagi pseudoalhagi* (a leguminous plant), total plant weight increases at low salinity (50mM NaCl) but decreases at high salinity (100 and 200mM NaCl).

Khan *et al*. (1999) have reported that when *Halopyrum mucronatum* (a perennial grass found on the coastal dunes of Karachi, Pakistan) is treated with 0, 90, 180, and 360mM NaCl in sand culture, fresh and dry mass of roots and shoots peaks at 90mM NaCl, a

further increase in salinity inhibits plant growth, ultimately resulting in plant death at 360mM NaCl.

Experimental evidence shows that in a salt non secretor mangrove *B. parviflora* the plant growth is optimal at 100mM NaCl under hydroponic culture, whereas further increase in NaCl concentration retards plant growth and 500 mM NaCl is found to be lethal in this species (Parida *et al*., 2004).

On the other hand a salt secretor mangrove *Aegiceras corniculatum* can tolerate upto 250mM NaCl and 300mM is found lethal in this case (Mishra and Das, 2003).

Increasing salinity is accompanied by significant reductions in shoot weight, plant height, number of leaves per plant, root length and root surface area per plant in tomato (Mohammad *et al*., 1998).

Increased NaCl levels results in a significant decrease in root, shoot, and leaf growth biomass and increase in root/shoot ratio in cotton (Meloni *et al*., 2001).

In sugar beet leaf area, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected (Ghoulam *et al*., 2002).

Fisarakis *et al*. (2001), working with sultana vines recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photo assimilates in favor of roots. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

2.5 Effect of salt stress on plant physiological attributes

Photosynthesis is the major physiological process for plant survival and greatly influenced by environmental factors. As salinity reduces water potential and increases accumulation of Na^+ and Cl^- ions in the chloroplast, the rate of photosynthesis gets inhibited (Hasanuzzaman *et al*., 2013).

According to the experiment conducted by Arfan *et al*. (2007), exposure to salt stress reduced the transpiration rate, net CO_2 assimilation rate, stomatal conductance, and

substomatal CO_2 concentration of both cultivars. Similarly, net photosynthetic rate, transpiration rate, stomatal conductance, and substomatal CO_2 concentration were decreased significantly at 150 mM NaCl stress (Wahid *et al*., 2007).

Tammam *et al*. (2008) reported that amount of photosynthetic pigments were significantly deceased in seedlings under 320 mM NaCl stress. Reduction of stomatal conductance and transpiration rate were also reported by (Guo *et al*., 2015).

Under salinity, the CO_2 assimilation rate (as a function of CO_2) was shown to be better maintained by a salt tolerant species, *Eutrema salsugineum*, compared with a sensitive-species, *Arabidopsis* (Stepien and Johnson, 2009).

However, under salinity stress, leaf expansion, associated with changes in leaf anatomy (smaller and thicker leaves), is reduced, resulting in higher chloroplast density per unit leaf area, which can lead to a reduction in photosynthesis as measured on a unit chlorophyll basis (Munns and Tester, 2008).

The decrease in chlorophyll content under salt stress is reported by Yasar *et al.* (2006). In sensitive genotypes of pumpkin chlorophyll decreased by 24.11- 59.67% when compared to control plants while, tolerant genotypes protected their chlorophyll content. The reduction in chlorophyll substance might be due to increased activity of chlorophyll degrading enzyme “chlorophyllase” (Sevengor *et al*., 2011).

Senguttuvel (2014) reported that salinity level of 120 mM and 60 mM considerably reduced the chlorophyll content up to 60.8% and 30.9% respectively, compared to control.

In *O. sativa* leaves, the reduction of chlorophyll a and b contents of leaves was observed after NaCl treatment (200 mM NaCl, 14 days) where chlorophyll b content of leaves (41 %) was affected more than the chlorophyll a content (33 %) (Amirjani, 2011).

Saha *et al*. (2010) observed a linear decrease in the levels of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, and xanthophylls as well as the intensity of chlorophyll fluorescence in *Vigna radiata* under increasing concentrations of NaCl treatments.

Kalaji *et al*. (2011) reported that salinity stress affects growth of barley by altering chlorophyll fluorescence (PS II) and function of oxygen evolving complex.

Availability of moisture in plants is a crucial factor for all physiological and metabolic processes of plants. Higher salt concentrations induce osmotic stress to plants, which ultimately causes low water potential. Relative water content (RWC) declined by 3.5 and 6.7%, compared to their controls in the salt-tolerant and salt-sensitive cultivars, respectively, after 6 d of 100 mM NaCl exposure (Mandhania *et al*., 2006). They also reported lowering of osmotic potential with increasing salt concentrations.

Arfan *et al*. (2007) showed reduced water use efficiency (WUE) of both sensitive and tolerant cultivars under saline condition. 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). Percentage of water content decreased in root, but increased in shoot and spike of Banysoif 1 cultivar of wheat (Tammam *et al*., 2008). Lv *et al*. (2016) recorded lower RWC in leaves of *T. monococcum* seedlings exposed to salt stress of 320 mM NaCl.

Higher accumulation of Na^+ and Cl^- ions interferes with the uptake of other necessary ions which disturbs plant processes. Salt-sensitive cultivars tend to uptake more Na^+ compared to the tolerant one 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). They also reported higher uptake of Na^+ and Cl^-, and reduced uptake of K^+ and Ca^{2+} by salt stressed wheat seedlings.

Asgari *et al*. (2012) recorded significant decrease of K^+ uptake under saline condition (15–16 dSm^{-1}). Under medium salinity, higher accumulation of both Na^+ and Cl^-, and lower uptake of K^+, $Ca^{2+,}$ and Zn^{2+} ions were reported by Guo *et al*. (2015).

2.6 Effect of salt stress on reproductive development of plants

Successful reproductive development is the limiting factor for crop yield and reproductive growth is more sensitive than vegetative growth to environmental salt (Baby *et al*., 2016).

In experiments with wheat (Maas and Poss, 1989a), sorghum (Maas *et al*., 1986) and cowpea (Maas and Poss, 1989b), investigators found that these crops were most sensitive during early reproductive stages, less sensitive during flowering and least sensitive during the seed filling stage.

In salt-sensitive chickpea (*Cicer arietinum*) plants, for example, treatment with 50 mM NaCl stimulates flower and pod abortion and reduces seed number (Kotula *et al*., 2015).

Anther and pollen development are crucial for male reproduction and are coordinately regulated by many external and internal cues, which are highly sensitive to abiotic stresses (Deng *et al*., 2012). A study by Grunberg *et al*. (1995) showed that the main cause of the salinity-induced decrease in tomato (*Solanum lycopersicum*) fruit yield is a reduction of pollen number rather than pollen fertility.

In rice (*Oryza sativa*), however, the seed yield decreases under salinity have been found to be caused by the reduction of pollen fertility and to be directly attributable to toxic ion accumulation in plants (Khatun and Flowers, 1995).

In grapevine (*Vitis vinifera*), salinity negatively affects fruit set and is associated with poor pollen tube growth in the style (Baby *et al*., 2016).

Furthermore, salinity has been reported to inhibit grain filling in wheat (*Triticum aestivum*) and reduce the receptivity of stigmas (Khan and Abdullah, 2003).

In the case of the edible halophyte *Crithmum maritimum*, salinity significantly reduces the numbers of inflorescences and flowering branches, and there are differences between genotypes in the timing of flowering initiation (Ventura *et al*., 2014).

Reduced grain production in rice has been linked with reduced production of photo assimilates in leaves and their decreased translocation to the reproductive organs (Sultana *et al*., 1999).

Reproductive failure in drought-stressed maize has been associated with reduced photosynthesis and sugar supply to developing ears, as elegantly demonstrated by the

provision of sucrose via stem-infusion, which enhanced reproductive growth (Zinselmeier *et al.*, 1995a).

Suaeda salsa is an annual herbaceous euhalophyte that grows in saline soil environments with an optimal salt concentration for both vegetative and reproductive growth of 200 mM NaCl (Guo *et al*., 2018).

The reproductive growth of *V. radiata* was also affected by salinity as the number of pods per plant substantially decreased with increasing salinity levels. An application of 250 mM NaCl reduced 77, 73 and 66 % yield in *V. radiata* cv. BARI mung-2, BARI mung-5 and BARI mung-6, respectively over control (Nahar and Hasanuzzaman, 2009).

2.7 Effect of salt stress on yield attributes and yields of plants

Yield is a very complex character which comprises of many components and these yield components are related to final grain yield which are also severely affected by salinity.

Yield contributing components of rice like length of panicle, spikelet number per panicle, number of grain per spikelet and grain yield were significantly affected by salt treatments (Zeng and Shannon, 2000).

Sterility was considered the major cause for reduction in yield under salt stress condition as observed by Shereen *et al.* (2005).

However, Siband *et al*. (1999) observed that newly formed yield component decreased when the earlier formed component increased. This phenomenon was found in non stresses condition but in stress condition a compensatory relationship was observed in maize (*Zea mays* L.) between two successively formed yield components.

Munns, (2002) discussed that the magnitude of salt induced yield losses could not be attributed only to single factor rather different physiological, biochemical factors at different stages of rice plants may be involved.

Nahar and Hasanuzzaman (2009) showed an application of 250 mM NaCl decreased 77, 73 and 66% yield in BARI mung-2, BARI mung-5 and BARI mung-6, respectively over control.

The reduction of yield and its components under salt stress condition may be attributed to low production, expansion, senescence and physiologically less active green foliage (Wahid *et al*., 1997) and thus reduced photosynthetic rate might be a supplementary effect (Seemann and Critchley, 1985).

In rice varieties, grain yield, which is the ultimate product of yield components, is greatly influenced by salinity levels. The loss of grain yield due to 150mM salinity was 50, 38, 44 and 36% over control for the cultivars BR11, BRRI dhan41, BRRI dhan44 and BRRI dhan46, respectively (Hasanuzzaman *et al*., 2009).

The severe inhibitory effects of salts on fertility may be due to differential competition in carbohydrate supply between vegetative growth and constrained supply of these to the developing panicles (Murty and Murty, 1982). Also reduced viability of pollen under stress condition could result in failure of seed set (Abdullah *et al*., 2001).

Greenway and Munns (1980) observed that at 200 mM NaCl, sugar beet (a salt-tolerant species) might have a reduction of only 20% in dry weight, cotton (a moderately tolerant) might have a 60% reduction, and as a sensitive species soybean might be dead.

In contrast, a halophyte such as *Suaeda maritima* (L.) might be growing at its optimum rate under salinity (Flowers *et al*., 1986).

In one of the recent studies on *F. vulgare*, it has been shown that yields and plant growth parameters including plant height, fresh weight yield and biomass were affected significantly by increasing irrigation water salinities (Semiz *et al*. 2012).

2.8 Effect of salt stress on wheat plants

Kahrizi *et al*. (2013) carried out an experiment with three salinity levels as control, 60 and 120 mM and ten durum wheat cultivars including Boomer, PGS, 71135, 61130, 605, C1351, KND, KDM, Haurani and G1252. Results showed that interaction of salinity and cultivars was only significant for number of grains per spike and grain weight per spike. It means that any stress during vegetative growth stages can affect yield through reduction in source to sink ratio.

Turki *et al*. (2012) conducted an experiment among 55 varieties and accessions of common and durum wheat (16 winter wheat varieties and 39 spring wheat accessions). The results showed that salt treatment (100 mM of NaCl solution) depressed growth and yield production in 45 common and durum wheat varieties. 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.

Hameed *et al*. (2009) conducted an experiment with two wheat genotypes differing in salt tolerance and observed that the 3 days old wheat seedlings were subjected to 5, 10 and 15 dSm^{-1} NaCl salinity for 6 days; application of low salinity ($5dSm^{-1}$) growth was suppressed even in tolerant genotype.

Rahman *et al*. (2008) conducted an experiment with four cultivars of wheat (*Triticum aestivum* L.) to NaCl salinity treatments measuring 0.00, -2.457, -4.914 and -14.742 bars at germination and early seeding growth stage. They observed that water uptake and germination decreased in all cultivars. Increased salt concentration also affected the early seedling growth.

Flagella *et al*. (2000) evaluated the effect of salinity on grain yield and yield components of durum wheat cv. Duilio subjected to the salinity levels of 0.5, 6, 12, 18 and 24 dSm^{-1} in a growth chamber. With regard to photosynthesis and grain yield, durum wheat was moderately resistant to salinity showing significant damages only when irrigation water with EC of 12 dsm^{-1} or higher was used.

Tammam *et al*. (2008) conducted a pot experiment with salt tolerance wheat cv. Banysoif-1. Seedlings were irrigated by different saline waters (0, 60, 120, 180, 240 and 320 mM NaCl). They observed that fresh and dry weight of roots was unchanged up to the level of 120 mM NaCl then a significant reduction obtained at 240 and 320 mM NaCl. In shoots and spikes, dry matters were either unchanged or even stimulated to increase toward 180 mM NaCl then a quick reduction was observed.

Islam and Salam (1996) conducted a pot experiment with the variety Pokkali, BINA 19, BINA 13 and IRATOM 24 were grown in nutrient solutions with different salinity levels

(control, 0.9% NaCl). The biomass of BINA 19 was not affected with increased salinity. The biomass of Pokkali and IRATOM 24 decreased with increase in salinity.

Gupta and Shrivastava (1989) also observed in a sand culture trial that the effects of ionic osmotic stress alone and in combination with NaCl, to wheat cultivars differed significantly. They observed Karicha-65 (tolerant) was superior to Kalayansona (susceptible) in maintaining higher leaf area and root growth under both types of stress. They had the opinion the salinity stress was less injurious than osmotic ionic stress.

2.9 Effect of mannitol on growth of plants

Mannitol is a six-carbon, non-cyclic sugar-alcohol having a role in storage of energy, regulation of coenzymes and osmoregulation. It is naturally synthesized in many plant species, while is absent in wheat (Stoop *et al*., 1996). It is known to function as scavenger of reactive oxygen species (ROS); therefore, it overcomes the peroxidation of lipids and consequent cell damage (Stoop *et al*., 1996).

The improved growth of the *mtlD* gene-contained transgenic wheat genotype plants can be explained also on the basis of the bacterial, *E. coli, mtlD* gene-accumulating mannitol and soluble sugars (Ramadan *et al*., 2013).

Studies using transgenic tobacco (*Nicotiana tabacum*) and Arabidopsis also showed improved growth of mannitol- accumulating plants under stress (Thomas *et al*., 1995).

Slama *et al*. (2007) demonstrated that mannitol increases the total carbohydrates (source of energy) and mineral content of *Sesuvium portulacastrum*, a halophyte, so that plant growth parameters especially total leaf area and leaf number also increases.

2.10 Effect of mannitol in improving salt stress tolerance

Mannitol has a dual function in stress protection, both by facilitating osmotic adjustment and by supporting redox control (Rathinasabapathi, 2000). Under stress, soluble sugars and sugar-like compounds (e.g. mannitol, sorbitol, etc.) may assist in osmotic adjustments as well as in membrane and protein stabilization (Amiard *et al*., 2003). The

production of mannitol may also confer higher carbon utilization efficiency (Stoop *et al*., 1996), increased oxidative stress tolerance (Keunen *et al*., 2013) and salt stress tolerance.

Biosynthesis and accumulation of mannitol and sugars in plants are correlated with salt-stress tolerance of plants (Matros *et al*., 2015). These solutes are believed to function as protectors or stabilizers of enzymes or membrane structures that are sensitive to dehydrations or ionically induced damage.

Various transgenic plant species accumulating varying levels of mannitol and soluble sugars in their tissues have been shown to be tolerant to different abiotic stress types, including salinity (Van den Ende and El-Esawe, 2014), assuring that the levels of mannitol and soluble sugar fractions synthesized and accumulated in the transgenic tissues were abundant enough to impart osmoprotection via compatible solute mechanism.

Chiang *et al*. (2005) indicated that fructose, glucose and sucrose are important substrates in plant metabolism to enhance tolerance of the high mannitol containing plants to salt-stress.

Hu *et al*. (2005) pointed out that the transgenic plants were better able than wild-type plants to maintain cell membrane integrity under salt stress, which supports the hypothesis that proline, mannitol and soluble sugars serve as a protective function.

Tarczynski *et al*. (1993) demonstrated that the mannitol- accumulating, transformed tobacco exhibited greater salt tolerance than non transformed tobacco. Growth of the non transformed tobacco, in the presence of 250 mM NaCl in hydroponic nutrient culture, was strongly reduced compared to that of transformed mannitol- containing tobacco. These experiments clearly and unequivocally establish that mannitol can promote salt tolerance in higher plants.

Cellular accumulation of mannitol as a result of the expression of the *mtl*D gene protects transgenic wheat (*Triticum aestivum* L.) from the harmful effects of both soil waterlogging and salinity (Abebe *et al*., 2003).

A bacterial mannitol-1-phosphate dehydrogenase gene was targeted to tobacco chloroplasts and the resistance to oxidative stress in transgenic tobacco was improved, evidently as a result of mannitol accumulation (Shen *et al*., 1997*a*).

Exogenous application of organic compounds such as mannitol have been found to be beneficial in ameliorating the adverse effects of salt stress on leaf photosynthetic pigments coupled with enhanced biomass production (Ali *et al*., 2007).

Mitoi *et al*. (2009) reported that foliar applications of mannitol and thiourea are effective in reducing the adverse effects of oxidative stress on biological membranes by acting as a scavenger of ROS (reactive oxygen species).

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during 17 November, 2018 to 6 March, 2019. The materials and methods those were used and followed for conducting the experiment have been presented under the following headings.

3.1 Description of the experimental site

3.1.1 Experimental site

This study was conducted under the shed house of Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The location of the experimental site is 23°74′ N latitude and 90°35′ E longitude at an altitude of 8.6 meter above the sea level.

3.1.2 Characteristics of soil

The soil of the experimental area belongs to the Modhupur Tract under AEZ No. 28. The characteristics of the soil under the experiment were analyzed in the Laboratory of Soil Resources Development Institute (SRDI), Dhaka (Appendix II).

3.1.3 Climate

The climate of the experimental site was subtropical, characterized by the winter season from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October.

3.2 Experimental details

3.2.1 Planting materials

The variety BARI Gom-25 was used as planting material. The seeds were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

3.2.2 Experimental design and lay out

The experiment was laid out in a Randomized Complete Block Design (RCBD) having one factor with three replications. The treatments of the experiment were assigned at random into 27 pots of size with 30cm × 20cm.

3.2.3 Treatments

1. T_1 = Control
2. T_2 = Mannitol 15 mM (M_1)
3. T_3= Mannitol 30 mM (M_2)
4. T_4 = 4 dS m^{-1} NaCl (S_1)
5. T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)
6. T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)
7. T_7 = 8 dS m^{-1} NaCl (S_2)
8. T_8 = 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)
9. T_9 = 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

3.2.4 Collection and preparation of soil

The soil of the experiment was collected from Sher-e-Bangla Agricultural University (SAU) farm. The soil was non-calcarious Red Brown Terrace soil with loamy texture belonging to the AEZ Madhupur Tract. The collected soil was pulverized and inert materials, visible insect pest and plant propagules were removed. The soil was dried in the sun, crushed carefully and thoroughly mixed.

3.2.5 Application of manure and fertilizer in the pots

The collected soil was well pulverized and dried in the sun and required amount of decomposed cowdung was mixed with the soil. Each pot was filled up with 12 kg soil. A basal dose of triple super phosphate (TSP), muriate of potash (MoP) and gypsum were used as the source of phosphorus, potassium and sulphur applied at the rate of 140kg ha^{-1}, 40kg ha^{-1} and 110kg ha^{-1}, respectively at the time of final pot preparation. Urea @ 180kg ha^{-1} was applied as 3 equal splits. One-third of urea and the whole amount of other fertilizers were incorporated with soil at final pot preparation before sowing. Rest of the

nitrogen were applied in two equal splits one at 30 days after transplanting (DAT) and second at 45 days after transplanting (DAT). Thereafter, the pots containing soil were moistened with water.

3.2.6 Preparation of nursery bed and seed sowing

Seedbed was prepared with 1m wide adding nutrients as per the requirements of soil. The seed were soaked with water for 24 hours, washed thoroughly in clean water, and incubated for sprouting and after 5 days the germinated seeds were sown in the wet seed bed. Seeds were sown on 17 October, 2018 in order to have seedlings of 30 days.

3.2.7 Transplanting of seedlings on pots and application of salinity stress

Thirty day's old seedlings were transplanted in the experimental plot on the 17 November, 2018. Healthy seedlings were uprooted carefully from the seed bed and two seedlings were transplanted in the respective pots. There were two hills in each pot. Two weeks after transplanting, the salt solutions were applied in required pot only. To avoid osmotic shock, salt solutions were added in three equal installments on alternate days until the expected conductivity was reached. The electrical conductivity (EC) of required pot was measured everyday with an EC meter and necessary adjustments were made by adding water.

3.2.8 Preparation and application of Mannitol solution

To prepare 15 mM mannitol (M_1) solution; 1.366 g mannitol was dissolved in 500 ml of water and for 30 mM mannitol (M_2) solution; 2.7326 g mannitol was dissolved in 500 ml of water. 1st spray with mannitol was done after 35 days of seedling transplanting and 2nd spray was done after 55 days of seedling transplanting.

3.3 Intercultural operations

After transplanting of the seedlings, different intercultural operations were carried out for better growth and development of the plant.

3.3.1 Weeding

Sometimes there were some small aquatic weeds observed in pots that were removed by hand pulling.

3.3.2 Irrigation and drainage

During cultivation, irrigation with saline water was done one day interval in required pot for creating salinity stress.

3.3.3 Plant protection measures

Plant protection measures were done whenever it was necessary.

3.3.4 Harvesting, threshing and cleaning

The wheat plant was harvested depending upon the maturity of grains and harvesting was done manually from each pot. Ten pre-selected hills per pot from which different data were collected and areas from middle portion of each pot was separately harvested and bundled, properly tagged and then brought to the threshing floor. Enough care was taken for harvesting, threshing and also cleaning of wheat seed. Then the plant samples were carried out to the laboratory. Fresh weight of grain and straw were recorded pot wise. Finally the weight was adjusted to a moisture content of 14%. The straw was sun dried and the yields of grain and straw pot^{-1} were recorded.

3.4 Collection of data

Data on the following parameters were recorded during the course of the experiment.

1. Plant height (cm)
2. Tillers number plant^{-1}
3. Leaf chlorophyll content (SPAD reading)
4. Filled grains spike^{-1}
5. Unfilled grains spike^{-1}
6. 1000- seed weight (g)
7. Dry weight of plant at harvest (g)
8. Grain yield plant^{-1} (g)

3.5 Procedure of sampling for growth parameters

3.5.1 Plant height

Plant height was measured from the ground level to the tip of the longest leaf/flag leaf by taking the average value of ten random samples, but before heading it was measured from base to tallest leaf tip

3.5.2 Tillers number plant^{-1}

Tillers number plant^{-1} was counted at 30, 60 and 90 DAT.

3.5.3 Leaf chlorophyll content (SPAD reading)

SPAD meter reading was collected at 60 and 90 DAT which indicates probable chlorophyll number present in a leaf. Three leaves were randomly selected from each pot. The top and bottom of each leaves were measured with SPAD meter as at leaf value, then it was averaged and total chlorophyll content was measured.

3.5.4 Filled grains spike^{-1}

Filled grains spike^{-1} of each plant was counted manually and averages and data were recorded from each pot.

3.5.5 Unfilled grains spike^{-1}

Unfilled grains spike^{-1} of each plant was counted manually and averages and data were recorded from each pot

3.5.6 1000-seed weight

1000-seed were counted, which were taken from the seed sample of each pot separately and then weighed in an electrical balance and data were recorded.

3.5.7 Dry weight of plant at harvest (g)

The total dry weight was recorded by drying the plants at 70 ± 2^{o}C for 72 hours and calculated from summation of leaves, stem, roots weight as observed in an electronic balance.

3.5.8 Grain yield plant^{-1} (g)

The grains from each plant were weighed by using an electrical balance with sun dried and oven dried.

3.6 Statistical analysis

The data in respect of growth, yield contributing characters and yield were statistically analyzed to find out the statistical significance of the experimental results. The means for all the treatments were calculated and the analyzed with the statistical software package Statistix-10. The significance of the difference among the means was evaluated by the least significant difference test (LSD) at 5% level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprised presentation and discussion of the results obtained from the study on the effect of mannitol on morphophysiology and yield of wheat plant under salinity. Data on different growth, yield contributing characters and yield of wheat were recorded. The results have been presented and discussed in different tables and graphs and possible interpretations are given under the following headings:

4.1 Plant height (cm)

The effect of mannitol on plant height (cm) of wheat in saline and non-saline conditions is shown in the table 1. From the experiment, it was found that mannitol had significant effect on plant height in saline treated plants. Results obtained that at 30 DAT the highest plant height (27.13 cm) was observed at treatment T_2 (Mannitol 15 mM) which was statistically similar to T_1 (control) and T_3 (Mannitol 30 mM) treatment. At 60 and 90 DAT the highest plant height (67.43 and 92.97 cm) was shown by T_3 (Mannitol 30 mM) which was similar to T_1 (control) and T_2 (Mannitol 15 mM) treatment. At harvest tallest plant (91.07 cm) was observed at T_1 (control) treatment which was also similar to T_2 (Mannitol 15 mM) and T_3 (Mannitol 30 mM) treatment.

The plant height was decreased under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment compared to the control plants in 30, 60, 90 DAT and at harvest stage. So, that's why the lowest plant height (16.20, 48.87, 71.47 and 69.40 cm at 30, 60, 90 DAT and at harvest respectively) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. Cell division, cell elongation and finally plant growth (plant height) become inhibited due to salinity in crop (Munns and Tester, 2008). Islam *et al*. (2011) also reported the decreased plant height as well as plant growth due to salinity inclusion.

The foliar application of mannitol (M_1 and M_2) to salinized wheat plants (S_1 and S_2) at various DAT caused a significant increase in plant height though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1)

for mitigating the salt stress induced reduction of plant height of wheat. These results were similar with the results found by Pujni *et al*. (2007) who demonstrated that under salt stressed conditions both *in vitro* and *in vivo* growth conditions rice seedling height was increased due to mannitol accumulation in different transgenic lines.

Table 1: Effect of mannitol on plant height of wheat in saline and non-saline conditions at different days after transplanting

Treatments	**Plant height (cm) at different days after transplanting (DAT)**			
	30 DAT	**60 DAT**	**90 DAT**	**At harvest**
T_1 (Control)	27.00 a	67.00 a	92.87 a	91.07 a
T_2 (M_1)	27.13 a	66.00 a	92.13 a	89.30 a
T_3 (M_2)	26.57 a	67.43 a	92.97 a	90.60 a
T_4 (S_1)	20.53 d	53.87 d	76.90 f	72.30 d
T_5 (S_1+M_1)	22.53 c	59.50 b	85.17 c	84.13 b
T_6 (S_1+M_2)	24.42 b	60.90 b	88.33 b	83.57 b
T_7 (S_2)	16.20 e	48.87 e	71.47 g	69.40 d
T_8 (S_2+M_1)	19.50 d	53.47 d	80.37 e	79.53 c
T_9 (S_2+M_2)	20.53 d	56.53 c	82.00 d	81.67 bc
LSD (0.05)	**1.82**	**2.25**	**1.22**	**3.15**
CV (%)	**4.64**	**2.20**	**0.83**	**2.21**

In a column, figures, bearing same letter(s) do not differ significantly at 5% level of significance

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.2 Tillers number plant^{-1}

Table 2 shows the influence of mannitol on tillers number plant^{-1} of wheat under both saline and non-saline conditions. From the experiment, it was observed that mannitol had significant effect on tillers number in saline treated plants. Results demonstrated that at 30 DAT the maximum number of tillers plant^{-1} (3.37) was obtained at treatment T_2 (Mannitol 15 mM) which was statistically similar to T_1 (control) and T_3 (Mannitol 30 mM) treatment. At 60 and 90 DAT the maximum number of tillers plant^{-1} (6.43 and 6.50) was obtained at treatment T_3 (Mannitol 30 mM) which was similar to T_1 (control) and T_2 (Mannitol 15 mM) treatment.

Number of tillers plant^{-1} was decreased under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment compared to the control plants in 30, 60 and 90 DAT. So, that's why the minimum number of tillers plant^{-1} (1.83, 2.40 and 2.30 at 30, 60 and 90 DAT respectively) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. This result agrees with Iqbal (2003) who also observed reduced number of tillers at higher level of soil salinity. Shazia *et al*. (2001) also found that the number of fertile tillers in wheat was significantly reduced with increasing salinity.

The foliar application of mannitol (M_1 and M_2) to salinized wheat plants (S_1 and S_2) at various DAT caused a significant increase in tillers number plant^{-1} though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for the improvement of salt stress induced reduction of tillers number plant^{-1} of wheat. These results were similar with the results of Abebe *et al*. (2003) who found that mannitol improves tillers number of wheat under salinity stress both at the callus and whole plant level.

Table 2: Effect of mannitol on tillers number plant^{-1} of wheat in saline and non saline conditions at different days after transplanting

Treatments	Tillers number plant^{-1} at different days after transplanting (DAT)		
	30 DAT	**60 DAT**	**90 DAT**
T_1 (Control)	3.20 a	6.37 a	6.43 a
T_2 (M_1)	3.37 a	6.30 a	6.37 a
T_3 (M_2)	3.30 a	6.43 a	6.50 a
T_4 (S_1)	2.37 c	3.00 e	2.93 e
T_5 (S_1+M_1)	2.83 b	5.03 c	5.13 c
T_6 (S_1+M_2)	2.87 b	5.30 b	5.47 b
T_7 (S_2)	1.83 d	2.40 f	2.30 f
T_8 (S_2+M_1)	2.40 c	4.50 d	4.70 d
T_9 (S_2+M_2)	2.40 c	4.47 d	4.57 d
$LSD_{(0.05)}$	**0.22**	**0.21**	**0.29**
CV (%)	**4.52**	**2.50**	**3.48**

In a column, figures, bearing same letter(s) do not differ significantly at 5% level of significance

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.3 Leaf chlorophyll content (SPAD reading)

The data pertaining to effect of mannitol on leaf chlorophyll content (SPAD reading) at various DAT (60 and 90 DAT) of wheat in saline and non-saline conditions is depicted in figure 1. Statistically significant variation was observed for leaf chlorophyll content due to application of mannitol in saline treated plants at various DAT. Results indicated that at 60 DAT the maximum leaf chlorophyll content (53.67 SPAD reading) was observed at treatment T_2 (Mannitol 15 mM) which was statistically similar to T_1 (control) and T_3 (Mannitol 30 mM) treatment. At 90 DAT the maximum leaf chlorophyll content (47.00

SPAD reading) was observed at T_1 (control) treatment which was also similar to T_2 (Mannitol 15 mM) and T_3 (Mannitol 30 mM) treatment.

Leaf chlorophyll content was decreased under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment compared to the control plants in 60 and 90 DAT. So, that's why the minimum leaf chlorophyll content (33.67 and 30.67 SPAD reading at 60 and 90 DAT respectively) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. Leaf chlorophyll content decreased with increased level of salinity. The inhibitory effect of the accumulated ions of salts on the biosynthesis of the different chlorophyll fractions might be reason behind the reduction in chlorophyll content. The result was in line with that of Jiang *et al*. (2017) who also reported the decreasing trend of leaf chlorophyll content (SPAD reading) with an increase in salinity levels. Ashraf and McNeilly (1988) also stated that the salinity significantly reduces the total chlorophyll content and the degree of reduction in total chlorophyll depends on salt tolerance of plant species and salt concentrations.

The foliar application of mannitol (M_1 and M_2) to salinized wheat plants (S_1 and S_2) at various DAT (60 and 90 DAT) caused a significant increase in leaf chlorophyll content of wheat though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for the increment of salt stress induced reduction of leaf chlorophyll content of wheat. Similarly, Kaya *et al*. (2013) found that exogenously applied mannitol increased leaf chlorophyll contents in the salt-stressed plants compared to those of the salt stressed plants which were not supplied with mannitol.

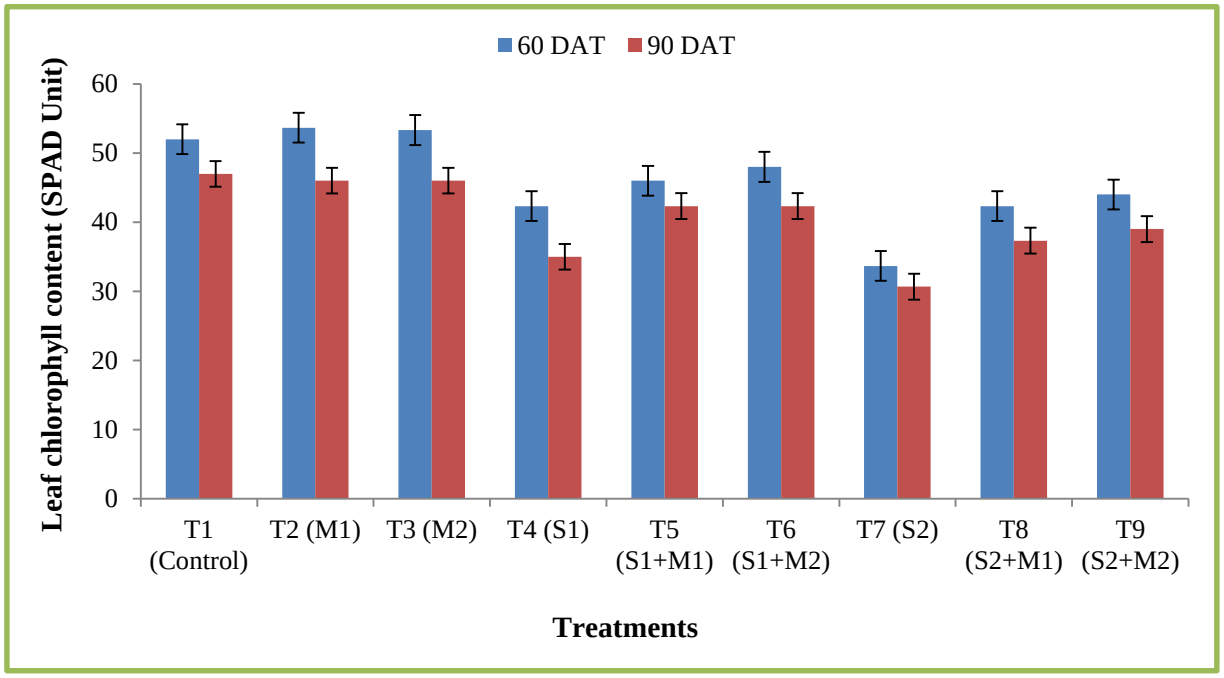

Figure 1: Effect of mannitol on leaf chlorophyll content of wheat in saline and non-saline conditions at different days after transplanting (LSD $_{(0.05)}$ = 1.22 and 1.05 at 60 and 90 DAT respectively)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.4 Filled grains spike^{-1}

Figure 2 shows the effect of mannitol on filled grains spike^{-1} of wheat under both saline and non-saline conditions. Statistically significant variation was observed for filled grains spike^{-1} due to application of mannitol in saline treated plants. Results showed that the maximum number of filled grains spike^{-1} (52.03) was obtained at T_1 (control) treatment which was statistically similar to T_2 (Mannitol 15 mM) and T_3 (Mannitol 30 mM) treatment.

Filled grains spike^{-1} was decreased under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment compared to the control plants. For this reason the minimum number of filled grains spike^{-1} (30.43) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. Similar result was found by Ashraf and Parveen (2002) who also reported that increase in NaCl concentration decreased the number of filled grains per spike. Bilkis *et al*. (2016) also showed that filled grains spike^{-1} was decreased due to salinity stress.

Mannitol application as foliar spray at different doses (M_1 and M_2) to salinized wheat plants (S_1 and S_2) caused a significant increase in filled grains spike^{-1} of wheat plants though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for mitigating the salt stress induced reduction of filled grains spike^{-1} of wheat. These results were similar with the results of El-Yazal *et al*. (2016) who found that mannitol accumulated transgenic plants showed increased number of grains spike^{-1} under salinity stress compared to the plants without mannitol accumulation.

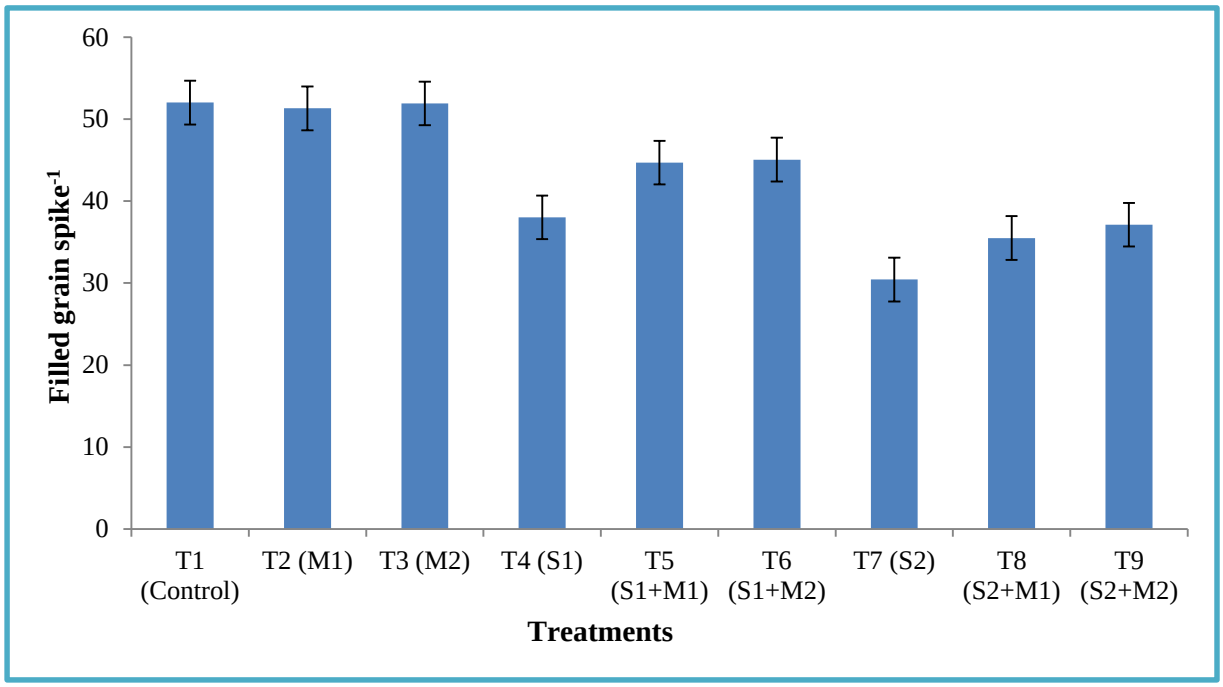

Figure 2: Effect of mannitol on filled grains spike^{-1} of wheat in saline and non-saline conditions (LSD $_{(0.05)}$ = 1.05)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.5 Unfilled grains spike^{-1}

The effect of mannitol on unfilled grains spike^{-1} of wheat under both saline and non-saline conditions is presented in figure 3. Statistically significant variation was observed for unfilled grains spike^{-1} due to application of mannitol in saline treated plants. Results showed that the minimum number of unfilled grains spike^{-1} (5.00) was obtained at T_1 (control) treatment which was statistically similar to T_2 (Mannitol 15 mM) and T_3 (Mannitol 30 mM) treatment.

Unfilled grains spike^{-1} was increased under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment. For this reason the maximum number of unfilled grains

spike^{-1} (21.87) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. Mannitol application as foliar spray at different doses (M_1 and M_2) to salinized wheat plants (S_1 and S_2) caused a significant decrease in unfilled grains spike^{-1} of wheat plants though the values were still higher than those of the control plants.

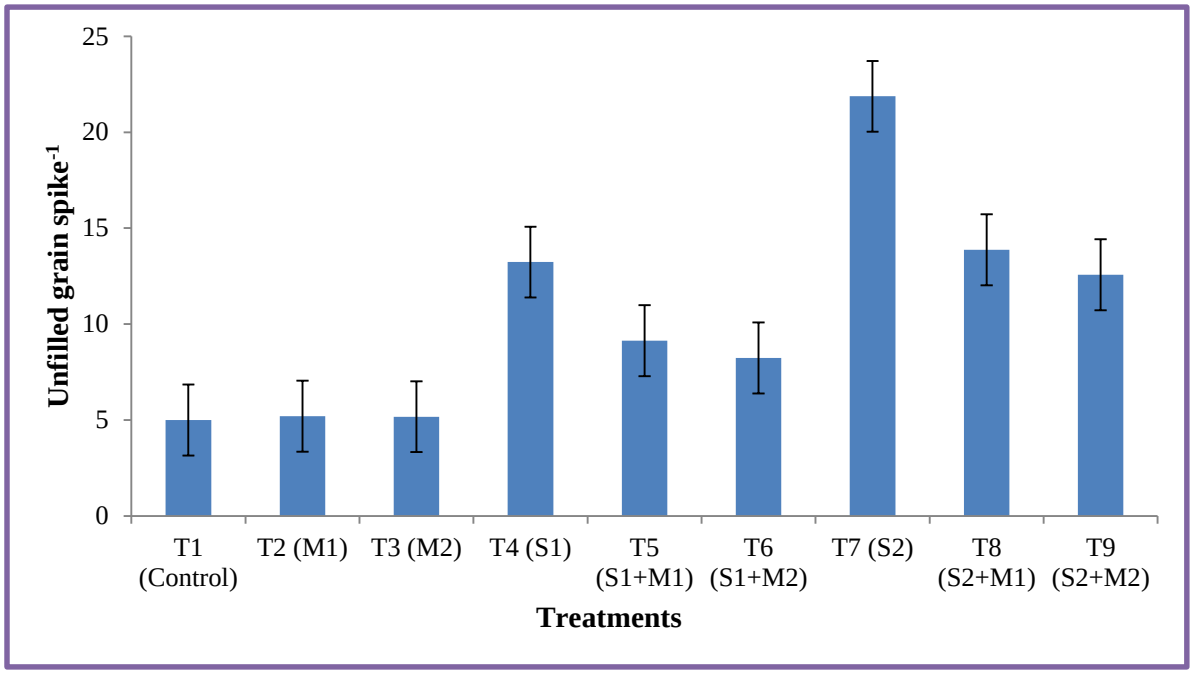

Figure 3: Effect of mannitol on unfilled grains spike^{-1} of wheat in saline and non-saline conditions (LSD $_{(0.05)}$ = 2.17)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.6 1000- seed weight (g)

Data regarding the effect of mannitol on 1000 seed weight (g) of wheat under both saline and non-saline conditions is placed in Figure 4. Statistically significant variation was found for 1000 seed weight due to application of mannitol in saline treated plants. Results indicated that the highest value of 1000 seed weight (46.67 g) was recorded from treatment T_2 (Mannitol 15 mM) which was statistically similar to T_1 (control) and T_3 (Mannitol 30 mM) treatment.

Under saline conditions at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment, 1000 seed weight was decreased compared to the control plants. For this reason the lowest value of 1000 seed weight (27.60 g) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. A significant decrease in 1000-grain weight was reported in both tolerant and sensitive cultivars of wheat seedlings under salinity conditions (Afzal *et al*., 2013). Hassan (2010) also found that grain mass was reduced with the increased salinity level.

Application of mannitol as foliar spray at different doses (M_1 and M_2) to salinized wheat plants (S_1 and S_2) caused a significant increase in 1000 seed weight of wheat plants though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for the improvement of salt stress induced reduction of 1000- seed weight of wheat. Similarly, it was found that 1000 grain weight of wheat was increased under salinity stress due to mannitol accumulation in plants than the plants without mannitol accumulation at the same stress (El-Yazal *et al*., 2016).

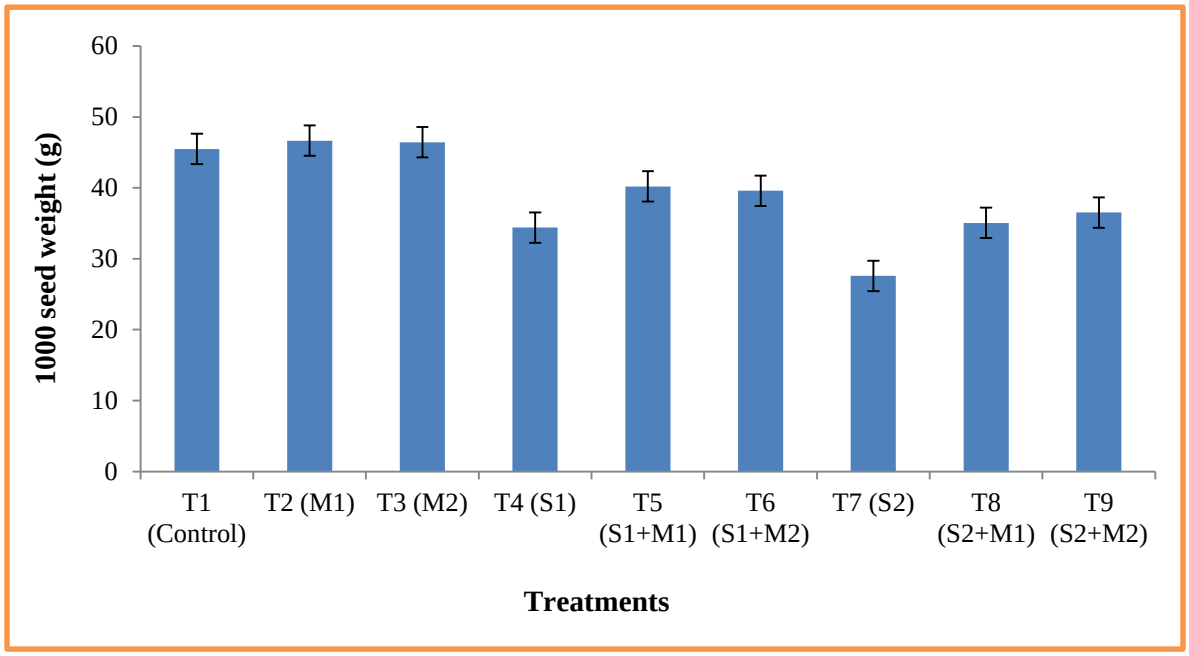

Figure 4: Effect of mannitol on 1000 seed weight of wheat in saline and non-saline conditions (LSD $_{(0.05)}$ = 1.30)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.7 Dry weight of plant at harvest (g)

Data regarding the effect of mannitol on dry weight (g) of wheat plant under both saline and non-saline conditions is depicted on figure 4. Statistically significant variation was obtained for dry weight of plant due to application of mannitol in saline treated plants. Results indicated that the highest dry weight of plant (17.20 g) was recorded from treatment T_2 (Mannitol 15 mM) which was statistically similar to T_1 (control) and T_3 (Mnannitol 30 mM) treatment.

Under saline conditions which was observed at T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment, dry weight of plant was decreased compared to the control plants. For this reason the lowest dry weight of plant (11.17 g) was recorded from T_7 (8 dS m^{-1}

NaCl) treatment. Similar results was found by Asgari et al. (2012) who also reported that dry weight of plant declined with the increasing level of salinity. Chhipa and Lal (1985) conducted a pot experiment and observed that salinity stress affected dry weight of wheat.

Application of mannitol as foliar spray at different doses (M_1 and M_2) to salinized wheat plants (S_1 and S_2) caused a significant increase in dry weight of plants though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for the increment of salt stress induced reduction of dry weight of wheat at harvest. The findings were similar with the findings of Anjum *et al*. (2011) who obtained that foliar application of mannitol significantly mitigated the salt stress-induced decrease in biomass production in maize plants.

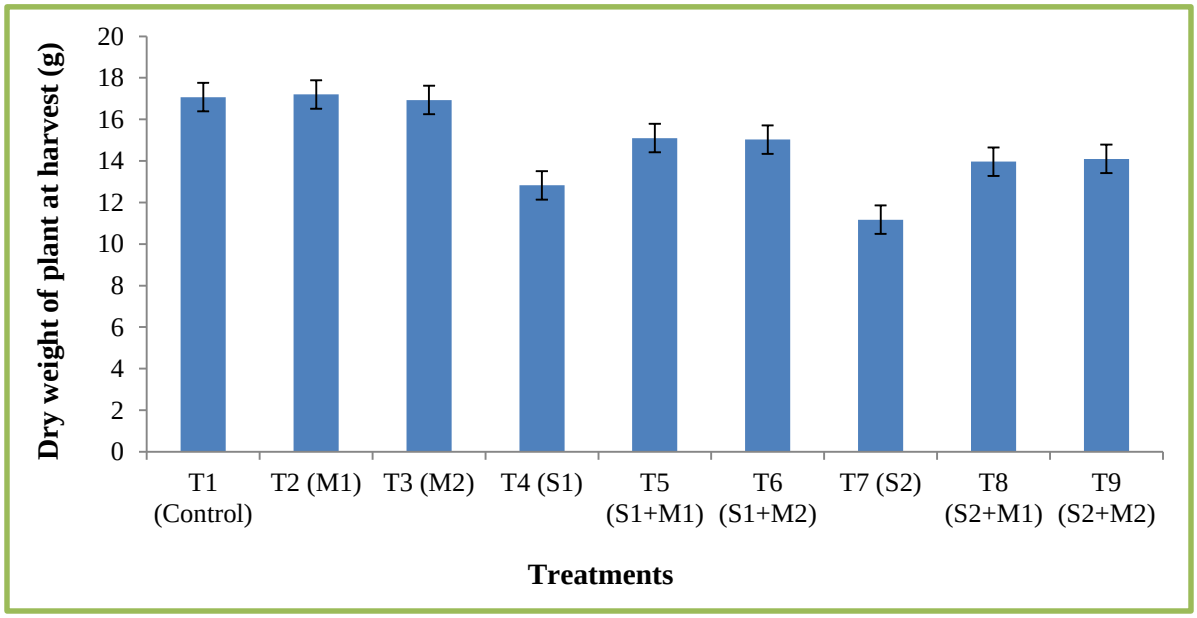

Figure 5: Effect of mannitol on dry weight of wheat at harvest in saline and non-saline conditions (LSD $_{(0.05)}$ = 0.35)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

4.8 Grain yield plant^{-1} (g)

The data pertaining to effect of mannitol on grain yield plant^{-1} (g) of wheat under both saline and non-saline conditions is depicted in figure 6. Statistically significant variation was obtained for grain yield plant^{-1} due to application of mannitol in saline treated plants. Results obtained that the maximum grain yield plant^{-1} (12.87 g) was obtained at T_1 (control) treatment which was statistically similar to T_2 (Mannitol 15 mM) and T_3 (Mannitol 30 mM) treatment.

When salinity was imposed to plants @ T_4 (4 dS m^{-1} NaCl) and T_7 (8 dS m^{-1} NaCl) treatment, grain yield plant^{-1} was decreased under this conditions compared to the control plants. For this reason the minimum grain yield plant^{-1} (4.50 g) was recorded from T_7 (8 dS m^{-1} NaCl) treatment. Similarly, Chinnusamy *et al*. (2005) also indicated that above the threshold level of salinity, wheat yield can reduce at a rate of 7.1% per dS m^{-1} increase of salinity. Abbas *et al*. (2013) also concluded that grain yield decreased significantly by saline conditions as compared to non saline conditions.

Mannitol application as foliar spray at different doses (M_1 and M_2) to salinized wheat plants (S_1 and S_2) caused a significant increase in grain yield plant^{-1} of wheat though the values were still lower than those of the control plants. Under the stress of S_1 (4 dS m^{-1} NaCl) and S_2 (8 dS m^{-1} NaCl), application of mannitol @ 30mM (M_2) was found superior over 15mM (M_1) for mitigating the salt stress induced reduction of grain yield plant^{-1} of wheat. Similarly, Ramadan *et al*. (2013) found that wheat plants grown in the presence of NaCl significantly reduced the yield but salinity did not affect the yield of mannitol accumulated transgenic plants.

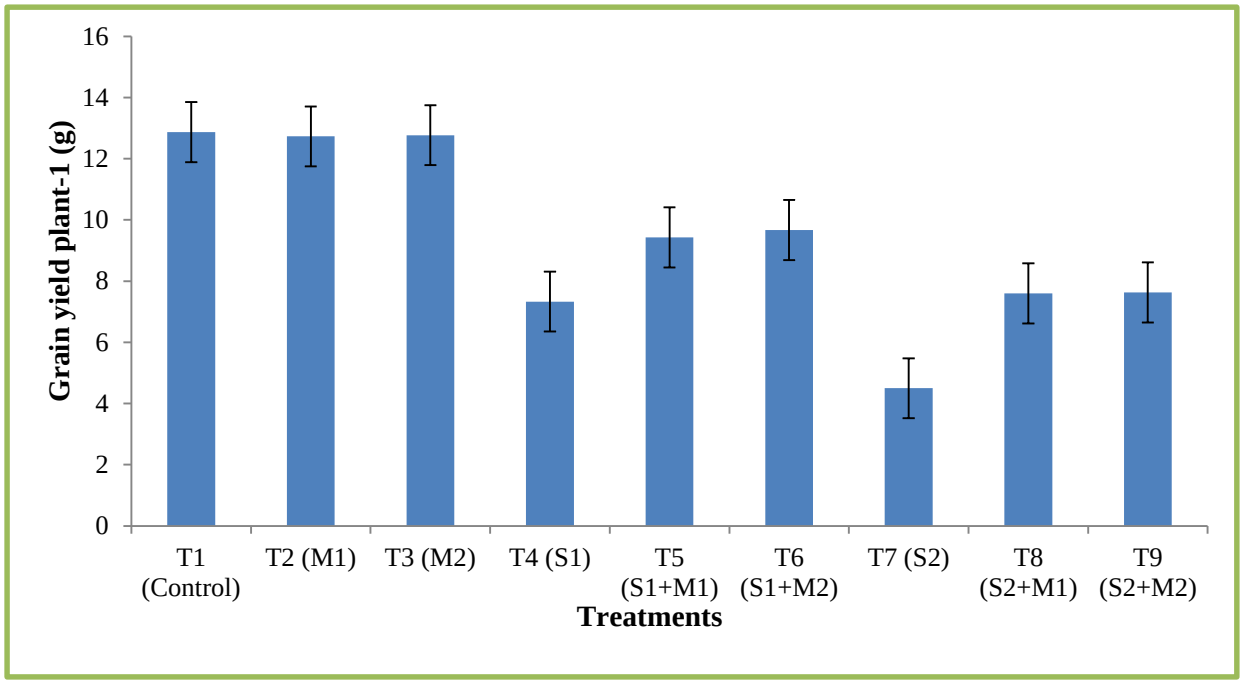

Figure 6: Effect of mannitol on grain yield plant^{-1} of wheat in saline and non-saline conditions (LSD $_{(0.05)}$ = 0.57)

T_1= Control

T_2= Mannitol 15 mM (M_1)

T_3= Mannitol 30 mM (M_2)

T_4= 4 dS m^{-1} NaCl (S_1)

T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1)

T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2)

T_7= 8 dS m^{-1} NaCl (S_2)

T_8= 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1)

T_9= 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2)

CHAPTER V

SUMMARY AND CONCLUSION

The present work was conducted in the net house of the Department of Agricultural Botany of Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period of November-March, 2018-2019 to find out the effect of mannitol on morphophysiology and yield of wheat plant under salinity. BARI Gom-25 was used as a test crop. The experiment consists of nine treatments viz. T_1 = Control, T_2 = Mannitol 15 mM (M_1), T_3= Mannitol 30 mM (M_2), T_4 = 4 dS m^{-1} NaCl (S_1), T_5 = 4 dS m^{-1} NaCl + Mannitol 15 mM (S_1+M_1), T_6 = 4 dS m^{-1} NaCl + Mannitol 30 mM (S_1+M_2), T_7 = 8 dS m^{-1} NaCl (S_2), T_8 = 8 dS m^{-1} NaCl + Mannitol 15 mM (S_2+M_1) and T_9 = 8 dS m^{-1} NaCl + Mannitol 30 mM (S_2+M_2). The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data on different growth, physiological and yield contributing parameters of BARI Gom-25 were recorded. The collected data were statistically analyzed for the evaluation of the treatment effect. The observation was made on plant height (cm), tillers number $plant^{-1}$, leaf chlorophyll content (SPAD reading), filled grains $spike^{-1}$, unfilled grains $spike^{-1}$, 1000-seed weight (g), dry weight of plant at harvest (g) and grain yield $plant^{-1}$ (g).

In this experiment, wheat plants were exposed to different levels of salinity. Plants exposed to maximum levels of salinity resulted in the lowest value of the studied parameters. Plants in non-saline and control treatments showed the highest value of the studied parameters. Mannitol was applied in both saline and non-saline treated plants. In non-saline treated plants, the effect of mannitol (M_1 and M_2) on different growth, physiological, yield contributing and yield parameters of BARI Gom-25 was found statistically similar effect to the effect of control treatments. In saline treated plants, application of mannitol (M_1 and M_2) significantly increased the value of the studied parameters which were decreased under salinity stress. Mannitol application @ 30mM (M_2) was found best over 15mM (M_1) for mitigating the salt stressed reduction in the value of studied parameters of wheat.

At 30 DAT the highest plant height (27.13 cm) was observed at treatment T_2. At 60 and 90 DAT the highest plant height (67.43 and 92.97 cm) was shown by treatment T_3. At harvest tallest plant (91.07 cm) was observed at T_1 (control) treatment. The lowest plant height (16.20, 48.87, 71.47 and 69.40 cm at 30, 60, 90 DAT and at harvest respectively) was recorded from T_7 treatment. At 30 DAT the maximum number of tillers plant^{-1} (3.37) was obtained at treatment T_2. At 60 and 90 DAT the maximum number of tillers plant^{-1} (6.43 and 6.50) was obtained at treatment T_3. The minimum number of tillers plant^{-1} (1.83, 2.40 and 2.30 at 30, 60 and 90 DAT respectively) was recorded from T_7 treatment. At 60 DAT the maximum leaf chlorophyll content (53.67 SPAD reading) was observed at treatment T_2. At 90 DAT the maximum leaf chlorophyll content (47.00 SPAD reading) was observed at T_1 (control) treatment. The minimum leaf chlorophyll content (33.67 and 30.67 SPAD reading at 60 and 90 DAT respectively) was recorded from T_7 treatment. The maximum number of filled grains spike^{-1} (52.03) was obtained at T_1 (control) treatment. The minimum number of filled grains spike^{-1} (30.43) was recorded from T_7 treatment. The minimum number of unfilled grains spike^{-1} (5.00) was obtained at T_1 (control) treatment. The maximum number of unfilled grains spike^{-1} (21.87) was recorded from T_7 treatment. The highest value of 1000 seed weight (46.67 g) was recorded from treatment T_2. The lowest value of 1000 seed weight (27.60 g) was recorded from T_7 treatment. The highest dry weight of plant (17.20 g) was recorded from treatment T_2. The lowest dry weight of plant (11.17 g) was recorded from T_7 treatment. The maximum grain yield plant^{-1} (12.87 g) was obtained at T_1 (control) treatment. The minimum grain yield plant^{-1} (4.50 g) was recorded from T_7 treatment.

Conclusions

Considering the above results it may be concluded that different growth, physiological, yield contributing and yield parameters of BARI Gom-25 was decreased by the increase of salinity levels. Application of mannitol to salinized wheat plants resulted in significant improvement of the studied parameters. But the effect of mannitol to non-salinized plants was similar to the effect of control plants.

Recommendation

Mannitol application @ 30mM performed best over 15mM for mitigating the damaging effect of salinity on different growth, physiological, yield contributing and yield parameters of wheat.

CHAPTER VI

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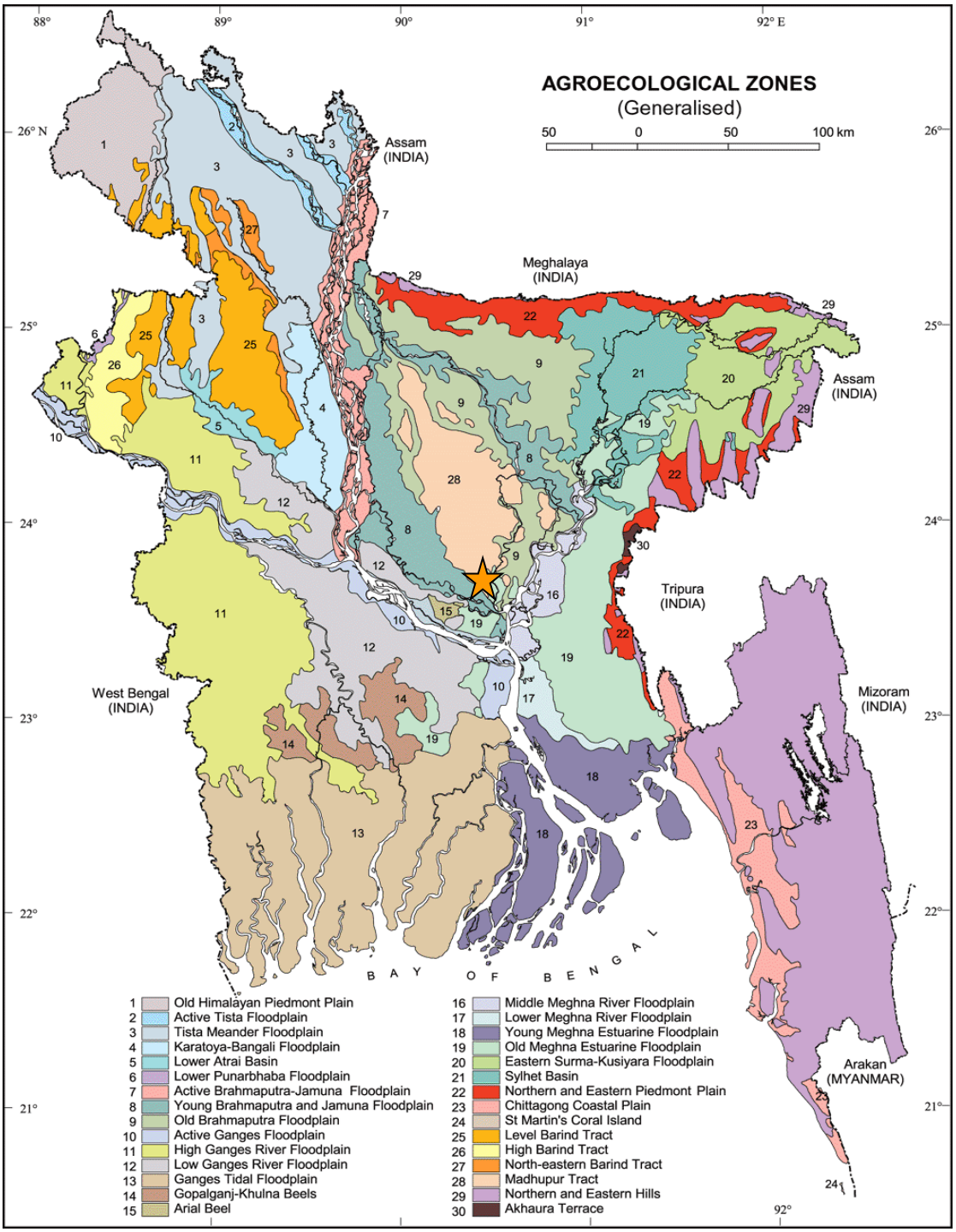
APPENDICES

Appendix I: Map showing the experimental sites under study



Appendix II: Characteristics of soil of experimental field

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Sher-e-Bangla Agricultural University Research Farm, Dhaka
AEZ	AEZ-28, Madhupur Tract
General Soil Type	Shallow Red Brown Terrace Soil
Land type	Medium high land
Soil series	Tejgaon
Topography	Fairly leveled

B. The initial physical and chemical characteristics of soil of the experimental site (0 - 15 cm depth)

Physical characteristics	
Constituents	**Percent**
Sand	25
Silt	43
Clay	29
Textural class	Silty clay
Chemical characteristics	
Soil characters	**Value**
pH	6.1
Organic carbon (%)	0.48
Organic matter (%)	0.86
Total N (%)	0.09
Available P (ppm)	21.56
Exchangeable K (me/100 g soil)	0.14

Source: Soil Resource and Development Institute (SRDI), Farmgate, Dhaka

Appendix III: Monthly record of air temperature, relative humidity and total rainfall of the experimental site during the period from November 2018 to April 2019

Month (2018-19)	Air temperature (degrees Celsius)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
November	29.20	18.75	45.12	38
December	30.10	17.56	44.11	40
January	31.56	19.56	49.33	45
February	32.79	23.38	63.48	166
March	34.07	25.42	67.83	185
April	34.75	26.70	74.57	375

Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka- 1212

Appendix IV: Analysis of variance of the data on plant height of wheat as influenced by the effect of mannitol under salinity

Source of variation	Degrees of freedom	Mean square of plant height (cm)			
		30 DAT	60 DAT	90 DAT	At harvest
Replication	2	8.3981	7.314	31.041	5.379
Treatment	8	44.2304**	132.370**	174.650**	177.785**
Error	16	1.1090	1.695	0.498	3.325

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix V: Analysis of variance of the data on tillers number plant^{-1} of wheat as influenced by the effect of mannitol under salinity

Source of variation	Degrees of freedom	Mean square of tillers number plant^{-1}		
		30 DAT	60 DAT	90 DAT
Replication	2	0.24481	0.37444	0.19444
Treatment	8	0.80037**	6.31417**	6.82750**
Error	16	0.01523	0.01486	0.02944

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VI: Analysis of variance of the data on leaf chlorophyll content of wheat as influenced by the effect of mannitol under salinity

Source of variation	Degrees of freedom	Mean square of leaf chlorophyll content	
		60 DAT	90 DAT
Replication	2	36.704	3.3704
Treatment	8	125.759**	93.2037**
Error	16	0.495	0.3704

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix VII: Analysis of variance of the data on yield contributing and yield parameters of wheat as influenced by the effect of mannitol under salinity

Source of variation	Degrees of freedom	Mean square of yield contributing and yield parameters				
		Dry weight of plant (g)	Filled grain spike^{-1}	Unfilled grain spike^{-1}	1000 Seed weight (g)	Grain yield plant^{-1} (g)
Replication	2	2.9233	16.018	4.4404	0.241	0.2248
Treatment	8	12.6908**	191.696**	92.2731**	123.993**	25.9415**
Error	16	0.0421	0.372	1.5704	0.567	0.1098

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

PLATES

Plate 1: Tillering stage

Plate 2: Heading and flowering stage

Plate 3: Experimental pots with tag

Plate 4: Ripening and maturity stage

Plate 5: Data collection

Plate 6: Harvesting