MANNITOL INDUCED SALT TOLERANT CAPACITY OF WHEAT UNDER SALT STRESS CONDITION

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CERTIFICATE

This is to certify that the thesis entitled, "**Mannitol Induced Salt Tolerant Capacity of Wheat Under Salt Stress Condition**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **Master of Science in Agronomy,** embodies the result of a piece of bona fide research work carried out by **Mst. Rumana Rahman** bearing Registration No. 11-04243 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.

Date:

Place: Dhaka, Bangladesh

Prof. Dr. Md. Abdullahil Baque
Research Supervisor


Dedicated To
My Beloved Parents

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The Authoress

MANNITOL INDUCED SALT TOLERANT CAPACITY OF WHEAT UNDER SALT STREES CONDITION

ABSTRACT

The experiment was conducted at The Central Laboratory of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from August to November 2016. Two experiments were conducted to study the mannitol induced salt tolerant capacity of wheat under salt stress condition. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Three wheat genotypes *viz*. V_1 (BARI Gom-28), V_2 (ESWYT-5) and V_3 (ESWYT-6) were used as test crop. Mannitol was used as priming chemicals and salt (NaCl) were used for salinity stress. Alcohol was used as surface sterilizer of wheat seeds. The data were recorded on germination percentage, root length, shoot length, root dry weight and shoot dry weight, fresh weight, turgid weight, relative water content, water retention capacity and vigour index. Data were analyzed using a computer software MSTAT-C. For the first experiment four levels of mannitol such as 2%, 4%, 6% and 8% were used for osmopriming and water was used for hydropriming. Among the genotypes, V_2 (ESWYT-5) gave the best results on studied parameters. Results revealed that the V_2 showed the highest germination rate (96.67%), shoot length (148.40 mm), root length (109.95 mm), shoot dry weight (9.65 mg), root dry weight (6.42 mg), fresh weight (264.55 mg), turgid weight (282.65 mg), relative water content (93.21%) and vigour index (43.04) primed with 2% mannitol solution for 9 hours where seeds without priming showed lowest results in respected parameters with the genotype V_1 (BARI Gom-28). In the second experiment, 2% mannitol primed (9 hours) seeds of wheat genotypes above mentioned with and without salt (NaCl) stress condition was evaluated. The salt stress levels (0 dS/m), 5dS/m, 10 dS/m, 15 dS/m and 20 dS/m were used during experimentation. It was observed that the genotype V_2 (ESWYT-5) with primed seeds placed without salt; (control) gave the best performance on respected parameters but under salinity stress, the highest germination rate (93.33%), shoot length (142.85 mm), root length (115.20 mm), shoot dry weight (11.66 mg) and root dry weight (8.01 mg), fresh weight (215.65 mg), turgid weight (268.55 mg), relative water content (88.03%) and vigour index (238.9) were achieved from V_2 primed seeds placed with 5 dS/m NaCl where V_1 primed seeds placed with 20 dS/m NaCl showed lowest results in respected parameters. Priming with water was more effective than control with regard to enhanced germination and growth parameters. From the result of the study, it was observed that seeds primed 2% mannitol for 9 hours showed the best result to compare with water primed and non-primed seed under salt stress condition.

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ABBREVIATIONS AND ACRONYMS

%	=	Percentage
AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSIR	=	Bangladesh Council of Scientific and Industrial Research
Ca	=	Calcium
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
e.g.	=	exempli gratia (L), for example
et al.,	=	And others
etc.	=	Etcetera
FAO	=	Food and Agriculture 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 square
mg	=	Miligram
ml	=	Mililitre
NaOH	=	Sodium hydroxide
No.	=	Number
^oC	=	Degree Celsius
P	=	Phosphorus
ROS	=	Reactive Oxygen Species
SAU	=	Sher-e-Bangla Agricultural University
USA	=	United States of America
USDA	=	United States Department of Agriculture
var.	=	Variety
viz.	=	Namely
WHO	=	World Health Organization
µg	=	Microgram

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) 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 ranks first in area (213600 thousand hectares) and third in production (576317 thousand metric tons) among the grain crops in the world (FAO, 2000). It is a staple food crop for more than one third of the world population (Shirazi *et al*., 2001). In Bangladesh, the area under wheat cultivation during 2015-2016 was about 1061602 acres producing 1302998 M. tons with an average yield of 1233 kg $acre^{-1}$ (BBS, 2016).

Various environmental stresses such as drought, cold, salinity causes heavy losses in agricultural production due to disruption in physiological and biochemical processes in plant. Salinity is major abiotic stressors which disrupt crop production. It creates an adversely impacts on the socio-economic condition of many developing countries like Bangladesh. In Bangladesh, over 30% of the net cultivable areas lies in the coastal zone close to the Bay of Bengal of which about 53% areas are affected by varying degrees of salinity (Haque, 2006). It has been reported that more than 1 million hectares of the coastal areas have been seriously affected by salinity (Rahman, 2007). Ali (2011) showed that the salt-affected areas in the coastal region of Bangladesh increased day by day. Agricultural land use in salt affected areas is very poor in respect of crop production (Petersen and Shireen, 2010). Most of the high yielding salt sensitive crop might not be adaptable 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 *et al*., 2008). Wheat crop is mainly cultivated in the north and north-western part of Bangladesh. A large area of cultivable land of the coastal region remains fallow and the dominant cropping pattern is fallow-aman-fallow. Introduction of wheat into the existing cropping pattern in the saline soil may become a costly effort to exploit these lands to meet up the food and nutritional balance of the over increasing population of Bangladesh.

Salinity reduces the growth of wheat plant by reducing the plants ability to absorb water from soil as root growth decreased under salt stress condition. Salinity also disturbs the physiology of plants by changing the metabolism of plants (Garg *et al.*, 2002). Wheat under saline conditions increases the concentration of proline and sugar resulting in significant increase of electrolyte leakage at 10 and 15 dSm^{-1} (Khatkar *et al.*, 2000). It has been reported that increase in salinity concentration brings about decrease in relative growth rate, net assimilation rate, K^+ and Ca^{2+} concentration, and grain yield of wheat, but causes an increase in Na^+ and Cl^- levels, this might be due to increase in Na^+/K^+ ratio in grain and straw at tillering stage (El-Hendawy *et al.*, 2005). Salinity affects wheat seedling growth by changing phytohormone levels (Shakirova *et al.*, 2003). Furthermore, salinity induction reduces in photosynthetic rate and stomatal conductance in wheat. Moreover, increased salinity induces a considerable reduction in height, number of fertile tillers and dry weight of shoots in wheat (Iqbal *et al.*, 2005). 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).

Water-deficit and salt affected soil are the major abiotic stresses, reducing wheat and rice crop productivity by more than 50% world-wide (Mahajan and Tuteja, 2005). Plant growth and developmental processes in terms of biochemical, physiological and morphological characteristics are inhibited by both water deficit and salt stresses (Parida and Das, 2005). Osmotic stresses derived from salt affected soil and water deficit conditions are well established in crop species (Luo *et al.*, 2005; Castillo *et al.*, 2007). In tolerant plants, there are many defense mechanisms such as osmoregulation, ion homeostasis, antioxidant and hormonal systems, helping plants to stay alive and development prior to their reproductive stages (Mahajan and Tuteja, 2005; Ashraf, 2010).

Mannitol, a member of sugar alcohols, is an osmotic adjustment chemical to control osmotic potential in the culture media or nutrient solutions in order to induce water deficit conditions for protein expression (Zang and Komatsu, 2007). Furthermore, sodium chloride salt, a small molecule with rapid dissolving and oxidizing by water into Na^+ and Cl^-, is generally selected as salt stress pressure (Vaidyanathan *et al.*, 2003; Cha-um et al., 2004; Cha-um et al., 2007a). As well as, the mannitol and NaCl

induced iso-osmotic stress in rice and wheat crop has been established (Hien *et al.,* 2003; Morsy *et al.,* 2007).

Salt stress leads to the suppression of plant growth and development, membrane leakage, ion imbalance or disequilibrium, enhanced lipid per oxidation and increased production of reactive oxygen species which are scavenged by both enzymatic and non-enzymatic reactions (Summart *et al.,* 2010; Zebarjadi *et al.,* 2010). 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; Ghasempour and Kianian, 2007).

In these perspective, the present study was carried out to evaluate the effect of mannitol on improving wheat salt tolerance in order to spread saline agriculture through wheat production.

Objectives of the research

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

1. To evaluate the effects of mannitol on changes of germination behavior, growth and morpho-physiology of wheat genotypes.
2. To investigate the effects of salinity and mannitol on changes of germination behavior, growth and morpho-physiology of wheat genotypes.

CHAPTER II

REVIEW OF LITERATURE

Salinity stress is a great problem in the coastal region of Bangladesh, where a vast area remains fallow for long time. Wheat is an important cereal crops in Bangladesh and it is a great source of carbohydrate and protein. The scientists of Bangladesh are conducting different experiments to adopt different crops in the saline area; wheat is one of them. Different treatments were applied before at different locations to overcome salt stress. External use of mannitol is one of them. Very limited research works have been conducted to adapt wheat in the saline area of Bangladesh. An attempt has been made to find out the performance of wheat at different levels of salinity. To facilitate the research works different literatures have been reviewed in this chapter under the following headings.

2.1 Effects of salinity on different wheat genotypes

Kahrizi *et al*. (2013) carried out a factorial experiment based on completely randomized design with three replications because of importance of durum wheat in human nutrition, identification of morphological and agronomic traits affecting tolerance to salt stress in order to use in selecting tolerant cultivars is essential. Treatments were salinity with three 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. Boomer was the tolerant cultivar in all salinity levels according to final grain weight and C1351 was the most sensitive one. On the other hand, PGS can be grown under sever saline soils because of it high performance under salinity, but under normal conditions does not produce high yield.

Turki *et al*. (2012) found that Salinity is a big constraint to crop quality and production. In the major wheat growing region of the world, wheat growth, yield and quality are affected by salinity. To solve this problem it is necessary to breed tolerant varieties through selection and breeding techniques. An experiment was conducted to determine the salinity impact on grain yield, protein content and thousand kernel

weight (TKW) 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. While 6 varieties of durum wheat, 3 accessions of durum wheat and 1 accession of common wheat were insignificantly affected by salinity. 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. In addition the results showed that winter wheat is more tolerant to salt stress than spring wheat and that durum type of wheat showed more tolerance than common wheat. TKW also decreased in all 10 varieties and accessions regardless of the species by salinity effect.

El Hendawy *et al*. (2011) proved that salinity did not affect final germination percentage, while seeds subjected to 80 and 160 mM Nacl treatment. Salinity affected shoot growth more severely than root growth of seedlings. Height and dry weight of shoot of the genotypes ranked in the same order as their salt tolerance ranking in terms of grain yield, whereas root dry weight did not. So, the measurement of shoot growth may be one of the effective criteria for screening wheat genotypes for salt tolerance at early growth stages. Barma *et al*. (2011) reported that two lines named BARI GOM 25 and BARI GOM 26) were selected for commercial production in the southern belt. BARI GOM 25 showed a good level of tolerance to salinity.

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. The cv. Lu-26, exhibited a better protection mechanism against salinity as indicated by lower salt induced proteolysis, higher biomass accumulation and protein contents than the relatively sensitive cv. Pak-81.

Datta *et al*. (2009) undertook an experiment with five varieties of wheat viz., HOW-234, HD-2689, Raj-4101, Raj-4123, and HD-2045 varying the salinity levels to (0, 25, 50, 75, 100, 125, 150mM NaCl). They observed that different level of salinity significantly affected the growth attributes by reducing root and shoot length for

salinity below 125mM. Fresh weight and dry weight of root and shoot were reduced significantly with subsequent treatment. Maximum germination was found in variety HD2689 in all the treatments and maximum inhibition was found to be in case of HOW234 variety at 150mM salinity level.

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. Among the cultivars under investigation Zarlasht cultivar appeared to be more sensitive at germination stage.

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. Akhtar *et al*. (2002) conducted an experiment for the screening of wheat and wheat *Thinopyrum amphiploids* that can produce good yields under saline and water logged conditions.

Rajpar and Sial (2002) conducted a pot experiment with eight varieties of wheat such as Khar-chia-65, Anmol, NIAB-20, PAI-81, TW161, Bakhtwar, KTDH-19 and SARC-l. They observed that under salinity condition up to EC 19 dSm^{-1}, plant height, shoot dry weight and root length were decreased. Singh *et al*. (2000) reported from a study that seeds of 20 wheat varieties were subjected to salinity stress during seedling growth along with the control. The salinity levels used were 0.0% (control) and 0.5% with corresponding EC values of 2.8 and 20.8 dSm^{-1} respectively. Seedling growth declined under salinity stress. The genotypes Raj-3077 and Kharchia-65 were tolerant to salinity with respect to seedling vigour while Raj-4530 and Raj-3934 were most susceptible genotypes under salinity.

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. The changes in photosynthetic activity were

not related to changes in leaf turgor. 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.

Rahman *et al*. (1989) conducted an experiment in the glasshouse of BINA to screen out tolerant cultivars of wheat & barley. Results of the experiment indicated that all the crops, particularly wheat cultivars "Akbar" & "Kanchan," produced higher dry matter yield in varying degrees of salinity conditions created by mixing a saline soil of Shatkhira region. They reported that all these crops can be successfully grown in the salt affected areas of Bangladesh. Chopra *et al*. (1997) conducted a field experiment with 6 wheat cultivars which were irrigated with water having salinity levels of 4.0 (control), 6.0, 7.0 and 12.0 dSm^{-1}. Grain yield decreased with increasing salinity level. The cv. Kharachia- 65 and IID-2189 were found the most salt tolerant.

Kumar *et al*. (1988) conducted a pot experiment where wheat areas grown in saline soil and irrigated with normal or saline water (8 or 12 irrigation with water containing caber ion ratios mmhos cm^{-1}) at Cl: S04 ratio of 1:1, 9:l Increasing salinity of the soil with water at l:l ratio gave markedly higher yield than that crop irrigation with water containing other ion ratios. Akram *et al*. (2002) studied in a pot experiment the effect of salinity (10, 15, 20 dSm^{-1}) on the yield and yield components of salt tolerant (234/2), medium responsive (243/1), and susceptible (Fsd 83) wheat varieties. They reported that salinity reduced the spike length, number of spikelets $spike^{-1}$, number of grains $spikelet^{-1}$, 1000-grains weight, and yield per plant of all the varieties but the susceptible variety was affected the most adversely.

Noaman (2000) conducted a pot experiment with four durum wheat, (*Triticum turgidum* lines .133. 146, 56 and 83) with kanal transferred from *Tritcum aestivum* cv. Sakha-8 (control), *Hordeum vulgarae* cv. Giza (control), *Triticum turgidum* cv. Langdon (LDN) and recombinant DS4D (LDN4B), which were grown at 3 levels of salinity (2, 4 and 8g $liter^{-1}$). He reported that increasing salinity affected plant height in most lines (24.5% reduction). Increasing salinity levels had no significant effect on the number of days from planting to booting, heading or flowering, even though differences among genotypes were significant. The DS4D (LDN 413) had the highest biological yield and grain yield under all salinity than the lines 133, 146 and 83 of

Triticum turgidum cv. Langdon (LDN) which showed the greatest sensitivity to salinity.

Ehsan *et al*., (1994) conducted a pot experiment having salinity levels of 2.0, 7.5, 15 and 22.5 mmhos cm^{-1} where they used wheat cv. Chenab-79, V-5444 and Layllpur-73 as a test crop. They reported that increasing salinity resulted reduction in plant height, dry matter and grain yield. All the cultivars failed to set seeds at the highest salinity level. Khan (2007) conducted an experiment and observed that maximum plant heights, shoot fresh and dry weight were high at control salinity level and at high salinity level ($10dSm^{-1}$) had a negative effect on these parameters. Yield and yield components of various genotypes were significantly reduced due to the exposure of plants to various salinity levels. Among genotypes, SR-40 and SR-23 performed better than the other genotypes under study when exposed to various salinity levels.

Barrett-lennard (1988) conducted a greenhouse experiment with wheat and observed that under moderately saline soil, 7days of water logging condition increased Na content by >200 percent in shoot. In a second experiment wheat was grown under either drained or water logged condition for 33 days with 0, 22 or 120 mM NaCl. A visual assessment showed that drained plants were healthy even with 120 mM NaCl.

Gawish *et al*. (1999) studied the responses of status and translocation of Na, Cl, N and production for both shoots and roots of two wheat varieties differing in salt tolerance, Giza-164 as a relatively salt tolerant and Sakha-69 as a relatively salt sensitive variety to salinity. The plants were treated with NaCl, CaCl or their mixture at a level of 50, 750, 1500 or 3000 ppm, after the 1st leaf had emerged. The status of Na and Cl positively responded in shoots. The rate of translocation for the different ions was higher under salinity conditions, particularly in relatively salt tolerant plants presumably due to osmotic adjustment as to reduce the adverse effect on root growth.

Halim *et al*. (1988) conducted a pot experiment with Maxipak wheat growth in soil salinized by the addition of MgSo4: NaCl: $CaCl_2$ (5: 2: 3respectively). The salinity level of EC 1.7, 4.2, 5.8, 9.4 and 11.0 dSm^{-1} were used at 25, 50 and 75 percent level of available soil moisture depletion. They observed that soil water decreased the soil salinity increased, the dry matter per plant, plant height, tiller or spike number per

plant were decreased at all the growth stages. Grain yield, grain number and root dry matter decreased. Root growth show the greatest sensitivity to soil salinity.

Kemal-ur-Rahim (1988) carried out an experiment with 4 winter wheat cultivars grown in a culture solution where he failed to observe any adverse effects of salinity, up to 75 mM NaCl but greater than 120 mM NaCl was sufficient to jeopardize survival of the crop in salt sensitive cultures. Salinity had little effect on photosynthesis but a large effect on grain yield and dry matter production was noticed. It increased Root: Shoot ratio, stomatal density and specific leaf weight.

Bouaounia *et al*. (2000) studied the salt tolerance of durum wheat (*Triticum turgidum*). They observed decreased growth of whole plants, delayed emergence of new leaves and limited K^+ and Ca^{++} accumulation in these organs under NaCl treated soil salinity. Moreover, Na^+ accumulation decreased from older to younger leaves. Cellular dry matter production was not much affected in spite of a drop in cellular water content. Depressive effects of K^+ and Ca^{++} accumulation were evident while Na^+ cellular accumulation increased with NaCl concentration. These results suggest that wheat has mechanisms to restrict Na^+ transport and accumulation in younger leaves.

Gupta and Shrivastava (1989) also observed in a sand culture trial that the effects of ionic osmotic stress alone and in combination with NaCl, tow 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.

Islam and Salam (1996) conducted a pot experiment. 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.

Mohammad *et al*. (1995) conducted an experiment with five wheat lines (PK-15869, PK-15885, PL-16171, PK-16172 and PK-16187) under saline condition. These lines were tested for salt tolerance in the presence of specific ions (Na^+, Ca^{++}, Cl^-, and

$SO4^{--}$). The seeds were germinated on agar medium containing varying salt concentrations (EC 0, 5, 10, 20, 25 and 30 dSm^{-1}). The genotypes PK-16171 showed the highest percentage germination, shoot length, plant fresh weight and dry matter yield under different salinity levels. Fresh and dry weights of plants were reduced in the presence of salinity in majority of the trails. Two genotypes, PK-15885 and PK-16171 showed salt tolerance.

2.2 Effect of seed priming against salt stress

Aymen *et al.* (2014) conducted an experiment to evaluate the effects of NaCl priming on growth traits and some biochemical attributes of safflower *(Carthamus tinctorius* L. cv Safola) in salinity conditions. Seeds of safflower were primed with NaCl (5 g L^{-1}) for 12 h in 23°C. Primed (P) and non-primed (NP) seeds were directly sown in the field. Experiments were conducted using various water concentrations induced by NaCl (0, 3, 6, 9 and 12 g L^{-1}) in salinity experiment. They found that growth (plant height, fresh and dry weight) and biochemical (chlorophyll, proline and proteins content) of plants derived from primed seeds were greater of about 15 to 30% than that of plants derived from non-primed seeds.

Abdoli (2014) set an experiment to evaluate the effects of seed priming on certain important seedling characteristic and seed vigor of fennel *(Foeniculum vulgare L.)* at Department of Agronomy and Plant Breading ,Faculty of Agriculture, Maragheh University in Maragheh state, Iran. Treatment included untreated seeds (control) and those primed in water (H_2O), sodium chloride (NaCl, 100 mM) and polyethylene glycol 6000 (PEG-6000,water potential-1.6MPa), in darkness for 18 hrs . Among them unsoaked seed (control) and hydropriming treatments had the lowest plumule, radicle and seedling length, seedling dry weight and seedling vigor index. PEG and NaCl in all of traits were better than the water priming treatments, respectively. PEG-6000 (1.6 MPa) is the best treatment for breaking of fennel seed dormancy.

Rastin *et al.* (2013) conducted an experiment in 2011 in Arak, Iran, to evaluate the effect of seed priming treatments on the seed quality of red bean. The experiment was conducted in split plot in the form of a randomized complete block design with three replications and two factors. The first factor was primary seed priming, in which seeds were or were not treated with water, for 14 hours. The second factor was complementary seed priming which was conducted after drying the seeds treated in

the first stepand water, 100 ppm KCl, 0.5% $CaCl_2.2H_2O$, 50 ppm KH_2PO_4 and 20 ppm GA_3 were used to treat seeds for 14 hours. They found that Primary seed priming had no significant effect on none of the measured traits but complementary seed priming significantly affected plant dry matter, grain yield, 100 grain weight and the number of pods. The highest plant dry matter (53.06 g) and the highest grain yield (5.98 t/ha) were achieved when seeds were first treated with water (as the primary seed priming) and after drying were treated with GA3 (as the complementary seed priming).

Meena *et al.* (2013) conducted an experiment for two consecutive years 2010-11 and 2011-12 to evaluate the influence of hydropriming on the water use efficiency and grain yield of wheat *(Triticum aestivum* L.) under moisture stress. The hydroprimed and pre-germinated seeds established earlier than dry seeds leading to better crop establishment under optimum, sub optimum soil moisture as well as dry soil conditions leading to higher tillering and grain yield.

Ajirlo *et al.* (2013) reported that Germination and early growth under prevailing environmental conditions improves by seed priming technique. Their result showed that all the priming treatments significantly affect the fresh weight, shoot length, number of roots, root length, vigor index, time to start emergence, time to 50% emergence and energy of emergence of forage maize. The interactive effect of varieties and priming techniques were not significant for mean emergence time and coefficient of uniformity of emergence.

Kisetu *et al.* (2013) conducted a field study to assess the effects of priming okra *(Abelmoschus esculentus* L.) seeds var. clemson spineless in tap-water, di ammonium phosphate (DAP) and Minjingu (M) Mazao fertilizers at varying hours from non-primed (absolute control) to 48 h at an interval of 12 h. The priming materials used contained 0.115 g L^{-1} DAP, 1 g L^{-1} M-Mazao, and 1 L tap-water. Seeds primed with DAP for 36 h gave the highest number of pods (6) as compared with the absolute control (3), tap-water (5) at 36 h and M-Mazao (5) at 12 h. The highest yield (4.52 t/ha) was obtained for DAP at 36 h compared with M-Mazao (3.32 t ha^{-1}) at 12 h, tap-water (3.16 t ha^{-1}) at 36 h and absolute control (1.88 t ha^{-1}).

Menon *et al.* (2013) conducted an experiment on seed priming with boron to observe the efficacy of priming on germination and growth related attributes of the broccoli

seedlings. Broccoli seeds (cultivar Marathon) of SAKATA Seed Company were soaked in boric acid solution at 0.01, 0.05, 0.5 and 1% (w/v) for 18 hours. Seeds were also soaked in distilled water (hydropriming) and unprimed seeds were taken as control. The results showed that Germination percentage (GP), Mean germination time (MGT), Germination index (GI), Seedling vigor index (SVI), Chlorophyll content, Shoot and root related attributes were significantly influenced by primed seeds as compared to unprimed seeds. The highest germination index (6.289), seedling vigor index (1753.3), chlorophyll content (4.137 mg ml^{-1}) and less mean germination time (3.23 days), maximum length of shoot (5.97 cm), root (11.57 cm), weight of the shoot (15.35 g) and root (2.68 g) were observed from the treatment where seeds were primed with boron solution at the lowest concentration of 0.01%.

Miraj *et al.* (2013) set a field experiment to assess the effect of different phosphorus priming sources on seedling growth and yield of maize. Phosphorus concentration (1% P), using potassium dihydrogen phosphate (KH2PO4), single super phosphate (SSP) and di-ammonium phosphate (DAP) along with amended solutions of SSP (20 gl^{-1} KOH, 15 gl^{-1} NaOH and 12.5 gl^{-1} Na_2CO_3) were included in the experiment. Water primed and non-primed seeds were also used as controls. Seeds were primed for 16 h and were then air-dried for 30 minutes. The nutrient uptake of seedling was increased four times due to 1 % P solution priming with KH_2PO_4. Yield of maize was also increased in response to P priming showing significant results in cobs yield, grain and straw yields. Phosphorus content of grain was also enhanced as compared to control. Priming maize with SSP + 20 gl^{-1} KOH showed almost the same effect as that of KH_2PO_4. Dorna *et al.* (2013) reported that when primed and non-primed onion seeds stored in air-tight plastic containers for 6 and 12 months at 4 and 20°C the number of seeds infested with *Botrytis* spp. significantly decreased after priming and storage, especially at 20°C.

Shabbir *et al.* (2013) conducted a field experiment to investigate the effect of different seed priming agents on growth, yield and oil contents of fennel during winter 2010-11. Priming techniques used in the experiment were hydropriming with distilled water, osmopriming with $CaCl_2$ (2.2%), KCl (2.2%), moringa leaf extract (3.3%), salicylic acid (50ppm) and ascorbic acid (50ppm). Unprimed seeds were used as control treatment. They found that priming techniques significantly affected the parameters relating to seedling emergence. The CaCl2 and KCl treatments

showed exactly similar results for time taken to start seedling emergence (TTSE) as both took minimum TTSE (7 days). Mean emergence time and time taken to 50% seedling emergence were minimum in $CaCl_2$ (2.2%) treatment. Highest final emergence percentage and germination index were also recorded when seeds were primed with $CaCl_2$ (2.2%). The priming techniques also significantly affected the parameters regarding growth and yield (plant height, number of leaves per plant, fresh and dry weight per plant, number of umbels per plant, seeds per umbel, 1000-seed weight, seed yield, biological yield and harvest index). The seed primed with $CaCl_2$ (2.2%) produced the maximum seed yield 492.6 kg ha^{-1} that was at par with KCl treatment.

Seed priming, a controlled hydration process followed by re-drying is pragmatic approach to counteract the salinity effects in many crops because of its simplicity, low cost and effectiveness (Wahid *et al.,* 2007; Afzal *et al.,* 2011). It improved the germination percentage and uniformity of growth following reduced emergence time and increased yields are reported in many field crops including rice (Farooq *et al.,* 2006b; Afzal *et al.,* 2006; Afzal *et al.,* 2011). But such enhancements are often found under non-saline conditions (Farooq *et al.,* 2006a; 2006b) and few studies are available for alleviation of adverse salinity effects in rice during germination and early seedling growth by seed priming (Xu *et al.,* 2011).

Patade *et al.* (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions. The higher germination percentage in seeds primed with CaCl2 is according to Ashraf and Rauf (2001) for wheat and Afzal *et al*. (2008b) for maize who reported an increase in germination percentage of plants raised from seeds primed with calcium salt under salinity stress. Short term seed priming with a low NaCl concentration also increases germination rate, field emergence and acquired stress tolerance (Nakaune *et al.,* 2012).

Sun *et al*. (2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination. It was reported

seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition (Demir Kaya *et al.,* 2006).

Osmopriming with PEG was described as a good technique for improving seed germination of Bromus seeds under salt and drought stress (Tavili *et al*., 2011) and for increasing the germination percentage and seedling vigor of bersim *(Trifolium alexandrinum)* seeds (Rouhi *et* al., 2010). In soybean too, seed priming with PEG was successfully carried out by Khalil *et al*. (2001). Osmopriming with PEG results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach (Chen and Arora, 2011). Osmo conditioning of Italian ryegrass *(Lolium multiflorum)* and sorghum *(Sorghum bicolor)* seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions (Hur, 1991).

According to Posmyk and Janas (2007), hydropriming and hydropriming along with proline can be used as a safe priming method for improving seed germination and growth of *Vigna radiata* seedlings at low temperature and also allowing fast repair of injuries caused by stress. More uniform germination and emergence were observed in primed seeds on canola *(Brassica compestris)* (Zheng *et al.,* 1994), wheat *(Triticum aestivum)* (Nayyar *et al.,* 1995) and rice *(Oryza sativa)* (Lee and Basra *et al.,* 2003) who described improved germination rate and percentage in seeds subjected to hydropriming and seed hardening for 24 h (Farooq *et al.,* 2006b).

Janmohammadi *et al*. (2008) presented hydropriming as a suitable, cheap and easy seed invigoration treatment for inbreed lines of maize, especially when germination is affected by salinity and drought stress. Hydropriming has been shown to result in the earlier germination of desert cacti (Dubrovsky 1996), *Allium* porrum (Ashraf and Bray 1993), pyrethrum *(Tanacetum cinerariifolium)* (Li *et* al., 2011), and coriander (Rithichai *et* al., 2009). Osmotic seed priming of maize caryopses resulted in more homogenous and faster seed germination as compared to the control was reported by Fotia, *et al.,* (2008). Priming with KNO_3 can be used to increase watermelon germination (Demir and Mavi, 2004) and in tomato, seed priming with KNO_3

increased germination percentage, germination index, root length, shoot length and seedling fresh weight (Nawaz *et al*., 2011). It was reported that osmo and hydropriming of chickpea seeds with mannitol and water alleviated the adverse effects of water deficiency and salt stress on seedling growth. The treatment of seeds with water, 2 and 4 % mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions (Kaur *et al*., 2005).

Priming of chickpea seeds with mannitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Previous studies on tomato (Cuartero *et al.,* 2006) and melon (Sivritepeet *al.,* 2003), showed that seed priming improves seed germination, seedling emergence and growth under saline conditions. Farhoudi and Sharifzadeh (2006) and Sarwar *et al.* (2006) while working with canola and chickpea, respectively, reported salt priming-induced improvement in seed germination, seedling emergence and growth under saline conditions.

Priming of seeds with water promoted seedling vigour, yield and crop establishment of chickpea, maize and rice in India (Harris *et.al.,* 1999). It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity affects during early seedling growth (Ashraf and Harris, 2004). Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solution of KCl or potassium sulfate (K_2SO_4) significantly increased plant height, yield attributes, and grain yield in wheat. The beneficial effects of gibberellic acid (GA_3) on germination are well known (Khan *et al.*, 2002).

ABA-primed seeds of *Brassica napus* exhibited earlier (2-7 days) germination and higher final percent radicle protrusion than non-primed control seeds, under salt (100 mM NaCl) or water stress (20 % PEG 8000) and at a low temperature (8 LC) (Gao *et al*., 2002). Kulkarni and Eshanna (1988) stated that pre-sowing seed treatment with IAA at 10 ppm improved root length, rate of germination, and seedling vigor. Kathiresan *et al*. (1984) also found similar findings and reported maximum root and shoot growth; seedling height and field emergence in sunflower seeds in response to priming with $CaCl_2$. Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells Kaya *et al*. (2006).

The increased shoot and root length in primed plants can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduction of lag time between inhibition and radicle emergence (Bradford *et al.*, 1990). Treated seeds had stronger embryos that were able to more easily emerge from seeds (Harris *et al.*, 2005). Sekiya and Yano (2009) also found that enhanced root and shoot length of seedlings obtained from P enriched seeds. To contribute to plant growth and development seed priming has been widely reported technique. Ajouri *et al.* (2004) reported a stimulation of P and Zn uptake, as well as an improved germination and seedling growth in barley after soaking seeds in water and in solutions containing 5-500 mMP.

PEG is frequently used to simulate drought stress (Chen *et al.*, 2010; Farahani *et al.*, 2010) as an inert osmoticum in germination tests (Dodd and Donovan, 1999) and is a non-penetrating solute (Almansouri *et al.*, 2001), which results in osmotic stress that inhibits seed germination through the prevention of water uptake. However, it has been reported that the inhibitory effect of PEG on germination may not be solely related to water imbibition (Almansouri *et al.*, 2001).

Wang *et al.* (2009a) have observed that the fresh weight and the length of the roots and shoots of two alfalfa cultivars (Xinmu No.1 and Northstar) were significantly inhibited by 35% PEG treatment. For a potential medicinal plant, *Matricaria chamomilla*, both the seed germination rate and seedling growth have been found to be reduced with the PEG- mediated increasing osmotic potential of the growth medium (Afzali *et al.*, 2006). Rouhi *et al.* (2011) also suggested that different priming techniques (hydro and osmo priming) had a varying effects on germination on each of the four grass species *(Bromus inermis, Festuca arundinacea, Agropyron e/ongatum* and *Festuca ovina)* and the result showed that, for most evaluated germination parameters, osmopriming treatment (with PEG) was more useful technique to reduce abiotic stress than hydropriming treatment.

Although priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions, the effectiveness of different priming agents varies under different stresses and different crop species (Iqbal and Ashraf, 2005). Patade *et al.*, (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane.

Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions. Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solutions with KCl or K_2SO_4 significantly increased plant height, grain yield and its components in wheat genotypes. Priming of chickpea seeds with mannitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Seed treatment with water and mannitol is also useful under water deficit stress and primed chickpea seeds gave high yield as compared to non-primed seeds (Kaur *et al.,* 2002).

Musa *et al.* (1999) reported that overnight priming of chickpea seeds gave better crop production in Bangladesh. Priming with H_2O_2 failed to improve emergence and seedling growth in rice cultivars which is inconsistent with Wahid *et al*. (2007) who reported improved salt tolerance in wheat by alleviation of salt stress and oxidative damage by H_2O_2 pre-treatment. Harris *et al*. (2004) reported that higher plant dry weight and seed yield following seed priming. The increase in the dry matter and grain yield of mungbean was due to better emergence and better performance per plant. In basil *(Ocimum basilicum* L.) under saline conditions, the seedling vigor, germination percentage and seedling dry weight was found to increase due to hydropriming (Farahani and Maroufi, 2011).

Sivritepe *et al.* (2002) evaluate the effect of salt priming on salt tolerance of melon seedling and reported that total emergence and dry weight were higher in melon seedlings derived from primed seeds and they emerged earlier than non-primed seeds. They also observed that total sugar and proline accumulation and prevented toxic and nutrient deficiency effects of salinity because less Na but more K and especially Ca was accumulated in melon in melon seedlings.

Post-harvest seed enhancement treatments improve germination and seedling vigour (Taylor, 1998). Maiti *et al*. (2009) studied the effect of priming on seedling vigour and productivity of tomato, chili, cucumber and cabbage during post-rainy seasons demonstrating that priming improved germination and seedling development and yield of these vegetable species. Seed priming significantly improved the germination rate and vigour of the mungbean seedlings (Umair *et al.,* 2010). It is also reported that seed priming improve the antioxidant enzymes activity which

decrease the adverse effects of Reactive Oxygen Species (ROS) (Del Ryo *et al.*, 2002).

Afzal *et al.* (2005) also found that the priming-induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants. Primed crops grew more vigorously, flowered earlier and yielded higher. This technique used for improvement of germination speed, germination vigour, seedling establishment and yield (Talebian *et al*., 2008).

It has been reported that primed seeds showed better germination pattern and higher vigour level than non- primed (Ruan *et al.*, 2002a). It has been also reported invigorated seeds had higher vigour levels (Ruan *et al.*, 2002b), which resulted in earlier start of emergence as high vigour seed lots performed better than low vigour ones (Hampton and Tekrony, 1995). Seed priming techniques such as hydropriming, hardening, osmo conditioning, osmo hardening and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al.*, 2002), sunflower (Kaya *et al.*, 2006) and cotton (Casenave and Toselli, 2007).

Various works have shown that hydropriming of seeds have many advantages as compared to non-primed seeds. Hydropriming has resulted in 3 to 4-fold increases in root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition (Kaur *et al*., 2002). This phenomenon was explained to be due to faster emergence of roots and shoots, more vigorous plants, better drought tolerance under adverse conditions (Lee-suskoon *et al*., 1998).

Fujikura *et al.* (1993) presented hydropriming as a simple and inexpensive method of seed priming and according to Abebe and Modi (2009), it is a very important seed treatment technique for rapid germination and uniform seedling establishment in various grain crops. Priming of seeds with water promoted seedling vigor, yield and crop establishment of chickpea, maize and rice in India. Harris *et al.* (1999) also found that hydropriming enhanced seedling establishment and early vigour of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields.

Chiu *et al.* (2006) reported that KNO_3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties. Salt priming with KNO_3, is an effective way to improve seed and seedling vigour of sunflower and cucumber (Ghassemi-Golezani and Esmaeilpour, 2008).

Hydropriming improved the early and vigorous crop establishment in maize (Nagar *et al.,* 1998) and *Heiichrysum bracteatum* L. (Grzesik and Nowak, 1998). However, other studies resulted in poor emergence from hydroprimed Kentucky bluegrass seeds under field conditions (Pill and Necker, 2001).

Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale *et al.,* 2003).

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from August to November 2016 to study the mannitol induced salt tolerant capacity of wheat under salt stress condition. The materials and methods describes a short description of the experimental site, climatic condition of the culture room, experimental materials, treatments and design, methods of the study, data collection procedure and data analysis. The detailed materials and methods which were used to conduct the study are presented below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

This study was implemented in the Central Laboratory of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.1.2 Duration of the study

The experiment was conducted during the period from August to November 2016.

3.1.3 Laboratory condition

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study period of the culture room was 27^0C to 34^0C respectively and the average minimum and maximum relative humidity were recorded 52% and 88%, respectively.

3.2 Test crops

Three wheat genotypes namely- BARI Gom-28, ESWYT 5 and ESWYT 6 were used for this experiment (Hasan *et a*l., 2017). Seeds were collected from Wheat Reasearch Cenre, Dinajpur and Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The collected wheat genotypes were free from any visible defects, disease symptoms and insect infestation.

3.3 Experimental materials

Different equipments such as 4-digit electric balance, petri dish, filter paper, micro pipette, forceps, magnetic stirrer, shelf for placing petri dish, oven etc. were used for this study.

3.4 Chemicals for seed priming

Mannitol, and distilled water were used for priming wheat genotypes. Alcohol was used to sterilize the surface of seed. NaCl was used for inducing salt stress.

3.5 Experimental design

The single factor experiment was laid out in a Completely Randomized Design (CRD) with five replications.

3.6 Experimental treatments

The experiment comprises of

i) Six levels of priming agent concentrations viz. 0 (control), water, 2%, 4%, 6% and 8% mannitol solution
ii) Priming time 9 hours with mannitol and
iii) Six levels of salinity stress viz. (0 dS/m), 5 dS/m, 10 dS/m, 15dS/m and 20 dS/m NaCl.

3.7 Steps of the experiment

This experiment was completed in two steps. In the 1st step, the best mannitol concentration was investigated through the response of genotypes and in 2nd step, the best result under salt stress condition was detected with 9 hours mannitol primed seeds.

3.7.1 First experiment: Study on the effect of different concentrations of mannitol on the germination and growth behavior of wheat.

3.7.1.1 Treatments: One factor experiment considering three wheat genotypes with six levels of seeds priming (0, water, 2%, 4%, 6% and 8% mannitol) solutions for 9 hours was done.

Three wheat genotypes; one wheat variety and two lines were as follows:

1. V_1 = BARI Gom-28
2. V_2 = ESWYT-5
3. V_3 = ESWYT-6

3.7.1.2 Surface treatment

Seeds were firstly treated with 40% alcohol solution for 5 min for surface sterilization. These sterilized seeds were rinsed with distilled water for 3 times to remove the residual effect of alcohol from the seed surface. After that seeds were dried in room temperature to regain the normal weight

3.7.1.3 Priming solutions

0 (control), distilled water (DW), and 2%, 4%, 6% and 8% mannitol solutions were used as priming solutions.

3.7.1.4 Preparation of priming solutions

Mannitol solutions (2%, 4%, 6%, 8%):

For preparing 2% mannitol solution**,** 5 g of mannitol was dissolved in 250 ml of DW. Similarly, 10g, 15g, and 20g mannitol were dissolved in 250 ml of DW to prepare 4%, 6%, and 8% of mannitol respectively.

3.7.1.5 Priming technique

Both hydro and osmopriming techniques without mannitol and with mannitol were applied to our experiments. The surface sterilized seeds were sub-sampled into two groups. One of the sub-sample was considered as control (unprimed) and also used for hydropriming with distilled water and the other sub-samples were used for priming with priming chemicals (osmopriming). For osmopriming seeds were divided into four sub-sample and treated with mannitol of 2%, 4%, 6%, and 8% concentrations for 9 hours. Priming was done in different plastic and glass containers covered with lids to prevent evaporation loss. All priming seeds were taken from the

priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for several times and taken it to blotting paper for drying lightly and finally air dried near to gain original weight (Umair *et al*., 2011) in room temperature for 24 hours for back to the original level of moisture.

3.7.1.6 Germination of seeds

Thirty random seeds were selected from each of the treatment and it placed in 90 mm diameter petri dishes on whatman No.1 filter paper which was moist with distilled water. In that case, whatman No.1 filter paper were used as growth media for germination of wheat seeds. Experimental units (90 Petri dishes) were arranged in a completely randomized design with five replications. During the test, filter papers were kept moist condition with water in the Petri dishes. Seeds were kept at room temperature 25±1°C under normal light to help germination for 10 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and this process was continued up to 10 days. The seedlings which was short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were rejected during counting. At the end of germination test (10 days), 5 random seedlings from each of the treatments were selected and their roots and shoots were cut from the cotyledons and length was measured and transferred to brown paper. Then these shoots and roots were dried in an oven at 75±2°C for 72 hours.

Achievement from the first experiment: From the first experiment, 2% mannitol solution gave the best result. So, 2% mannitol solution was used for the next experiment to evaluate salt tolerant capacity by using 9 horus priming time.

3.7.2 Second experiment: Germination and growth behavior of mannitol primed seeds under salt (NaCl) stress condition

3.7.2.1 Treatments: Single factor experiment with primed seeds of three wheat genotypes under five levels of salt concentration (0 dS/m, 5 dS/m, 10 dS/m , 15 dS/m, and 20 dS/m NaCl) was done.

3.7.2.2 Surface treatment

Seeds were firstly treated with 40% alcohol solution for 5 min for surface sterilization. These sterilized seeds were rinsed with distilled water for 3 times. After that Seeds were dried in room temperature to regain the normal weight.

3.7.2.3 Priming solutions and time

The best priming solution (2 % mannitol) as of the 1st experiment was considered for salt stress test of the genotypes under study.

3.7.2.4 Preparation of priming solutions

Mannitol solutions (2%): 5g of mannitol was dissolved in 250 ml of water to prepare 2% mannitol solution.

3.7.2.5 Preparation of stress solutions with NaCl

In 250 ml of distilled water, 0.731 g of sodium chloride (Nacl) was dissolved for preparing 5 dS/m solution of salt (Nacl). Similarly, 1.436 g, 2.18 g, 2.925 g sodium chloride (Nacl) was dissolved in 250 ml of distilled water for preparing 10 dS/m, 15 dS/m, 20 dS/m solution of NaCl, respectively.

3.7.2.6 Priming technique

Seeds were pretreated with mannitol for osmopriming at a concentration of 2% and hydropriming with distilled water for 9 hours. Priming is done in different plastic and glass containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. All priming seeds were taken from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for several times and taken it to blotting paper for dried lightly and finally air dried near to gain original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original level of moisture.

3.7.2.7 Germination of seeds

The germination test was done by placing randomly selected 30 seeds in 90-mm-diameter petri dishes on whatman No.1. filter paper. Petri dishes containing primed and control seeds were watered with salt solutions as mentioned. In that case, whatman No.1 filter paper were used as growth media for germination. Experimental

units (90 Petri dishes) were designed in a completely randomized design with five replications. During the test, filter papers in the Petri dishes were kept saturated condition. Seeds were kept at room temperature 25±1°C under normal light to help germination for 10 days. Germination was occurred to have considered when radicles were 2 mm long (Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and this process continued up to10 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were discarded during counting. At the end of germination test (10 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and length was measured and transferred to brown paper. Then these shoots and roots were dried in an oven at 75±2°C for 72 hours.

3.8 Data collection

Data on seedling emergence of three wheat genotypes were collected from 1 to 10 days after seed placement. Normal seedlings were counted and percent of seedling emergence was recorded upto 10 days after placing of seeds. Seedling mortality rate was also counted upto 10 days after seed placement in petridishes. The uprooted seedlings were cleaned with tap water and excess water was removed with tissue paper.

The following data were measured and weighted:

1. Rate of germination (%)
2. Shoot length (mm)
3. Root length (mm)
4. Shoot dry weight (mg)
5. Root dry weight (mg)
6. Fresh weight (mg)
7. Turgid weight (mg)
8. Relative water content (%)
9. Water retention capacity
10. Vigour index

3.9 Procedure of recording data

3.9.1 Rate of germination (%)

The number of germinated seeds was counted every day. Germination was recorded at 24 hrs interval and continued up to 10^{th} days. More than 2 mm long plumule and radicle was considered as germinated seed.

The germination rate was calculated by using following formula of Othman et al. (2006).

$$\text{Rate of germination (\%)} = \frac{Total\ Number\ of\ germinated\ seeds}{Total\ number\ of\ seed\ placed\ for\ germination} \times 100$$

3.9.2 Shoot length (mm)

The shoot length of five seedlings from each petrit dish was measured finally at10 DAS. Measurement was done by using the unit millimeter (mm) by a meter scale.

3.9.3 Root length (mm)

The root length of five seedlings from each petri dish was measured finally at 10 days after placement. Measurement was done using a meter scale and unit was expressed in millimeter (mm).

3.9.4 Dry weight of shoot and root (mg)

The dry weight of shoot and root of the five seedlings from each petridish was measured at finally at 10 days after placement. Dry weight was recorded by drying the sample in an oven at 70°C until attained a constant weight.

3.9.5 Turgid weight (mg)

After recording the fresh weight of leaf, each seedling placed into ptridishes for 24 hrs soaking with distilled water then turgid weight was recorded by using electric balance.

3.9.6 Relative water content (%)

Relative water content was measured using following formula of Matin *et al.* (1989).

$$\text{Relative water content (WRC) (\%)} = \frac{Fresh\ weight - Dry\ weight}{Tugid\ weight - Dry\ weight} \times 100$$

3.9.7 Water retention capacity

Water retention capacity was measured by using following formula

$$\text{Water retention capacity (WRC)} = \frac{Turgid\ weight}{Dry\ weight}$$

3.9.8 Vigour index

Vigour index was calculated by using following formula of Abdul-Baki and Anderson (1970).

$$\text{Vigour index} = \frac{Total\ germination \times Seedling\ length\ (mm)}{100}$$

3.10 Statistical Analysis

Data obtained for different parameters were statistically analyzed to observe the significant difference among the treatment. The data were analyzed using ANOVA technique with the help of computer package programme "MSTAT-C" and mean difference among the treatments were adjudged with Least Significant Difference (LSD) as described by Gomez and Gomez (1984). Drawings were made by using Excel software.

CHAPTER IV

RESULTS AND DISCUSSIN

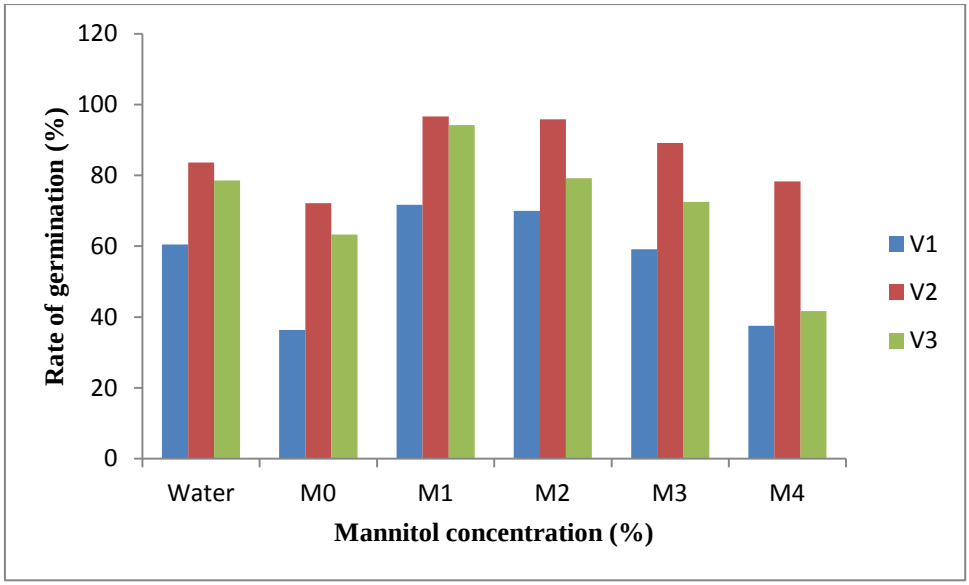
This chapter encompasses the presentation and discussion of the results obtained from the study to investigate mannitol induced salt tolerant capacity of wheat under salt stress condition. The results of the germination and growth parameters of wheat genotypes as influenced by different concentrations of priming agent (mannitol) with priming time in salt stress condition have been presented and discussed in this chapter.

4.1 First experiment: Study on the effect of different concentrations of mannitol on the germination and growth behavior of wheat.

Results obtained from the present study regarding the effects of different concentrations of mannitol on the germination rate of wheat varieties have been presented, discussed and compared in this chapter.

4.1.1 Rate of germination (%)

Significant variation was found in terms of germination rate due to varietal performance and seed priming with mannitol at different concentration (Figure 1 and Appendix II). Results indicated that the genotype, V_2 (ESWYT-5) showed the highest germination rate at all concentration of mannitol including water and control treatment followed by V_3 (ESWYT-6) where genotype V_1 (BARI Gom-28) showed the lowest germination rate at all mannitol concentration. Among all the test sample, the highest germination rate (96.67%) was found from V_1 (ESWYT 5) primed with 2% mannitol solution for 9 hours which was statistically identical with V_3 (ESWYT-6) primed with 2% mannitol solution for 9 hours. The lowest germination rate (36.36%) was observed from V_1 (BARI Gom-28) from without priming treatment followed by 2% mannitol solution for 9 hours primed seeds with the same variety. These findings are consistent of the results of Mohseni *et al*. (2010) where they observed the highest germination percentage with water, and the least germination percentage with 4% KCL and 2% KNO_3. Ajirlo *et al.* (2013) reported that germination improves by seed priming technique. Ali *et al.* (2013) reported that seed priming treatments reduced the mean emergence time and promoted germination.



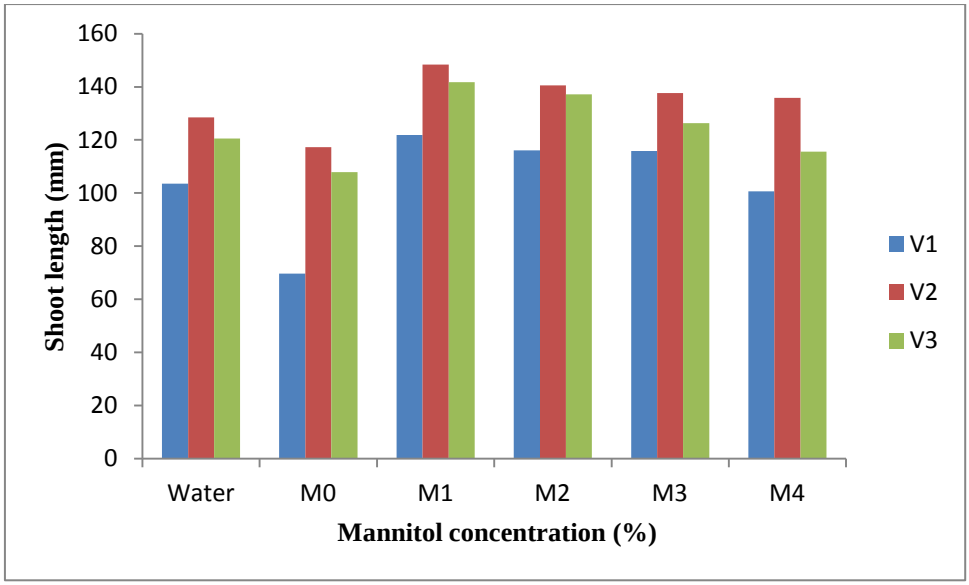
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 1: Effect of different mannitol concentration on germination rate of wheat genotypes

4.1.2 Shoot length (mm)

Significant variation was observed on shoot length among the test genotypes priming with different concentration of mannitol including control treatment (Figure 2 and Appendix III). It was found that the genotype, V_2 (ESWYT-5) showed the highest shoot length in all priming concentration where genotype V_1 (BARI Gom-28) showed the lowest shoot length. It was also observed that the maximum shoot length (148.40 mm) was recorded for V_2 (ESWYT-5) primed seeds with 2% mannitol solution for 9 hours followed by V_3 (ESWYT-6). The lowest shoot length (69.71 mm) was recorded from V_1 (BARI Gom-28) without priming. Primed seed showed increase shoot length of seedlings that is supported by the findings of Basra *et al.* (2005) and Iqbal and Ashraf (2007) in wheat. Rafiq *et al.* (2006) reported that priming with $CaCl_2$ significantly enhanced shoot length under both saline and non-saline conditions.



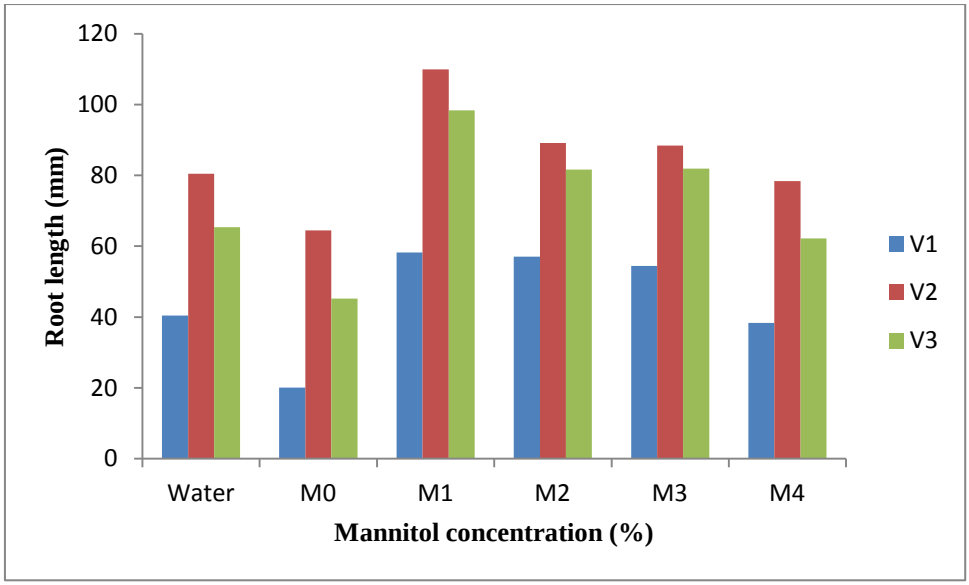
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 2. Effect of different mannitol concentration on shoot length of wheat genotypes

4.1.3 Root length (mm)

Root length was significant varied among the test genotypes primed with different concentration of mannitol including control (Figure 3 and Appendix IV). It was found that the genotype, V_2 (ESWYT-5) showed the highest root length in all mannitol concentration where genotype V_1 (BARI Gom-28) showed the lowest root length. It was also observed that the maximum root length (109.95 mm) was recorded for V_2 (ESWYT-5) primed seeds with 2% mannitol solution for 9 hours followed by seeds primed with 2% mannitol with the genotype V_3 (ESWYT-6). The lowest root length (20.13 mm) was observed from V_1 (BARI Gom-28) using control treatment followed by V_3 (ESWYT-6) genotype using without priming treatment. The root length of seedlings obtained from primed seeds was increased significantly compared to non-primed seeds. The root length of seedlings obtained from primed seeds was increased significantly compared to unprimed seeds. This improvement as affected by priming is supported by the findings of Basra*et al*. (2005) and Iqbal and Ashraf (2007) with wheat. Rafiq *et al.* (2006) reported that priming with $CaCl_2$ significantly enhanced root length under both saline and non-saline conditions.



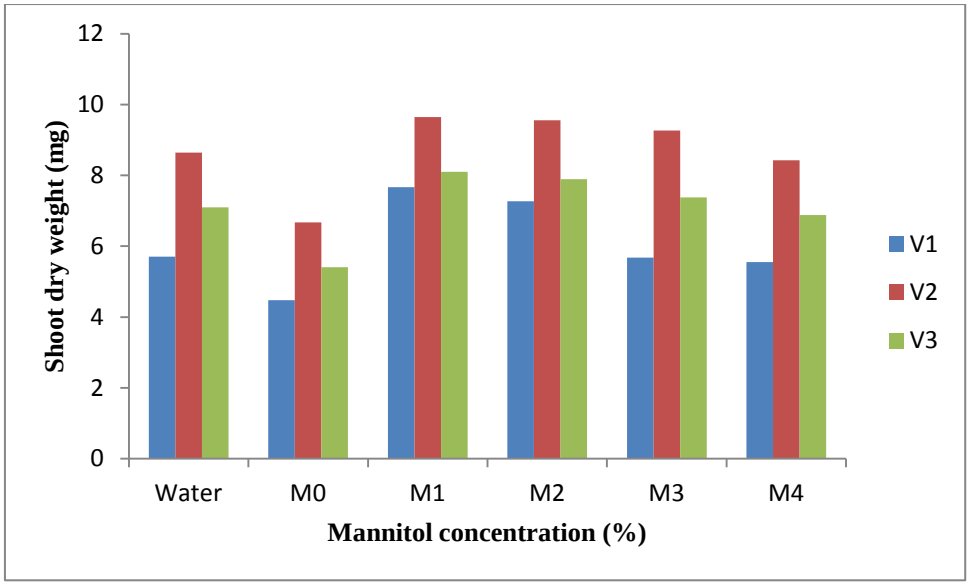
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 3. Effect of different mannitol concentration on root length of wheat genotypes

4.1.4 Shoot dry weight (mg)

Statistically significant variation was found in case of shoot dry weight of different genotypes of wheat due to priming with different mannitol concentrations including control treatment (Figure 4 and Appendix V). Dry weight of shoot was highest with 2% mannitol primed seeds for 9 hours priming time, it was observed that shoot dry weight decreased with the increasing mannitol concentration and also with control (without priming) treatment. Results revealed that the highest shoot dry weight (9.65 mg) was recorded in V_2 (ESWYT-5) primed seeds with 2% mannitol solution for 9 hours priming treatment. The lowest shoot dry weight (4.48 mg) was observed from V_1 (BARI Gom-28) in control.



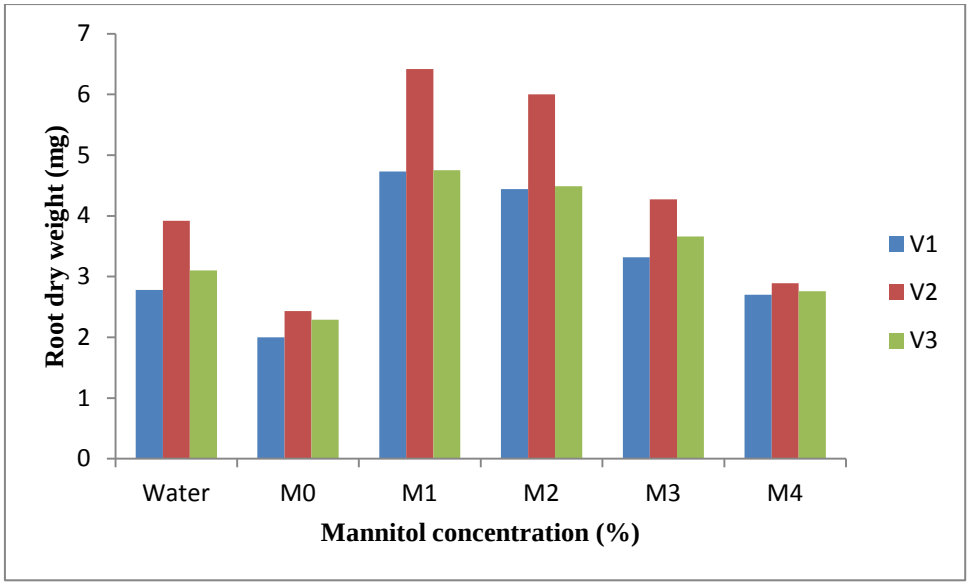
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure. 4. Effect of different mannitol concentration on shoot dry weight of wheat genotypes

4.1.5 Root dry weight (mg)

Significant variation was found in terms of root dry weight of different genotypes of wheat due to priming with different mannitol concentrations including control (Figure 5 and Appendix VI). Results revealed that the highest root dry weight (6.42 mg) was recorded in V_2 (ESWYT-5) primed with 2% mannitol solution for 9 hours followed by Seeds primed with 4% mannitol solution for 9 hours with the same genotype. The lowest root dry weight (2.00 mg) was observed from V_1 (BARI Gom-28) without priming. The root dry weight of seedlings obtained from primed seeds was increased significantly compared to unprimed seeds and this improvement as affected by priming is supported by the findings of Basra *et al*. (2005) and Iqbal and Ashraf (2007) in wheat.



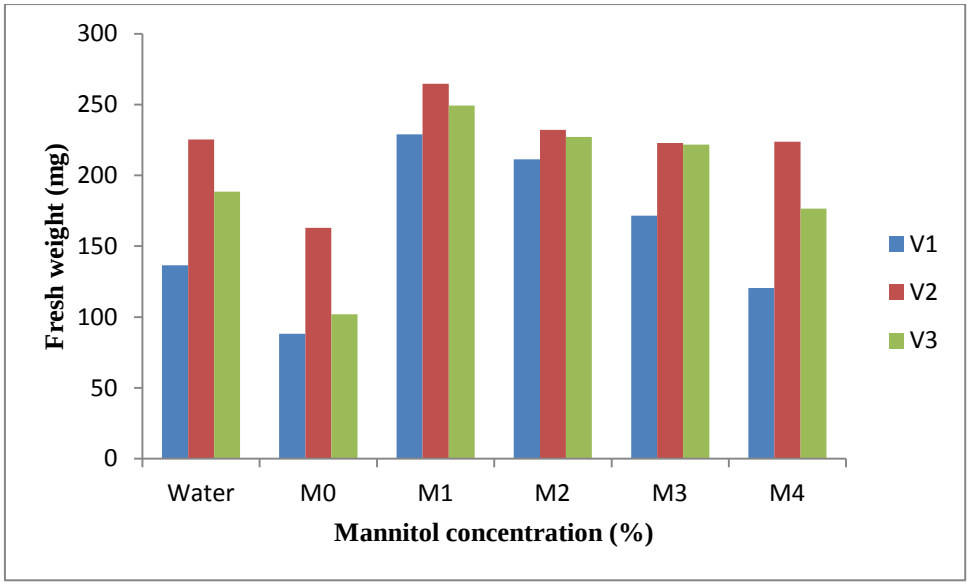
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 5. Effect of different mannitol concentration on root dry weight of wheat genotypes

4.1.6 Fresh weight (mg)

Significant variation was observed on fresh weight among the test genotypes priming with different concentration of mannitol including control treatment (Figure 6 and Appendix VII). It was found that the genotype, V_2 (ESWYT-5) showed the highest fresh weight in all priming concentration where genotype V_1 (BARI Gom-28) showed the lowest fresh weight. It was found that the maximum fresh weight (264.55 mg) was recorded for V_2 (ESWYT-5) primed seeds with 2% mannitol solution for 9 hours followed by V_3 (ESWYT-6) with 2% mannitol solution for 9 hours (249.28 mm). The lowest fresh weight (88.28 mg) was recorded from V_1 (BARI Gom-28) without priming.



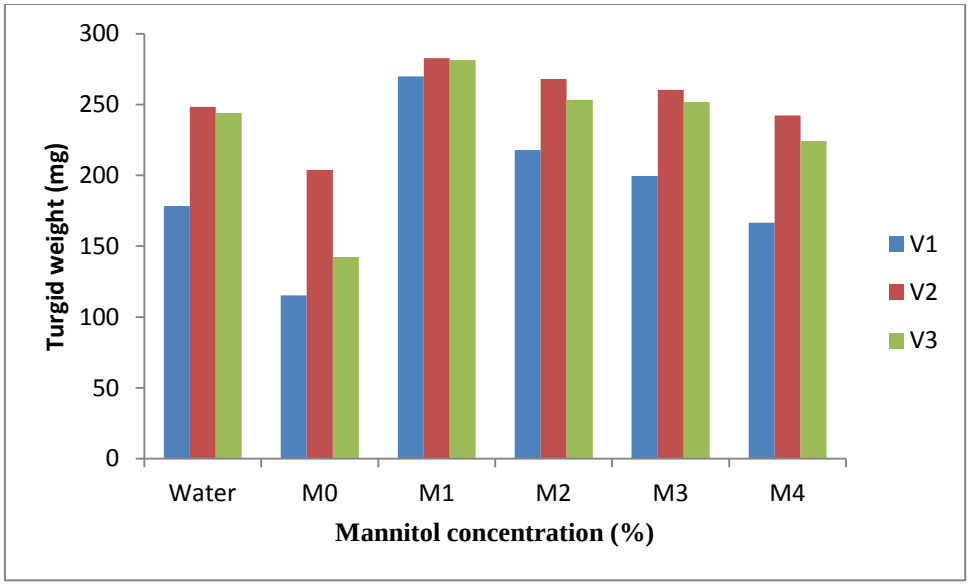
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 6. Effect of different mannitol concentration on fresh weight of wheat genotypes

4.1.7 Turgid weight

Significant variation was observed on turgid weight among the test genotypes priming with different concentration of mannitol including control treatment (Figure 7 and Appendix VIII). Results revealed that the genotype, V_2 (ESWYT-5) showed the highest Turgid weight in all priming concentration where genotype V_1 (BARI Gom-28) showed the lowest. It was also observed that the maximum turgid weight (282.65 mg) was recorded for V_2 (ESWYT-5) primed seeds with 2% mannitol solution for 9 hours which was identical with V_3 (ESWYT-6) with 2% mannitol solution for 9 hours (281.40 mg). The lowest turgid weight (115.20 mg) was recorded from V_1 (BARI Gom-28) without priming.



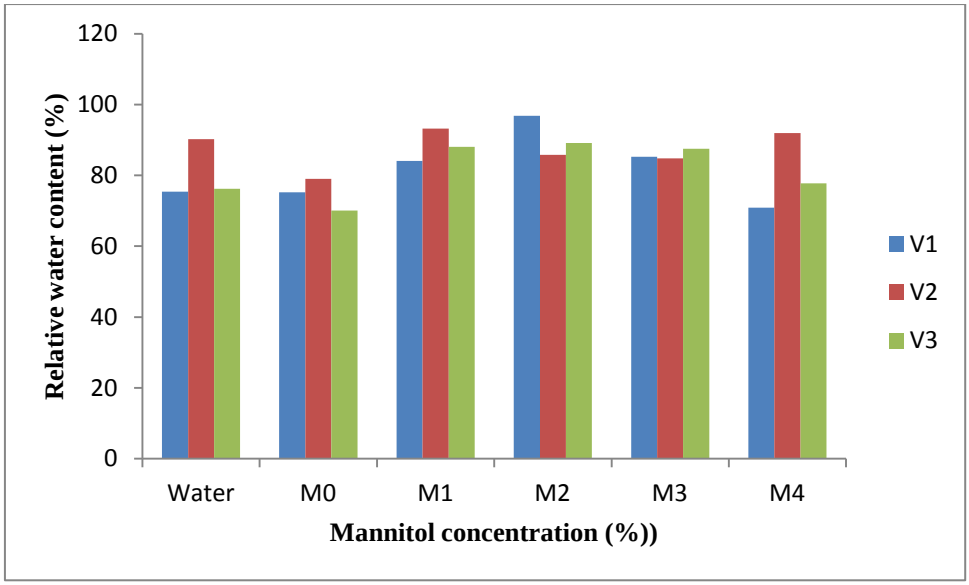
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure.7. Effect of different mannitol concentration on turgid weight of wheat genotypes

4.1.8 Relative water content (%)

Relative water content of different genotypes of wheat showed statistically significant variation due to different concentrations of mannitol solutions including control (Figure 8 and Appendix IX). Among the different genotypes, V_2 (ESWYT-5) gave the best relative water content at all priming treatments. Results indicated that the highest relative water content (93.21%) was recorded in V_2 (ESWYT-5) primed with 2% mannitol solution for 9 hours followed by seeds primed with 4% mannitol solution for 9 hours with the same genotype where the lowest relative water content (70.04%) was observed from V_3 (ESWYT-6) without priming followed by Seeds primed with 8% mannitol with V_1 (BARI Gom-28). Hajer *et al.* (2006) found similar finding and they observed that relative water content (% RWC) decreased with the decrease in osmotic potential of PEG 6000 and NaCl.



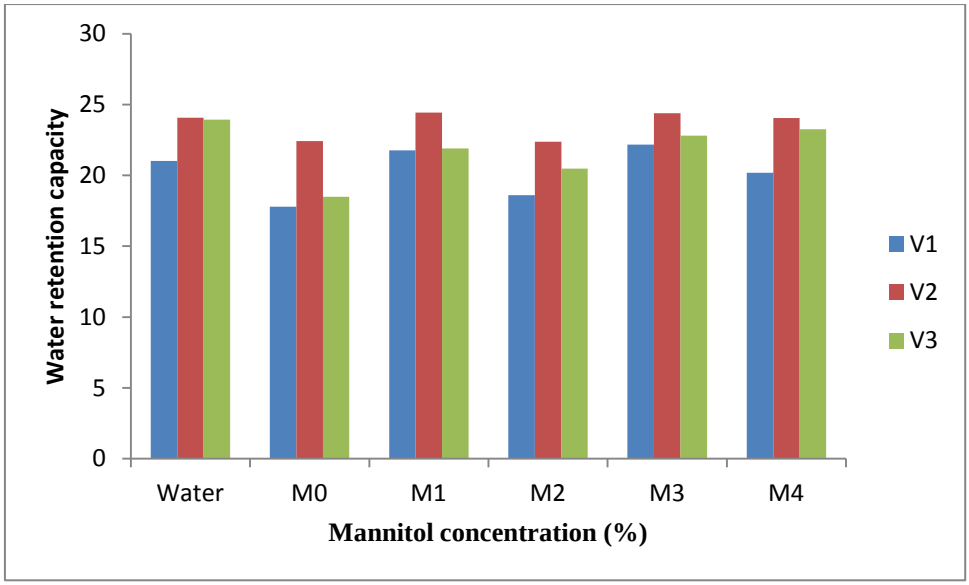
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol M_4 = 8% mannitol

Figure. 8. Effect of different mannitol concentration on relative water content (%) of wheat genotypes

4.1.9 Water retention capacity

Water retention capacity of different genotypes of wheat was not significantly influenced by different priming solution of mannitol priming including control (Figure 9 and Appendix X). But the results showed that the highest water retention capacity (23.25) was recorded in V_3 (ESWYT-6) primed with 8% mannitol solution for 9 hours where the lowest water retention capacity (17.23) was observed from V_2 (ESWYT-5) 2% mannitol solution. Ali *et al.* (2013) reported that seed priming improves irrigation water use efficiency which helps to increase higher water retention capacity.



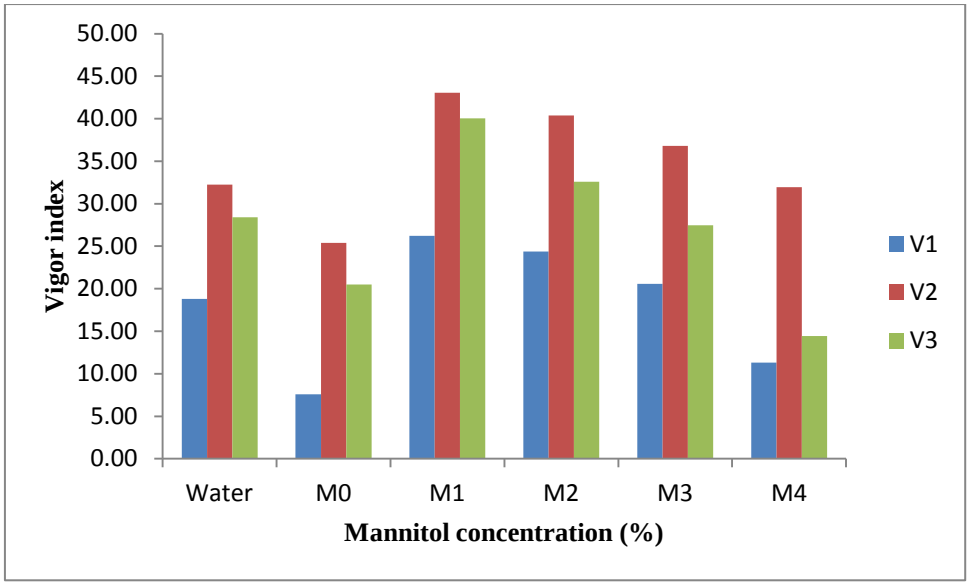
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 9. Effect of different mannitol concentration on water retention capacity of wheat genotypes

4.1.10 Vigour index

Significant influence was found in terms of vigour index of different wheat genotypes due to different priming solution of mannitol and control treatment (Figure 10 and Appendix XI). Among the different genotypes, V_2 (ESWYT-5) gave the best performance on vigour index with all priming treatments where V_3 (ESWYT -6) gave the lowest performance on vigour index with all priming treatments. Results revealed that the highest vigour index (72.13) was recorded in V_2 (ESWYT-5) with water priming seed where the lowest water vigour index (11.32) was observed from V_1 (BARI Gom-28) with 8% mannitol priming seeds. Afzal *et al.* (2008) observed that the priming-induced salt tolerance was associated with improved seedling vigor in wheat. Abdoli (2014) observed that unsoaked seed (control) and hydropriming treatments had the lowest vigor index where PEG and NaCl in all of traits were better than the water priming treatments, respectively. They also observed that PEG-6000 (1.6 MPa) is the best treatment for breaking seed dormancy. Ajirlo *et al.* (2013) reported that germination improves by seed priming technique. They found that all the priming treatments significantly affect the vigor index, time to start emergence.



V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

M_0 = 0% mannitol, M_1 = 2% mannitol, M_2 = 4% mannitol, M_3 = 6% mannitol, M_4 = 8% mannitol

Figure 10. Effect of different mannitol concentration on vigor index of wheat genotypes

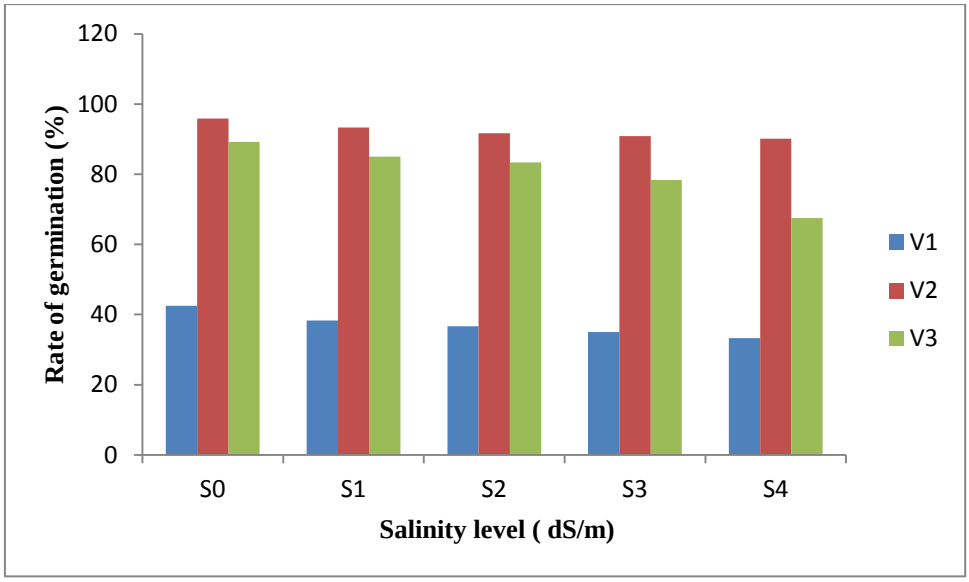
4.2 Second experiment: Germination and growth behavior of mannitol primed seeds (wheat) under salt (NaCl) stress condition

This experiment was conducted under laboratory condition. Three wheat genotypes were primed in 2% mannitol solution and water for 9 hours. Dry seed used as control and was exposed to 0 (water), 5, 10, 15 and 20 dsm^{-1} NaCl induced salt stress conditions in petridishes. The results have been presented and discussed under the following headings:

4.2.1 Rate of germination (%)

Different salinity levels revealed significant variation in respect of germination rate (Figure 11 and Appendix XII). Result exposed that the germination from primed seeds decreased significantly with increasing salinity level. It was found that the variety, V_1 (ESWYT- 5) gave the promising result on seed germination at all salt concentration and germination rate was highest in no salinity level. But under salinity level the highest germination rate was found in Primed seeds placed with 5 dsm^{-1} NaCl and thereafter gradually decreased germination rate was found with

increased salinity levels. It was observed that the highest germination rate (95.83%) was in V_2 (ESWYT-5) under primed seeds placed without salt and after that the second highest germination rate (93.33%) was in V_2 (ESWYT-5) with Primed seeds placed with 5 dsm^{-1} where the lowest germination rate (33.33%) was obtained from V_1 (BARI Gom-28) with Primed seeds placed with 2 dsm^{-1} NaCl. Edalat-Pisheh *et al*. (2010) declared that total germination percentage in wheat seeds decreased when salinity of both primed and unprimed (control group) treatments increased. Kaya *et al*. (2006) and Khajeh-Hosseini *et al*. (2003) also find that reduction in total germination was significantly lower for non- primed seeds, compared to primed seeds and this may be due to the toxic effects of Na+ and Cl- in the process of germination.



V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

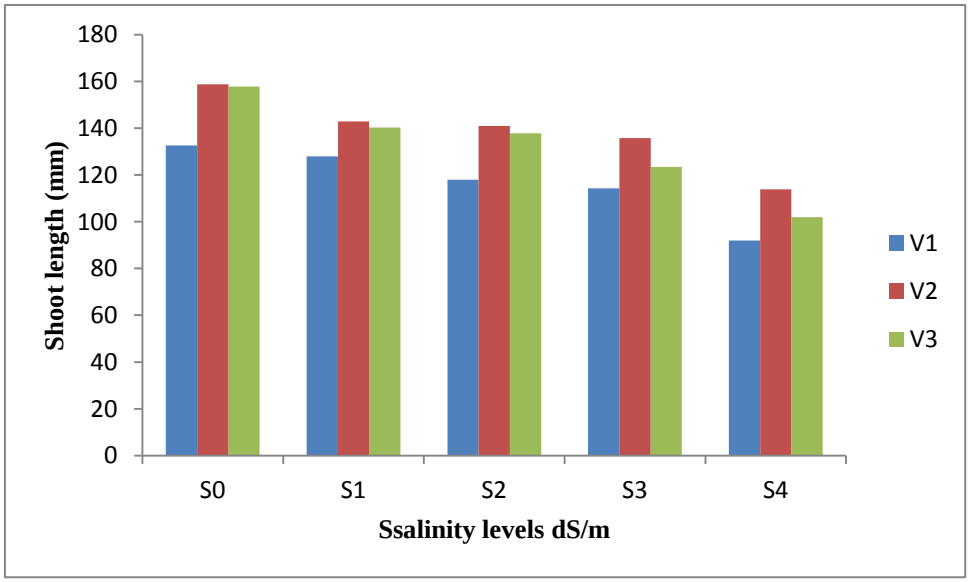
S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 11. Effect of different salinity levels on germination rate of mannitol primed wheat seeds

4.2.2 Shoot length (mm)

Shoot length of different wheat genotypes was significantly influenced by different salinity levels (Figure12 and Appendix XIII). Result exposed that the shoot length from primed seeds decreased significantly with increasing salinity level regarding all

tested genotypes. Results revealed that the highest shoot length (158.70 mm) was observed from V_2 (ESWYT-5) under primed seeds placed without salt. But under salinity stress V_2 (ESWYT-5) gave highest shoot length (142.85 mm) with Primed seeds placed with 5 dsm^{-1} NaCl where the lowest shoot length (91.95 mm) was observed from V_1 (BARI Gom-28) Primed seeds placed with 20 dsm^{-1} NaCl. Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita 2000). Similarly, toxic ion accumulation (Na^+ and Cl^-) negatively affect plant metabolism (Grieve and Fujiyama 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim*etal*. 2008).



V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

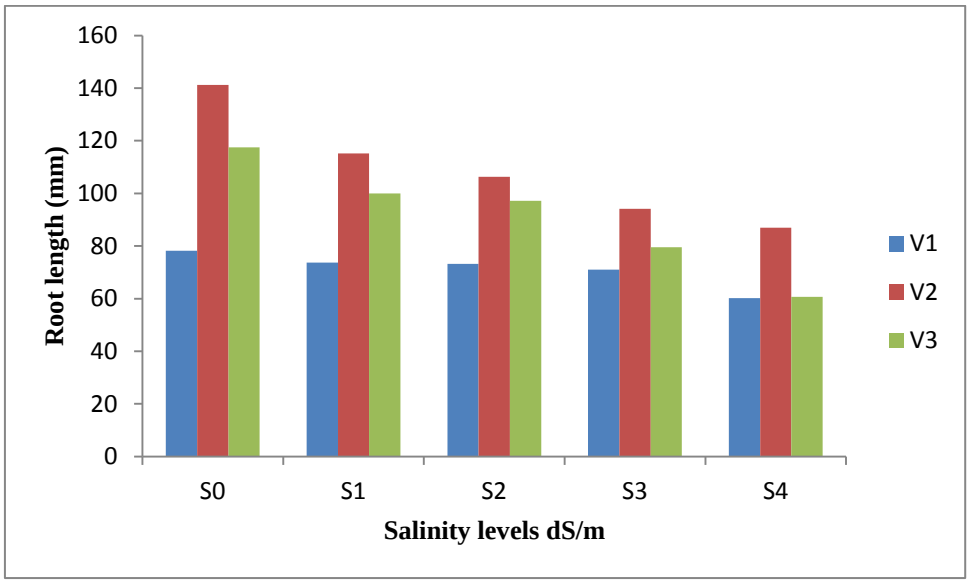
S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 12. Effect of different salinity levels on shoot length of mannitol primed wheat seeds

4.2.3 Root length (mm)

Root length of different wheat genotypes was significantly influenced by different salinity levels (Figure 13 and Appendix XIV). Result exposed that the root length from primed seeds decreased significantly with increasing salinity level where no salinity stress gave highest root length for all the genotypes. Results indicated that the highest root length (141.15 mm) was observed in V_2 (ESWYT-5) primed seeds

placed without salt. Under salinity stress, the highest root length (115.20 mm) was found from V_2 (ESWYT-5) under primed seeds placed with 5 dsm^{-1} NaCl solution and thereafter decreasing trend was observed with increasing salinity level. The lowest root length (60.25 mm) was observed from V_1 (BARI Gom-28) under primed seeds placed with 20 dsm^{-1} NaCl solution. Significant improvement in root and shoot length may be attributed to earlier germination induced by primed over non-primed seeds (Farooq *et al*. 2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds.



V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

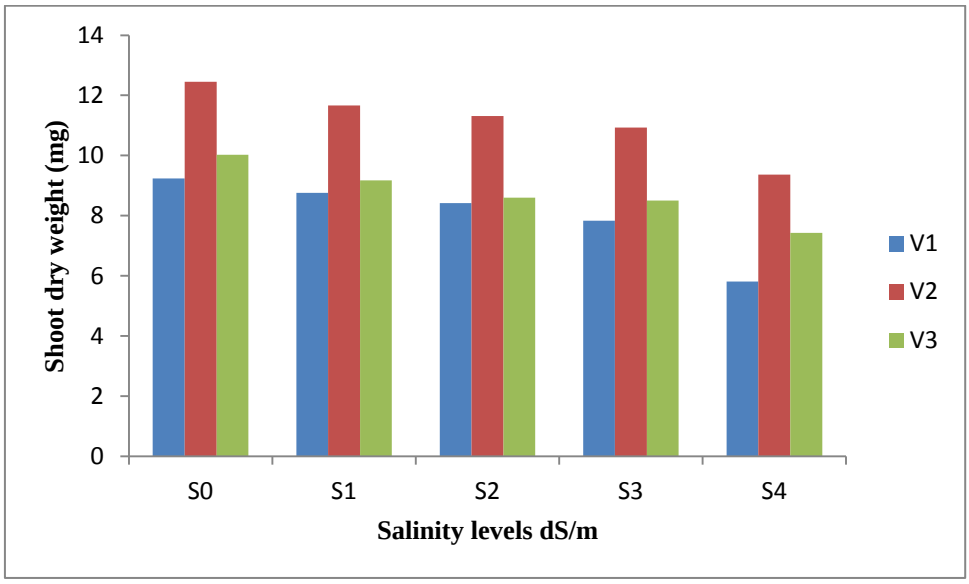
S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 13. Effect of different salinity levels on root length of mannitol primed wheat seeds

4.2.4 Shoot dry weight (mg)

Significant variation was found for shoot dry weight of different wheat genotypes affected by different salinity levels (Figure 14 and Appendix XV). Decreased shoot dry weight was observed with increased salinity level where no salinity level gave highest shoot dry weight. The results showed that that the highest shoot dry weight (12.45 mg) was observed from V_2 (ESWYT-5) primed seeds placed without salt but under salinity stress, V_2 (ESWYT-5) also showed highest shoot dry weight (11.66

mg) under primed seeds placed with 5 dsm^{-1} NaCl solution where the lowest shoot dry weight (5.81 mg) was observed from V_1 (BARI Gom-28) under primed seeds placed with 20 dsm^{-1} NaCl solution.



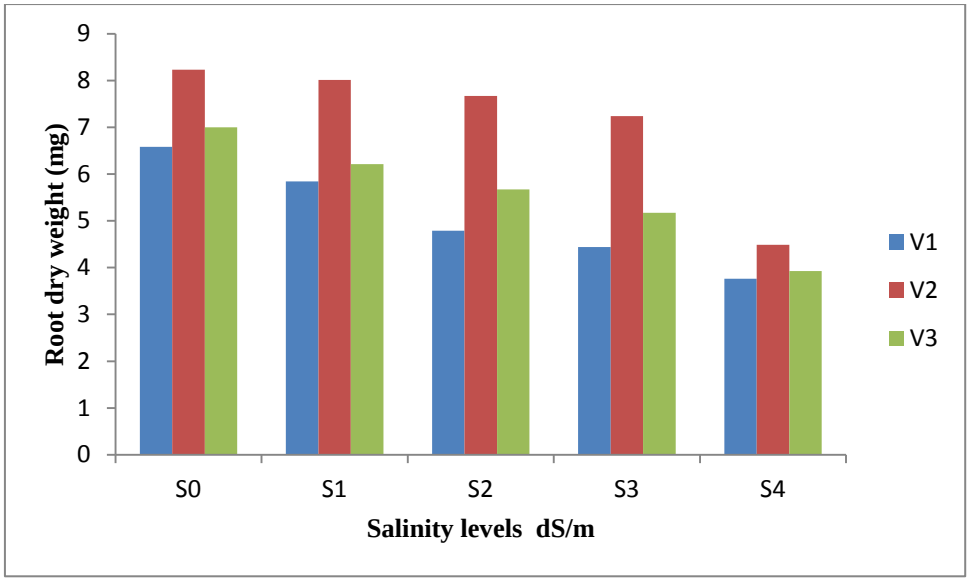
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 14. Effect of different salinity levels on shoot dry weight of mannitol primed wheat seeds

4.2.5 Root dry weight (mm)

Significant variation was also found for root dry weight of different wheat genotypes affected by different salinity levels (Figure 15 and Appendix XVI). Decreased root dry weight was observed with increased salinity level where no salinity level gave highest root dry weight. Results showed that that the highest root dry weight (8.23 mg) was observed from V_2 (ESWYT-5)) where primed seeds placed without salt. Under salinity stress, the highest root dry weight (8.01 mg) was found from V_2 (ESWYT-5) under primed seeds placed with 5dsm^{-1} NaCl solution. The lowest root dry weight (3.76 mg) was found from V_1 (BARI Gom-28) under Primed seeds placed with 20 dsm^{-1} NaCl.



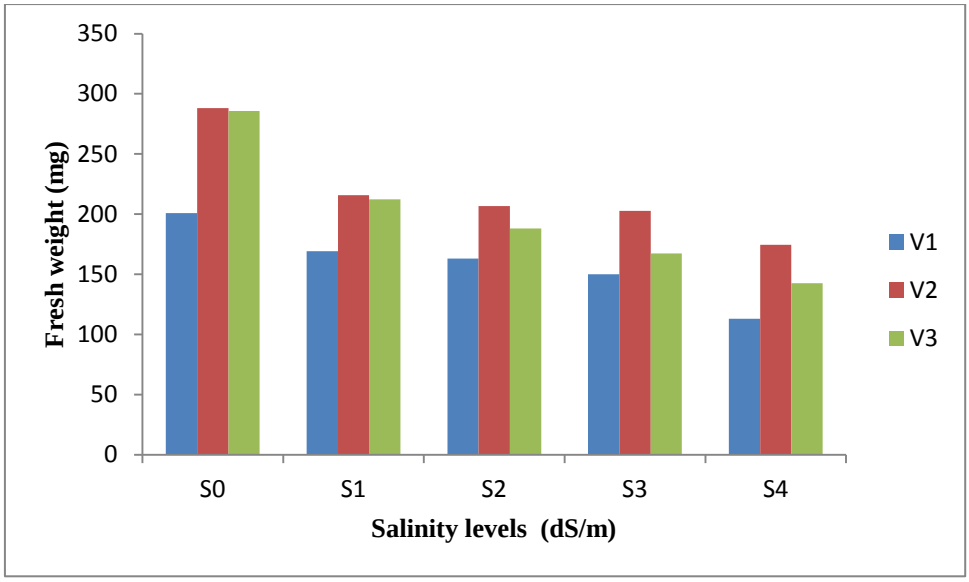
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 15. Effect of different salinity levels on root dry weight of mannitol primed wheat seeds

4.2.6 Fresh weight (mg)

Significant variation was also found for fresh weight of different wheat genotypes affected by different salinity levels (Figure 16 and Appendix XVII). Decreased fresh weight was observed with increased salinity level where no salinity level gave highest fresh weight. Results showed that that the highest fresh weight (288.03 mg) was observed from V_2 (ESWYT-5) without salt stress. Under salinity stress, the highest fresh weight (215.65 mg) was found from V_2 (ESWYT-5) under Primed seeds placed with 5 dsm^{-1} NaCl solution. The lowest fresh weight (113.18 mg) was found from V_1 (BARI Gom-28) under Primed seeds placed with 20 dsm^{-1} NaCl solution.



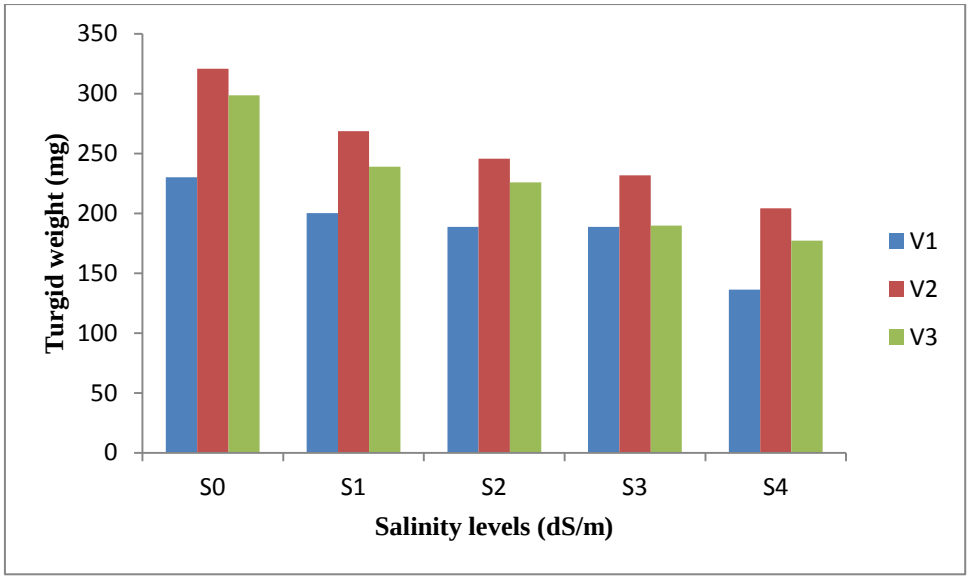
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 16. Effect of different salinity levels on fresh weight of mannitol primed wheat seeds

4.2.7 Turgid weight (mg)

Turgid weight of different wheat genotypes was significantly influenced by different salinity levels (Figure 17 and Appendix XVIII). Result exposed that the turgid weight from primed seeds decreased significantly with increasing salinity level regarding all tested genotypes. Results revealed that the highest turgid weight (320.60 mm) was observed from V_2 (ESWYT-5) under primed seeds placed without salt. But under salinity stress V_2 (ESWYT-5) gave highest turgid weight (268.55 mg) with primed seeds placed with 5 dsm^{-1} NaCl solution. Where the lowest turgid weight (136.23 mg) was observed from V_1 (BARI Gom-28) primed seeds placed with 20 dsm^{-1} NaCl solution.



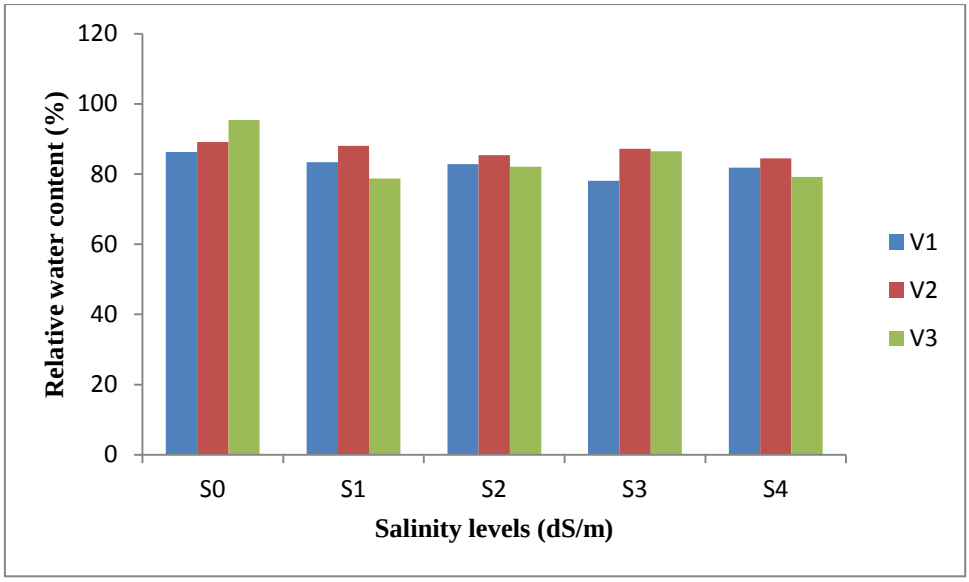
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 17. Effect of different salinity levels on turgid weight of mannitol primed wheat seeds

4.2.8 Relative water content (%)

Relative water content of different wheat genotypes was significantly influenced by different salinity levels (Figure 18 and Appendix XIX). Result exposed that the relative water content from primed seeds decreased significantly with increasing salinity level. Results indicated that the highest relative water content (89.14%) was observed from V_3 (ESWYT-6) where primed seeds without salt. Under salinity stress, the highest relative water content (88.03%) was found from V_2 (ESWYT-5) under primed seeds placed with 5 dsm^{-1} NaCl solution. The lowest relative water content (78.05%) was observed from V_1 (BARI Gom-28) under Primed seeds placed with 15 dsm^{-1} NaCl solution.



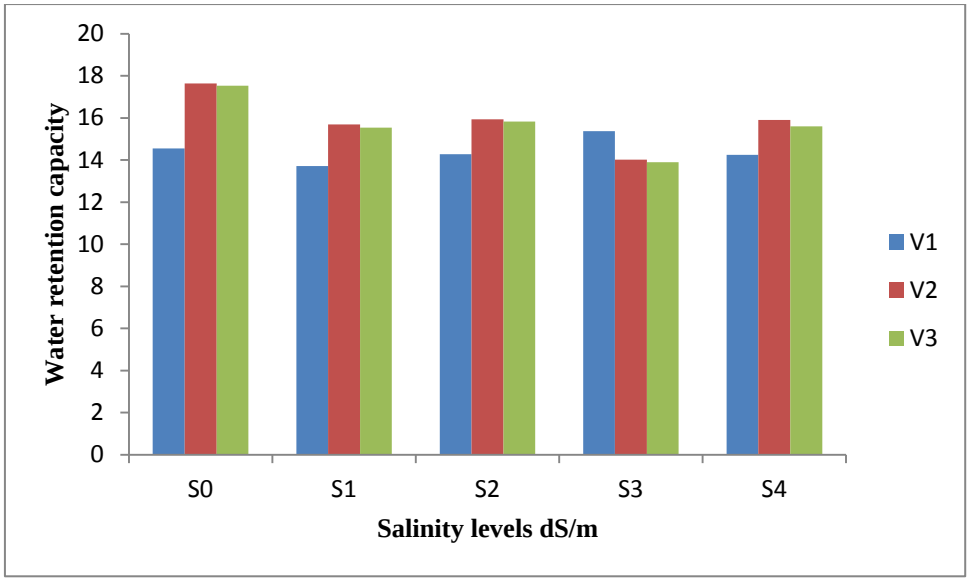
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure. 18. Effect of different salinity levels on relative water content (%) of mannitol primed wheat seeds

4.2.9 Water retention capacity

Significant influence was found for water retention capacity of different wheat genotypes affected by different salinity levels (Figure 19 and Appendix XX). But the results showed that the highest water retention capacity (17.53) was observed from V_3 (ESWYT-6) where primed seeds placed without salt but under salinity stress, the highest water retention capacity (15.54) was found from V_3 (ESWYT-6) under Primed seeds placed with 5 dsm^{-1} NaCl solution. The lowest water retention capacity (12.75) was observed from V_2 (ESWYT-5) primed seeds placed with 15 dsm^{-1} NaCl solution.



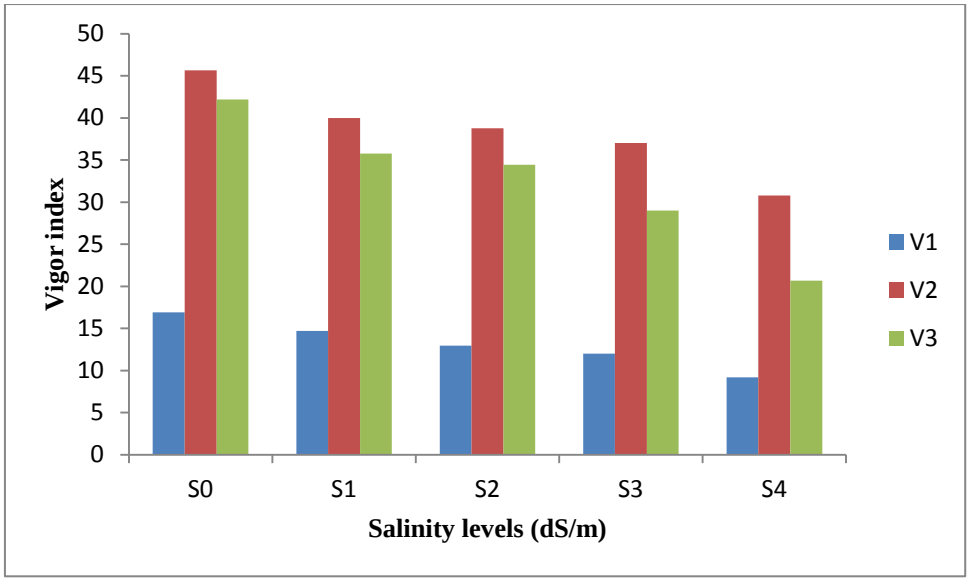
V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 19. Effect of different salinity levels on water retention capacity of mannitol primed wheat seeds

4.2.10 Vigour index

Significant influence was found for vigour index of different wheat genotypes affected by different salinity levels (Figure 20 and Appendix XXI). Result exposed that the vigour index from primed seeds decreased significantly with increasing salinity level and no salinity stress gave highest vigour index. It was found that the highest vigour index (45.63) was observed from V_2 (ESWYT-5) with primed seeds without salt stress. Under salinity stress, the highest vigour index (40.00) was found from V_2 (ESWYT-5) under 5 dsm^{-1} NaCl solution. The lowest vigour index (9.20) was observed from V_1 (BARI Gom-28) under 20 dsm^{-1} NaCl.



V_1 = BARI Gom-28, V_2 = ESWYT-5, V_3 = ESWYT-6

S_0 = 0 dS/m, S_1 = 5 dS/m, S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m

Figure 20. Effect of different salinity levels on vigor index of mannitol primed wheat seeds

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at Central Laboratory of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from August to November 2016. Two experiments were conducted to study the mannitol induced salt tolerant capacity of wheat under salt stress condition. The experiment was laid out in a Completely Randomized Design (CRD) with five replications.

Three wheat genotypes *viz*. V_1 (BARI Gom-28), V_2 (ESWYT-5) and V_3 (ESWYT-6) were used as test crop. Different priming chemicals such as mannitol and water were utilized for osmopriming and hydropriming respectively. Alcohol was used to sterilize surface of seed. NaCl was used as salt stress inducing chemical.

Priming was done in room temperature and all the primed seeds were removed from the priming solution at the same time. Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter Petri dishes on whatman No.1 filter paper and filter paper was moistened distilled water.

Germination was considered to have occurred when radicles were 2 mm long. Germination progress was inspected and data were collected at every 24 h intervals and this process was continued up to 10 days. The seedlings which was short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds. These types of abnormal or dead seedlings were rejected during counting.

The data recorded on germination percentage, root length, shoot length, root dry weight, shoot dry weight, fresh weight, turgid weight, relative water content, water retention capacity and vigour index. Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the LSD at 1% level of probability.

5.1 First experiment

The first experiment was carried out to find the effect of different concentrations of mannitol on the germination and growth behavior of three wheat genotypes (BARI Gom-28, ESWYT-5 and ESWYT-6) without any stress condition. Four levels of mannitol such as 2%, 4%, 6% and 8% were used for osmopriming and water as hydropriming agent for 9 hours. Seeds without priming (control) also took as treatment.

Among the genotypes, V_2 (ESWYT-5) gave the best results on studied parameters. Results revealed that the V_2 (ESWYT-5) showed the highest germination rate (96.67%), shoot length (148.40 mm), root length (109.95 mm), shoot dry weight (9.65 mg), root dry weight (6.42 mg), fresh weight (264.55 mg), turgid weight (282.65 mg), relative water content (93.21%) and vigour index (43.04) primed with 2% mannitol solution for 9 hours where seeds without priming showed lowest results in respected parameters with the genotype V_1 (BARI Gom-28).Seed soaked with water also gave better result than without priming but lower result than 2% mannitol primed seed.

5.2 Second experiment

In the second experiment germination and growth behavior of primed seeds of wheat genotypes (BARI Gom-28, ESWYT-5 and ESWYT-6) with and without salt (NaCl) stress condition was evaluated. Mannitol solution 2% were used as priming solutions and 9 hours as priming time and salt stress levels; without salt but primed (control), S_0 = 0 dS/m, S_1 = 5 dS/m , S_2 = 10 dS/m, S_3 = 15 dS/m, S_4 = 20 dS/m were used in this experiment.

Results revealed that the genotype V_2 (ESWYT-5) with primed seeds placed without salt; control gave the highest germination rate (95.83%), shoot length (158.70 mm), root length (141.15 mm), shoot dry weight (12.45 mg), root dry weight (8.23 mg), fresh weight (288.03 mg), turgid weight (32.060 mg) and vigour index (45.63) where V_3 (ESWYT-6) gave highest relative water content (95.41%). But under salinity stress, the highest germination rate (93.33%), shoot length (142.85 mm), root length (115.20 mm), shoot dry weight (11.66 mg) and root dry weight (8.01 mg), fresh

weight (215.65 mg), turgid weight (268.55 mg), relative water content (88.03%) and vigour index (238.9) were achieved from V_2 (ESWYT-5) primed seeds placed with 5 dsm^{-1} NaCl where V_1 (BARI Gom-28) primed seeds placed with 20 dsm^{-1} NaCl showed lowest results in respected parameters.

From the result of above study arrived at a judgment that the performance of wheat genotype ESWYT-5 which was 2% mannitol primed gave better response in respect of germination and growth parameter. Wheat seeds primed with 2% mannitol and distilled water promoted germination behavior and seedling growth up to 5 dsm^{-1} induced salt stress condition, after that significantly decreased with increasing salt stress. Reduction of germination and seedling growth was more profound in control seeds than primed seeds under salt stress condition. Thus the priming may be effective method to meet the demands of farmer during the setting of culture in the field under salt stress condition. For this reason, further studies are needed to assess the efficacy of seed priming during the later stage of the culture.

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APPENDICES

Appendix I. Monthly records of Temperature, Rainfall, and Relative humidity of the experiment site during the period of November 2016

Year	Month	Air Temperature (^{0}c)			Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
		Maximum	Minimum	Mean			
2015	November	28.5	19.5	24.0	66.8	0.0	231.2

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

Appendix II. Effect of different mannitol concentration on germination rate of wheat genotypes

Source of variation	df	Mean square of Rate of germination at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	132.85**	148.12**	113.71**	122.38**	105.61**	82.49**
Error	8	6.824	5.59	8.82	7.08	6.40	4.12

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix III. Effect of different mannitol concentration on shoot length of wheat genotypes

Source of variation	df	Mean square of Shoot length at different mannitol concentration					
		Water	0% mannitol	2 % mannitol	4% mannitol	6% mannitol	8% mannitol
Treatment	2	26.312**	21.344**	28.389**	18.641**	16.322**	11.642*
Error	8	5.661	6.435	5.652	5.987	4.858	3.512

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix IV. Effect of different mannitol concentration on root length of wheat genotypes

Sources of variation	df	Mean square of root length at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	48.723**	42.303**	36.827**	32.874**	24.205**	18.676*
Error	8	4.288	5.309	6.536	4.811	4.144	3.273

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix V. Effect of different mannitol concentration on shoot dry weight of wheat genotypes

Sources of variation	df	Mean square of shoot dry weight at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	3.216**	3.077**	5.666**	3.265*	2.705*	2.312**
Error	8	0.208	0.214	0.136	0.122	0.064	0.028

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix VI. Effect of different mannitol concentration on root dry weight of wheat genotypes

Sources of variation	df	Mean square of root dry weight at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	3.057**	2.627**	3.421**	3.204**	2.312**	1.751**
Error	8	0.114	0.104	0.197	0.088	0.075	0.046

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix VII. Effect of different mannitol concentration on fresh weight of wheat genotypes

Sources of variation	df	Mean square of fresh weight at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	7.312**	8.071**	12.621**	10.429**	8.741*	7.520**
Error	8	0.204	0.232	0.384	0.212	0.104	0.048

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix VIII. Effect of different mannitol concentration on turgid weight of wheat genotypes

Sources of variation	df	Mean square of turgid weight at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	76.552**	87.82**	120.67**	110.34**	67.49**	58.83**
Error	8	8.214	7.53	9.81	5.79	5.69	3.87

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix IX. Effect of different mannitol concentration on relative water content (%) of wheat genotypes

Sources of variation	df	Mean square of relative water content at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	58.712**	52.439*	68.128**	53.961**	40.897**	32.958**
Error	8	6.344	7.184	10.449	6.478	4.877	3.704

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix X. Effect of different mannitol concentration on water retention capacity of wheat genotypes

Sources of variation	df	Mean square of water retention capacity at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	24.74**	18.332*	26.036**	13.795*	10.386*	6.621**
Error	8	0.204	0.168	0.211	0.186	0.152	0.104

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XI. Effect of different mannitol concentration on vigor index of wheat genotypes

Sources of variation	df	Mean square of vigor index at different mannitol concentration					
		Water	0% mannitol (M_0)	2 % mannitol (M_1)	4% mannitol (M_2)	6% mannitol (M_3)	8% mannitol (M_4)
Treatment	2	172.67**	164.53**	201.27**	148.36**	126.24**	136.24*
Error	8	6.57	8.455	10.637	6.219	3.714	2.211

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XII. Effect of different salinity levels on germination rate of mannitol primed wheat seeds

Sources of variation	df	Mean square of rate of germination at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	92.84**	102.60**	76.36**	68.42**	52.59**
Error	8	5.56	7.741	6.72	3.69	4.81

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XIII. Effect of different salinity levels on shoot length of mannitol primed wheat seeds

Sources of variation	df	Mean square of shoot length at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	64.073**	72.134**	48.227**	42.381**	37.178**
Error	8	6.763	6.311	5.838	3.354	3.261

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XIV. Effect of different salinity levels on root length of mannitol primed wheat seeds

Sources of variation	df	Mean square of root length at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	33.088**	36.052**	25.433**	18.711*	12.215**
Error	8	3.215	4.114	2.316	1.144	1.023

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XV. Effect of different salinity levels on shoot dry weight of mannitol primed wheat seeds

Sources of variation	df	Mean square of shoot dry weight at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	1.148**	2.766**	2.142**S	1.074**	0.853**
Error	8	0.102	0.171	0.058	0.044	0.026

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XVI. Effect of different salinity levels on root dry weight of mannitol primed wheat seeds

Sources of variation	df	Mean square of root dry weight at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	0.661**	1.718**	1.017**	1.228**	0.508**
Error	8	0.022	0.036	0.011	0.012	0.008

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XVII. Effect of different salinity levels on fresh weight of mannitol primed wheat seeds

Sources of variation	df	Mean square of fresh weight at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	27.117*	32.054**	28.228**	18.317**	129.433**
Error	8	3.182	4.488	2.822	1.314	1.039

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XVIII. Effect of different salinity levels on turgid weight of mannitol primed wheat seeds

Sources of variation	df	Mean square of turgid weight at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	49.311**	52.466**	47.814**	32.532**	22.149**
Error	8	5.389	7.463	4592	4.179	2.325

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XIX. Effect of different salinity levels on relative water content (%) of mannitol primed wheat seeds

Sources of variation	df	Mean square of relative water content at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	36.255**	46.63**	58.411**	20.314**	16.049**
Error	8	4.009	7.806	6.345	4.214	3.472

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XX. Effect of different salinity levels on water retention capacity of mannitol primed wheat seeds

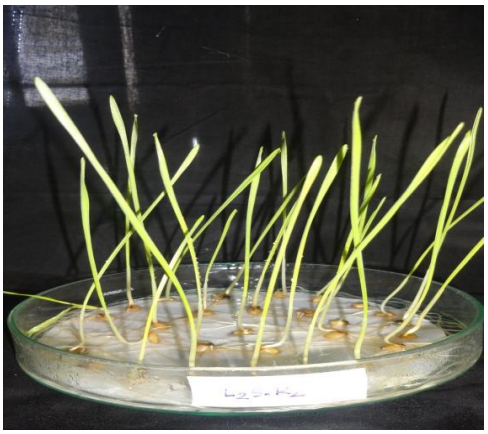
Sources of variation	df	Mean square of water retention capacity at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	21.144*	26.367**	21.524*	25.312*	12.153**
Error	8	1.207	2.234	2.268	1.219	0.659

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

Appendix XXI. Effect of different salinity levels on vigor index of mannitol primed wheat seeds

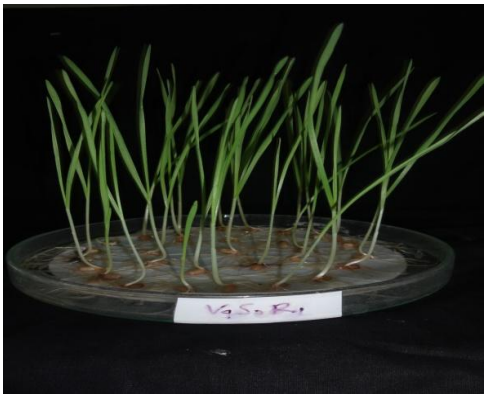
Sources of variation	df	Mean square of vigor index at different salt concentration				
		0 dS m^{-1} (S_0)	5 dS m^{-1} (S_1)	10 dS m^{-1} (S_2)	15 dS m^{-1} (S_3)	20 dS m^{-1} (S_4)
Treatment	2	74.86**	83.68**	80.31**	38.44**	27..88**
Error	8	6.36	10.863	5.311	4.806	4.892

**Significant at 1% level of significance
*Significant at 5% level of significance
NS Non significant

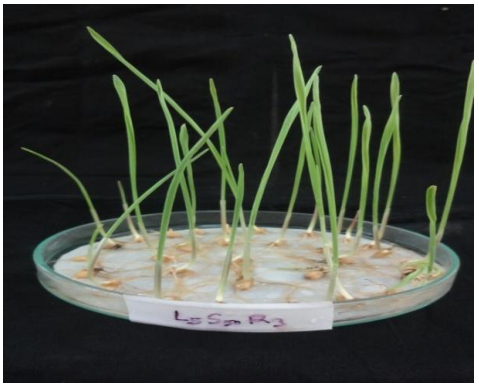


Water

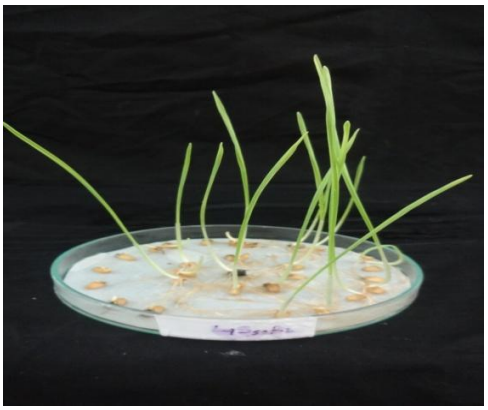
control



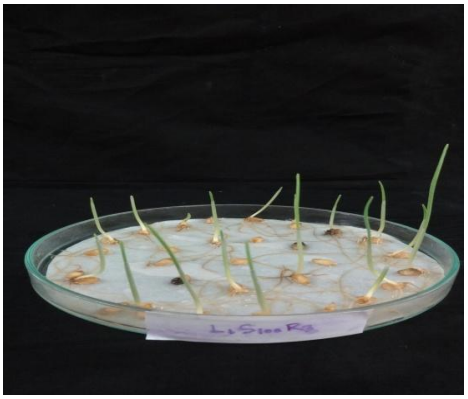
2% mannitol



4% mannitol

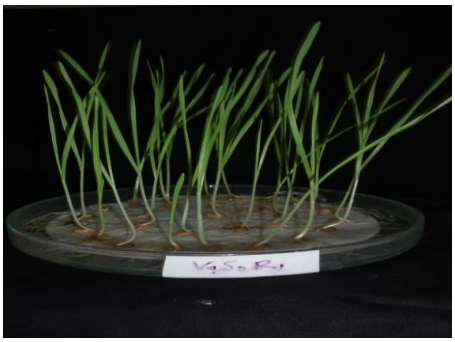


6% mannitol

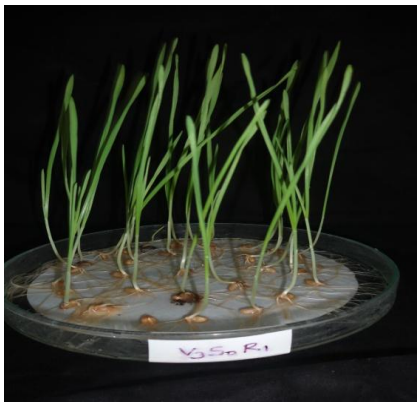


8% mannitol

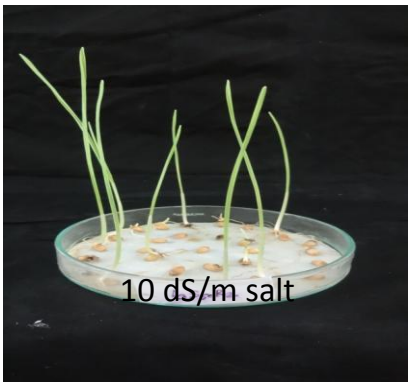
Plate 1: Effect of different concentration of priming solution on germination behavior of wheat genotypes



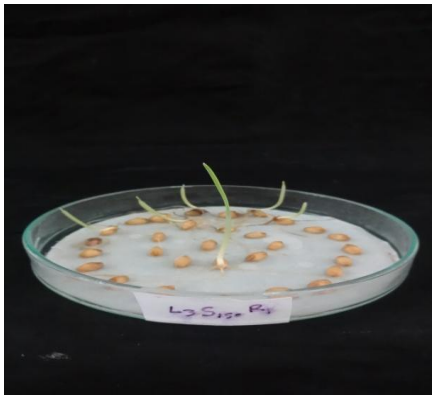
Water



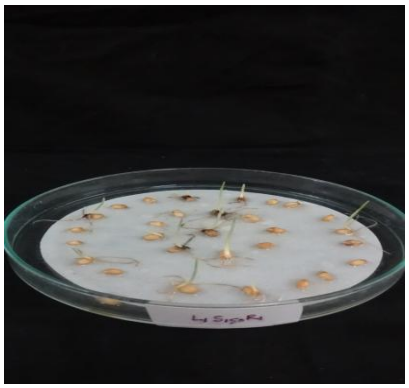
5 dS/m salt



10 dS/m salt



15 dS/m salt



20 dS/m salt

Plate 2: Effect of different salinity level on wheat growth