INDUCTION OF SALT TOLERANCE CAPABILITY IN WHEAT (*Triticum aestivum L.)* THROUGH HYDROGEN PEROXIDE (H_2O_2) PRIMING

BY

MARIAM AKTER ORTHY

DEPARTMENT OF AGRONOMY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

SHER-E-BANGLA NAGAR, DHAKA, BANGLADESH

JUNE, 2017

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BY

MARIAM AKTER ORTHY

Registration No. 11-04246

A Thesis

Submitted to
The Department of Agronomy,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (MS)
IN
AGRONOMY

SEMESTER: JANUARY-JUNE, 2017

Approved by:

Prof. Dr. Md. Abdullahil Baque	**Prof. Dr. H. M. M. Tariq Hossain**
Supervisor	**Co-supervisor**

Prof. Dr. Md. Shahidul Islam

Chairman

Examination committee

CERTIFICATE

This is to certify that the thesis entitled, "**INDUCTION OF SALT TOLERANCE CAPABILITY IN WHEAT (*Triticum aestivum L.)* THROUGH HYDROGEN PEROXIDE (H_2O_2) PRIMING**" 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 **MARIAM AKTER ORTHY** bearing Registration No. 11-04246 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

ACKNOWLEDGEMENTS

The author seems it a much privilege to express his enormous sense of gratitude to the almighty Allah for there ever ending blessings for the successful completion of the research work.

The author feels proud to express his deep sense of gratitude, sincere appreciation and immense indebtedness to his supervisor Prof. Dr. Md. Abdullahil Baque, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, for his continuous guidance, cooperation, constructive criticism and helpful suggestions, valuable opinion in carrying out the research work and preparation of this thesis, without his intense co-operation this work would not have been possible.

The author feels proud to express his deepest respect, sincere appreciation and immense indebtedness to his co-supervisor Prof. Dr. H. M. M. Tariq Hossain, Department of Agronomy, SAU, Dhaka, for his scholastic and continuous guidance during the entire period of course, research work and preparation of this thesis.

The author expresses his sincere respect to Prof. Dr. Md. Shahidul Islam (Chairman), Prof. Dr. Md. Fazlul Karim, Prof. Dr. Md. Jafar Ullah, Prof. Dr. A. K. M. Ruhul Amin and Prof. Dr. Tuhin Suvra Roy, Department of Agronomy, SAU, Dhaka, for their valuable suggestions and cooperation during the study period.

The author expresses his sincere appreciation to his father and beloved mother, sister, brother, his all roommates and his all friends.

The author also expresses his thanks to BAS-USDA, PALS, project no. CR-09, Bangladesh Academy of Science, National Science and Technology Museum Bhaban, Agargaon, Dhaka-1207, Bangladesh.

The Author

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Abstract

An experiment was conducted at the Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from October 2016 to December 2016. Three different experiments were conducted in a Completely Randomized Design (CRD) with five replications. Three wheat genotypes namely- ESWYT 5, ESWYT 6, and BARI Gom 28 were used as test crop and different priming chemicals such as of H_2O_2 and distilled water were utilized for chemical and hydro priming and NaCl was used to induce salt stress. The data on germination parameters of wheat like germination percentage and growth parameters like root length, shoot length, dry weight and vigor index were reviewed. Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the Least Significant Difference (LSD) at 1% level of probability. The first experiment was carried out to find out the effect of different concentrations of H_2O_2 on germination and growth behavior of three wheat genotypes (ESWYT 5, ESWYT 6 and BARI Gom 28) without any stress condition. It was found that ESWYT 5 showed the highest rate of germination (93.40%), shoot length (162.40 mm), root length (146.20 mm), shoot dry weight (0.04220g), root dry weight (0.03480g), relative water content (93.08%), water retention capacity (20.80), coefficient of velocity of germination (17.66) and vigor index (253.90) when the seeds were primed with different concentrations 0ml, 2ml, 4ml, 6ml ,8ml and 10 ml of H_2O_2 per 1000ml H_2O solution in 24 hours. The second experiment was carried out to optimize the priming time on the germination and growth behavior of wheat genotypes. It was found that ESWYT 5 primed with 2ml H_2O_2 for 9 hours gave the highest germination rate (94.20%), shoot length (169.30 mm), root length (146.0 mm), shoot dry weight (0.04340g), root dry weight (0.03820g), relative water content (91.14%), water retention capacity (17.76), coefficient of velocity of germination (18.08) and vigor index (303.1). In the third experiment germination and growth behavior of primed seeds of wheat genotypes (ESWYT 5, ESWYT 6 and BARI Gom 28) under 5 different salt (NaCl) concentrations (0, 5, 10, 15, 20 dSm^{-1}) were evaluated. It was observed that highest germination rate (93.60%), shoot length (151.70 mm), root length (127.5 mm), shoot dry weight (0.04480g), root dry weight (0.03580g), relative water content (92.92%), water retention capacity (15.57), coefficient of velocity of germination (17.52) and vigor index (264.1) were obtained from ESWYT 5 when the primed seeds are placed without salt. But under salinity stress, the highest germination rate (91.80%), shoot length (143.0 mm), root length (118.10 mm), shoot dry weight (0.04140g) and root dry weight (0.03220g), relative water content (86.50%), water retention capacity (14.18), coefficient of velocity of germination (17.27) and vigor index (239.80) were achieved from ESWYT 5 under primed seeds placed with 5 dSm^{-1}.There were a slow reduction observed with the increasing of salt concentration from 0 to 20 dSm^{-1}.The result of the study suggest that exogenous H_2O_2 application effectively alleviated the adverse effect of salt stress.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF FIGURES (CONT'D)

LIST OF PLATES

LIST OF APPENDICES

LIST OF APPENDICES (CONT'D)

ABBREVIATIONS AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSIR	=	Bangladesh Council of Scientific and Industrial Research
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agriculture Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
LSD	=	Least Significant Difference
m^2	=	Meter squares
ml	=	Millilitre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celceous
%	=	Percentage
NaOH	=	Sodium hydroxide
GM	=	Geometric mean
mg	=	Milligram
P	=	Phosphorus
K	=	Potassium
Ca	=	Calcium
L	=	Liter
µg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization
J	=	Journal
Res	=	Research
Agric	=	Agricultural

CHAPTER 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop and ranks first globally and third in Bangladesh both in terms of production and acreage (FAO, 2014). It is a staple food crop for more than one third of the world population (Shirazi *et al*., 2001). By 2050 the world population will be about 9.10 billion, which will be 34% higher from today and we need to feed another 2.30 billion people with limited resources. Food production must need to be increased about 70% and to meet this huge demand cereal production will need to increase about 3 billion metric tons from 2.10 billion metric tons today. But in a dilemma, the world agriculture in 21st century faces versatile challenges (SRDI, 2010). Wheat is a grass widely cultivated for its seed, a cereal grain which is a worldwide staple food (Shewry, 2009). There are many species of wheat which together make up the genus Triticum; the most widely grown is common wheat *(T. aestivum). Triticum* has 10 species six are cultivated and four which are not. The most economically important species, *T. aestivum*, has five subspecies. Wheat is grown on more land area than any other food crop (220.4 million hectares, FAOSTAT, 2014). World trade in wheat is greater than for all other crops combined (Curtis *et al.*, 2002). In 2016, world production of wheat was 749 million tones, making it the second most-produced cereal after maize (FAOSTAT, 2014). Since 1960, world production of wheat and other grain crops has tripled and is expected to grow further through the middle of the 21st century (Godfray, 2010). Global demand for wheat is increasing due to the unique viscoelastic and adhesive properties of gluten proteins, which facilitate the production of processed foods, whose consumption is increasing as a result of the worldwide industrialization process and the westernization of the diet (Day *et al.*, 2006).

Globally, it is the leading source of vital protein in human food, having a protein content of about 13%, which is relatively high compared to other major cereals but relatively low in protein quality for supplying essential amino acids (CORDIS, 2016). Wheat is an important source of carbohydrates. When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber (Shewry and Hey, 2015).

In Bangladesh, the area under wheat cultivation during 2013-2014 was about 1061602 acres producing 1302998 M tons with an average yield of 1233 kg acre-1 (BBS, 2014). The present population of Bangladesh will progressively increase to 223 million by 2030 requiring 48.0 million tons of food grains (Karim *et al.*, 1990). Owing to population pressure the cultivable area is decreasing in the country day-by-day, and this problem will gradually but soon be acute.

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop yield (Munns and Tester, 2008). Salinization affects many irrigated areas mainly due to the use of brackish water. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Munns and Tester, 2008). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa *et al.*, 2000). Together, these effects reduce plant growth, development and survival. Bangladesh is also not beyond this threat. In Bangladesh the salinity affected area was 83.3 million ha in 1973, 102 million ha in 2000 and in 2009 it has reached up to 105.5 million ha and the area is being expanded with times being reported by Soil Resource and Development Institute (SRDI, 2010). The dramatic increasing of saline area is caused by rise of the sea levels due to global warming. Salinity is a major abiotic stress, which hinder crop production. It creates and adversely impacts the socio-economic condition of many developing countries including Bangladesh. In Bangladesh, over 30% of the net cultivable areas lie in the coastal zone close to the Bay of Bengal of which approximately 53% are affected by varying degrees of salinity (Haque, 2006). Ali (2011) showed that the salt-affected areas in the coastal region of Bangladesh increased sharply, by 26.71%, to 950,780 hectares in 2009 from 750,350 hectares in 1973. Agricultural land use in salt affected areas is very poor in respect of crop production (Petersen and Shireen, 2001). Most of the high yielding salt sensitive crop might not be suitable for cultivation in the existing cropping pattern. At low salt concentrations, yields are mildly affected or not affected at all (Maggio *et al.*, 2001). As the concentrations increase, the yields move towards zero, since most plants,

glycophytes, including most crop plants, will not grow in high concentrations of salt and are severely inhibited or even killed by 100-200 mM NaCl. The reason is that they have evolved under conditions of low soil salinity and do not display salt tolerance (Munns and Termaat, 1986). High salinity affects plants in two main ways: high concentrations of salts in the soil disturb the capacity of roots to extract water, and high concentrations of salts within the plant itself can be toxic, resulting in an inhibition of many physiological and biochemical processes such as nutrient uptake and assimilation (Hasegawa *et al.*, 2000, Munns and Tester, 2008). Together, these effects reduce plant growth, development and survival.

Wheat is cultivated over a wide range of environments, because of wide adaptation to diverse environmental conditions. It is a moderately salt-tolerant crop (Moud and Maghsoudi, 2008).

Salinity reduces the growth of wheat plant by reducing the plants ability to absorb water from soil. Salinity 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). Salinity affects wheat seedling growth by changing phytohormone levels (Shakirova *et al*., 1997). Furthermore, salinity induces reduction in photosynthetic rate and stomatal conductance in wheat. Adding more NaCl increases the action of superoxide dismutase and peroxidase and reduces the transpiration rate in *Triticum aestivum* (Sharma *et al*., 2005). Moreover, increased salinity induces a considerable reduction in height, number of fertile tillers and dry weight of shoots in wheat (Iqbal *et al*., 2005). It has been demonstrated that about 61% reduction of seed germination and 23-25% yield loss can be occurred when wheat seeds were cultivated under salt stress condition (AL-Musa *et al*., 2012). Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results into low agricultural production. Salinity causes a variety of biochemical, physiological, and metabolic changes in most of the crop plants (Xiong and Zhu, 2002), which may result in oxidative stress and affect plant metabolism, stand establishment and thereby the yield (Shafi *et al*., 2009). Plant growth and development are regulated by a number of intrinsic and extrinsic

factors, which can be modified in various ways. There are different approaches to mitigate the salt hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahloowalia *et al*., 2004), in vitro selection, use of plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H_2O_2) and osmoprotectants as foliar application and seed treatment (Farooq *et al*., 2009).

Study of wheat to salinity stress response may be helpful in breeding salt tolerant varieties. With the above facts keeping in view the present investigation was undertaken with following objectives:

- To evaluate the effect of seed treatment with H_2O_2 (Hydrogen Peroxide) on germination behavior of wheat.
- To optimize the priming time on germination behavior of wheat.
- To evaluate the effect of Hydrogen peroxide on germination and vigor of wheat under salt stress.

CHAPTER 2

REVIEW OF LITERATURE

Wheat is an important food crop in our country. Most of the areas of the southern part of Bangladesh are affected by salt condition and farmers cannot cultivate wheat in the salt affected area due to lack of efficient salt tolerant variety or lack of proper management strategy. Again, priming of seeds can reduce the water requirement and increase total productivity of crops. In regions where water is scarce people have to grow wheat as it requires much less water than boro rice. The findings from this review will help to cultivate wheat under salt affected areas.

Salinity stress is one of the most deleterious abiotic stresses reducing crop production across the world. It is one of the most important stresses limiting crop production in arid and semiarid regions (Saboora and Kiarostami, 2006) and it 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 are conducting many experiments to adopt different crops in the saline area; wheat is one of them. Some of the countries like Australia, USA, Bangladesh, Pakistan, Sri Lanka etc. are having acute problem with the management of salinity and sustainable crop production. However, soil salinity is not harmful in similar manner for all wheat cultivars. Genetic improvement of salinity tolerance in crop plants is of high importance throughout the world. 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 Effect of salinity

Soil salinity is one of the most important abiotic stress and limiting factor for worldwide plant production (Koyro, 2006). Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Muhling and Lauchli, 2003). Under salt stress, plants have to cope with stress imposed by the low external water potential and with ion toxicity due to accumulation of ions inside the plants (Romero-Aranda *et al*., 2006). Differences in salt tolerance exist not only among

different genera and species, but also within the different organs of the same species (Ismail, 2003). Comparing the response of cultivars of one species to salinity provides a convenient and useful tool for unveiling the basic mechanisms involved in salt tolerance.

2.1.1 Effect of salinity on growth parameters

A pot experiment was conducted by Ewase (2013) to observe the effect of salinity stress on plants growth of Coriander (*Coriandrum sativum* L.). He used four treatments of different concentrations of NaCl namely 0, 1000, 2000, 3000 and 4000 ppm. The Obtained results showed that plant length, number of leaves, roots number and length were reduced by increasing the NaCl concentration and Coriander plants were found to resist salinity up to the concentration of 3000 ppm NaCl only.

Nawaz *et al*. (2010) reported that applications of salt in the growth medium caused reduction in shoot length of sorghum cultivars. Under saline conditions 50 mM proline was more effective to reduce the effect of NaCl than 100 mM proline in both cultivars. Proline level 50 mM showed 11.78% increased shoot length as compared to NaCl stresses plants. However, high concentration of proline (100 mM) was not so much effective as compared to low concentration i.e. 50 mM.

BINA (2008) studied the screening of wheat varieties for growth and yield attributes contributing to salinity tolerance and reported that wheat varieties of high yielding and tolerant group recorded a higher value of number of effective tillers plant.

Liu *et al*. (2008) reported significant reduction in the dry biomass of halophyte Suaeda salsa when exposed to different concentration of NaCl under different water regimes.

2.1.2 Effect of salinity on physiological attributes of plant

Salt stress induces stomatal closure, which affects CO_2 fixation. Exposed to salt and water stresses over days cause reduction in photosynthesis (Munns *et al.,* 2000). In addition to reduced CO_2 diffusion through the stomata, both stresses also result in an apparent reduced CO_2 diffusion through the leaf mesophyll, i.e. in a reduced mesophyll conductance to CO_2 (Flexas *et al.*, 2004). Reduced rate of photosynthesis induces production of ROS, which can cause strong photoinhibition and interrupt photochemical processes in thylakoids (Sairam and Tyagi, 2004).

Analysis of chlorophyll fluorescence showed that applied salt doses did not disturb the

light phase of photosynthesis in all cultivars under study. Maximal PSII quantum yield (Fv/Fm) which reflects efficiency of PSII electron transport was unaffected in salt stressed condition (Plazek *et al.*, 2013).

The ability of plants to survive under saline conditions varies among different species of halophytes and glycophytes. The halophytes adapted to live, support growth and reproduce in soils containing high concentration of salt (above 200 mM NaCl), by adapting various tolerance mechanisms (Bose *et al*., 2013). Unlike the halophytes, the glycophytes cannot tolerate more than 25% of the salinity level of sea water without short changing their growth and yield. Unfortunately, most of the modern crops including wheat, rice and barley are glycophytes. The growth response of glycophytes to salinity (>40 mM NaCl) occurs in two phases: (i) a rapid response to increase in external salt known as- "osmotic phase" and (ii) slower response with accumulation of Na^+ ions in vacuoles refer to as "ionic phase". At both phases, the growth and yield of crops are significantly reduced (Munns and Tester, 2008). Leaf injury and death is associated with high salt loadings in the leaf to levels that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Munns *et al*., 2006). The trade-off between the rate at which the leaves die and the rate at which new leaves are produced would determine the tolerance status of the plant under salt stress. Plants are unable to cope, tolerate and survive in saline conditions long enough to supply sufficient photosynthate to the reproductive organs and produce viable seeds, if the former process progresses faster than the latter. Based on this two-phase concept, the osmotic effect exerted by salts in the medium around the roots would cause the initial growth reduction in both salt tolerant and salt sensitive genotypes (i.e., *Osmotic Phase*). However, the salt-sensitive genotypes are much more affected at the ionic phase, because of their inability to prevent Na+ build-up in transpiring leaves to toxic levels (Munns *et al*., 2006). Because of this development, crops have been classified into two categories: (i) salt-includers and, (ii) salt-excluders. Salt-includers take up Na+ and translocate it to the shoot, where it is sequestered and used as vacuolar osmoticum (tissue tolerance), whereas the salt-excluders adapt to saline stress by avoiding Na+ uptake (Mian *et al*. 2011). The salt sensitive genotypes can be differentiated from the salt-tolerant ones at ionic phase, and

the effect of salinity on crops may also be as a result of the combination of osmotic and ionic salt effect. The ionic phase has been associated with the reduction in the stomatal conductance, photosystem II efficiency, decrease in photosynthesis capacity, reduced biomass and poor yield in plants (Tester and Davenport, 2003).

2.1.3 Effect of salinity on yield and yield contributing parameters

Soil salinity reduces crop biological yield by affecting all aspects of plant physiology, growth and development, such as germination potential, vegetative growth and the reproductive growth stages, due to the complex interactions among morphological, physiological and biochemical processes (Akbarimoghaddam *et al*., 2011).

Saberi *et al*. (2011) conducted an experiment and found that increased salinity significantly reduced forage dry yield from 44.09 gm plant-1 in the control to 32.76 g plant-1 at salinity with 15 dSm^{-1}. For every one unit increase in salinity, the forage yield decreased by 5.2 units and for every one unit increase in water stress (irrigation frequency), the forage yield decreased by 3.6 units.

Ali *et al*. (2005) conducted a pot experiment with three salinity levels (0, 6 and 9 dSm^{-1}) and observed that 1000-seed weight decreased with increased salinity level in sesame. Again, Thakral *et al*. (1996) studied six B. carinatus species under 0-125 meq L-1 chloride solution and observed that siliqua plant-1, 1000-seed weight and seed yield decreased under salinity.

The decrease in crop yield may be partly due to in ion (Na^+ and Cl^-) toxicity (Chinnusamy *et al.,* 2006). Salinity can also upset the nutrient balance in the plant and/or interfere with the uptake of some nutrients (Blaylock, 1994). Reports have indicated that uptake of nutritive cations - potassium and calcium (Asch *et al.,* 2000) and anions - nitrate and phosphate (Song *et al.,* 2009) by plants are significantly decreased under soil salinity conditions. The adverse effects of salt stress have also been observed on crop plant at physiological and biochemical levels (Munns and James, 2003), as well as at the molecular level (Tester and Davenport, 2003). Salt stress increases the formation of reactive oxygen species (ROS) in plant (Miller *et al*., 2008). The ROS main primary production sites in plant are chloroplasts, mitochondria, and peroxisomes (Asada, 2006). These important organs are very sensitive to ROS. Excessive ROS formation is often considered as the initial process that leads to cellular damage of

these organs in plant under salt stress. ROS are toxic and damages the cellular membranes, membrane bound structures, enzymes and DNA especially in mitochondria and chloroplasts, and can therefore severely impair plant growth and/or survival (Allen, 1995) and consequently, reduction crop yield. Reduction in crop yield of up to 76% has been reported due to salt stress (Rajpar *et al*., 2006).

2.2.1 Effect of Seed Priming

Meriem *et al*. (2014) carried out an experiment to evaluate the interactive effect of salinity and seed priming on coriander. The experiment was carried out in completely randomized design with three replications consisting of four coriander genotypes (Tunisian cv, Algerian cv, Syrian cv and Egyptian cv) at two seed conditions (seed priming with 4 g L-1 NaCl for 12h or no seed priming). Results showed that seed priming and salinity had significantly ($p<0.05$) affected all the parameters under study. Seed priming with NaCl had diminished the negative impact of salt stress in all cultivars and primed plants showed better response to salinity compared to unprimed plants.

Dalil (2014) reported that during seed priming in medicinal plants seeds are partially hydrated, so that pre-germinative metabolic activities proceed, while radicle protrusion was prevented, then were dried back to the original moisture level. Primed seeds are physiologically closer to germination and growth after planting than unprimed seeds. Aymen and Cherif (2013) reported that with increasing salinity, emergence traits (total emergence, mean emergence time), growth parameters (plant height, shoot fresh and dry weight) and mineral contents (K+ and Ca2+) decreased, but to a less degree in primed seeds. At different salinity levels, primed seeds possessed higher emergence and growth rate than control.

2.2.2 Effect of plant growth regulator

The depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones such as gibberedllic acid or kinetin (Debez *et al.*, 2001) and there are many reports that presoaking seeds with optimal concentrations of phytohormones (Ungar, 1985) increased the germination rate and emergence under saline conditions. For example, alleviation of salinity stress by GA3, Kin and IAA on seed germination of Brassica campestris L. was reported. They found that growth regulators

significantly increase germination under salt stress compared with controls receiving no growth regulators. GA3 was more effective than kinetin or IAA. Sangkuk *et al*. (1997) treated four cultivars of rice with higher concentrations of ABA and kinetin and observed their alleviating effect on NaCl injury during rice germination. Kinetin treatment increased germination except in cv. Dasanbyeo. However, some studies showed that ABA also accelerated germination.

Growth promoting hormone (kinetin) promoted germination of three plant species (tomato, barley and cotton) by alleviating the osmotically-induced dormancy by NaCl (Bozcuk, 1981). Similarly, germination studies with lettuce (Kaufman and Ross, 1970) also indicated that kinetin can alleviate dormancy induced by osmotic stress in seeds.

2.3 Improved gas exchange with non-enzymatic antioxidant system:

The production of reactive oxygen species (ROS) is a normal event of oxidative metabolism in plants but their generation is further enhanced in response tos various biotic and abiotic stresses, such as salinity (Moller *et al*. 2007). Salinity is a limiting environmental factor, which impairs plant growth and development. It affects approximately 20% of the world's cultivated area and nearly half of the world's irrigated lands (Sairam and Tyagi, 2004). In excess, ROS can damage DNA, proteins, chlorophyll and membrane functions. The main ROS produced are hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and hydroxyl (OH^-) radicals (Azevedo *et al.*, 2008).

Cell growth and photosynthesis are among the primary processes affected by salinity (Munns *et al.*, 2006). Accordingly, the decline in productivity observed for many salt stressed plant species is often related to direct or indirect reductions in photosynthetic capacity (Meloni *et al.*, 2003). Direct effects include the decreased CO_2 availability because of diffusion limitations through the stomata and the mesophyll Flexas *et al.*, 2007) and/or the alterations of photosynthetic metabolism (Lawlor and Cornic, 2002). Indirectly, photosynthetic rate can be reduced by the harmful effects of ROS on the photosynthetic machinery (Ort, 2001). Until recently, H_2O_2 was seen as a toxic cellular metabolite (Azevedo *et al.*, 2005); nevertheless, it is toxic only at high concentrations (Uchida *et al.*, 2002). According to Quan *et al.* (2008), the H_2O_2 is the most stable compound among ROS and the most feasible molecule for ROS-mediated signal transduction. H_2O_2 is produced in response to stress and mediates crosstalk

between signaling pathways. Therefore, the H_2O_2 is a probable signaling molecule that contributes to the phenomenon of "cross-tolerance", whose exposure of plants to one stress (for instance, H_2O_2) may provide protection towards another stress (such as salinity) (Neill *et al.*, 2002).

It has been reported that the exogenous application of H_2O_2 prior to salt exposition induced salinity tolerance in plants by activation of enzymatic antioxidant defense system (Uchida *et al.*, 2002; Azevedo *et al.*, 2005). Additionally, Gao *et al.* (2010) reported that H_2O_2 application improved the tolerance to oxidative and heat stress of tobacco and cumcumber plants, respectively. However, most of these researches are related to H_2O_2 application in seeds and root system, and there are few studies that examined its exogenous application through spraying leaves in plants under abiotic stress.

We tested the hypothesis that the non-enzymatic defense system confers an additional protection against oxidative damage in H_2O_2-induced salt tolerance in maize plants. In a previous study, the behavior of enzymatic defense pathway was examined (Gondim *et al.*, 2012). Therefore, this study investigated the effects of H_2O_2 leaf spraying in maize plants under salt stress on the non-enzymatic defense system (ascorbate and glutathione) and its relationship with some physiological processes, such as plant growth, relative chlorophyll content, relative water content and gas exchanges.

2.4 Effects of H_2O_2 treatment:

Several studies have shown the beneficial effects of H_2O_2 pretreatment on salt tolerance in monocotyledonous plants (Uchida *et al.*, 2002; Azevedo *et al.*, 2005 and Wahid *et al.*, 2007). In our previous study (Gondim *et al.*, 2012), we also demonstrated that H_2O_2 pretreatment could reverse the harmful effects of salinity on growth by alleviating salinity-induced membrane damage, which was associated to the ability of H_2O_2 to induce antioxidant enzymatic defenses, especially catalase activity. Herein, we described that the leaf H_2O_2 spraying was effective to reduce the salinity deleterious effects on growth and gas exchange of maize plants, which was not closely related to a better non-enzymatic antioxidant system.

Salinity stress has been shown to reduce the overall growth and productivity of plants by disturbing several physiological and biochemical processes like photosynthesis, ion homeostasis and enzyme activities (James *et al.*, 2006). In fact, our results are in

agreement with those previously reported for different crops such as cowpea (Freitas *et al.*, 2011), sorghum (Lacerda *et al.*, 2003; Freitas *et al.*, 2011), maize (Azevedo *et al.*, 2005), cotton (Freitas *et al.*, 2011) and pea (Noreen and Ashraf, 2009). In this study, the chlorophyll content was negatively affected by salinity only in water sprayed plants after 14 days of treatment. According to Singh and Dubey (1995), the loss of chlorophyll content acts as a cellular marker of salt stress, and it could be related to photo inhibition or ROS formation. Therefore, the pretreatment with H_2O_2 was effective to reduce the detrimental effects of salinity in chlorophyll content.

The prevented chlorophyll degradation due to H_2O_2 supplying may be assigned to maintain higher RWC and lower hydrogen peroxide content in leaves under salt stress (Chakraborty *et al*., 2012). Additionaly, the higher RWC in H_2O_2-sprayed stressed plants can be explained by the ability of H_2O_2 to induce mechanisms that allow the plant to obtain and preserve high water content, as well as accumulate high contents of ions and compatible solutes under saline conditions, which resulted in better growth of stressed plants.

The most common response to salt stress is a decrease in photosynthesis, and it may be caused by salt-induced changes like ROS formation, water status alteration and reduction in chlorophyll content and CO_2 diffusion through stomatal guard cells (Munns and Tester, 2008). It has been reported that reduction in photosynthesis by low stomatal conductance, which causes CO_2 availability, occurs during early exposure to salt stress, while biochemical limitations arise due to long-term NaCl exposure (Silva *et al.*, 2011). Thus, the reduction of photosynthesis in maize plants was caused by stomatal closure, decreasing the intercellular CO_2 concentration for Rubisco activity (Shahbaz *et al.*, 2010).

Some studies correlate the maintenance of gas exchange with salt tolerance in plants (James *et al.*, 2006; Munns and Tester, 2008). In this work, it should be emphasized that all gas exchange parameters were less affected by salinity in plants previously treated with H_2O_2. Therefore, our data indicate that H_2O_2 supplying increased gs, which enabled high photosynthetic rate and improved shoot dry mass. In addition, the lower leaf H_2O_2 accumulation induced by the H_2O_2 pretreatment in NaCl stressed plants is an evidence that plants were able to control oxidative damages caused by ROS in the photosynthetic

machinery and maintain leaf gas exchange.

Similarly, Wahid *et al.* (2007) observed that the H_2O_2-pretreatment in wheat seeds caused increases in plants subjected to salinity when compared to non-treated seedlings. A soluble compound ubiquitously present in photosynthetic organisms, acting as an important antioxidant in plant cells (Nakano and Asada, 1981).

According to Noctor and Foyer (1998), the ascorbate could react directly with hydroxyl radicals, superoxide, and singlet oxygen and thus promote oxidative protection against several stresses. Moreover, it has been reported that the changes in ascorbate redox state are directly correlated with stress tolerance in plant species (Noctor and Foyer, 1998). Conversely, the leaf amount ascorbate redox state did not act in the ROS scavenging and salt stress acclimation here in.

2.5 Soil salinity

Salinization of arable land has continued to increase in recent times and, is particularly detrimental to irrigated agriculture, which provides one third of the global food supply. Soil salinity can be determined by measuring the electrical conductivity (EC) of the soil saturation extract. According to the standard definition, a soil is said to be saline if the EC ≥ of 4 dSm^{-1} (equivalent to about 40 mM NaCl), while soils with EC's exceeding 15 dSm^{-1} are considered strongly saline (SSSA, 1997). Traditionally, saline irrigation water is grouped into 4 categories: *slightly saline* (EC< 2 dSm^{-1}); *moderately saline* (2–6 dSm^{-1}); *highly saline* (6–15 dSm^{-1}), and *extremely saline* (EC>15 dSm^{-1}) (FAO, 2008). The salinity of soils is associated with the excessive presence of primary cationic species (*i.e.*, Na^+, Ca^{2+}, and Mg^{2+}) and anionic (*i.e.*, Cl^-, SO_4^{2-}, HCO_3^-, and CO_3^-) species in the soil. However, Na^+ and Cl^- are the most important ions (Hasegawa *et al*., 2000), because they not only cause degradation of soil physical structure but also impair plant growth and development. Thus, soils are said to be *saline*, *sodic* and/or *saline-sodic* based on the total concentration of salt and the ratio of Na^+ to Ca^{2+} and Mg^{2+} in the saturated extract of the soil (Dudley, 1994). The diverse ionic composition of salinized soils would result in a wide range of physiochemical properties.

2.6 Salinization of arable lands

Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Zhao *et al*., 2007). It may occur naturally in the top soil or may be introduced by man. The natural soil salinization is caused by either the shallow saline water table or weathering of parent rock materials which releases salts in the soil, while the human-induced soil salinity arises from human activities and improper irrigation/poor cultural practices, such as., the use of saline water for irrigation, deforestation, overgrazing and poor drainage of irrigated fields (Yadav *et al*., 2011). Salinity is becoming more extensive due to land clearing and unsustainable irrigation practices and through pressures for bringing marginal land into production (Munns and Gilliham, 2015). According to the FAO (2008), over 6% of the world's land is affected by salinity, accounting for more than 800 million ha of land. Salinity is already widespread in many regions and has continued to increase due to the changing climate. It has been estimated that 950 million ha of salt affected lands occur in arid and semi-arid regions, which is about 33% of the arable land area of the world. Globally, 20% of irrigated land (450,000 km^2) is afflicted by salinity, with 2,500-5,000 km^2 of lost production lands every year as a result of salinity (UNEP, 2008). The distribution of saline land world-wide, with the affected areas predominantly located in the wheat producing countries including Central and West Asia, Australia, Northern Africa and some parts of South and Northern America. Jamil *et al*. (2011) has predicted that more than 50% of the arable would be salinized by the year 2050. The global annual losses in agricultural production from salt-affected land are in excess of US$12 billion and rising (Qadir *et al.*, 2008). In view of this development, concerted efforts must be taken to manage the arable lands (especially those prone to salinity) to minimize the impact of salinity on crop yield by adopting practices that curtail further soil degradation.

2.7 Mechanisms of salinity tolerance

Several reports have shown wide spectrum of responses to salinity in plants that warrant wide range of adaptations at the whole plant level (Jones and Gorham, 1983). Over the years, plants have evolved several mechanisms that allow them to adapt, grow and

reproduce under high salinity conditions. According to Roy *et al.* (2014), these mechanisms are grouped into three main categories: (i) osmotic stress tolerance, which is controlled by long distance signals that reduce shoot growth and is triggered before shoot Na+ accumulation; (ii) Na^+ or Cl^- exclusion, that tend to prevent Na^+ and Cl^- uptake and transport processes in roots in order to reduce the accumulation of these ions to a toxic concentration within leaves and, (iii) tolerance of tissue to accumulated Na^+ or Cl^-, where Na^+ or Cl^- that succeeded in getting into the plants are compartmentalized in the leaf vacuole to prevent salt injury to the sensitive thylakoid membrane of the chloroplasts. These three mechanisms have also been reported by Tester and Davenport (2003) and Kumari *et al.* (2014). Although the information available for the plant tolerance to the "osmotic phase" still remain vague, Mittler *et al.* (2011) have suggested that this process may be linked to the rapid, long-distance signaling via processes such as ROS waves, Ca^{2+} waves (Simon Gilroy, personal communication), or the long distance electrical signaling (Maischak *et al.*, 2010). This alludes to the fact that the differences of plants in osmotic tolerance may be due to the differences in the long-distance signaling and/or in the initial salt stress perception and/or in the response to the signals existing among plants. However, further studies are needed to gain a clearer understanding of osmotic tolerance in plants. The most researched aspect of salt tolerance mechanism is the "ionic phase", which is due to Na^+ and Cl^- accumulation in the leaf blade. The ion toxicity in plants during the ionic phase can be minimized by reducing the accumulation of toxic ions (Na^+ and Cl^- exclusion) in the leaf blades and/or by increasing the ability of crops to cope with salts that succeeded in gaining entry into the shoot (tissue tolerance) via compartmentation in the vacuoles. Tissue tolerance, which entails Na^+ exclusion from the cytosol and compartmentalization in the vacuole before the ion has a detrimental effect on cellular processes (Roy *et al.*, 2014), may be essential in the synthesis of compatible solutes and higher level controls to coordinate transport and biochemical processes, thus plays a role in both osmo-protection and osmotic adjustment in plants (Munns and Tester, 2008). Munns *et al*. (2012) and Roy *et al.* (2014) have suggested that these three mechanisms of salt tolerance are not mutually exclusive. In other words, the occurrence of one does not prevent the other. However, it might be possible that each of these tolerance mechanisms is more effective in a particular circumstance and/or

genotype and growth stage dependent. For instance, Na^+ exclusion may be more effective in higher salinity (Munns *et al*., 2012), while at moderately saline conditions, "osmotic tolerance" may be much more pronounced. In view of this, salinity tolerance is considered a complex trait, being controlled by many genes and physiological factors. Thus, a probable reason why breeding for salt tolerance through introgression using traditional breeding has not been successful (as measured by the lack of commercial products), as has been usually been attributed to the multi-genic nature of salt stress tolerance in plants (Flowers and Yeo, 1995).

The improvement of salt tolerance in glycophytic crops have been achieved by the development of cultivars with low Na^+ in shoot or high K^+/Na^+ ratio (Tester and Davenport, 2003; Munns and Tester, 2008 and Munns *et al*., 2012). The ability of plants to maintain high K^+/Na^+ is a key feature of salt tolerance because high K^+/Na^+ is required for normal cellular functions and ion-homeostasis. When the plant roots are exposed to salinity, the K^+/Na^+ ratio in the plant is reduced significantly (Tester and Davenport, 2003), because Na^+ competes antagonistically with K^+ uptake via K^+/Na^+ co-transporters, which may block the K^+-specific transporters of root cells under saline conditions (Zhu, 2003) and result in the accumulation of Na^+ to toxic levels in the plant tissues. This means that salt tolerance status of any plant mainly depend on its ability to exclude the Na^+ ions, through preferential absorption of K^+ over Na^+. Amtmann and Sanders (1999) have demonstrated that glycophytes exhibit poor Na^+ exclusion potentials, which would disrupt the ion homeostasis and inhibit cellular growth and functions.

2.7.1 Na^+ transport in crop plants

AS has been described previously, the ability of plants to adapt under high salinity depend on the extent at which they can: prevent Na^+ initial entry, maximize Na^+ efflux transport, minimize loading to the xylem or maximize retrieval before reaching the shoot, maximize intracellular compartmentation or allocation to particular parts of the shoot (e.g. pith cells or old leaves), extrude, mobilize Na^+ ions and secrete salt onto the surface of the leaf (Tester and Davenport, 2003). The Na^+ transporter genes have been reported to perform these functions (Tester and Davenport, 2003). For instance, the overexpression of vacuolar Na^+/H^+ antiporter (*NHX1*) increased salinity tolerance of Arabidopsis (Apse

et al., 1999). The Na^+/H^+ is involved in the intracellular compartmentation of Na^+ via pumping Na^+ into the vacuole and, its activity was increased upon Na^+ application in Barley (Gabarino and DuPont, 1989) and tomatoes (Wilson and Shannon, 1995) and, the $Na^{+/}H^+$ expression was significantly higher in salt tolerant species, *Plantago maritima*, than in the salt-sensitive species, *P. media* (Staal *et al*., 1991). The Na+ transporters are members of the monovalent cation proton antiporter-1 (CPA1) family that were derived from bacteria, yeast, plants and animals (Kumari *et al*., 2014). They play a role in cytoplasmic pH regulation, pumping out H^+ generated by metabolism, K^+ homeostasis and salt tolerance due to Na^+ influx into vacuoles (Waditee *et al*., 2001). The ability of these transporters to prevent Na^+ entry into the plant root cells or facilitate the pumping out of Na^+ that have gained entry into the plant back to the soil solution are important adaptive features of plant under saline conditions. The control sites of two important Na^+ transporters - *Nax1* and *Nax2* genes in protecting plants from salinity stress. While, *Nax1* mediate Na^+ unloading from the xylem into the sheath under salinity stress; thus, preventing Na^+ over accumulation in leaves, to protect the photosynthetic organs**,** the *Nax2* unloads Na^+ from the xylem in roots.

The loading of Na^+ into xylem is essential process for salt tolerance in plant. This process leads to increased Na^+ concentrations in leaves (Shi *et al*., 2002). The leaf blade appears to be more sensitive to salinity than the roots (Munns and Tester, 2008). Karley *et al*. (2000) have demonstrated that Na^+ accumulation is more in the older leaves than younger due of differential distribution of various nonselective cation channels in different cell types. High-affinity K^+ transporter1 (*AtHKT1)* is also associated with Na^+ transport from the shoot into the phloem and also in the unloading of Na^+ into stelar cells (Kumari *et al*., 2014).

2.7.2 K^+ transport in crop Plants

Optimal K^+ uptake is very crucial for salt tolerance in plants (Greenway and Munns, 1980). K^+ plays an important role in plant metabolism and functions including enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance (Wang *et al*., 2013a) and, K^+ influx in plant is inhibited under saline conditions (Ahanger *et al*., 2014).

Low K^+ concentration at the binding sites would activate the hyperpolarization of membrane potential; but depolarization would occur when the K^+ concentration is high (Kumari *et al*., 2014). Several genes encoding K^+ channels/transporters have been linked to K^+ transport in plants. They include: *KUP/HAK/KT*, *HKT*, *Shakers*, *TPK*, *Kir-like* and *CPA* sub-families (Kumari *et al*., 2014).

This K^+ transporter gene family is homologous to bacterial *kup* (*K^+ up*take) and has been cloned from barley (Santa-Maria *et al*., 1997) and, it plays important roles in cell expansion and plant development (Kumari *et al*., 2014) because K^+ is a major cellular solute. Reduction of K^+ uptake impairs K^+ homeostasis, leading to weak cell turgor and reduction in the rate of cell expansion.

High-affinity K^+ (HKT) transporters (HKT) gene families regulate K^+ transport in plants (Rubio *et al*., 1995; Roy *et al*., 2014) and play vital role in salt tolerance (Maser *et al*., 2002). Two classes of HKT transporters exits- the *HKT1* [which mediate relative Na^+ selective uniporters (Maser *et al*., 2002) and *HKT2* [which mediate $Na^{+/}K^+$ cotransport activity and homeostasis (Rubio *et al*., 1995)] transporters. Of the two classes, the *HKT1* group is perhaps of greatest potential for improving the salinity tolerance of crops, frequently appearing as the most likely candidate for quantitative trait loci when phenotyping for salt tolerance and/or Na^+ exclusion in mutant and mapping populations (James *et al*., 2006) and, has been located on 2AL. Munns *et al*. (2012) have demonstrated that the incorporation of novel *HKT1;5* gene from the salt-tolerant wheat relative *Triticum monococcum* into susceptible commercial durum wheat (*Triticum turgidum* ssp. durum) increased grain yield by 25% on saline soil. Moreover, the *HKT2* has been reported to increase salinity tolerance, but not through Na^+ exclusion. Mian *et al.* (2011) indicated that the over-expression of *HvHKT2;1* would increase the Na^+ uptake, Na^+ concentrations in the xylem sap, and enhance translocation of Na^+ to leaves under saline conditions, suggesting that another way plant increase salt tolerance is rather not to translocate Na^+ to the shoot but rather to compartmentalize Na^+ in leaf tissues.

2.8 Improvement of salt tolerance in wheat

The use of wide range of genetic materials for comparative phenotype and physiology screening for salt stress tolerance and ion uptake in cereals have progressed steadily with the identification three mechanisms which may contribute to salt tolerance, such as *osmotic tolerance, ion exclusion* and *tissue tolerance*. This offers strong indication that salt tolerance in wheat can be improved via pyramiding and/or incorporation of useful alleles that are associated with the above mentioned mechanisms. Several breeding strategies have been adopted to achieve these objectives.

2.8.1 Conventional Breeding

Genetic variation in Na^+ for both exclusion and $K^{+/}Na^+$ discrimination exists amongst wheat genotypes, wheat progenitors, wild relatives (Gorham *et al*., 1987), and in the halophytic species in the *Triticeae* (Garthwaite *et al*., 2005). In the past, screening of a large collection of wheat germplasm for salt tolerance identified genotypes that can sustain growth and produce seeds under saline soil conditions. However, only few of the identified salt tolerant genotypes have been successfully released. They include Indian *KRL1–4* and *KRL 19* (from Central Soil Salinity Research Institute at Karnal), *LU26S* and *SARC- 1* (released by the Saline Agriculture Research Centre at University of Agriculture, Faisalabad), *Sakha 8* (from the Agricultural Research Centre at Giza) and *Kharchia 65* (from India). Among them, *Kharchia 65* was the most widely and globally exploited salt tolerance "donor parent" that has been used to contribute positive alleles in many breeding programs (Munns *et al.*, 2006). *Kharchia 65* was developed via selection by Indian farmers on sodic-saline soils of the Kharchi-Pali area of Rajasthan (Rana, 1986). The tolerant genotype *KRL1-4*, derived from a cross between *Kharchia 65* and *WL711*, has performed well on the saline soils of northern India, but it was not successful in Pakistan due to the problem of water logging and soil texture (Hollington, 2000). Also, *KTDH 19* which was developed in UK by Quarrie and Mahmood from a cross between *Kharchia 65* and *TW161* (a line identified with exceptional Na+ exclusion) performed well in Spain (Hollington *et al*., 1994); but in India and Pakistan, it was found to be highly tolerant in terms of total dry matter but the grain yield was very low due to it maturing around 2 weeks later than local genotypes (Hollington, 2000). In addition, the cross of *LU26S*, *Kharchia-65* and two salt-tolerant genotypes, produced two salt-tolerant

genotypes, *S24* and *S36* (selected from the F3 populations) at salinity levels of 24 and 36 dSm^{-1}, respectively (Ashraf and O'Leary, 1996). S-24 showed positive transgressive inheritance for salt tolerance when compared to its parents- *Kharchia-65*, *LU26S* and *SARC-1*, due to its low accumulation of Na^+ in leaves (Ashraf, 2002). It also exhibited higher grain yield potentials than most wheat cultivars (Shahbaz *et al.*, 2008).

2.8.2 Mutation breeding

Mutation breeding techniques have been used to generate a vast amount of genetic variability among genotypes for salinity tolerance. It has played a significant role in plant breeding and genetics and has been used to develop thousands of novel crop varieties which have been released to farmers for cultivation. It is cost effective, quick, proven, robust, non-hazardous and environmentally friendly. It is based on selfing mutants until the induced character has a stable expression in the advanced mutant generations. Mutation breeding has been used to reduce the maturity time by 3 weeks without adverse effects on yield at 150 mM NaCl (Mahar *et al*., 2003).

According to Chijioke (2017), four salt tolerance mutant wheat varieties have been officially released for commercial use as referenced in the mutant varieties database. They include: *Jiaxuan 1* (released in 1974), *Changwei 19* (released in 1978), *Emai 9* (released in 1980), and *H6765* (released in 2004).

2.8.3 Modern Breeding for salt tolerance using molecular markers

The development of molecular markers for the exploitation of DNA polymorphisms in plant systems is one of the most significant developments in the field of molecular biology and biotechnology (Soto-Cerda and Cloutier, 2012). DNA marker is a portion of DNA situated on a chromosome and tightly linked to a known gene controlling trait variation in a given population. Because salt tolerance is polygenic in nature and is largely influenced by environment and genotype, it is difficult to breed using conventional methods. Thus, the use of DNA marker systems have gained prominent in plant breeding, because of the absence of genotype x environment interaction, epistatic effect, and also ease in the picking up of homozygous plants which can be greatly distinguished from the others at an early generation (Kumar *et al*., 2015). Once a marker is found to be linked to gene/QTL contributing to the trait (i.e., salt tolerance) variation in

the crop species, such marker can be used "as surrogate" to incorporate the gene into the commercial crop varieties using either marker assisted selection (MAS) or transgenic approach. MAS has been successfully used to incorporate the Na^+ exclusion gene *HKT1;5* into the susceptible commercial durum wheat (Munns *et al*., 2012). DNA marker systems have been used to tag/map several genes or QTL contributing to salt tolerance in cereals**.** The association and application of the indirect selection markers which are genetically linked with the trait(s) of interest is a well-known approach for improvement of the crop having difficult complex traits such as salt stress tolerance (Im *et al*., 2014). This approach has contributed immensely on deciphering the genetic basis of salt tolerance in many crops.

There are several strategies to increase wheat production in the salt affected areas (such as leaching, drainage etc), the cultivation of tolerant genotypes is recognized as the most effective way to overcome the limitations. The prerequisite is the identification of wheat genotypes with proven wide adaptation under saline conditions. The cultivar, *Kharchia 65* is one of the very few reputed donors of salt tolerance (ST) in wheat and has been extensively used in breeding for ST cultivars globally (Chatrath *et al*., 2007). Thus, there is an urgent need to identify new sources of ST to broaden the gene base and to provide donor parents in locally adapted genetic backgrounds. An imminent task is the efficient characterization of wheat plants for tolerance towards salt stress. The most valuable agronomical traits might serve as good surrogates to discriminate among genotypes under salt stress conditions. Munns and James (2003) consider biomass yield as a useful criterion because it permits the direct estimation of economic return under saline conditions. Moreover, it has been reported that shoot growth is more sensitive to salt stress than the root growth firstly, because the reduction in leaf area development relative to the root growth leads to a decrease in water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation of the salt concentration in the soil, and secondly, due to the accumulation of Na^+ and/or Cl^- at toxic concentration levels affects the photosynthetic capacity resulting in less supply of carbohydrates to the young leaves, that further reduces the shoot growth rate (Munns and Tester, 2008). The ST status of plants can be assessed as the percent biomass production in saline versus control conditions (Genc *et al*., 2007) over a prolonged period of time. Selection of plants with

high ST values would allow breeders to identify genotypes better adapted to the salinized arable lands. Screening for chlorophyll fluorescence characteristics has also gained increasingly interest in plant abiotic stress research. Salinity stress has negative impact on photosynthesis by inhibiting photosystem II (PSII) activity and destruction of chlorophyll pigments due to the accumulation of toxic ions. The relationship between the PSII operating efficiency and CO_2 assimilation in leaves allows fluorescence to be used to detect differences in the response of plants to environmental challenges and, consequently, to screen for tolerance to environmental stresses (Baker and Rosenqvist, 2004).

Tolerance to salt stress is a complex biological phenomenon governed by several physiological and genetic factors and it is growth stage specific (Haq *et al*., 2010). Little effort has been made so far to simultaneously characterize the wheat germplasm across different growth stages. Experiments carried out under controlled conditions were not exposed to those conditions that prevail in salt affected soil such as spatial and temporal heterogeneity of soil chemical and physical properties, high diurnal temperature variations, low humidity, and presence of drought stress (Munns and James, 2003). These could be one of the reasons why breeding for ST has not gained significant progress up till now.

2.9 Findings from salt stress test

Salt-water flooding method as described by the Association of Official Seed Analysts (AOSA, 2009) was adopted to evaluate the genotypes germination ability under two salt types (NaCl and Na_2SO_4) and several concentrations: 100, 150, 200 mM for NaCl and 75, 100 mM for Na_2SO_4 plus control (without salt). Twenty-five seeds of each genotype, in three repetitions, were sown in 29 x 22.5 cm plastic transparent boxes containing blotting paper (ALBET Lab Science, Germany) soaked in 75-ml of each salt treatment solution. Thereafter, the boxes were placed in a growth chamber with white fluorescent light (600 $\mu mol\ m^{-2}\ s^{-1}$; 14 h light/10 h dark) at 15±1°C, and relative humidity of 65±8%. Ten days after sowing, the germination potentials of each genotype were determined with the scale from 0 to 9 as described by Mano *et al*. (1996).

Most of the ST estimates at germination stage were significant and negatively correlated with ST estimates at seedling stage. The mechanisms of salt stress response are highly

growth stage-specific and change during the plant life cycle (Walia *et al*., 2005).

The germination vigor, dry shoot weight and grain yield were negatively affected by salt stress as already reported (Munns and Tester, 2008).

Ion analysis revealed that the accumulated K^+ in the stem after stress was significantly higher than that accumulated in the 3rd leaf and RLP but, no significant difference was found between K^+ concentration in the 3rd leaf and RLP. This was in line with the findings in maize (Kobaissi *et al*., 2014) and barley (Booltink and Verhagen, 1997). In contrast, there was no significant difference among the accumulated Na^+ in 3rd leaf, stem and RLP, although highest and lowest amounts were found in the stem and 3rd leaf, respectively. The high K^+ observed in the stem indicates that the ion is transported preferentially through the stem channels to other plant parts under salt stress conditions. The K^+ accumulation in the 3rd leaf, stem and RLP were positively correlated among each other, an indication that K^+ is mobile within the plant and, can be transported from the stem to the other shoot parts. The increase in the shoot K^+ was accompanied by a significant decline in the shoot Na^+, showing antagonism between K^+ and Na^+ (Elhamid *et al*., 2014). Antagonism exits between K^+ and Na^+ in the site of ion uptake due to direct competition of both ions for absorption in the plants (Epstein, 1966).

The rate of root and shoot water loss due to salt stress correlated positively with each other, suggesting that shoot water loss is a direct consequent of the decreased water absorption capacity of root systems due to high osmotic potential exerted by salt stress around the plant rooting zone. The shoot K^+ concentrations increased with the decrease in the rate of root and shoot water loss, an indication that maintaining optimum K^+ status is favorable for water conservation in plant and would ultimately improve the plant growth and survival under salt stress. Reports have also indicated that sufficient K^+ status would contribute to greater water retention in plant tissues, due to its vital role in the osmotic adjustment and turgor regulation during stomatal movement that affects transpiration and photosynthetic rates and xylem hydraulic conductance (Wang *et al*., 2013b).

Some of the genotypes analyzed in this study have been previously reported to be resilient to different abiotic and biotic stresses. Four genotypes with high ST estimates, have been shown to be resistant to different stresses: Gerek-*79* and *Altay-2000* to drought, salt and cold resistant genotypes (Akfirat and Uncuoglu, 2013), *Katia* to zinc and drought tolerance (ICARDA, 2005) and *Demir 2000* to lodging, cold, stripe and leaf

rust resistant (Mazid *et al*., 2009), have shown to be resistant to different stresses. However, the salt stress sensitive genotype *Bobur* is susceptible to stripe rust at seedling and mature stages (Ziyaev *et al*., 2013). These findings may suggest cross-tolerance among these stress factors in wheat. Mantri *et al.* (2010) reported that plant responses to fungal infection (*Ascochyta blight*) are similar to high-salinity stress.

The chlorophyll fluorescence transients (*Fo*, *Fj*, *Fi*, *Fm* and *Fv*) in both tolerant and sensitive genotypes declined under saline conditions but the sensitive genotypes were more severely affected. The decrease in Fo due to salt stress indicates an increased thermal dissipation (Guidi *et al.*, 2002), while the decrease in Fv may be attributed to the pigment losses due to salt injury. Salinity stress reduces photosynthesis by inhibiting photosystem II complex (PSII) at both acceptor [QA] and donor side (oxygen evolving complex OEC) and destruction of chlorophyll pigments by accumulation of toxic ions (Chen and Murata, 2011). However, the higher fluorescence transients observed in the tolerant genotypes can be attributed to higher number of deactivating PSII and PSI associated with increase in the excitation energy (increased energy trapping capacity of PSII) and decrease in the photochemical quenching coefficient (Guidi *et al.*, 2002). Baker (2008) suggested the use of fluorescence induction parameters to detect metabolic perturbations by abiotic stresses.

Under saline conditions, crops exhibit slower growth rates, increased leaf senenses, reduced tillering and, over months, the reproductive development is affected (Munns and Tester, 2008), resulting in significant grain yield reduction. The effect of salinity on crops is due to osmotic stress caused by the accumulation of Na^+ and Cl^- ions to toxic levels within the plant cells and its interference with the uptake of mineral nutrients (Mba *et al*., 2007). The mechanism of plant response to salt stress is a *complex phenomenon that involves several* genetic, physiological and environmental factors occurring at different levels including cellular, tissue and whole plant level. The cell-based synthesis of osmoprotectants and the mechanisms of ion-homeostasis are essential determinants for salt tolerance (Borsani *et al*., 2003). As the specialization of plant cell progress during ontogeny, the adaptive mechanisms to tolerate salt stress start to differentiate, giving rise to the coordination of all the cellular, tissue and organ responses which are needed for proper tolerance response. It has been suggested that salt tolerance (ST) is developmental

growth stage dependent (Haq *et al.*, 2010), but there may exist the possibility of salt-stress response mechanisms that are active across all the different plant growth stages. The discovery of key genetic switches associated with genes controlling ST at various growth stages would allow not only for characterization of the genetic architectures of salt stress responses, but would also facilitate breeding for improved ST.

Genetic diversity for salinity tolerance has been limited in bread wheat. One land race Kharcia 65 played a major role in salt tolerant varietal development in India where the cultivars *KRL1-4* and later *KRL 19* emerged (Ogbonnaya *et al*., 2013). Dreccer *et al*. (2004) identified synthetic hexaploid wheat that possessed considerable variation for ST based on Na+ exclusion. Similarly, Colmer *et al*. (2006) reviewed the potential of wild relatives to contribute towards improving salinity tolerance. The salinity tolerance of bread wheat is based on a relatively high ability to exclude Na^+ from the leaf blades and an overall increase in the $K^{+/}Na^+$ ratio, in some cases associated with increased K^+ uptake. Several studies have reported on the genetic variation for ST at various growth stages in wheat, providing great opportunity for ST improvement (Rahnama *et al*., 2011). However, the drawback of these studies is their inability to simultaneously analyze the genetic variation for salt tolerance at three key growth stages using the same population. In addition, most of the efforts towards exploring the genetic variation to identify loci associated with salinity tolerance relied on the classical biparental linkage mapping that are characterized by poor resolution in QTL detection, costly, with considerable amount of time needed to develop appropriate mapping population and results in identifying limited number of alleles that can be studied simultaneously at any given locus (Flint-Garcia *et al*., 2003). Genome-wide association studies (GWAS) has emerged as an alternative approach that is maximizing recent advances in genomic tools and statistical methods by exploiting cumulative recombination and mutation events that occurred in a population and taking into account numerous alleles present in the population to identify significant marker-trait associations (MTAs). GWAS has proven to be useful tool to dissect the complex genetic mechanisms governing biotic (Jighly *et al*., 2015) and abiotic (Long *et al*., 2013) stress tolerance in many crops. The inclusion of population structure and kinship matrixes in GWAS model during analysis accounts for false positives and thus, improves its effectiveness and power to detect genetic variants for

the trait of interest. In wheat, there has been little research into the identification of large-scale salt tolerance loci using GWAS for different stages of growth within the same germplasm simultaneously. It is well known that several genes are differentially expressed in response to a range of biotic and abiotic stresses including drought, heat and salinity (He *et al*., 2015).

2.10 H_2O_2 and MDA contents

In both levels of applied Zn, H_2O_2 and MDA contents of plants increased significantly by increasing salinity ($p<0.05$). In saline conditions, application of concentration of 5 μM Zn reduced the H_2O_2 and MDA content of plants. The reduction in the H_2O_2 content were significant at both levels of salinity, but reduction in the MDA content was significant only at the concentration of 100 mM NaCl. In non-saline conditions, H_2O_2 and MDA content of plants were not affected significantly by Zn levels (Abedini, 2016).

Salt stress limits plant growth by adversely affecting numerous physiological and biochemical processes, including photosynthesis, antioxidant capacity and ion homeostasis (Ashraf and Harris, 2009).

The ameliorative effects of sufficient Zn application in improvement of detrimental effects of salinity on plants growth, that was seen obviously in 100 mM NaCl treated plants in this study, have been reported by several authors (Weisany *et al.,* 2012). Reduction in the photosynthetic pigment content in saline conditions is reported for numerous salt sensitive plants (Weisany *et al.*, 2011).

Carotenoids are the one of components required for salt tolerance in plant species (Hernandez *et al*., 1995), because they raise the antioxidant capacity of plant, in order to protect the photosynthetic systems (Perez-Rodriguez, 2009).

Enhancement of membrane permeability and ion leakage, which was seen at high salinity in this study, is a common phenomenon in salt stressed plants (Farhoudi *et al.,* 2015). Sufficient Zn application could deteriorate ion leakage in salt stressed plants; this result is similar to that obtained by Eker *et al.* (2013).

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted during the period from October 2016 to December 2016 to study the mitigation role of hydrogen peroxide (H_2O_2) in eliminating toxicity caused by salt (NaCl). This chapter includes materials and methods those were used in conducting the experiment are presented under the following headings.

3.1 Experimental site

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

3.2 Duration of the study

The experiment was conducted during the period from October 2016 to December 2016.

3.3 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 15.6^0C to 29.5^0C, respectively and average relative humidity was 69.5%.

3.4 Test crops

Three wheat genotypes namely- ESWYT 6, ESWYT 5 and BARI Gom 28 were used for this experiment. Seeds were collected from Wheat Research Centre, Nashipur, dinajpur and Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The collected wheat genotypes were free from any visible defects, disease symptoms and insect infestations and transported to the laboratory of the Department of Agronomy, SAU, Dhaka with careful handling to avoid any injury.

3.5 Experimental materials

Different equipments such as 4-digit electric balance, Petri dish, filter paper, micro pipette, UPS, refrigerator, growth chamber, conductivity meter, pH meter, spectrophotometer, magnetic stirrer, forceps, oven etc. were used for this study.

3.6 Chemicals for seed priming

Different priming chemicals such as H_2O_2 and distilled water were utilized for chemical and hydro priming. Alcohol was used to sterilize the seeds. NaCl was used to induce salt stress.

3.7 Experimental design

The experiment was laid in a Completely Randomize Design (CRD) with five replications.

3.8 Experimental treatments

The experiment comprises of

i) Seven levels of priming agent concentrations *viz*. distilled water, 0ml (control), 2ml, 4ml, 6ml, 8ml and 10ml H_2O_2 solution

ii) Six levels of priming time *viz*. 0, 3, 6, 9, 12,15 hours and

iii) Five levels of salinity stress *viz*. 0, 5, 10, 15, 20 dSm^{-1}NaCl

iv) Five Replication *viz.* R_1, R_2, R_3, R_4, and R_5.

3.9 Steps of the experiment

This experiment was completed in three steps. In the 1st step, the best H_2O_2 concentration with variety, in 2^{nd} step, the best priming time with variety and 3rd step the best result under salt stress condition was identified. On the basis of 1^{st} experiment result, 2^{nd} and 3^{rd} experiments were done.

3.9.1 First experiment

Study on the effect of different concentrations of H_2O_2 on the germination, seedling growth and water relation behavior of wheat.

3.9.1.1 Treatments

One factor experiment considering three wheat genotypes with seven levels of seeds priming for 24 hours was done.

Three wheat genotypes; one wheat variety and two advanced lines (Hasan *et ai., 2017;* Faijunnahar, 2017) were as follows:

i) V1 = ESWYT 5

ii) V2 = ESWYT 6

iii) V3 = BARI Gom 28

3.9.1.2 Priming solutions

0ml, 2ml, 4ml, 6ml, 8ml, 10ml H_2O_2 solution and distilled water were used as priming solutions.

3.9.1.3 Preparation of priming solutions

a) H_2O_2 solutions (2ml, 4ml, 6ml, 8ml, 10ml**)**

2ml H_2O_2 was dissolved in 1000 ml of distilled water to prepare 2ml solution of H_2O_2. Similarly, 4ml, 6ml, 8ml, 10ml H_2O_2 was dissolved in 1000 ml of distilled water to prepare 4ml, 6ml, 8ml, 10ml solution of H_2O_2, respectively.

b) Distilled water

Distilled water was collected from the laboratory of Sher-e-Bangla Agricultural University (SAU).

3.9.1.4 Priming technique

Two priming techniques viz., chemical priming and hydro priming were applied on wheat genotypes. One of the sub-samples was considered as control (unprimed) and the other sub-samples were primed with priming chemicals. For hydropriming seeds of a sub-sample were soaked in distilled water and for chemicalpriming seeds of another sub-sample were divided into another sub-samples and pretreated with H_2O_2 a six levels of concentration of 0ml, 2ml, 4ml, 6ml, 8ml, 10ml for 24 hours. Priming was done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.9.1.5 Experimental set up

75 petridishes were used during the experiment and 30 seeds were placed in each petridish.

Achievement from the first experiment: From the first experiment, 2ml solution of H_2O_2 gave the best result. So, 2ml H_2O_2 solution was used for the next experiment to evaluate best priming time.

3.9.2 Second experiment

Optimization of priming time for the germination, seedling growth and water relation behavior of wheat

3.9.2.1 Treatments

One factor experiment considering three wheat genotypes with six levels of seeds priming time (0, 3, 6, 9, 12 and 15 hours) by 2ml H_2O_2 solution was done. Three wheat genotypes; one wheat variety and two advanced lines were as follows:

i) V1 = ESWYT 5

ii) V2 = ESWYT 6

iii) V3 = BARI Gom 28

3.9.2.2 Priming solutions

2ml H_2O_2 solution was used for chemical priming.

3.9.2.3 Priming technique

For chemical priming the sample of seeds were divided into five sub-sample and pretreated with H_2O_2 for 0, 3, 6, 9, 12 and 15 hours. Priming is done in different plastic containers covered with lids to prevent evaporation loss. Seeds were removed from the priming solution at the required time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

Achievement from the second experiment: From the second experiment, 2ml H_2O_2 solution with 9 hours priming time gave the best result. So, 2ml H_2O_2 solution with 9 hours priming time was used for the next experiment to evaluate best result under salt stress condition.

3.9.3 Third experiment

Germination, seedling growth and water relation behavior of primed seed (wheat) under salt (NaCl) stress condition.

3.9.3.1 Treatments: One factor experiment considering primed seeds of three wheat genotypes under five levels of salt concentration (Control (0 dSm^{-1}), 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1} NaCl) was done.

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

i) V1 = ESWYT 5

ii) V2 = ESWYT 6

iii) V3 = BARI Gom 28

3.9.3.2 Priming solutions and time

2ml H_2O_2 solution and 9 hours priming time were used to test salt stress.

3.9.3.3 Preparation of stress solutions

Salt (Nacl) solutions (5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}):

0.731 g of sodium chloride (Nacl) was dissolved in 250 ml of distilled water to prepare 5 dSm^{-1} 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 to prepare 10 dSm^{-1}, 15 dSm^{-1}, 20 dSm^{-1} solution of NaCl, respectively.

3.9.3.4 Priming technique

Seeds of a sub-sample were soaked in distilled water for hydropriming and seeds of another sub-samples were pretreated with H_2O_2 for chemical priming at a concentration of 2ml for 9 hours, respectively. Priming was done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.10 Data collection

Data on seedling emergence of all the 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 was also counted upto 10 days after seed placing. The seedlings were washed with tap water and excess water was removed with tissue paper.

The following data were taken:

1. Germination rate (%)
2. Shoot length (mm)
3. Root length (mm)
4. Shoot dry weight (g)
5. Root dry weight (g)
6. Relative water content (%)
7. Water saturation deficit (%)
8. Water retention capacity
9. Coefficient of velocity of germination
10. Vigor index

3.11 Procedure of recording data

3.11.1 Germination percentage (%)

The number of sprouted and germinated seeds was counted daily commencing. Germination was recorded at 24 hours 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 using following formula:

Rate of germination (%)

= (Total Number of normal seedlings/Total number of seed placed for germination) × 100 (Othman *et al*., 2006).

3.11.2 Shoot length (mm)

The shoot length of five seedlings from each petrit dish was measured finally at 10 days after placement. Measurement was done using the unit millimeter (mm) by a meter scale.

3.11.3 Root length (mm)

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

3.11.4 Dry weight of shoot and root (g)

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 till attained a constant weight. Then the weight was converted to gram (g).

3.11.5 Relative water content (%)

Relative water content was measured using following formula:

Relative water content (WRC) (%) = {(Fresh weight-Dry weight)/ (Turgid weight- Dry weight) ×100. (Smart, 1974).

3.11.6 Water saturation deficit (%)

Water saturation deficit was recorded using following formula:

Water saturation deficit (WSD) (%) = 100- Relative water content (Sangakkara *et al*., 1996).

3.11.7 Water retention capacity

Water retention capacity was measured using following formula:

Water retention capacity (WRC) = Turgid weight/ Dry weight. (Sangakkara *et al*., 1996).

3.11.8 Coefficient of velocity of germination

Germination coefficient was measured using following formula:

Coefficient of velocity of germination (CVG) = {(N1+N2+...+Ni)/100} x (N1T1+...+NiTi).

Where, N is the number of seeds germinated every day and T is the number of days from seeding corresponding to N. (Al-Mudaris, 1998).

3.11.9 Vigor index

Vigor index was calculated using following formula:

Vigor index = (Total germination× seedling length$_{mm}$)/ 100. (Abdul-Baki and Anderson, 1970).

3.12 Statistical analysis

Data recorded for different parameters were compiled and tabulated in proper form for statistical analysis. CRD analysis was done for statistical test. The data were analyzed using “Analysis of Variance (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 Gomes and Gomez (1984).

CHAPTER 4

RESULTS AND DISCUSSION

This study was carried out to investigate the potential role of including a signaling molecule, H_2O_2 to improve the performance of growth of wheat plants under salinity conditions.

The results of the germination and growth parameters of wheat genotypes such as root length, shoot length, root dry weight, shoot dry weight etc. which is influenced by different concentrations of H_2O_2 and priming time under salt stress condition have been presented and discussed in this chapter. To strengthen the discussion, information are provided in the forms of graphs.

4.1 First experiment: Study on the effect of different concentrations of H_2O_2 on the germination, seedling growth and water relation behavior of wheat.

Results obtained from the present study regarding the effects of different concentrations of H_2O_2 on the germination rate of different wheat genotypes have been presented, discussed and compared in this chapter. The analytical results have been presented in Figure 1 to 10 and Appendices II to XI.

4.1.1 Germination percentage (%)

Significant variation was observed on germination percentage among the test genotypes priming with water and different concentration of H_2O_2 including control treatment (Figure 1 and Appendix II). Results indicated that the genotype, V_1 (ESWYT 5) showed the highest germination percentage in all seed priming concentration where genotype, V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest germination percentage. Among all the genotype, the highest germination percentage (93.4%) was scored by V_1 (ESWYT 5) with 2ml H_2O_2 concentration whereas the minimum germination percentage (56.81%) was recorded from wheat genotype V_3 (BARI Gom 28) with 10ml H_2O_2 solutions. It was recorded that all the genotypes gave better performance at 2ml H_2O_2 concentration. H_2O_2 was able to promote germination (Christophe *et al.,* 2008) or formation and development of adventitious roots (Li *et al.,* 2009). Similar concentrations of H_2O_2 (50 − 200 uM H_2O_2) led to significant increase in the germination rate of the seeds of drought sensitive wheat cultivars whereas vice versa

with drought tolerant ones (Lu *et al*., 2013). Basra *et al*. (2003) and Farooq *et al*. (2006) observed that germination was improved by seed priming technique. This findings is also supported by (Liheng *et al.*, 2009) that seed priming with hydrogen peroxide solution stimulates germination and seed vigor since it improves the activity of peroxidase enzyme. Seiadat *et al*. (2012) reported that priming improves germination characteristics in many crops.

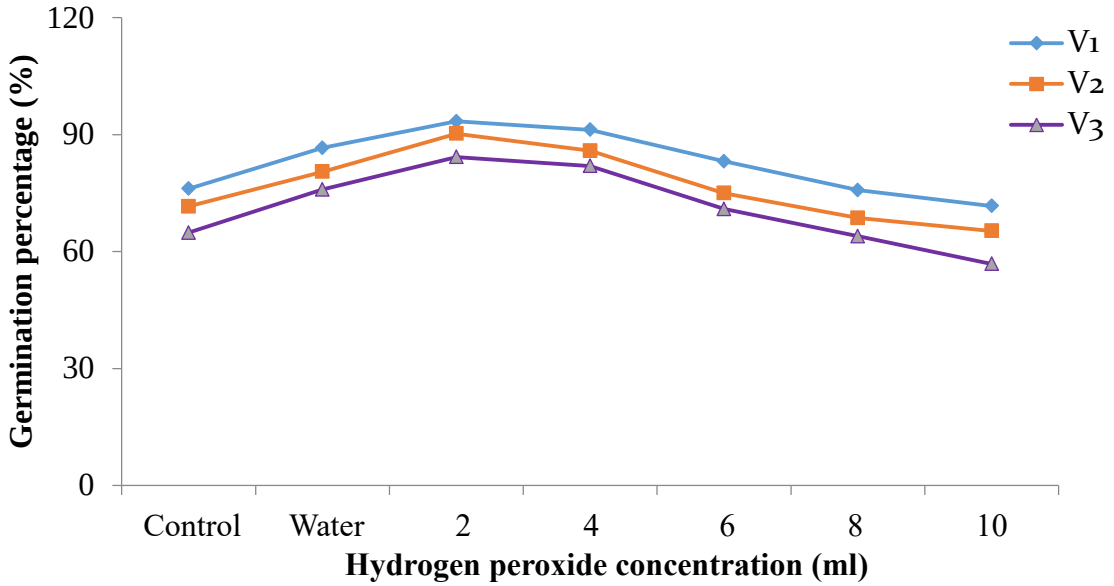


Figure 1. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the germination percentage of wheat cultivars (LDS $_{(0.01)}$ = 6.51, 6.2, 6.00, 4.51, 6.29, 4.95 and 4.54 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.2 Shoot length (mm)

Significant variation was observed on shoot length among the test genotypes priming with water and different concentration of H_2O_2 including control treatment (Figure 2 and Appendix III). It was found that the genotype, V_1 (ESWYT 5) showed the highest shoot length in all seed priming concentration where genotype, V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest shoot length. It was also observed that the maximum shoot length (162.4mm) was recorded for V_1 (ESWYT 5) primed with 2ml H_2O_2 solution and lowest shoot length (79.60mm) was observed V_3 (BARI Gom 28) primed with 10ml H_2O_2.

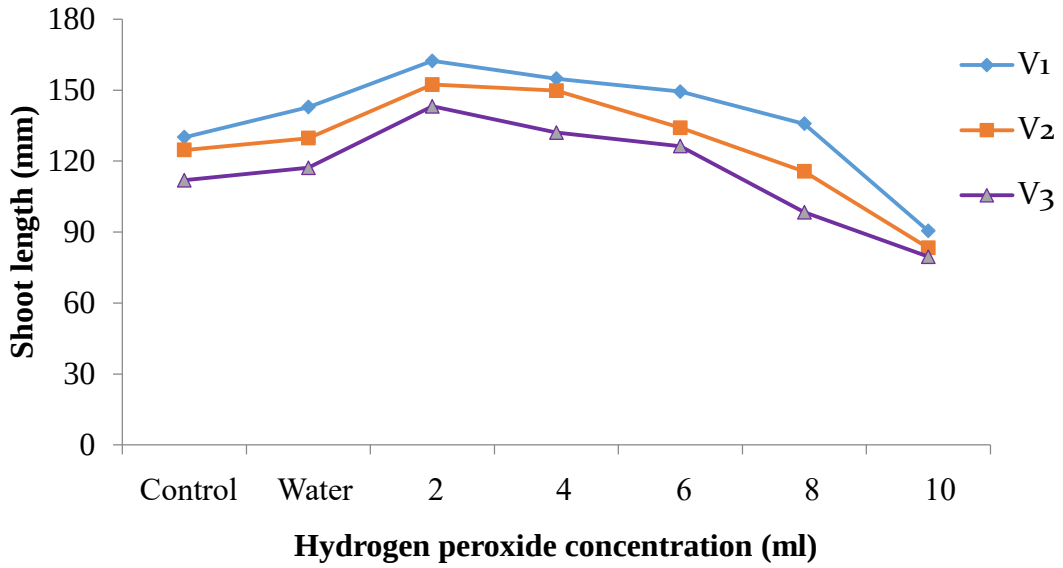


Figure 2. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the shoot length of wheat cultivars (LDS $_{(0.01)}$ =9.95, 11.39, 9.43, 10.70, 8.01, 11.52 and 6.38 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

In wheat, Gray and Steckel (1983) concluded that priming increased embryo length, which resulted in early initiation of germination and higher shoot length. 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.

4.1.3 Root length (mm)

Root length of wheat genotypes significantly affected by different H_2O_2 concentrations. Root length of wheat genotype increased in 2ml H_2O_2 and there was a gradual decreased with the increasing H_2O_2 concentration (Figure 3 and Appendix IV). The result of the experiment revealed that the maximum root length (146.2 mm) was scored by V_1 (ESWYT 5) with concentration 2ml H_2O_2 solution whereas the minimum root length (42.12mm) was recorded from V_3 (BARI Gom 28) primed with 10ml H_2O_2 concentration. Wheat genotype V_1 (ESWYT 5) performed the best, V_2 ((ESWYT 6) performed moderately and V_3 (BARI Gom 28) gave consistently poor performance under all the chemical and hydropriming solutions. Experiments conducted by Ashraf and Abu-shakra (1978) revealed that priming of wheat seed in osmoticum or water might improve germination, emergence and aggrandize vigorous root growth as a consequence root length of chemical and hydro primed seed exerted the highest length than non-primed

seed. Jisha *et al.* (2013) reported that overall growth of plants was enhanced due to the seed-priming treatments. Priming is a controlled hydration process followed by redrying that allow metabolic activities to proceed before radical protrusion (Khan, 1992; Sivritepe *et al*., 2003, 2005).

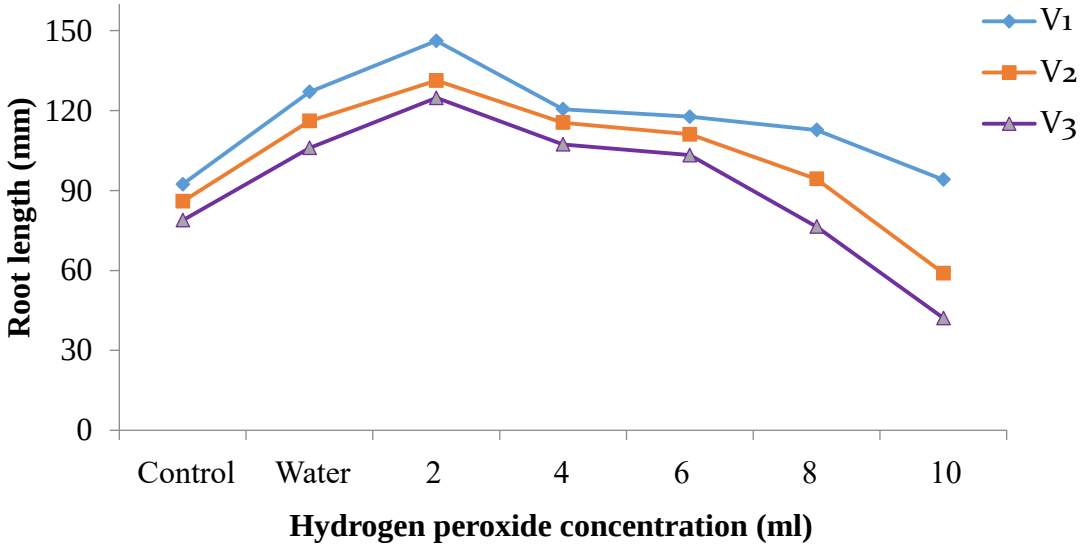


Figure 3. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the root length of wheat cultivars (LDS $_{(0.01)}$ = 7.34, 8.39, 12.01, 9.13, 8.69, 7.90 and 5.01 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.4 Shoot dry weight (g)

Shoot dry weight of wheat genotypes significantly varied by different concentrations of H_2O_2 solution and water priming including control (Figure 4 and Appendix V). The result of the experiment revealed that the highest shoot dry weight (0.04220 g) was scored by V_1 (ESWYT 5) with 2ml H_2O_2 concentration whereas the minimum shoot dry weight (0.01500 g) was recorded from wheat genotype V_3 (BARI Gom 28) with 10ml H_2O_2 solutions. Variety V_1, V_2, V_3, all gave good result at 2ml H_2O_2 concentration. Lee and Kim (2000) reported that, priming increased the metabolic activities of seed ultimately gained the substantial shoot length than unprimed seed. Ghassemi-Golezani *et al*. (2008) observed that hydropriming significantly improved shoot weights and Sarwar *et al.* (2006) also reported that shoot length and biomass of shoots were increased when treated with water and H_2O_2 .Basra *et al*. (2005) and Iqbal and Ashraf (2007) also observed that seed priming improved shoot dry weight significantly compared to unprimed seeds in wheat. It has been proved that seed priming intensifies seed reserve consumption and

depletion rate and seedling dry biomass (Ansari *et al*., 2012).

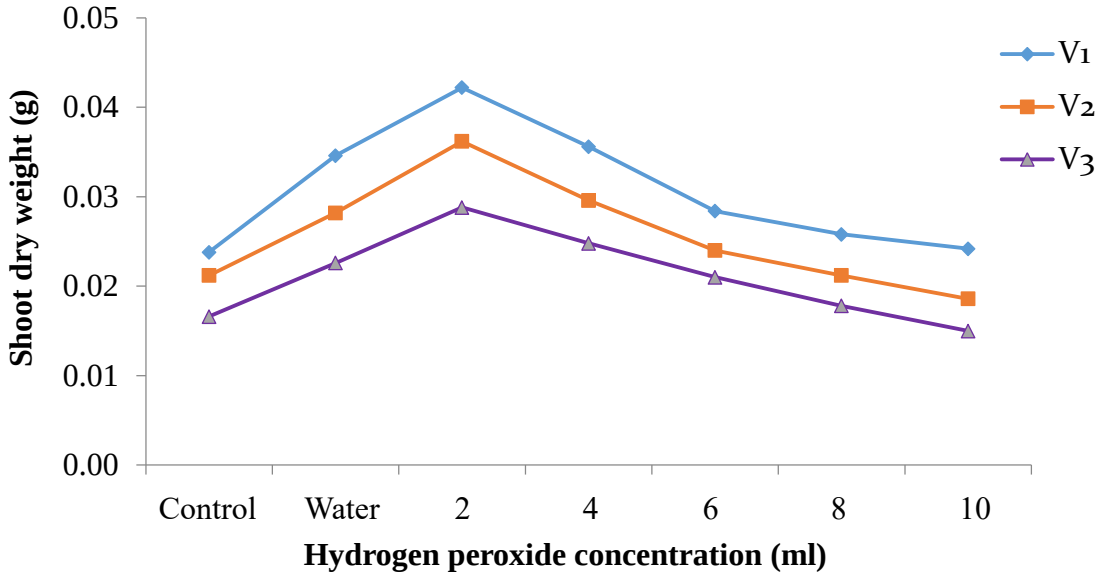


Figure 4. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the shoot dry weight of wheat cultivars (LDS $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006, 0.006, 0.006 and 0.006 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.5 Root dry weight (g)

Statistically significant variation was found in case of root dry weight of different genotypes of wheat due to priming with water and different concentrations of H_2O_2 including control treatment (Figure 5 and Appendix VI). It was found that the genotype, V_1 (ESWYT 5) showed the highest shoot length in all seed priming concentration where genotype V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom28) showed the lowest shoot length. The result of the experiment revealed that the maximum root dry weight (0.03480 g) was scored by V_1 (ESWYT 5) with 2ml H_2O_2 concentration whereas the minimum root dry weight (0.01040 g) was recorded from V_3 (BARI Gom 28) treatment. The result of the present study is also in line with the results of previous researchers: Basra *et al*. (2005) and Iqbal and Ashraf (2007) observed that dry matter yield increased in wheat with each increment of priming.

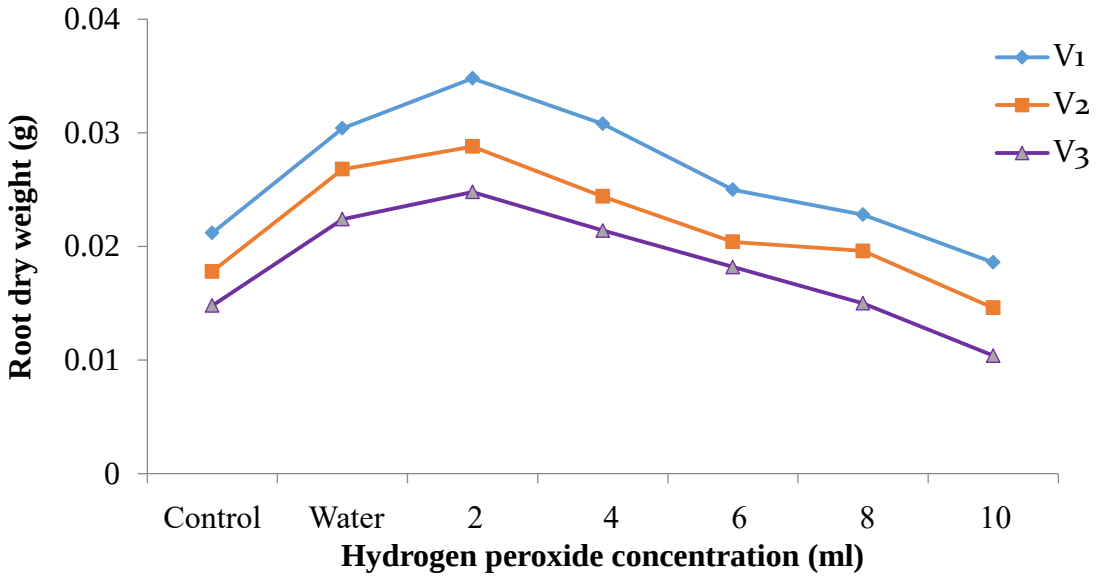


Figure 5. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the root dry weight of wheat cultivars (LDS $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006, 0.006, 0.006 and 0.006 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.6 Relative water content (%)

Relative water content could be the perfect indicator of plant hydrologic condition as it denotes the physiological consequences of cellular water deficit. A wide range of statistical difference was observed for the relative water content of wheat genotypes under different H_2O_2 solutions (Figure 6 and Appendix VII). Corresponding water content followed the similar trend as the previous parameters of wheat genotype. It was found that the genotype, V_1 (ESWYT 5) showed the highest relative water content where genotype V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest relative water content under all the chemical and hydro priming solutions. It was also observed that the maximum relative water content (82.50%) was recorded for V_1 (ESWYT 5) primed with 2ml H_2O_2 solution and lowest (43.39%) was observed V_3 (BARI Gom 28) primed with 10ml H_2O_2. Under stress condition, chemical and hydro primed seeds can thrive and provide better water use efficiency thus plant growth not hampered under stress condition than non-primed seeds (Flower *et al.* 1998). A similar finding was reported by Sairam *et al.* (2002). Mouradi *et al.* (2016) observed that alfalfa plants raised from primed seeds maintained the high ($P < 0.001$) relative water content values, compared to those raised from unprimed seeds.

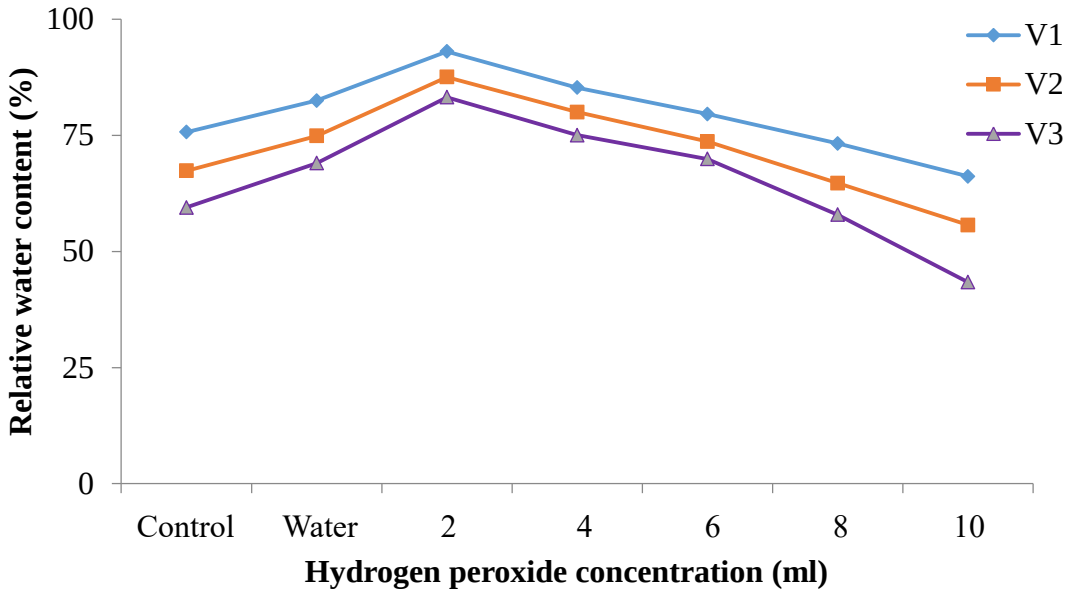


Figure 6. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the relative water content of wheat cultivars (LDS $_{(0.01)}$ = 6.25, 5.41, 3.50, 7.14, 5.79, 5.37 and 4.83 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.7 Water Saturation Deficit (%)

Water saturation deficit of different genotypes of wheat showed statistically significant variation due to different concentrations of H_2O_2 solutions and water priming including control (Figure 7 and Appendix VIII). It followed the opposite trend compared to the previously described parameter, *i.e.*, the water saturation deficit was maximum at control and gradually decreased up to 2ml H_2O_2 concentration and then steadily increased. Among the different genotypes, V_3 (BARI Gom 28) distinctly scored the maximum value of water saturation deficit at all priming treatments where V_1 (ESWYT 5) scored the minimum value of water saturation deficit with all priming treatments. The highest water saturation deficit (56.61%) was recorded from V_3 (BARI Gom 28) with 10ml H_2O_2 solution and the lowest (6.92%) were recorded from V_1 (ESWYT 5) 2ml H_2O_2 with solution. Due to lack of defense mechanism, the non-primed seedling failed to uptake enough water necessary for running the physiological process smoothly than the primed seedling. As a result, excessive amount of water deficit occurred in non -primed seed than the primed one. Ali *et al.* (2013) also observed that seed priming improves irrigation water use efficiency resulted lower water saturation deficit.

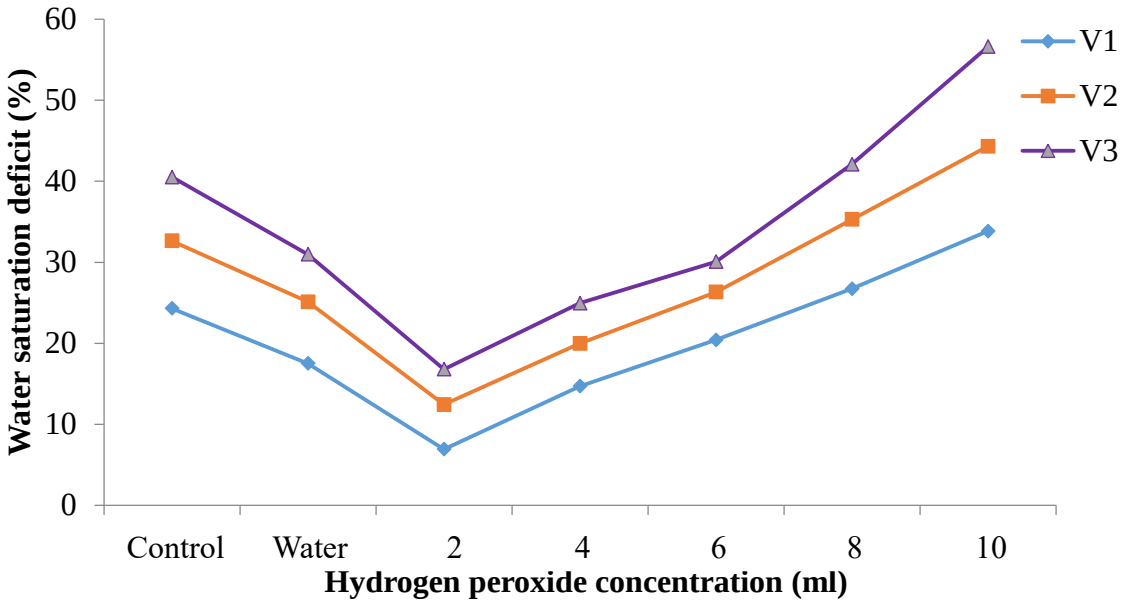


Figure 7. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the water saturation dificit of wheat cultivars (LDS $_{(0.01)}$ =2.91, 2.01, 0.95, 1.52, 1.33, 3.42 and 3.98 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.8 Water Retention Capacity

Water retention capacity of wheat genotypes influenced significantly by different H_2O_2 solution (Figure 8 and Appendix IX). V1 (ESWYT 5) performed poor under the all H_2O_2 concentrations and scored the highest value consistently under all chemical and hydro priming solution where V3 (BARI Gom 28) scored the minimum value for water saturation deficit under the all priming solutions. It was found that the maximum water retention capacity (20.80) was recorded for V1 (ESWYT 5) primed with 2ml H_2O_2 solution for 24 hours and lowest water retention capacity (5.152) was observed V3 (BARI Gom 28) primed with 10ml H_2O_2 solution for 24 hours. It was also recorded that all the genotypes had increased water retention capacity in 2ml H_2O_2 concentration. Priming helps to activate the metabolic enzymes which are responsible for germination of seedbefore germination takes place. As a result, primed seeds can uptake more water than the non-primed one and gained the maximum turgid weight and consequently attained the maximum water retention capacity. It is also supported by the finding of Ali *et al.* (2013) and they found that seed priming improves irrigation water use efficiency which helps to increase higher water retention capacity.

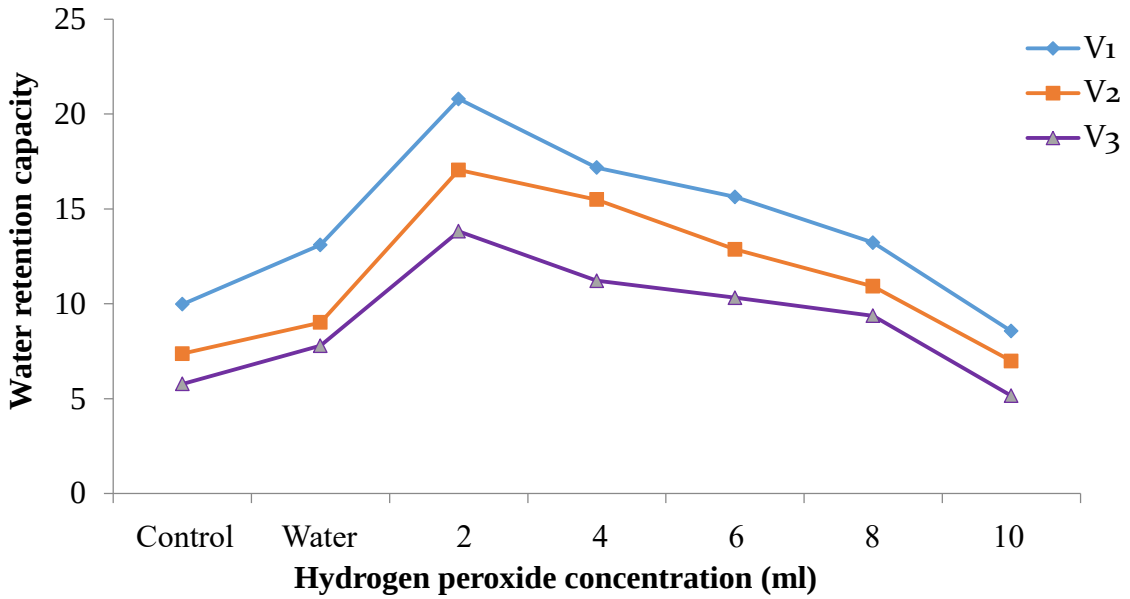


Figure 8. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the water retention capacity of wheat cultivars (LDS $_{(0.01)}$ = 0.78, 1.23, 1.78, 1.48, 1.68, 0.83 and 0.84 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.9 Coefficient of velocity of germination

Coefficient of velocity of germination of wheat genotypes significantly affected by different H_2O_2 concentrations. Coefficient of velocity of germination increased in 2ml H_2O_2 and there was a gradual decreased with the increasing H_2O_2 concentration (Figure 9 and Appendix X). The result of the experiment revealed that the maximum coefficient of velocity of germination (17.66) was scored by V_1 (ESWYT 5) with 2ml H_2O_2 concentration whereas the minimum coefficient of velocity of germination (9.628) was recorded from V_3 (BARI Gom 28) with primed with 2ml H_2O_2 solution for 24 hours. Wheat genotype V_1 (ESWYT 5) performed the best, V_2 (ESWYT 6) performed moderately and V_3 (BARI Gom 28) gave consistently poor performance under all the chemical and hydro priming solutions. Huns and Sung (1997) observed that seed priming resulted from anti-oxidant increment as glutathione and ascorbate in the seed. These enzymes trigger germination speed via reduction of lipid peroxidation activity; as a result coefficient of velocity of germination was higher in primed seed compare to that of non-primed one.

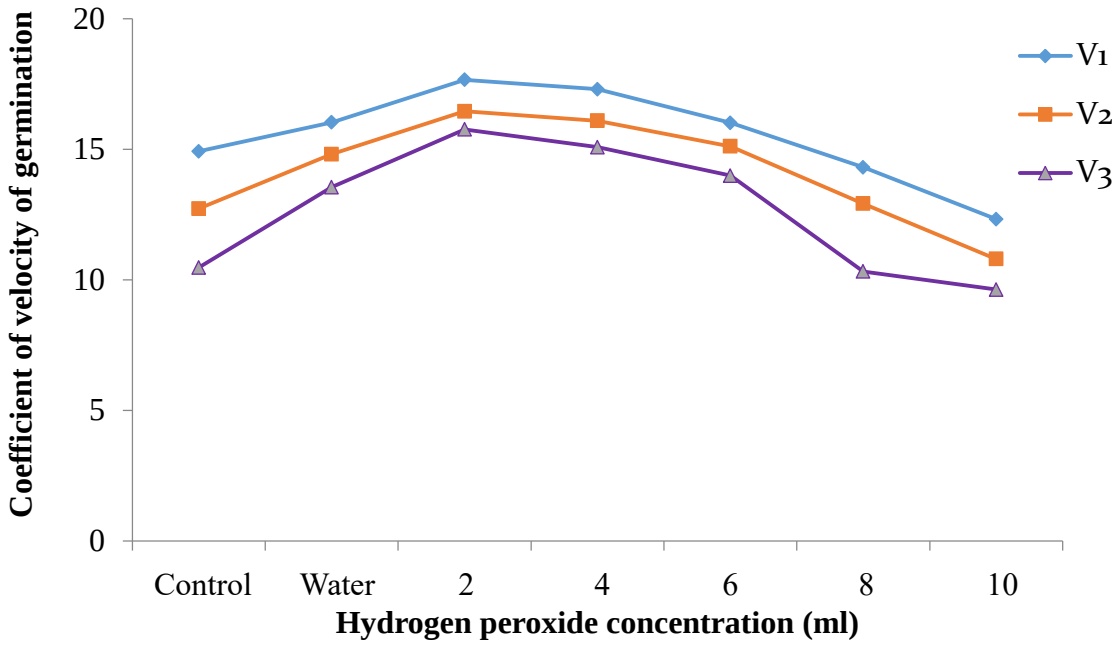


Figure 9. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the coefficient of velocity of germination of wheat cultivars ($LDS_{(0.01)}$ = 0.89, 1.25, 1.04, 1.31, 1.23, 1.52 and 1.16 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.1.10 Vigor Index

Figure 10 and Appendix XI showed that, the maximum vigor index (311.30) was accounted from wheat genotype V_1 (ESWYT 5) when the seed primed with 2ml H_2O_2 solution and minimum vigor index (75.87) was achieved from V_3 (BARI Gom 28) when the seeds were not primed either chemical or hydro priming solutions. It was also found that all the genotype had improved vigor index in 2ml H_2O_2 solution. It was recoded that H_2O_2 had positive effect on germination index and vigor index under different concentration in barley (Kilic and Kahraman, 2016).

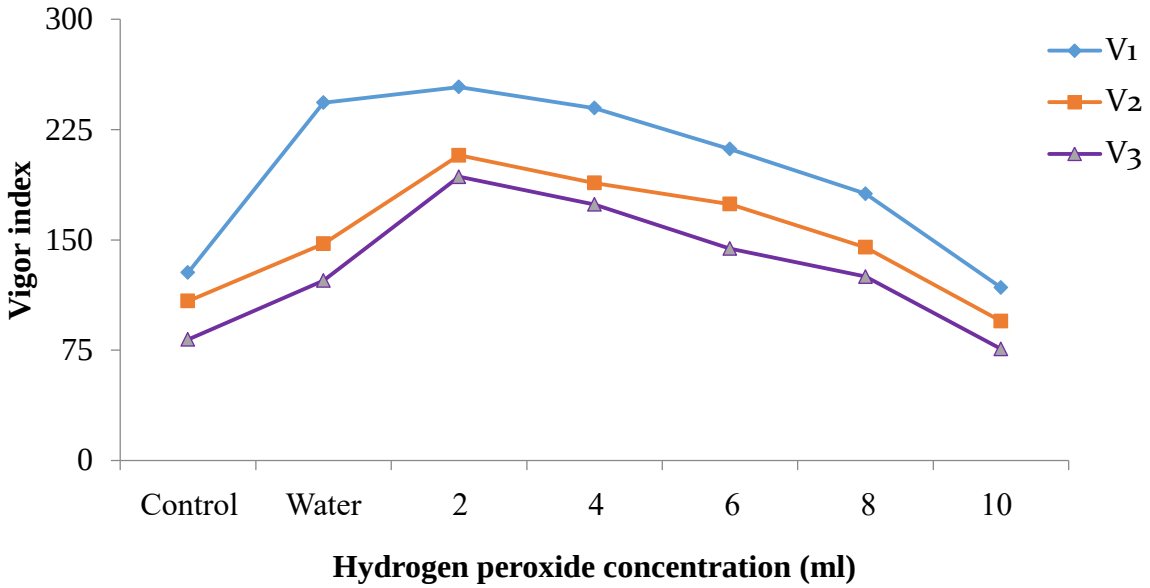


Figure 10. Effect of various concentrations of hydrogen-per oxide (H_2O_2) on the vigor index of wheat cultivars (LDS $_{(0.01)}$ = 7.62, 14.96, 17.13, 12.84, 15.29, 9.40 and 8.49 at control, water, 2ml, 4ml, 6ml, 8ml and 10ml, respectively)

4.2 Second experiment

Optimization of priming time on the germination, seedling growth and water relation behavior of wheat.

Results obtained from the present study regarding the effects of different priming time of H_2O_2 on the germination, seedling growth and water relation behavior of different wheat genotypes have been presented, discussed and compared in this chapter. The analytical results have been presented in Figures 11 to 20 and Appendices XII to XXI.

4.2.1 Germination percentage (%)

Priming time with 2ml H_2O_2 showed significant influence on germination percentage of different wheat genotypes at 0, 3, 6, 9, 12 and 15 hours priming time among the genotypes (Figure 11 and Appendix XII). The experiment revealed that genotype V_1 (ESWYT 5) gave the highest germination percentage (96.20%) from 9 hours priming time with 2ml H_2O_2 solution. The lowest germination percentage (69.11%) obtained from wheat genotype V_3 (BARI Gom 28) seed primed with 2ml H_2O_2 solution for 15 hours. Many researchers conducted research to explore the possiblity of improving late sown wheat performance by seed priming techniques to avoid chilling stress. Seed priming improved emergence, stand establishment, tiller numbers, allometry, grain and straw

yield and harvest index (Farooq *et al*., 2007). Hydropriming showed highest germination percentage (53%) when primed for 16 hours at 15^0 C (Omid and Farzad, 2012).

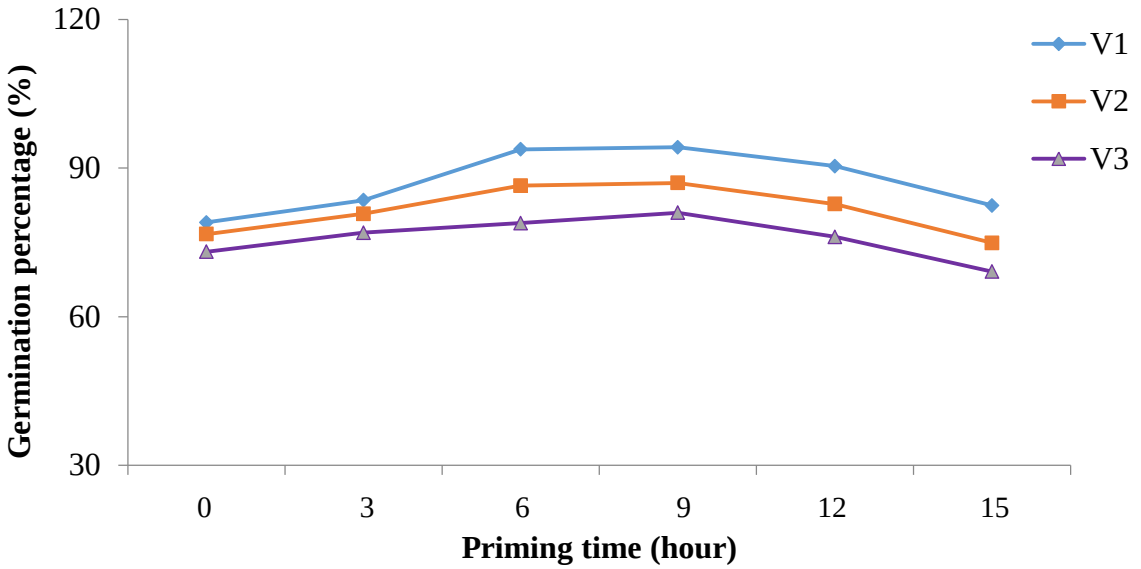
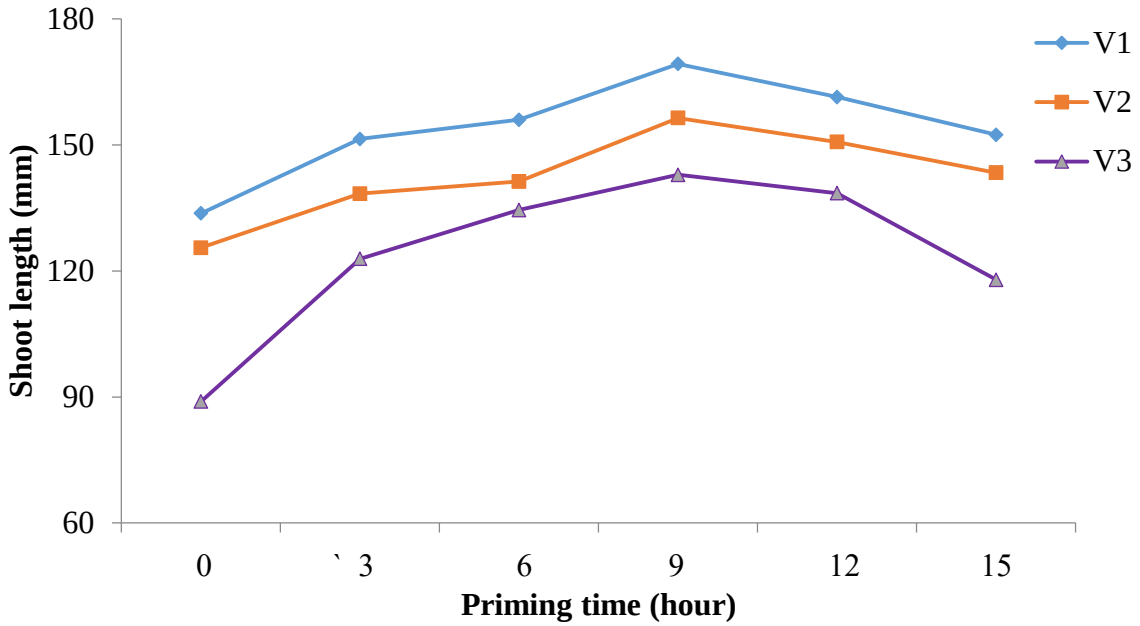


Figure 11. Effect of different priming time on the germination percentage of wheat cultivars (LSD $_{(0.01)}$ = NS, 5.94, 7.02, 8.22, 4.89 and 6.45 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.2 Shoot length (mm)

Shoot length of wheat genotypes significantly differed by different priming times. Shoot length increased with increasing priming time up to 9 hours and then gradually decreased (Figure 12 and Appendix XIII). It was found that the genotype, V_1 (ESWYT 5) showed the highest shoot length where genotype V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest shoot length under all priming time with 2ml H_2O_2 solution. It was also found that maximum shoot length (169.3mm) was scored by V_1 (ESWYT 5) from 9 hours priming time with 2ml H_2O_2 solution and minimum shoot length (88.92mm) was recorded by V_3 (BARI Gom 28)with 15 hours. Priming time increases enzymatic activities of seed and triggers the vigorous plant growth and in consequence up to 9 hours and then it decreases gradually. Over priming time facilitate the ageing of seed. Hamidreza *et al.* (2013) reported that shoot length significantly influenced by osmopriming times as 6 hours seed treatment had highest shoot length of wheat. Afzal *et al.* (2007) observed that the maximum shoot length (28

cm) was scored by hydro priming seeds followed by 24h of chilling.



Fgiure 12. Effect of different priming time on the shoot length of wheat cultivars (LSD $_{(0.01)}$ = 8.58, 12.07, 10.23, 11.80, 9.18 and 11.23 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.3 Root length (mm)

Statistically significant variation was found in case of root length different genotypes of wheat due to priming with different priming time at 2ml H_2O_2 solution (Figure 13 and Appendix XIV). It was found that the genotype, V_1 (ESWYT 5) showed the highest root length where genotype V_3 (BARI Gom 28) showed the lowest root length in different priming time. It was also observed that the maximum root length (146.0mm) was recorded for V_1 (ESWYT 5) primed with 2ml H_2O_2 solution for 12 hours and The lowest root length (100.9mm) was observed from V_3 (BARI Gom 28) with 15 hours priming time at 2ml H_2O_2 solution followed by V_3 (BARI Gom 28) genotype without priming . The root length of seedlings obtained from primed seeds was increased significantly compared to unprimed seeds. This result obtained from the present study is supported by the findings of Afzal *et al*. (2007) with wheat. Maximum aboveground biomass and photosynthetic pigments were recorded by Nouman *et al*. (2014) when the seeds were hydroprimed (12 hours) but maximum root length and number of roots were found in MLE (*Moringa* leaf extract) primed (12 hours) *Moringa olifera* plants.

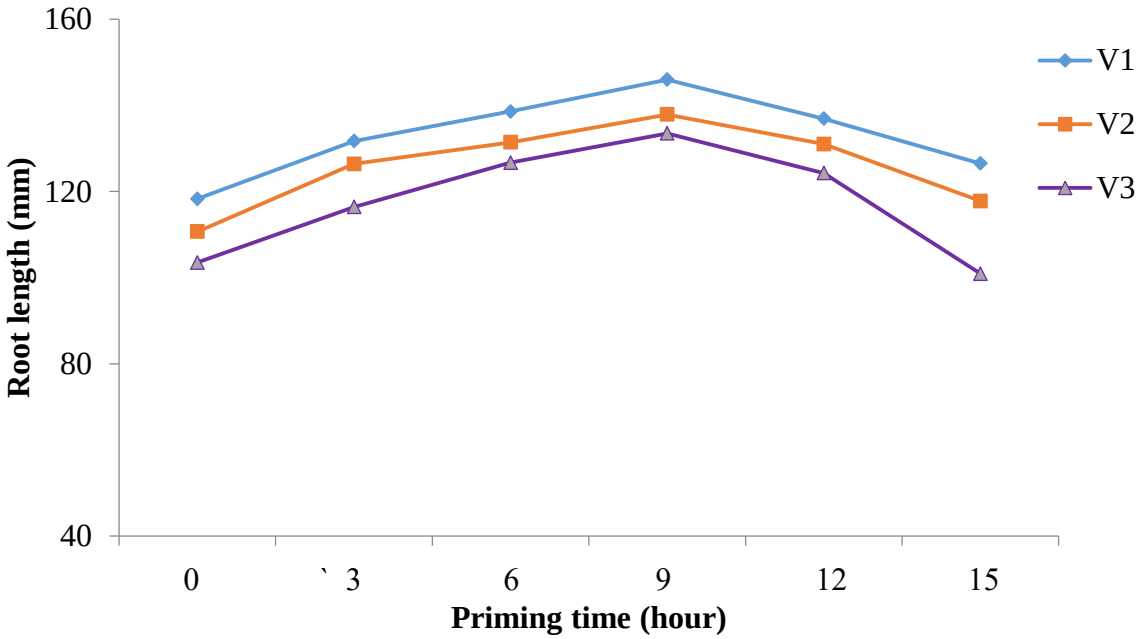


Figure 13. Effect of different priming time on the root length of wheat cultivars (LSD $_{(0.01)}$ = 8.47, 9.55, 11.31, 10.45, 12.33, 7.56 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.4 Shoot dry weight (g)

Shoot dry weight of wheat genotypes significantly differed by different priming times (Figure 14 and Appendix XV). Shoot dry weight increased with increasing priming time up to 9 hours and then gradually decreased. The highest shoot dry weight (0.04340 g) was recorded from wheat genotype V_1 (ESWYT 5) with seed primed with 2ml H_2O_2 solution for 9 hours while the lowest shoot dry weight (0.01200 g) was recorded from wheat genotype V_3 (BARI Gom 28) primed with 2ml H_2O_2 solution for 0 hour. Wheat genotype V_2 (ESWYT 6) respond intermediately under all the treating hours. The result of the present study supported by many researchers: Nahar *et al*. (2016a), Ajirloo *et al*. (2013), and Moghanibashi *et al*. (2012). Moghanibashi *et al.* (2012) revealed that, the effect of hydro priming for 24 hour increased shoot dry weight of sunflower than the non-primed one. Ghasemi *et al*. (2014) reported that different level of aging period (0, 3 or 6 days) and increased priming (hydropriming) time significantly increased the seedling dry weight of wheat.

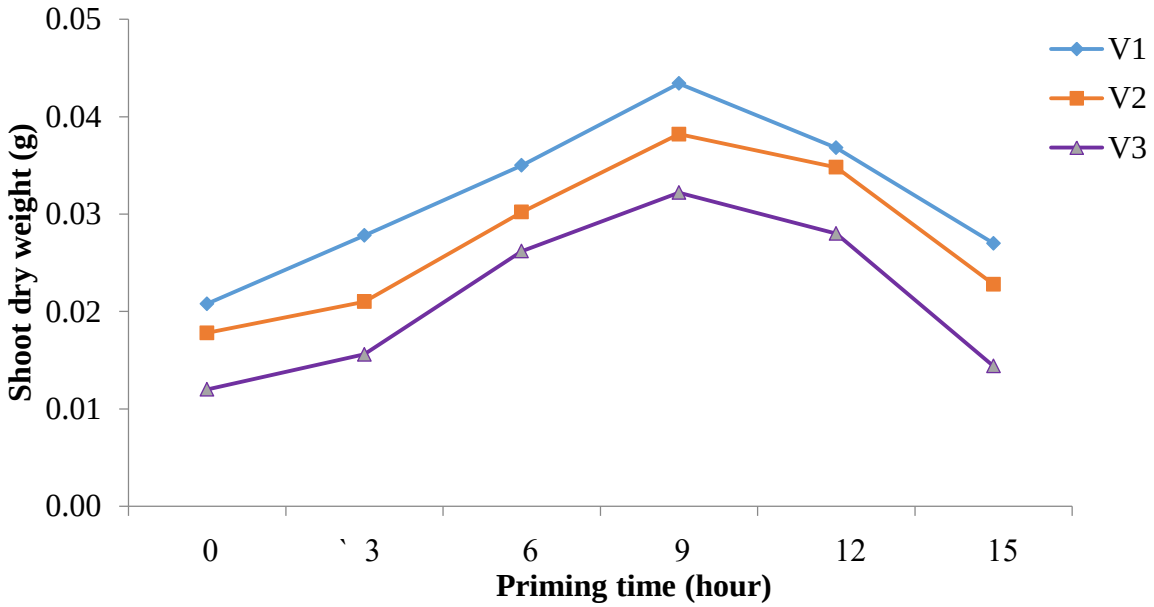


Figure 14. Effect of different priming time on the shoot dry weight of wheat cultivars (LSD $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006, 0.006 and 0.006 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.5 Root dry weight (g)

Priming time with H_2O_2 showed significant influence on root dry weight of different wheat genotypes at 0, 3, 6, 9, 12 and 15 hours (Figure 15 and Appendix XVI). It was observed that at all priming time treatment, V_1 (ESWYT 5) gave the best performance on root dry weight where V_3 (BARI Gom 28) showed the lowest results. It was also found that all genotypes gave their best performance on root dry weight under seeds primed with 2ml H_2O_2 solution for 9 hours. The highest root dry weight (0.03820 g) was found from V_1 (ESWYT 5) treated with 2ml H_2O_2 solution for 9 hours where the genotype, V_3 (BARI Gom 28) gave the lowest root dry weight (0.01200 g) under seeds primed with 2ml H_2O_2 solution for 15 hours). Nahar *et al.* (2016b) reported that, the maximum weight of wheat genotypes BARI Gom 27 was recorded when the seed primed with 10% PEG solution for 12 hours.

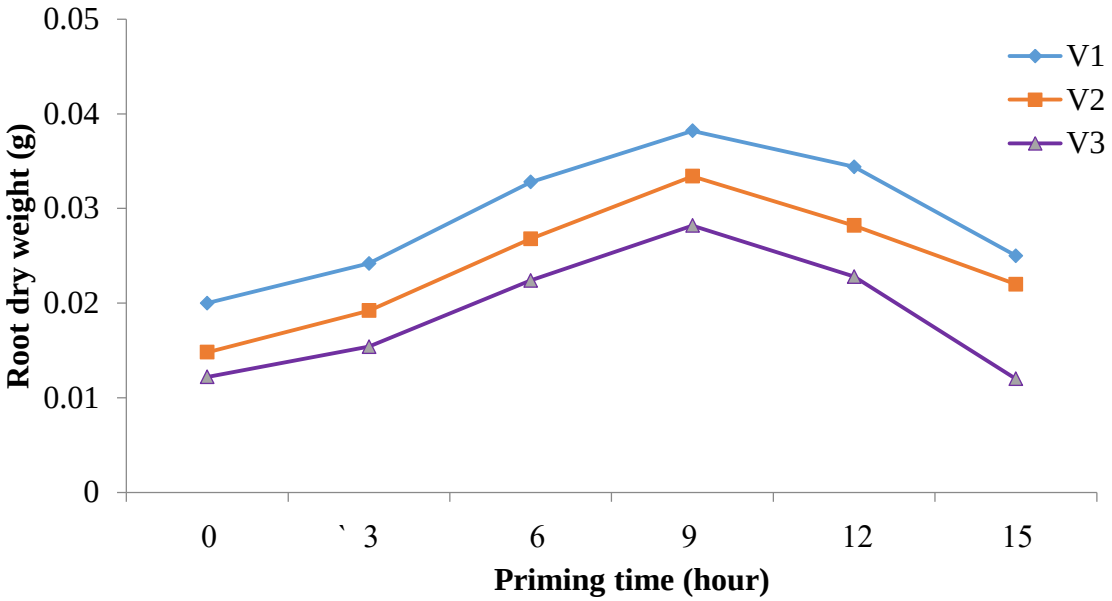


Figure 15. Effect of different priming time on the root dry weight of wheat cultivars (LSD $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006, 0.006 and 0.006 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.6 Relative water content (%)

Relative water content of wheat genotypes significantly varied by different priming times (Figure 16 and Appendix XVII). Increasing trend of relative water content was observed with increasing priming 0, 3, 6 time up to 9 hours and then gradually decreased with increasing priming time. The wheat genotype ESWYT 5 consistently gave the highest value for relative water content followed by ESWYT 6 and BARI Gom 28 gave the lowest value for relative water content under all priming time. The result of the experiment revealed that the highest relative water content (91.14%) was observed from wheat genotype ESWYT 5 with 9 hours priming while the lowest relative water content (61.41%) was observed from wheat genotype BARI Gom 28 primed with 2ml H_2O_2 solution for 15 hours.

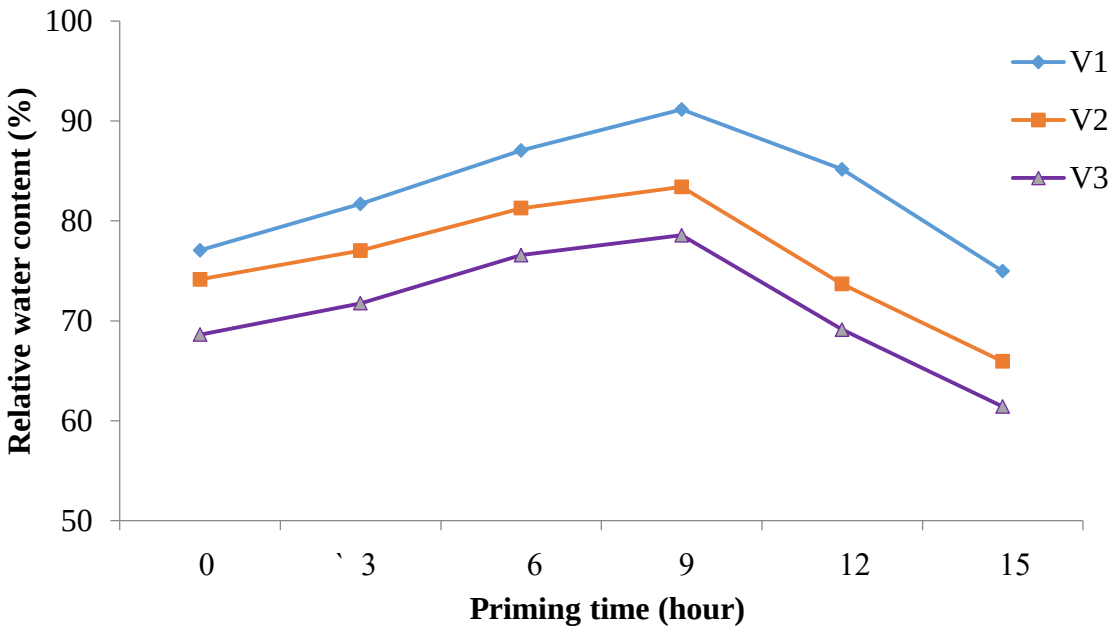


Figure 16. Effect of different priming time on the relative water content of wheat cultivars (LSD $_{(0.01)}$ = 6.38, 5.60, 5.79, 7.70, 4.39 and 7.67 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.7 Water Saturation Deficit (%)

The trend of water saturation deficit was quite opposite compare to the trend of rest of the parameters i. e. water saturation deficit decreased with the increasing priming time and gradually increased with increasing priming time (Figure 17 and Appendix XVIII). Wheat genotype BARI Gom 28 dominantly scored highest value at all the priming time where ESWYT 5 showed consistently lowest values regarding water saturation deficit. The maximum water saturation deficit (38.59%) was observed when seeds primed with 15 hours increased water saturation deficit scored by BARI Gom 28 when the seeds were primed with of 2ml H_2O_2 wheat. The minimum water saturation deficit (38.59%) was found seed priming with 2ml H_2O_2 solution for 9 hours.

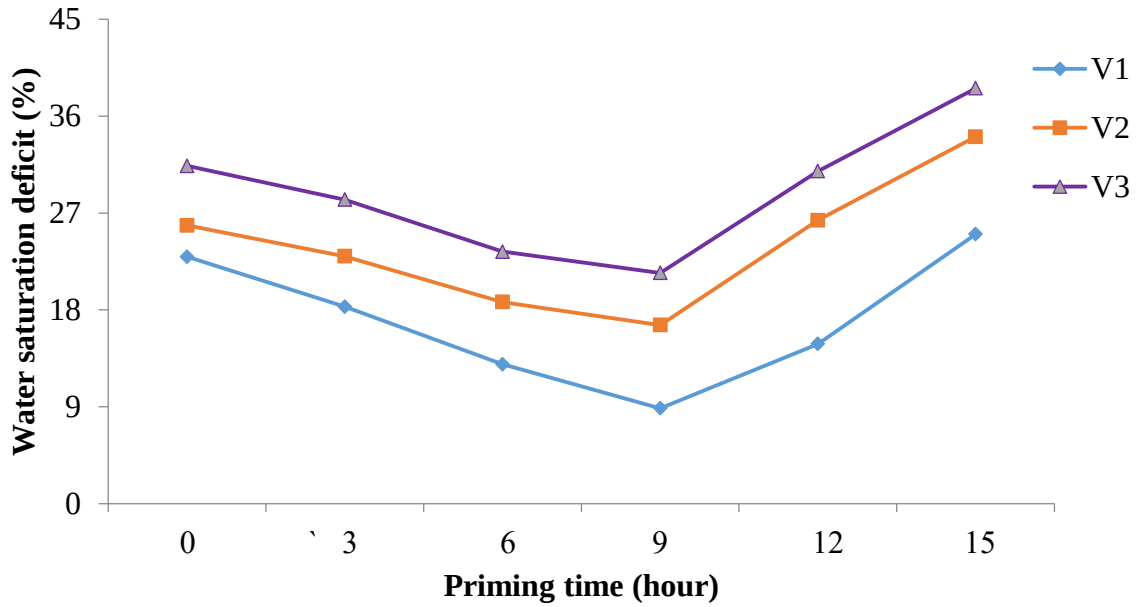


Figure17. Effect of different priming time on the water saturation deficit of wheat cultivars (LSD $_{(0.01)}$ = 2.79, 2.51, 1.87, 1.54, 1.83 and 3.15 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.8 Water retention capacity

All the wheat genotypes primed with 2ml H_2O_2 solution for 9 hours showed the best result (Figure 18 and Appendix XIX). Maximum water retention capacity (17.76) was accounted from wheat genotype V_1 (ESWYT 5) when the seed primed with 2ml H_2O_2 solution for 9 hours and minimum water retention capacity (7.184) was observed from V_3 (BARI Gom 28) when the seeds were primed with 2ml H_2O_2 solution for 15 hours. It has been reported by Wahid and Ghazanfar (2006) that H_2O_2 treatment improved the water relations of salinity-treated seedlings by turgor maintenance, which was comparable to the water control of seedlings.

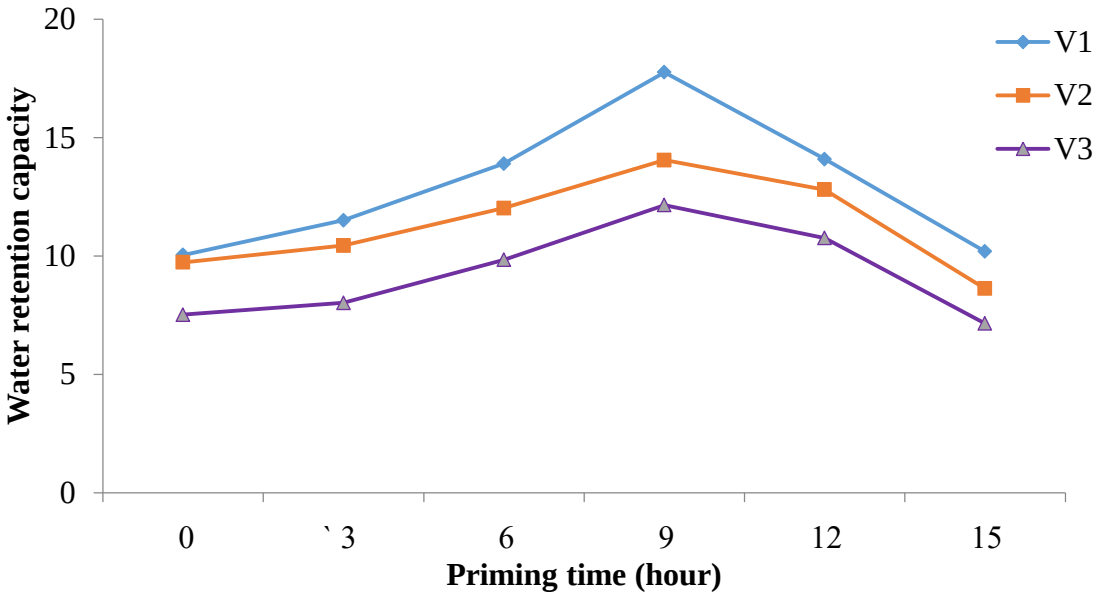


Figure 18. Effect of different priming time on the water retention capacity of wheat cultivars (LSD $_{(0.01)}$ = 1.14, 0.81, 1.15, 1.59, 1.33 and 0.92 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.9 Coefficient of velocity of germination

A wide range of statistical difference was observed due to different priming times (Figure 19 and Appendix XX). It was observed that the genotype, V1 (ESWYT 5) showed the highest coefficient of velocity of germination where genotype V2 (ESWYT 6) showed the intermediate result and variety V3 (BARI Gom 28) showed the lowest coefficient of velocity of germination under all priming time with 2ml H_2O_2 solution**.** It was also observed that all genotypes gave their best performance on coefficient of velocity of germination under seeds primed with 2ml H_2O_2 solution for 9 hours. Among the different genotypes, V1 (ESWYT 5) distinctly scored the maximum value of coefficient of velocity of germination (18.08) where V3 (BARI Gom 28) scored the minimum value (13.44) at 2ml H_2O_2 solution for 9 hours priming time. Time of priming signicantly influence the coefficient of velocity of germination and the finding of many researcher support this. Omid and Farzad (2012) found that in mountain rye (*Secale montanum*) highest coefficient of velocity of germination was attained from concentration of 9 bar PEG for 24 hours at 10° C and highest coefficient of velocity of germination was attained from hydropriming for 8 hours at 10° C as it was increased compared to the unprimed one.

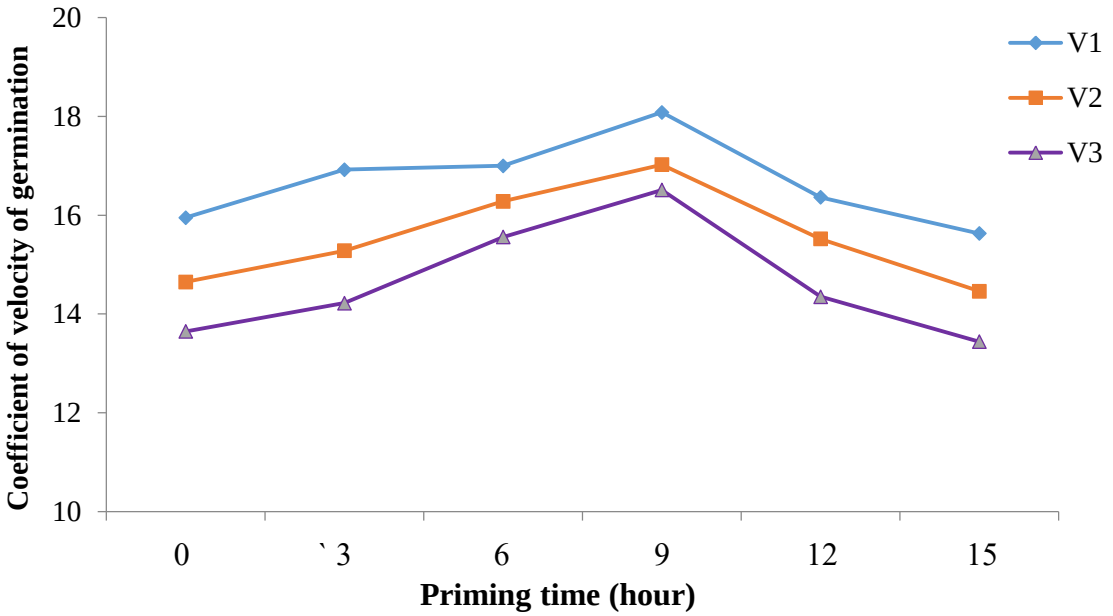


Figure 19. Effect of different priming time on the coefficient of velocity of germination of wheat cultivars (LSD $_{(0.01)}$ = 1.65, 1.45, 1.37, 1.52, 1.32 and 1.27 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.2.10 Vigor Index

Priming time with H_2O_2 showed significant influence on vigor index of different wheat genotypes (Figure 20 and Appendix XXI).Vigor index of wheat genotypes increased with the increasing of priming time up to 9 hours and gradually decreased with increasing time. It was also observed that the maximum vigor index (303.1) was recorded for V_1 (ESWYT 5) primed with 2ml H_2O_2 solution for 9 hours and the lowest Vigor Index (140.8) was observed from V_3 (BARI Gom 28) with 0 hour priming time at 2ml H_2O_2 solution followed by V_2 (ESWYT 6) genotype with 15 hour priming. Osmopriming with 15 bar PEG for 24 hour at 15^0 C increased germination percentage (80.5%) germination index (17.9) normal seedling percentage (45%) seedling vigor index (257.85) and seedling length (5.73 cm) as compared to the unprimed and other treatments of chemical priming. Also osmo and hydroprimig increased catalase and ascorbate peroxidase as compared to the unprimed seeds (Omid and Farzad, 2012).

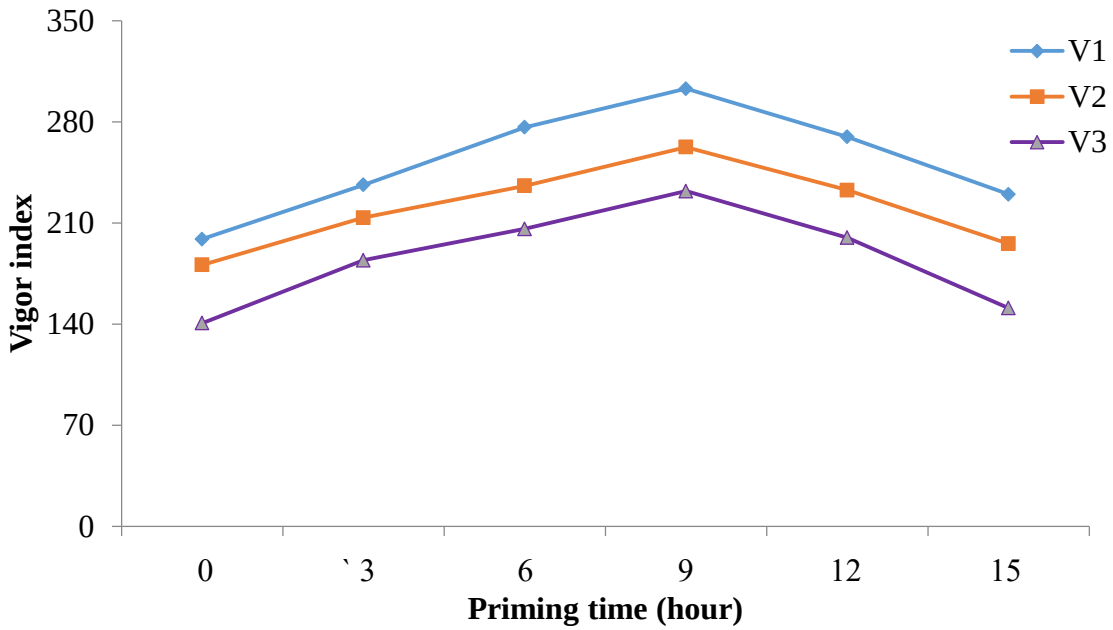


Figure 20. Effect of different priming time on the vigor index of wheat cultivars (LSD $_{(0.01)}$ = 15.54, 21.19, 20.73, 17.96, 16.62 and 21.97 at 0h, 3h, 6h, 9h, 12h and 15h, respectively)

4.3 Third experiment

Germination, seedling growth and water relation behavior of primed seed of wheat genotype under salt (NaCl) stress condition.

This experiment was conducted under laboratory condition. Three wheat genotypes were primed in 2ml H_2O_2 solution for 9 hours. Dry seed used as control and was exposed to 0, 5, 10, 15 and 20 dSm^{-1} NaCl induced salt stress conditions in petri dishes. The results have been presented separately in Figures 21 to 30 and Appendices XXII to XXXI under the following headings**:**

4.3.1 Germination percentage (%)

Different salinity levels revealed significant variation in respect of germination rate (Figure 21 and Appendix XXII). Result indicated that the germination of primed seeds decreased significantly with increasing salinity level. It was observed 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 stress, the maximum germination rate was found in primed seeds placed with 5 dSm^{-1} NaCl and thereafter gradually decreased germination rate was observed with the increasing salinity levels. It was also recorded that the highest germination rate (93.60%) was observed from V_1

(ESWYT 5) under primed seeds placed without salt and after that the second highest germination rate (91.80%) was in V_1 (ESWYT 5) with primed seeds placed with 5 dSm^{-1} where the lowest value (66.67%) was obtained from V_3 (BARI Gom 28) when the primed seeds are kept under 20 dSm^{-1} NaCl. Under stress conditions, primed seed induces the germination changes and which is to maintain the germination rate and uniformity in the seedling emergence (Ashraf and Fooland, 2005).

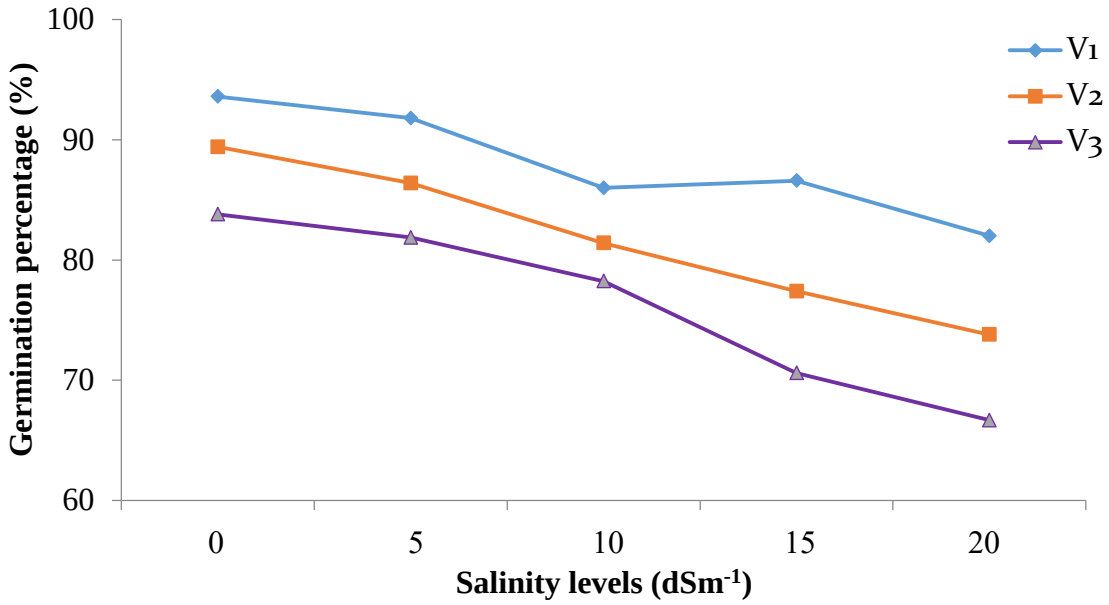


Figure 21. Effect of different salt concentration on the germination percentage of primed wheat cultivars (LSD $_{(0.01)}$ = 6.72, 8.43, 6.93, 6.10 and 6.46 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.2 Shoot length (mm)

Shoot length of different wheat genotypes was significantly influenced by different salinity levels (Figure 22 and Appendix XXIII). Result exposed that the shoot length from primed seeds decreased significantly with increasing salinity level. The highest shoot length was observed from V_1 (ESWYT 5) where primed seeds placed without salt. Under salinity stress, the highest shoot length (143.0mm) was observed from V_1 (ESWYT 5) under primed seeds placed with 5 dSm^{-1} NaCl. The lowest shoot length (74.28mm) was found from V_3 (BARI Gom 28) under primed seeds placed with 20 dSm^{-1} NaCl. Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point (McCue and Hanson, 1990). Salt stress decreased coleoptile length by 39%, 62%, and 76% in 0.25, 0.275, and 0.30 M salt

concentrations respectively (Kilic and Kahraman, 2016). According to, Hakim *et al.* (2010) any crops growing under salinity condition there may be a reduction in seedling length. Similarly in indica rice varieties there was a decreased seedling length with increased salinity condition (Anbumalarmathi and Mehta, 2013). It has been reported by Mouradi *et al.* (2016) osmopriming of seed with -0.6 Mpa of PEG6000 has positive effect on the increase of total plant biomass and nodulation under drought stress agrees with the results documented by Kaur *et al.* (2002, 2006) for chickpea and Tabatabaei (2013).

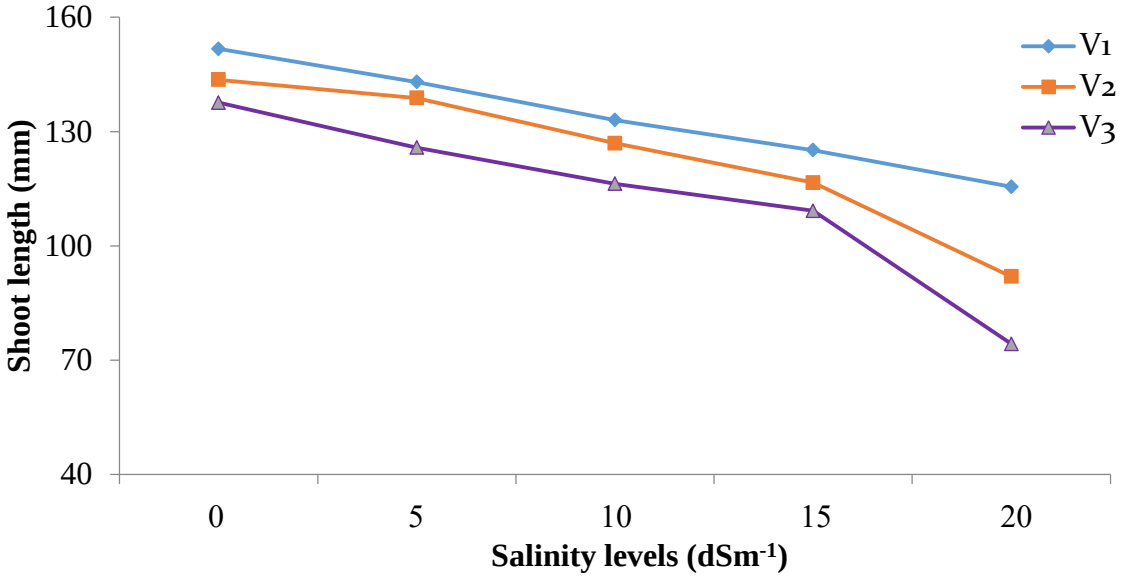


Figure 22. Effect of different salt concentration on the shoot length of primed wheat cultivars (LSD $_{(0.01)}$ = 12.46, 10.02, 12.68, 8.12 and 7.48 at 0 dSm^{-1},5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.3 Root length (mm)

Significant variation was observed on root length among the test genotypes primed seed under different salt concentrations (Figure 23 and Appendix XXIV). In this present study it was recorded that root length of wheat genotypes decreased with the increasing salt concentration where no salinity stress gave highest root length for all the genotypes. It was observed that all the genotypes showed good result when the seeds are primed with 5 dSm^{-1} NaCl. It was also found that among the genotype, V_1 (ESWYT 5) had the highest root length (127.5mm) without salt stress and V_3 (BARI Gom 28) had the lowest root length (46.96mm) at 20 dSm^{-1} NaCl. It was reported that Salt stress decreased radicle length by 82%, 84% and 85% in 0.25, 0.275 and 0.30 M salt concentrations, respectively (Kilic and Kahraman, 2016).

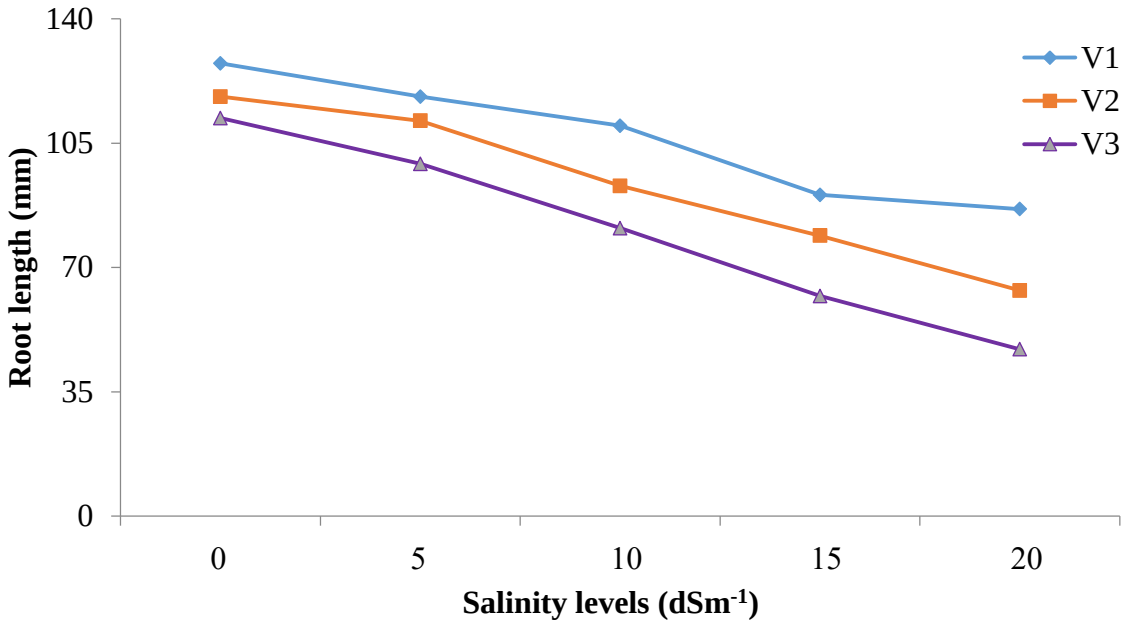


Figure 23. Effect of different salt concentration on the root length of primed wheat cultivars (LSD $_{(0.01)}$ = 6.55, 15.62, 8.35, 8.19 and 5.45 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.4 Shoot dry weight (g)

Salinity level had highly significant influence on shoot dry weight of different wheat genotypes (Figure 24 and Appendix XXV). The results of the experiment showed that the highest shoot dry weight (0.04480 g) was observed from V_1 (ESWYT 5) Primed seeds placed without salt stress, followed by primed seeds placed with 5 dSm^{-1} NaCl where the lowest shoot dry weight (0.01580 g) was observed from V_3 (BARI Gom 28) under primed seeds placed with 20 dSm^{-1} NaCl. Salt stress decreased fresh weight by 27%, 47%, and 66% in 0.25, 0.275, and 0.30 M salt concentrations (Kilic and Kahraman, 2016). Similar results were observed in rice and wheat, in which the seedling length and dry weight were decreased with increased salt stress (Jamil and Raha, 2007).

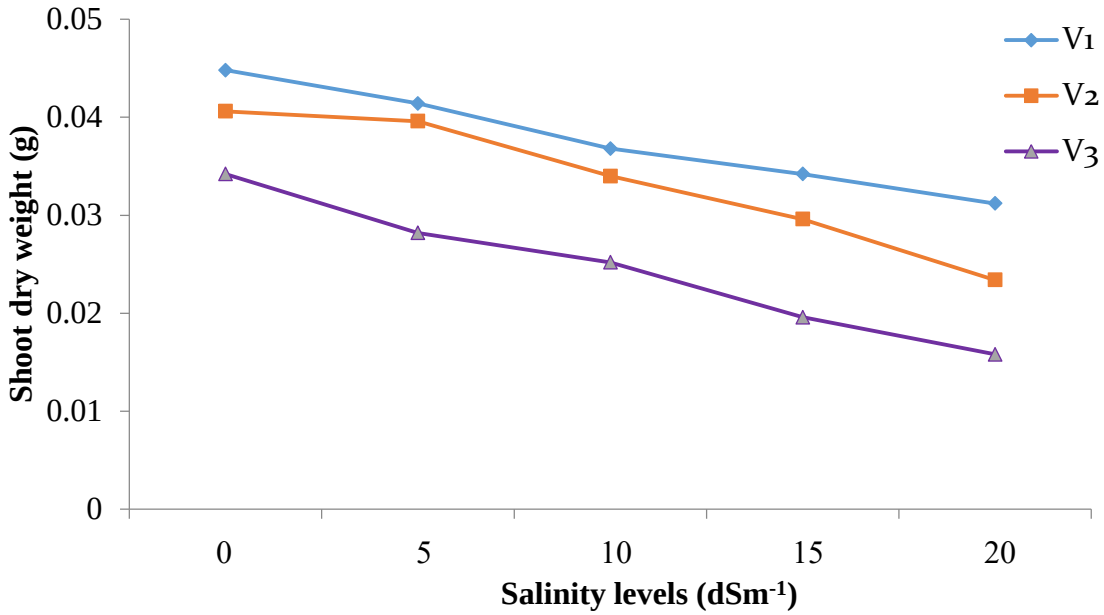


Figure 24. Effect of different salt concentration on the shoot dry weight of primed wheat cultivars (LSD $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006 and 0.006 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.5 Root dry weight (g)

Significan variation was found in terms of root dry weight of different wheat genotypes affected by different salinity levels (Figure 25 and Appendix XXVI). It was recorded that the genotype, V_1 (ESWYT 5) distinctly showed the highest root dry weight in different salinity level where genotype, V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest root dry weight under salinity stress. The result of the experiment indicated that maximum root dry weight (0.0358 g) was scored by V_1 (ESWYT 5) when primed seed placed without salt and the minimum root dry weight (0.013 g)was recorded from V_3 (BARI Gom 28) when primed seed placed in 20 dSm^{-1} NaCl solution. Excess of ions in root medium exerts effects like osmotic strain, ion specificity/toxicity, nutritional imbalances (Jones and Gorham, 2002), changes in cell metabolites levels (Rhodes *et al*., 2002; Wahid and Ghazanfar, 2006) and diminished growth and yield (Wahid *et al*., 2004 and Ahmad *et al*., 2005).

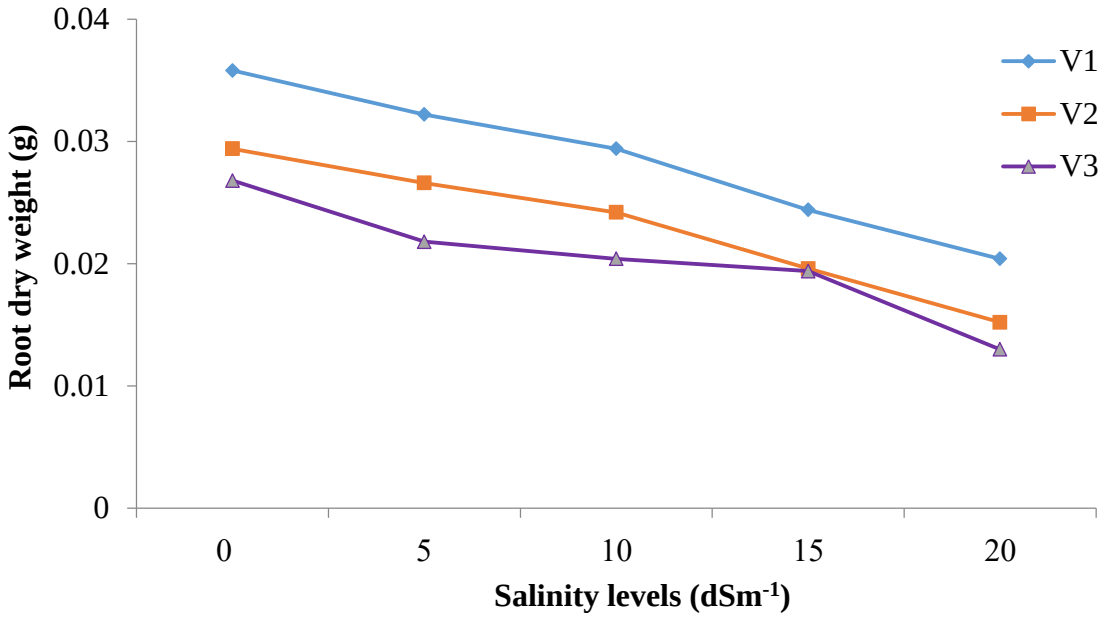


Figure 25. Effect of different salt concentration on the root dry weight of primed wheat cultivars (LSD $_{(0.01)}$ = 0.006, 0.006, 0.006, 0.006 and 0.006 at 0 dSm^{-1},5dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.6 Relative water content (%)

Relative water content of wheat genotypes significantly affected by different salt concentration (Figure 26 and Appendix XXVII). There was a gradual decreased observed with increasing the salt concentration. Wheat genotype V_1 (ESWYT 5) performed the best, V_2 (ESWYT 6) performed moderately and V_3 (BARI Gom 28) gave consistently poor performance under all salinity levels. The result of the present study revealed that the maximum relative water content (92.92%) was scored by V_1 (ESWYT 5) with primed seed placed without salt solution whereas the minimum relative water content (55.49%) was recorded from V_3 (BARI gom28) when primed seed placed with 20 dSm^{-1} NaCl solution. Chakraborty *et al.* (2012) reported that Pretreatment with H_2O_2 was effective to prevent chlorophyll degradation due to H_2O_2 supplying may be assigned to maintain higher relative water content and lower hydrogen peroxide content in leaves under salt stress.

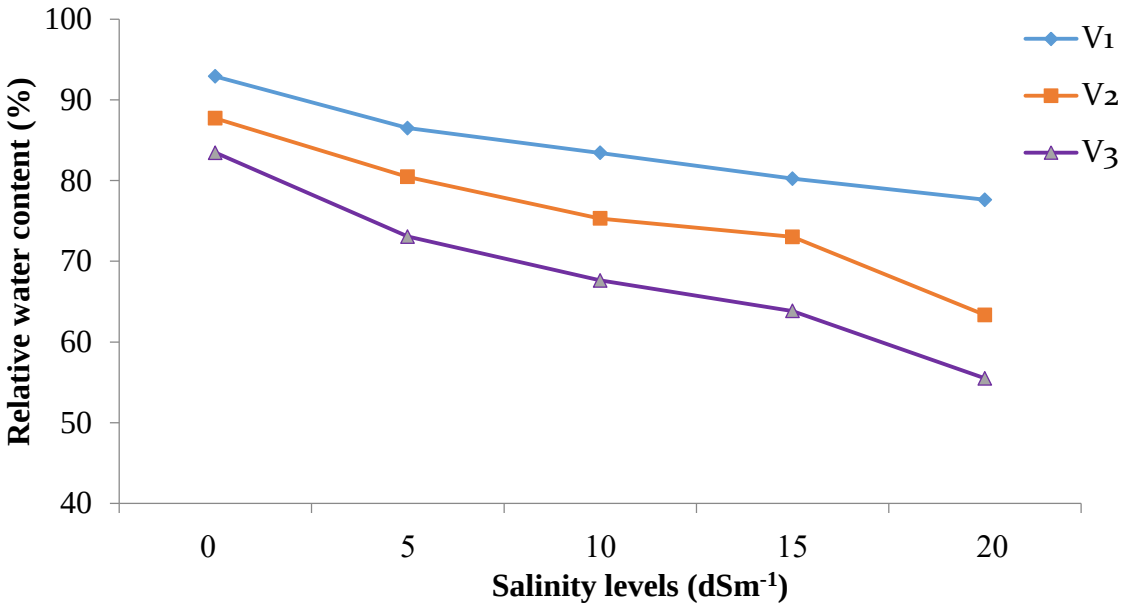


Figure 26. Effect of different salt concentration on the relative water content of primed wheat cultivars (LSD $_{(0.01)}$ = 6.29, 6.54, 6.63, 6.52 and 4.61at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.7 Water Saturation Deficit (%)

A wide range of statistical difference was observed due to different salt concentration (Figure 27 and Appendix XXVIII). Water saturation deficit increases with increased salt concentration. It was observed that the genotype, V_1 (ESWYT 5) showed the lowest water saturation deficit and the genotype V_3 (BARI Gom 28) showed the highest result. Under salinity stress, the performance of all the genotype is lower under 5 dSm^{-1} NaCl solution. The result indicated that maximum water saturation deficit (44.51%) was scored by V_3 (BARI Gom 28) when the primed seed are placed with 20 dSm^{-1} NaCl solution and minimum value (7.082%) was scored V_1 (ESWYT 5) where the primed seed are placed without salt stress.

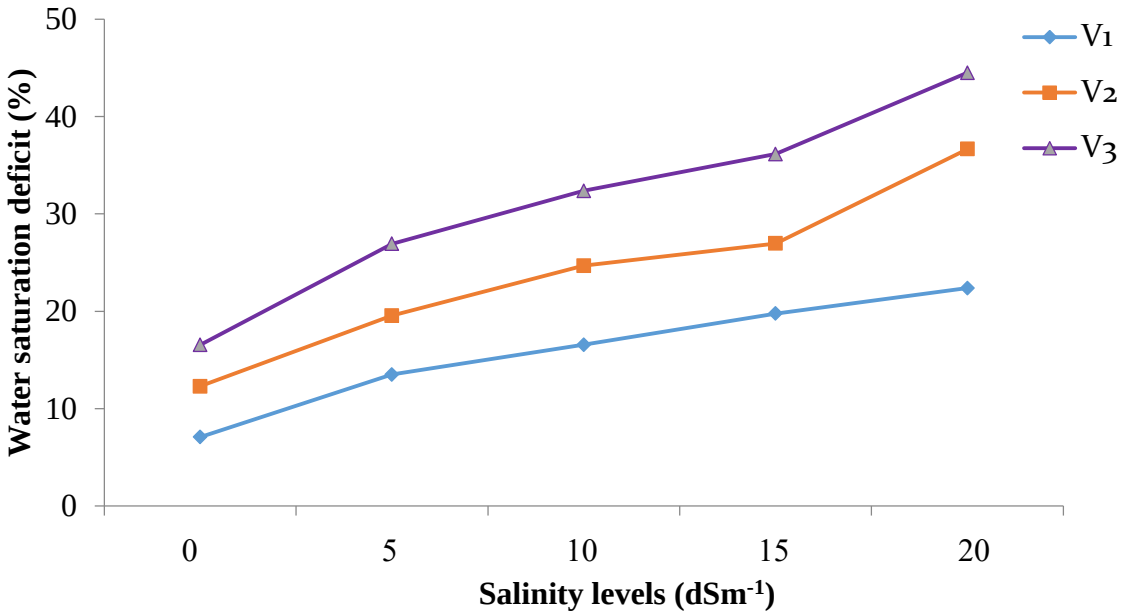


Figure 27. Effect of different salt concentration on the water saturation deficit of primed wheat cultivars (LSD $_{(0.01)}$ = 1.10, 1.82, 2.20, 2.40 and 3.29 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.8 Water retention capacity

Significant influence was found in terms of water retention capacity of different wheat genotypes affected by different salinity levels (Figure 28 and Appendix XXIX). The line graph which is maintained a decreasing tend. The result of present study exposed that highest water retention capacity (15.57) was accounted from wheat genotype V_1 (ESWYT 5) when the primed seed are placed without salt and lowest water retention capacity (6.816) was achieved from V_3 (BARI Gom 28) when the primed seeds are kept under 20 dSm^{-1} NaCl solution.

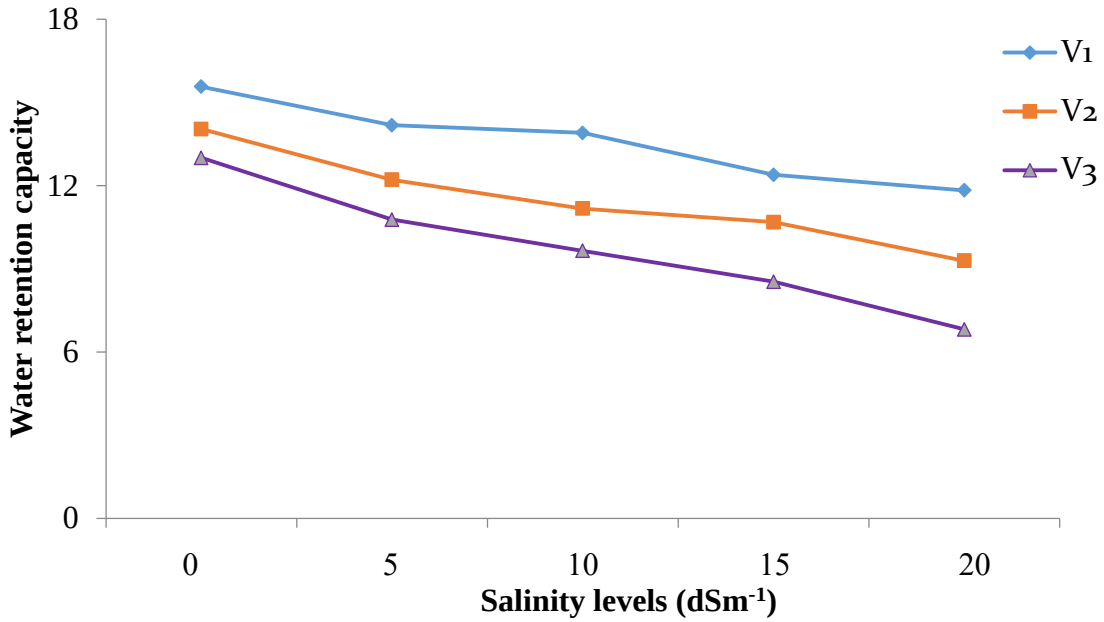


Figure 28. Effect of different salt concentration on the water retention capacity of primed wheat cultivars (LSD $_{(0.01)}$ = 1.13, 1.14, 0.90, 1.01 and 0.70 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.9 Coefficient of velocity of germination

Primed seed showed significant influence on coefficient of velocity of germination of different wheat genotypes under different salinity level (Figure 29 and Appendix XXX). Wheat genotype V_1 (ESWYT 5) dominantly scored highest value at all the salt level followed by V_2 (ESWYT 6) where V_3 (BARI Gom 28) showed consistently poor performance of wheat in terms of coefficient of velocity of germination. It was distinctly found that the maximum coefficient of velocity of germination (17.52) was achieved from V_1 (ESWYT 5) primed seed without salt stress whereas minimum coefficient of velocity of germination (11.70) was obtained from V_3 (BARI Gom 28) primed seed under 20 dSm^{-1} NaCl salt concentration. Wahida *et al.* (2007) reported that Seeds treated with 1–120 mM H_2O_2 for 8 hours and germinated in saline (150mM NaCl) medium curtailed the mean germination time (MGT) being even less than water controls.

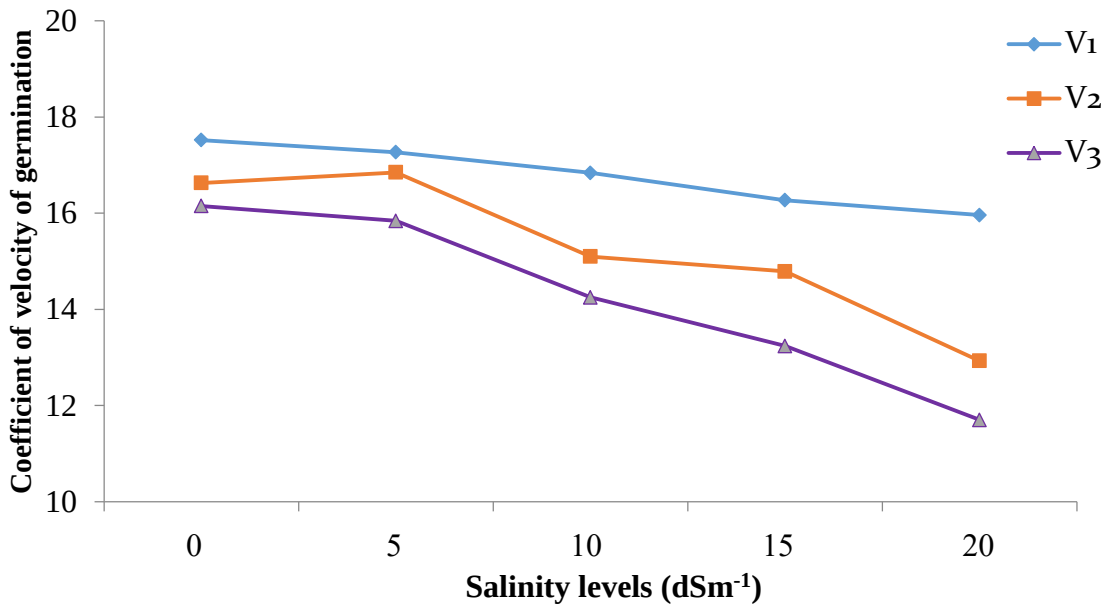


Figure 29. Effect of different salt concentration on the coefficient of velocity of germination of primed wheat cultivars (LSD $_{(0.01)}$ = NS, 1.34, 1.31, 1.55 and 1.11 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively)

4.3.10 Vigor Index

Statistically significant variation was found in case of vigor index of different genotypes of wheat due to primed seed placed with different concentrations of salt (Figure 30 and Appendix XXXI). There was a decreasing trend observed with increasing the salt concentration. Among the test sample, V_1 (ESWYT 5) showed the highest vigor index where genotype and V_2 (ESWYT 6) showed the intermediate result and variety V_3 (BARI Gom 28) showed the lowest vigor index under all salinity level. Vigor index was in no salinity stress followed by primed seeds placed with 5 dSm^{-1} NaCl solution. The result of experiment exposed that maximum vigor index (264.1) was accounted from wheat genotype V_1 (ESWYT 5) when the primed seed are placed without salt and minimum (80.89) was achieved from V_3 (BARI Gom 28) when the primed seeds are kept under 20 dSm^{-1} NaCl solution. H_2O_2 had positive effects on germination index (GI) and vigor index (VI). H_2O_2 increased vigor index by 56%, 33%, and 16% in increasing salt concentrations (0.25, 0.275, and 0.30 M) compared to control (Kilic and Kahraman, 2016).

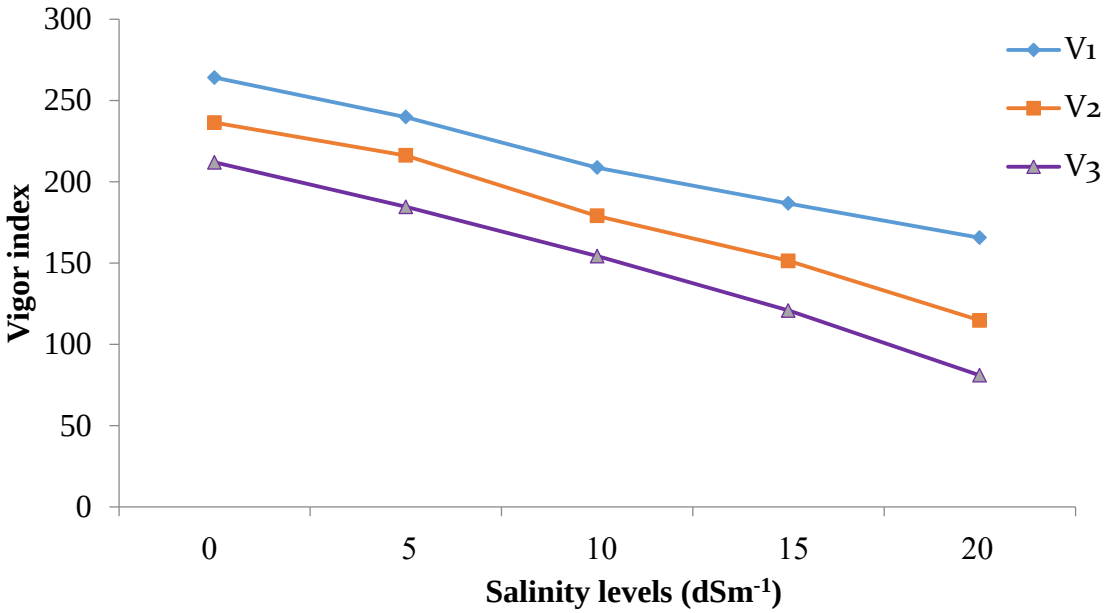


Figure 30. Effect of different salt concentration on the vigor index of primed wheat cultivars (LSD $_{(0.01)}$ = 21.65, 24.18, 14.24, 11.23 and 11.41 at 0 dSm^{-1}, 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1}, respectively).

CHAPTER 5

SUMMARY AND CONCLUSION

With a view to alleviate the negative effects of salt stress on different wheat genotype an experiment was conducted at Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from October 2016 to December 2016 to study the H_2O_2 induce changes in growth and physiology of wheat *(Triticum aestivum.L)* under salt stress. A set of experiment was conducted in three different experiments. The experiment was laid out in a Completely Randomized Design (CRD) with five replications.

Three wheat genotypes namely- ESWYT 5, ESWYT 6, and BARI Gom 28 were used as test crop. Different priming chemicals such as H_2O_2, and distilled water were utilized for chemical and hydro priming and NaCl was used to induce salt stress.

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 with 8 ml of distilled water.

Germination was measured to have occurred when radicles were 2 mm long. Germination progress was examined and data were collected at every 24 h intervals and continued up to 10 days. The abnormal or dead seedlings were excluded during counting. The data recorded on germination percentage, root length, shoot length, root dry weight, shoot dry weight, water saturation deficit, water retention capacity and vigor 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.

First experiment

The first experiment was carried out to find the effect of different concentration of H_2O_2 on germination and growth behavior of three wheat genotypes (ESWYT 5, ESWYT 6, and BARI Gom 28) without any stress condition. Six levels of H_2O_2 such as 0, 2, 4, 6, 8, 10ml and were used for chemical priming and distilled water used as hydro priming agent for 24 hours, respectively.

Among the genotypes, V_1 (ESWYT 5) gave the best results on studied parameters. Results revealed that V_1 (ESWYT 5) showed the highest germination rate (93.40%), shoot length (162.40 mm), root length (146.20 mm), shoot dry weight (0.04220 g), root dry weight (0.03480 g), relative water content (93.08%), water retention capacity (20.80), coefficient of velocity of germination (17.66) and vigor index (253.90) primed with 2ml H_2O_2 solution for 24 hours where seeds without priming showed lowest results in respected parameters with the genotype V_3 (BARI Gom 28).

Second experiment

The second experiment was conducted to optimization of priming time on the germination and growth behavior of wheat genotypes. Three wheat genotypes (ESWYT 5, ESWYT 6 and BARI Gom28) without any stress condition were considered. Six different priming times such as 0, 3, 6, 9, 12 and 15 hours for chemical priming were used using 2ml H_2O_2 solution.

The seeds of genotype V_1, (ESWYT 5) primed with 2ml H_2O_2 solution for 9 hours gave the highest germination rate (94.20%), shoot length (169.30 mm), root length (146.0 mm), shoot dry weight (0.04340 g), root dry weight (0.03820 g), relative water content (91.14%), water retention capacity (17.76), coefficient of velocity of germination (18.08) and vigor index (303.1). The lowest water saturation deficit (8.864 %) was also obtained from V_1 (ESWYT 5) seeds primed with 2ml H_2O_2 solution for 9 hours where seeds primed with H_2O_2 solution for 15 hours showed the highest results (38.59%) in respected parameters with the genotype V_3 (BARI Gom 28).

Third experiment

In the third experiment germination and growth behavior of primed seeds of wheat genotypes (ESWYT 5, ESWYT 6 and BARI Gom28) with and without salt (NaCl) stress condition was evaluated. H_2O_2 solution 2ml were used as priming solutions and 9 hours as priming time and salt stress levels; without salt (control), 5 dSm^{-1}, 10 dSm^{-1}, 15 dSm^{-1} and 20 dSm^{-1} were used in this experiment.

Results revealed that the genotype V_1 (ESWYT 5) with primed seeds placed without salt; control gave the highest germination rate (93.60%), shoot length (151.7 mm), root length (127.5mm), shoot dry weight (0.04480 g), root dry weight (0.03580 g), relative water

content (92.92%), water retention capacity (15.57), coefficient of velocity of germination (17.52) and vigor index (264.1). But under salinity stress, the highest germination rate (91.8%), shoot length (143.00 mm), root length (118.10 mm), shoot dry weight (0.04140 g), root dry weight (0.03220 g), relative water content (86.50 %), water retention capacity (14.18), coefficient of velocity of germination (17.27) and vigor index (239.8) were achieved from V_1 (ESWYT 5) primed seeds placed with 5 dSm^{-1} NaCl where V_3 (BARI Gom28) primed seeds placed with 20 dSm^{-1} NaCl showed lowest results in respected parameters.

Considering the findings of the present experiment following conclusions may be drawn:

- Performance of ESWYT 5 was better in respect of germination and growth parameters when the seed are primed with 2ml H_2O_2 concentration in 9 hours priming time.
- Seed priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of salt stress.
- Further studies are needed to assess the efficacy of seed priming during the later stages of the crop.
- More research should be conducted to observe the field performance.
- More Regional trial should be practiced.

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APPENDICES

Appendix 1. Monthly records of temperature, rainfall, and relative humidity of the experiment site during the period of October 2016

Year	Month	Air Temperature (^{0}c)			Relative humidity (%)	Rainfall (mm)	Sunshine (hour)
		Maximum	Minimum	Mean			
2016	October	29.5	18.5	24.0	69.5	0.0	233.2
2016	December	26.4	15.6	21.0	68.6	0.0	230.4

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

Appendix II. Mean squares value for germination percentage (%) of wheat genotypes at different level priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of germination percentage (%) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	160.158**	143.212**	110.867**	108.923**	194.317**	177.127**	277.855
Error	12	11.347	10.290	9.631	5.455	10.590	6.577	5.525

**Significant at 1% level of significance
^{NS} Non significant

Appendix III. Mean squares value for shoot length (mm) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of shoot length (mm) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	434.195**	816.821**	462.349**	715.261**	695.921**	1752.200**	152.899**
Error	12	26.517	34.756	23.841	30.668	17.188	35.579	10.915

**Significant at 1% level of significance
^{NS} Non significant

Appendix IV. Mean squares value for root length (mm) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of root length (mm) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	228.659**	547.213**	599.76**	221.846**	261.716**	1647.948**	3514.035**
Error	12	14.423	18.845	38.623	22.338	20.245	16.735	6.737

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

Appendix V. Mean squares value for shoot dry weight (g) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of shoot dry weight (g) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Error	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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

Appendix VI. Mean squares value for root dry weight (g) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of root dry weight (g) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Error	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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

Appendix VII. Mean squares value for relative water content (%) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of relative water content (%) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	328.623**	228.347	122.461**	131.213**	118.819**	295.462**	649.154**
Error	12	10.479	7.838	3.284	13.660	8.992	7.728	

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

Appendix VIII. Mean squares value for water saturation deficit (%) of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of water saturation deficit (%) at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	328.623**	228.347**	122.461**	131.213**	118.819**	295.462**	649.154**
Error	12	2.272	1.086	0.244	0.617	0.477	3.137	4.244

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

Appendix IX. Mean squares value for water retention capacity of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	df	Mean squares of water retention capacity at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	22.473**	38.702**	60.979**	47.338**	35.210**	18.858 **	14.512**
Error	12	0.161	0.403	0.850	0.590	0.760	0.185	0.188

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

Appendix X. Mean squares value for coefficient velocity of germination of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	df	Mean squares of coefficient velocity of germination at different priming solution						
		Control	Water	2ml	4 ml	6 ml	8ml	10 ml
Treatment	2	24.755**	7.726**	4.615**	6.166**	5.133**	20.488**	9.081**
Error	12	0.210	0.421	0.289	0.461	0.407	0.617	0.360

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

Appendix XI. Mean squares value for vigor index of wheat genotypes at different level of priming (H_2O_2 and Water) and non-priming (control) seeds

Sources of variation	Df	Mean squares of vigor index at different priming solution						
		Control	Water	2ml	4 ml	6ml	8ml	10 ml
Treatment	2	2609.51**	20397.920**	5079.328**	5904.93**	5730.63**	4093.61**	2177.722**
Error	12	15.554	59.981	78.631	44.169	62.624	23.685	19.334

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

Appendix XII. Mean squares value for germination percentage (%) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of germination percentage (%)at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	44.349**	54.447**	277.754**	187.985**	255.342**	223.817**
Error	12	16.730	9.442	13.220	18.083	6.410	11.131

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

Appendix XIII. Mean squares value for shoot length (mm) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of shoot length (mm) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12hours (T_4)	15 hours (T_5)
Treatment	2	2839.91**	1011.887**	605.156**	868.755**	659.651**	1599.251**
Error	12	19.724	39.044	28.066	37.304	22.591	33.804

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

Appendix XIV. Mean squares value for root length (mm) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of root length (mm) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	271.454**	301.714**	180.162**	198.426**	198.020**	842.870**
Error	12	19.207	24.440	34.262	29.266	40.725	15.309

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

Appendix XV. Mean squares value for shoot dry weight (g) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of shoot dry weight (g) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Error	12	15.554	0.000	0.000	0.000	0.000	0.000

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

Appendix XVI. Mean squares value for root dry weight (g) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	df	Mean squares of root dry weight (g) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Error	12	0.000	0.000	0.000	0.000	0.000	0.000

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

Appendix XVII. Mean squares value for relative water content (%) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of relative water content (%) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	91.792**	123.555**	137.687**	201.131**	341.853**	237.933**
Error	12	10.899	8.403	8.970	15.880	5.159	15.780

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

Appendix XVIII. Mean squares value for water saturation deficit (%) of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of water saturation deficit (%) at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours ((T_4)	15 hours (T_5)
Treatment	2	91.792**	123.555**	137.633**	201.131**	341.853**	237.873**
Error	12	2.081	1.688	0.936	0.637	0.899	2.659

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

Appendix XIX. Mean squares value for water retention capacity of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of water retention capacity at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	9.458**	16.019**	20.643**	40.612 **	14.095 **	11.571**
Error	12	0.349	0.177	0.351	0.673	0.472	0.226

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

Appendix XX. Mean squares value for coefficient velocity of germination of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of coefficient velocity of germination at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	6.673**	9.282**	2.614**	3.207**	5.078**	6.006**
Error	12	0.731	0.565	0.501	0.616	0.464	0.434

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

Appendix XXI. Mean squares value for vigor index of H_2O_2 primed seed of wheat genotypes on different priming time

Sources of variation	Df	Mean squares of vigor index at different priming time					
		O hour (T_0)	3 hours (T_1)	6 hours (T_2)	9 hours (T_3)	12 hours (T_4)	15 hours (T_5)
Treatment	2	4436.538**	3423.735**	6240.38**	6334.19**	6064.44**	7817.315**
Error	12	64.743	120.306	115.131	86.398	74.039	129.385

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

Appendix XXII. Mean squares value for germination percentage (%) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	df	Mean squares of germination percentage (%) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	120.867**	123.523 **	76.136**	322.400 **	294.163**
Error	12	12.100	19.027	12.867	9.967	11.173

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

Appendix XXIII. Mean squares value for shoot length (mm) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of shoot length (mm) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	251.235**	398.051 **	354.247 **	316.186**	2140.584**
Error	12	41.567	26.928	43.069	17.673	14.973

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

Appendix XXIV. Mean squares value for root length (mm) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of root length (mm) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	299.277**	457.748 **	1047.186**	1025.964**	1963.902**
Error	12	11.500	65.393	18.676	17.992	7.950

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

Appendix XXV. Mean squares value for shoot dry weight (g) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of shoot dry weight (g) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	0.000**	0.000**	0.000**	0.000**	0.000**
Error	12	0.000	0.000	0.000	0.000	0.000

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

Appendix XXVI. Mean squares value for root dry weight (g) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of root dry weight (g) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	0.000**	0.000**	0.000**	0.000 **	0.000**
Error	12	0.000	0.000	0.000	0.000	0.000

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

Appendix XXVII. Mean squares value for relative water content (%) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	df	Mean squares of relative water content (%) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	112.468**	226.263**	311.892 **	2487.48**	2273.88**
Error	12	10.605	11.451	11.784	8.59	5.88

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

Appendix XXVIII. Mean squares value for water saturation deficit (%) of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of water saturation deficit (%) at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	1715.67**	1937.64**	2328.34**	337.499 **	629.377 **
Error	12	8.54	10.86	9.76	11.372	5.685

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

Appendix XXIX. Mean squares value for water retention capacity H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	df	Mean squares of water retention capacity at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10 dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	8.377 **	14.663**	23.250 **	18.702 **	31.377**
Error	12	0.343	0.349	0.217	0.274	0.133

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

Appendix XXX. Mean squares value for coefficient of velocity of germination of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of coefficient of velocity of germination at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	2.392**	2.705**	8.706**	11.508**	23.998**
Error	12	0.629	0.480	0.457	0.642	0.328

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

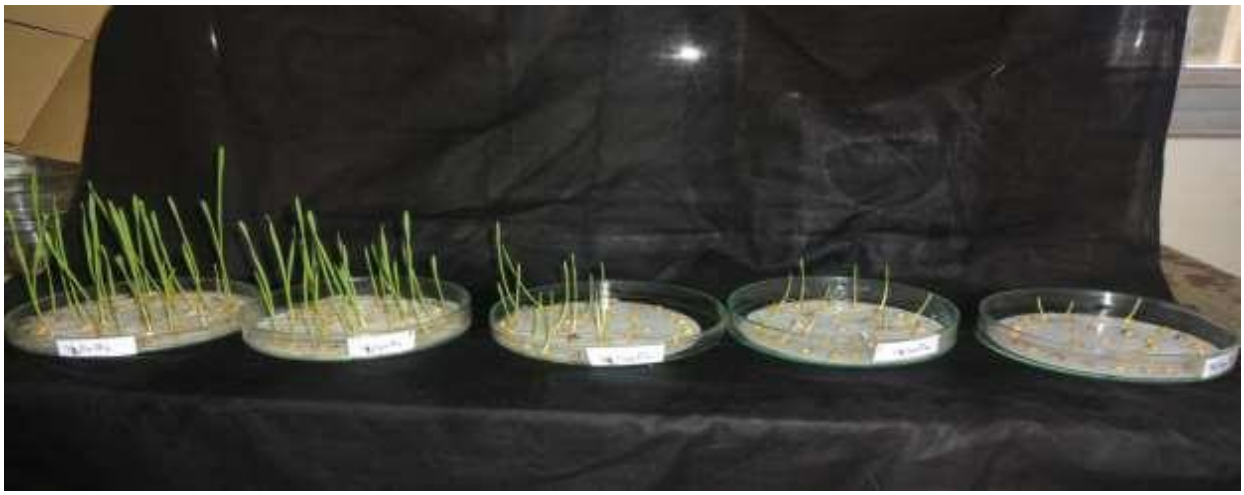
Appendix XXXI. Mean squares value for vigor index of H_2O_2 primed seed of wheat genotypes under different level of salinity

Sources of variation	Df	Mean squares of vigor index at different salt concentration				
		0 dSm^{-1} (S_0)	5 dSm^{-1} (S_1)	10dSm^{-1} (S_2)	15 dSm^{-1} (S_3)	20 dSm^{-1} (S_4)
Treatment	2	3406.197 **	3833.655**	3718.656**	5407.424**	9098.260**
Error	12	125.597	156.625	54.301	3 3.776	34.888

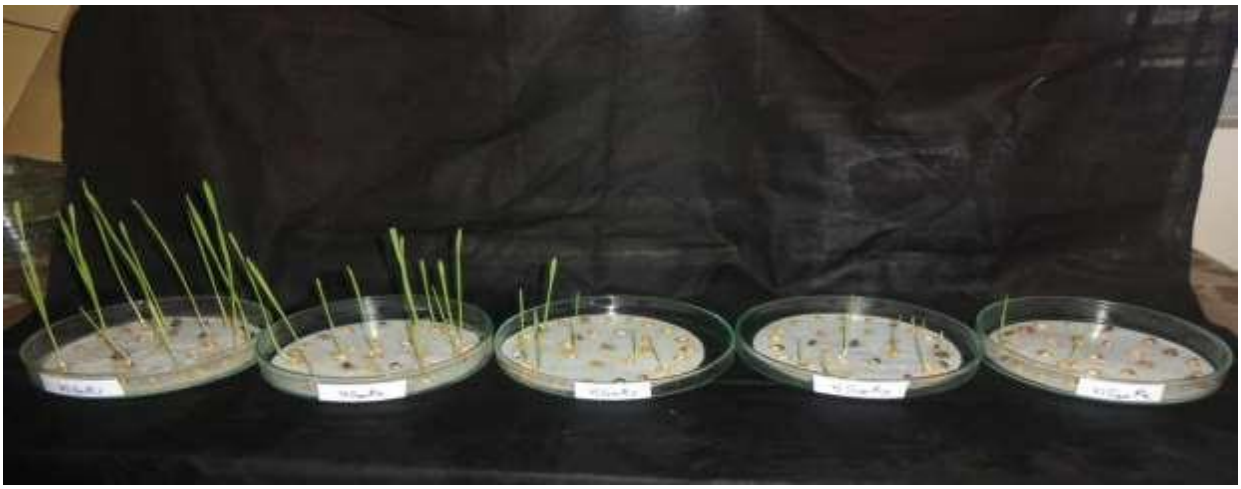
**Significant at 1% level of significance
NS Non significant

SOME PICTURES OF SALINITY EFFECT AT SEEDLING STAGE IN WHEAT

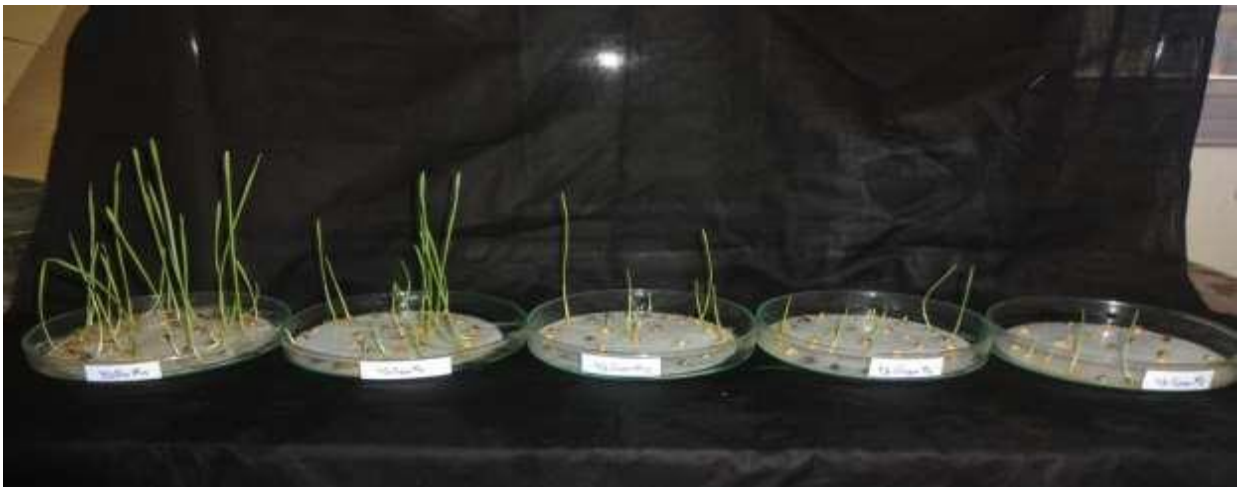
Plate 1: Experimental set up in the laboratory



(A)



(B)



(C)

Plate 2: Effect of different salinity level (0, 5, 10, 15 and 20 dSm-1) on H_2O_2 primed wheat seeds showing in the petri dishes: (A) genotype, V1 = ESWYT 5, (B) genotype, V2 = ESWYT 6 and (C) genotype, V3 = BARI Gom 28.

(A)

(B)

(C)

Plate 3: Seedling growth of H_2O_2 primed seeds of wheat at 0, 5, 10, 15, and 20 dSm-1 NaCl concentrations respectively from left side to right side: (A) genotype, V1 = ESWYT 5, (B) genotype, V2 = ESWYT 6 and (C) genotype, V3 = BARI Gom 28.