POLYETHYLENEGLYCOL (PEG) INDUCE CHANGES IN GERMINATION, SEEDLING GROWTH AND WATER RELATION BEHAVIOUR OF WHEAT UNDER SALT STRESS CONDITION

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By

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CERTIFICATE

This is to certify that the thesis entitled "POLYETHYLENEGLYCOL (PEG) INDUCE CHANGES IN GERMINATION, SEEDLING GROWTH AND WATER RELATION BEHAVIOUR OF WHEAT UNDER SALT STRESS CONDITION" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) IN AGRONOMY, embodies the results of a piece of bona fide research work carried out by MOST. FALJUNNAHAR Registration. No. 15-06968, under my supervision and guidance. No part of this 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 duly been acknowledged.

Dated: December, 2016 Dhaka, Bangladesh (Prof. Dr. Md. Abdullahil Baque) Supervisor

Dedicated To My Beloved Parents

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POLYETHYLENEGLYCOL (PEG) INDUCE CHANGES IN GROWTH AND PHYSIOLOGY OF WHEAT (*Triticum aestivum L.*) UNDER SALT STRESS

ABSTRACT

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. A set of experiment was conducted in three different experiments. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Four wheat genotypes namely-ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom 28 were used as test crop and different priming chemicals such as PEG, salt (NaCl) and distilled water were utilized for osmo and hydro priming. The data on germination parameters of wheat like germination percentage and growth parameters like root length, shoot length, dry weight and vigour index. Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the Least Significance Difference (LSD) at 1% level of probability. The first experiment was carried out to find the effect of different concentration of PEG on germination and growth behavior of four wheat genotypes (ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom 28) without any stress condition. It was found that ESWYT 5 showed the highest germination rate (95.30%), shoot length (168.20 mm), root length (158.30 mm), shoot dry weight (0.0522 mg), root dry weight (0.0432 mg), relative water content (95.05%), water retention capacity (16.18) and vigour index (311.30) with primed seeds with 10% PEG solution for 12 hours. The second experiment was conducted to optimization of pre-sowing priming time on the germination and growth behavior of wheat genotypes. It was observed that ESWYT 5 with 10% PEG primed seeds for 9 hours gave the highest germination rate (83.33%), shoot length (181.1 mm), root length (145.0 mm), shoot dry weight (0.0536 mg), root dry weight (0.0432 mg), relative water content (91.45%), water retention capacity (19.94) and vigour index (272.3). In the third experiment germination and growth behavior of primed seeds of wheat genotypes (ESWYT 5, ESWYT 6 and BARI gom 28) under salt (NaCl) stress condition were evaluated. It was observed that highest germination rate (93.81%), shoot length (157.80 mm), root length (124.6 mm), shoot dry weight (0.037 mg), root dry weight (0.0414 mg), relative water content (89.13%), water retention capacity (22.32) and vigour index (264.7) were obtained from ESWYT 5 under primed seeds placed without salt. But under salinity stress, the highest germination rate (87.62%), shoot length (154.20 mm), root length (118.50 mm), shoot dry weight (0.0349 mm) and root dry weight (0.0393 mg), relative water content (85.61 %), water retention capacity (21.40) and vigour index (238.9) were achieved from ESWYT 5 under primed seeds placed with 5 dsm⁻¹.

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ABBREVIATIONS AND ACRONYMS

| AEZ | = | Agro-Ecological Zone |
|---------|---|---|
| BBS | = | Bangladesh Bureau of Statistics |
| BCSRI | = | Bangladesh Council of Scientific Research Institute |
| 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 Agricultural Organization |
| g | = | Gram (s) |
| i.e. | = | id est (L), that is |
| Kg | = | Kilogram (s) |
| LSD | = | Least Significant Difference |
| m^2 | = | Meter squares |
| ml | = | MiliLitre |
| M.S. | = | Master of Science |
| No. | = | Number |
| SAU | = | Sher-e-Bangla Agricultural University |
| var. | = | Variety |
| °C | = | Degree Celceous |
| % | = | Percentage |
| NaOH | = | Sodium hydroxide |
| GM | = | Geometric mean |
| mg | = | Miligram |
| Ρ | = | Phosphorus |
| Κ | = | Potassium |
| Ca | = | Calcium |
| L | = | Litre |
| μg | = | Microgram |
| | | United States of America |
| WHO | = | World Health Organization |
| | | e |

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop and ranks first globally and second in Bangladesh both in terms of production and acreage (Anonymous, 2010). It is a staple food crop for more than one third of the world population (Shirazi *et al.*, 2001). In Bangladesh, the area under wheat cultivation during 2013-2014 was about 1061602 acres producing 1302998 M. tons with an average yield of 1233 kg acre-1 (BBS, 2014).

It contains carbohydrate (78.1%), protein (14.7%), minerals (2.1%), fat (2.1%) and considerable proportion of vitamins (Peterson, 1965). The crop is grown under different environmental condition ranging from humid to arid, subtropical to temperate zone (Saari, 1998).

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 increase but soon be acute.

The average yield of wheat in Bangladesh is 1.9 t ha⁻¹, which is very low in compared to other wheat growing countries like the Netherland, UK, France and Norway where average yield is 7.1, 5.9, 5.6 and 4.1 t ha⁻¹ respectively (FAO, 2001). The low yield of wheat in Bangladesh may be attributed to number of reasons like cultivation practices, poor knowledge, lack of improved varieties, improper fertilizer application, seed rate, water shortage soil salinity, cultural method, time of sowing etc.

Variety is an important factor affecting farmer's yields and is also among the factors given the highest priority for immediate technology transfer. Diffusion of new varieties ensures continuing increase in productivity through the increased

yield potential of new varieties; it reduces the investment, thereby increasing the returns and helps to maintain genetic resistance to diseases and pests (Heisey, 1990).

Salinity is one of the most important environmental stress which severely limits plant growth and productivity worldwide (Tester and Davenport, 2003). 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 Maghsoud, 2008). It is a suited crop for saline soils because it required irrigation water, which is necessary for reclamation of saline soils. Soils are considered saline if they contain soluble salts in quantities sufficient to interfere with the growth of most crop species. Salinity is a major threat to crop productivity in the southern and south-western part of Bangladesh, where it is developed due to frequent flood by sea water of the Bay of Bengal and on the other hand introduction of irrigation with saline waters. A vast area of cultivable land of the coastal region remains fallow (seasonal or complete) and the dominant cropping pattern of there is fallow-aman-fallow. Introduction of wheat into the existing cropping pattern in the saline soil may become a worthy effort to utilize these lands to meet up the food and nutritional balance of the over increasing population of Bangladesh. Saline soil covers our earth's surface, estimated to be from 400 to 950 million ha. The total world wide area of land affected by salinity is about 190 million ha (FAO, 2010).

It has been demonstrated that about 61% reduction of seed germination and 23-25% yield loss can be occurred when wheat seed 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).

Growth regulator has been reported successively on regulating various biological processes, including root formation (Zhang *et al.*, 2013), flowering and fruit ripening (Shi *et al.*, 2016), and leaf senescence (Wang *et al.*, 2012). Polyethylene glycol (PEG), being a high molecular weight ostmotic substance, has been used frequently as artificial stress inducer in many studies (Landjeva *et al.*, 2008). Hamayun *et al.* (2010) have also studied the effect of PEG induced stress on physio-hormonal attributes of soybean. Due to many reasons, PEG is considered superior to other solutes to induce different stress (Kaur *et al.*, 1998).

Seed priming is considered as a promising approach to increase stress tolerance capacity of crop plants including salinity. It has been found to be a reliable technology to enhance rapid and uniform emergence, high vigor, and better yields for vegetable and field crops (Rouhi *et al.*, 2011). In fact, this technique is a treatment that applied before germination in a specific environment that seeds are partially hydrated to a point where germination processes begin but radical emergence does not occur (Giri and Schilinger, 2003). On the other hand on seed priming the amount of water absorption is controlled so as necessary metabolic activities occurred for germination but radical emergence is prohibited. Seed priming can be accomplished through different methods such as hydropriming

(soaking in DW), osmopriming (soaking in osmotic solutions such asmannitol, PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth inducers (CCC, Ethephon, IAA) (Chiu *et al.*, 2002, Capron *et al.*, 2000).

Therefore, seed priming is a technology that enhances rapid emergence (7-10 d) and early establishment of mungbaen. Rapid and uniform field emergences are regarded as an essential prerequisite for both irrigated and rain fed conditions to reach the yield potential, quality and ultimately profit in annual crops (Cantliffe *et al.*, 1994).

Moreover, it is also important to study more about the performance of on the germination, vigour and other attributes of wheat. Therefore the present study on seed priming of wheat with PEG under salt stress was formulated with the following objectives,

- 1. To evaluate the effect of pre-sowing seed treatment with PEG on germination behavior of wheat in relation to salt tolerance.
- 2. To evaluate the effect of priming time with priming chemical on germination and growth behavior of wheat under salt stress.
- 3. To understand the physiological mechanism involve during seed germination in relation to water relation behavior of wheat.

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, riming 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.

2.1 Effect of salinity

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.

Milne (2012) studied on the effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L.), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined.

Saberi *et al.* (2011) conducted a pot experiment where two forage sorghum varieties (Speed feed and KFS4) were grown under salinity levels of 0, 5, 10 and 15 dSm⁻¹. Leaf area of plants were also reduced in response to salinity and

decreasing soil water availability, while the suppressive effect was magnified under the combined effect of the two factors. Salinity and water stress significantly affected the total leaf area of ratoon crop. The maximum total leaf area was obtained in the control treatment but with increasing salinity and infrequent irrigation, this parameter was found to decrease. Maximum leaf area of 1167 mm² plant⁻¹ was attained in plants with normal irrigation, without water stress. Under effects of salinity 5, 10 and 15 dSm⁻¹ the leaf area was reduced by 7, 12 and 17%, respectively.

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 26.58% and 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.

Jafari *et al.* (2009) studied the interactive effects of salinity, calcium and potassium on physio- morphological traits of sorghum *(Sorghum biclolor L.)* in a green-house experiment. Treatments included 4 levels of NaCl (0, 80, 160, and 240 mM NaCl), 2 levels of CaCh (0 and 20 mM), and 2 levels of KCl (0 and 20 mM). Salinity substantially reduced the plant growth as reflected by a decrease in the plant height, shoot and root weight.

Jampeetong and Brix (2009) and Gorai *et al.* (2010) reported that, various plant growths and development processes viz. seed germination, seedling growth, flowering and fruiting are adversely affected by salinity, resulting in reduced yield and quality.

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 plant⁴

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.

Munns and Tester (2008) observed that osmotic effect, which develops due to increasing salt concentration in the root medium, is a primary contributor in growth reduction in the initial stages of plant growth. This stage can be characterized by reduction in generation of new leaves, leaf expansion, development of lateral buds leading to fewer braches or lateral shoots formation in plants.

Memon *et al.* (2007) conducted a pot experiment on silty clay loam soil at Sindh Agriculture University, in Tando Jam, Pakistan. Sarokartuho variety of Sorghum *(Sorghum bicolor L.)* was continuously irrigated with fresh (control) and marginally to slightly saline EC 2, 3, 4 and 5 (dSm⁻¹) waters. Increasing water salinity progressively decreased plant height and fodder yield (fresh and dry weight) per plant.

Mortazainezhad *et al.* (2006) had observed that tiller number decreased with increasing salinity levels imposed at all growth stages in rice. Soil salinity affects the growth of rice plant. But the degree of deleterious effect may vary on the growth stages of plant. During germination rice is tolerant, but it becomes very sensitive during the early seedling stage. Similar result was also reported by many workers in rice (Karim, 2007).

Munns (2005); Munns and Tester (2008) reported that salt-induced osmotic stress is the major reason of growth reduction at initial stage of salt stress, while at later stages accumulation of Na+ occurs in the leaves and reduces plant growth. Parida and Das (2005) observed salt stress affects some major processes such as root/shoot dry weight and Na^+/K^+ ratio in root and shoot.

Sixto *et al.* (2005) stated that depending on increasing salinity levels, decrease in vegetative growth parameters has been observed in plants. Decrease in root, stem and shoot developments, fresh & dry stem and root weights; leaf area and number and yield have been observed in plants subject to salinity stress.

2.1.2 Effect of salinity on physiological attributes of plant

Eisa (2012) conducted an experiment where *Chenopodium quinoa* plants were grown in a hydroponic quick check system with 0, 100, 200, 300, 400, and 500 mM NaCl (equivalent to 0, 20, 40, 60, 80 and 100% seawater salinity). Higher salinity considerably reduced plant growth, with maximum reduction of 82% observed at 500 mM NaCl. The net photosynthesis rates were greatly decreased by high salinity, being 28% of initial control values at 500 mM NaCl. Salt- induced photosynthesis inhibition was accompanied with a decrease in transpiration rates but also with improved water use efficiency. Salt-induced growth reduction is presumably due to low photosynthate supply as a consequence of impaired photosynthetic capacity.

Haghighi *et al.* (2012) conducted a study to evaluate the effectiveness of salinity on seed germination and growth characteristics of tomato. The experiment was performed with two levels of salinity (25 and 50 mM NaCl) and 2 concentration of Si (1 and 2 mM) with 4 replications. The result showed that seed germination of *Lycopersicon esculentum* L. was significantly affected by salinity levels, Si and their interaction and germination characteristics of tomato decreased drastically increasing by NaCl concentrations. 1 mM Si had positive effects on seed germination characteristics and improved germination percentage, germination rate and mean germination time and Si alleviated the harmful effect of salinity stress on tomato seed germination at almost all germination characteristics. Akbarimoghaddam *et al.* (2011) evaluated salinity effects on seed germination and seedling growth of six bread wheat cultivars (*Triticum aestivum* L). They reported that water uptake by seeds have a direct relationship with increases in NaCl levels. By increasing NaCl concentration, seed germination delayed and decreased. Increasing NaCl concentrations adversely affected shoot dry weight, shoot dry weight fluctuated by varying NaCl concentration.

Bavei et al.(2011) studied the tolerance of sorghum varieties in terms of fresh weight, ion accumulations, proline content and peroxidase activity was analyzed in this study. Three sorghum varieties, Payam, Kimia, and Jambo, differing in salt tolerance, were grown in a greenhouse-hydroponic culture with a complete nutrition solution to which 0, 50, 100, 150 and 200 mM NaCl was added. Plant roots and leaves were harvested at 15 and 30 days after treatment and subjected to analysis. Clear decline in K+ and Ca²+ concentrations and increase in Na+ and proline contents were observed in the root and leaf tissues at each NaCl concentration in all varieties during the NaCl treatment.

Hamayun (2010) reported that, the adverse effects of NaCl induced salt stress on growth attributes of soybean and the result showed that Chlorophyll content was significantly decreased in response 70 mM and 140 mM concentrations of NaCl.

Patel (2010) reported that, Salinity induced a significant increase in Na+, Cl⁻ and proline concentrations, while reduced the accumulation of K^+ and Ca^{2+} in leaves of all the cultivars of cowpea.

Zuccarini (2008) studied the effect of Si on *Phaseolus vulgaris* L. under two level of salinity (30 and 60 mM). His results showed that salinity decreased stomatal conductance and net photosynthetic rate.

Memon *et al.* (2007) experimented on sarokartuho variety of sorghum that was continuously irrigated with fresh (control) and marginally to slightly saline EC 2,

3, 4 and 5 (dSm⁻¹) waters in a pot experiment.Saline water treated plants contained more Na+, less K+ and showed lower leaf K^+/Na^+ ratio.

Munns *et al.* (2006) suggested that Na+ exclusion in plants is attained by low up take of Na+ by the root cortex, controlled unloading of xylem by parenchyma in the stele. Initial step of transport of Na⁺ from soil to plant shoot is entrance of Na⁺ into root epidermis and cortex (Na⁺ influx).Numerous studies have revealed that salt stress can reduce K⁺, Ca²⁺ and N accumulation in different crop plants, e.g. wheat, sunflower (Akram *et al.*, 2007), radish, cabbage (Jamil *et al.*, 2007) and canola (Ulfat *et al.*, 2007). Salinity reduces nutrient availability as well as transport to the growing regions of the plant, thereby affecting the quality of both vegetative and reproductive organs. For example, higher concentrations of Na+ in soil decreased the Ca²+ activity in the external medium leads to limit its availability in *Celosia argentea* (Carter *et al.*, 2005).

2.1.3 Effect of salinity on yield and yield contributing parameters

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 $32.76 \text{ g plant}^{-1}$ 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.

Hamayun (2010) reported that, the adverse effects of NaCl induced salt stress on growth attributes and endogenous levels of gibberellins (GA), abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) soybean cv. Hwangkeumkong was showed. 1000 seed weight and yield significantly decreased in response 70 mM and 140 mM concentrations of NaCl.

Prakash and Chen (2010) observed that all the physiological properties and yield were negatively affected by increasing salinity levels due to less water use and radiation interception. Compared to the low salinity level, medium and high salinity levels reduced the above-ground dry weight of the crop at harvest by 40 and 41%, accumulated intercepted radiation by 23 and 37%, radiation use efficiency by 25 and 52%, water use by 18 and 35% and grain yield by 41 and 48%, respectively.

Rafat and Rafiq (2009) reported that, total chlorophyll content in tomato plant proportionally decreased with the increase in salinity levels up to 0.4% sea salt solution (EC 5.4 dSm^{-1}).

Karim (2007) conducted an experiment to investigate the effect of different salinity levels (0, 6, 9 and 12 dSm⁻¹) and reported that all parameters including panicle length decreased with increased salinity levels. Panicle length was adversely affected by soil salinity levels as reported by most of the researchers (Rana, 2007).

Karim (2007) reported that grain yield decreased with increased salinity levels. The yield was decreased due to production of decreased number of effective tillers hill⁻¹, decreased number of grains panicle⁻¹ and 1000-seed weight. Similar result was also reported by many researchers (Hossain*et al.*, 2006).

Rana (2007) carried out a pot experiment with 5 levels of salinity (0, 3, 6, 9 and 12 dS/m) of three rice varieties viz., BRR1 dhan-42, STM-1 and STM-2 and reported that plant height, number of tillers hill⁻¹, TDM hill⁻¹, leaf area hill⁻¹, root dry weight hill⁻¹ and yield contributing characters and yield decreased significantly with increase in salinity levels. Among the advanced rice lines BRRIdhan-42 showed more tolerance for all studied parameters compared to STM-1 and STM-2.

Hajer *et al.* (2006) conducted two different experiment separately on tomato under saline condition and reported the effect of NaCl salinity stress on the growth of tomato plants was reflected in lower fresh and as well as dry weights.

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.

El-Hendawy *et al.* (2005) reported that tiller number of wheat was affected more by salinity than leaf number and leaf area at the vegetative stage. Salinity decreased dry weight per plant significantly at all growth stages. Spikelet number on the main stem decreased much more with salinity than spike length, grain number and 1000-grain weight at maturity. They also concluded that an increase in tiller number per plant and spikelet number per spike will improve the salt tolerance of wheat genotypes in breeding programs.

Uddin *et al.* (2005) conducted an experiment to study salt tolerance of *B. napus* and *B. campestris* varieties under saline conditions (1.2-11.5 dSm⁻¹) and observed that siliqua number and seeds siliqua⁻¹ decreased with increased salinity.

Gain *et al.* (2004) studied the effect of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm^{-1}) on yield attributes and yield in rice and reported that number of spikelet panicle⁻¹, 1000-grain weight and dry mass decreased with increasing salinity levels but the decrement was less in salt tolerant varieties than salt susceptible varieties This statement was supported many workers (Hossain*et al.*, 2006).

Netondo *et al.* (2004) conducted an experiment to determine how salinity affects growth, water relations, and accumulation of cations of nutritional importance in various organs of grain sorghum. Two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. The 250 mM NaCl treatment significantly reduced the relative shoot

growth rates, measured 25 days after the start of salt application, by 75 and 73%, respectively, for Serena and Seredo, and stem dry weight by 75 and 53%.

2.2 Effect of Priming

Nowadays various strategies are employed to generate plants that can withstand these stresses. In recent years, seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. Seed priming is the induction of a particular physiological state in plants by the treatment of natural and synthetic compounds to the seeds before germination. In plant defense, priming is defined as a physiological process by which a plant prepares to respond to imminent abiotic stress more quickly or aggressively. Moreover, plants raised from primed seeds showed sturdy and quick cellular defense response against abiotic stresses. Priming for enhanced resistance to abiotic stress obviously is operating via various pathways involved in different metabolic processes. The seedlings emerging from primed seeds showed early and uniform germination. Moreover, the overall growth of plants is enhanced due to the seed-priming treatments. The main objective of this review is to provide an overview of various crops in which seed priming is practiced and about various seed-priming methods and its effects (Jisha, *et al.* 2013).

The effects of priming on seed germination properties have been well documented. High potential in improving field emergence and ensures early flowering and harvest under stress condition especially in dry areas and under drought stress. Patade *et al.* (2009) suggest that salt priming is an effective pregermination practice for overcoming salinity and drought induced negative effects in sugarcane. Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions. Priming led to an increased solubilization of seed storage proteins like the betasubunit of the 11S globulin and reduction in lipid peroxidation and enhanced antioxidative activity in seeds. Afzal *et al.* (2008) observed that the priming-induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na⁺ accumulation in wheat plants. Sivritepe *et al.* (2003) evaluate the effect of salt priming on salt tolerance of melon seedling and reported that total emergence and dry weight were higher in melon seedlings derived from primed seeds and they emerged earlier than non-primed seeds. They also observed that total sugar and proline accumulation and prevented toxic and nutrient deficiency effects of salinity because less Na but more K and especially Ca was accumulated in melon in melon seedlings

2.2.1 Effect of priming concentration

Seed performance under drought or salt stress is also affected by the concentration of priming materials. It has been reported that, NaCl priming generally requires longterm treatment periods using solutions with relatively high concentrations of NaCl; however, short term seed priming with a low NaCl concentration also increases germination rate, field emergence and acquired stress tolerance (Nakaune *et al.*, 2012). Sun *et al.*, (2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydropriming, while higher concentrations of PEG had negative effects on seed germination.

The concentration of the priming agent (osmotic potential) is also a critical factor. Afzal *et al.* (2007) studied the effect of halopriming with 10, 25 or 50 mM NaCl and CaCl₂ in wheat (*Triticum aestivum* cv. Auqab-2000) under saline conditions. The results showed that most priming agents were not effective in improving germination and seedling establishment under salt stress. However, primed seeds with 25 and 50 mM of CaCl significantly reduced the meangermination time and significantly increased the shoot length, and fresh and dry weight of seedlings more than all priming treatments. The shoot and root length, and fresh and dry weight of shoots and roots of seedlings obtained from primed seeds were increased significantly compared to unprimed seeds. This improvement as affected by priming is supported by the findings of Basra *et al.* (2005) and Iqbal and Ashraf (2007) with wheat. Afzal *et al.* (2008) reported that the growth of wheat increased significantly by using 50 mM CaCl₂ as seed priming treatment in saline medium. Moreover, Rafiq *et al.* (2006) reported that priming with CaC₂ significantly enhanced shoot and root length under both saline and non-saline conditions.

2.2.2 Effect of priming duration

Soaking period has been reported to be one of the key factors of determining the effectiveness of priming (Dezfuli et al., 2008). Yari et al. (2010) studied the effect of priming duration on germination and early growth of two wheat cultivars (Azar-2 and Sardari 101). Seeds were primed for 12, 24 and 36 h in several priming agents (PEG 10%, PEG 20%, KCl 2%, KCl 4%, KH₂PO₄ 0.5%, KH PO 1% and distilled water). The results showed that the greatestgermination percentage was recorded in cv. Azar-2 when the seed were primed with PEG 20% for 12 h and the greatest stem length was obtained with seeds primed with PEG 10% for 24 h. Moreover, Yari et al. (2012) tested the effect of soaking three wheat cultivars (Fajer, Sherodi and Taram) for 12, 24 and 36 h in 0.5 and 1% CaCl₂ priming solutions. They concluded that soaking for 24 hwas suitable for all three cultivars in terms of increasing the germination percentage. Many studies concluded that the duration of priming is different amoung the species and cultivars, for example, Rashid et al. (2006) reported that the best priming duration for soaking barley seeds was between 12 and 16h Furthermore, the best duration for maize and rice was 18 h (Harris et al., 1999).

Rashid *et al.* (2006) reported that hydropriming increased the germination rate, enhanced yields and provided good establishment of barley under saline and non-saline soils.

Golezani *et al.* (2008) claimed that hydropriming can enhance seedling emergence rate, emergence percentage and seedling establishment of lentil (*Lens culinaris* Medik.) as well as increase the length of roots. Hydropriming by soaking in water for 12, 24, 36 or 48 h improved seed germination and increased maximum length of the radicle of two genotypes of maize (Zea *mays* L.) including B73 and MO17 (Dezfuli *et al.* 2008). Numerous research efforts have concluded that treating crop seeds in water prior to sowing can enhance the resistance of crops to salinity (Zheng *et al.* 2002). By soaking maize in water, the resistance of seeds to salinity has been improved and gave better germination percentage compared with the control (Ashraf and Rauf, 2001). Afzal *et al.* (2007) studied the effects of several priming treatments (hydropriming, matriconditioning, chilling, osmopriming and hardening) on wheat (*Triticum aestivum* L.). They found that the maximum emergence percentage (61%) was obtained from hydropriming seeds followed by 24 h chilling. Also the maximum shoot length (18 cm) and root length (28 cm) were recorded by hydropriming seeds followed by 24 h of chilling.

2.2.3Effect 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 *et al.* (2014) conducted an experiment to evaluate the effects of NaCl priming on growth traits and some biochemical attributes of safflower (*Carthamus tinctorius* L. cv Safola) in salinity conditions. Seeds of safflower were primed with NaCl (5 g L⁻¹) for 12 h in 23 °C. Primed (P) and non primed (NP) seeds were directly sown in the field. Experiments were conducted using various water concentrations induced by NaCl (0, 3, 6, 9 and 12 g L-1) in salinity experiment. They found that growth (plant height, fresh and dry weight) and biochemical (chlorophyll, proline and proteins content) of plants derived from primed seeds were greater of about 15 to 30% than that of plants derived from non primed seeds.

Saleem *et al.* (2014) set an experiment to study the effect of seed soaking on seed germination and growth of bitter gourd cultivars. Three cultivars of bitter gourd Faisalabad Long, Jaunpuri and Palee were soaked in water for various soaking durations (4, 8, 12 and 16 hours) along with control to determine the optimal soaking duration and find out the best growing cultivar. The highest germination percentage (85.18%), number of branches plant-1 (8.64), fruits plant⁻¹ (20.70) were obtained when the bitter gourd seeds soaked for 12 hours. Earlier emergence (6.28) and earlier flowering (39.40) were recorded in plants where seeds soaked for 16 hours. Seed soaking in water for 12 hours has the potential to improve germination, seedling growth of bitter gourd cultivars.

Mehta *et al.* (2014) reported that presowing seed priming helps to improvegermination and stand establishment. Seeds of bitter gourd cultivar Solan Harawere hydro-primed at 20 C between wet germination papers for different durations keeping unprimed seeds as control. The plateau phase (Phase-II) with little change in water content from 53.3 to 57.3% (after 24 hours to 72 hours of seed priming) found as seed priming regime for bitter gourd. Significantly higher speed of germination, total% germination, seedling length, seedling dry weight, vigour index-I and II were recorded in hydro-priming for 72 hours as compared to other durations and control. Based on seed priming regime i.e. phase-II of seed germination and performance with respect to seed quality parameters it was found that 72 hours of seed priming is optimum in bitter gourd.

Abdoli (2014) set an experiment to evaluate the effects of seed priming on certain important seedling characteristic and seed vigor of fennel (*Foeniculum vulgare L.*) at Department of Agronomy and Plant Breading,Faculty of Agriculture, Maragheh University in Maragheh state, Iran.Treatment included untreated seeds (control) and those primed in water(H₂O),sodium chloride(NaCl, 100 mM) and polyethylene glycol 6000 (PEG-6000,water potential-1.6MPa), in darkness for 18 hrs.Among them unsoaked seed (control) and hydropriming treatments had the lowest plumule, radicle and seedling length, seedling dry weight and seedling vigor index. PEG and NaCl in all of traits were better than the water priming treatments, respectively. PEG-6000 (1.6 MPa) is the best treatment for breaking of fennel seed dormancy.

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

seeds treated in the first stepand water, 100 ppm KCl, 0.5% CaCl₂.2H₂O, 50 ppm KH₂PO₄ and 20 ppm GA₃ were used to treat seeds for 14 hours. They found that Primary seed priming had no significant effect on none of the measured traits but complementary seed priming significantly affected plant dry matter, grain yield, 100 grain weight and the number of pods. The highest plant dry matter (53.06 g) and the highest grain yield (5.98 t/ha) were achieved when seeds were first treated with water (as the primary seed priming) and after drying were treated with GA3 (as the complementary seed priming).

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

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

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 Ca^{2+}) decreased, but to a less degree in primed seeds. At different salinity levels, primed seeds possessed higher emergence and growth rate than control.

Dastanpoor *et al.* (2013) carried out an experiment to find out the influence of hydro priming treatments on seed parameters of *Salvia officinalis* L. (sage). Seeds of sage were treated by hydro priming at three temperatures 10, 20, 30°C for 0, 12, 24 and 48 h. Hydro priming clearly improved the final germination percentage (FGP), mean germination time (MGT) and synchronized the germination of seeds at each three temperature. All the treatments resulted in germination enhancement except hydro primed seeds for 48 h at temperature 30°C. Hydro priming (12 h at 30°C) was most effective in improving seed germination that FGP was increased by 25.5% as compared to that of non-primed seeds.

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

Ogbuehi *et al.* (2013) carried out a field experiment in 2012 at Teaching and Research Farm of faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri to assess the effect of hydro priming duration on performance of morphological indices of Bambara groundnut (*Vigna subterranean* (L.) Verdc). The treatments were 12hrs, 24 hrs, 36 hrs, 48 hrs and 0 hrs which served as control (untreated seeds). Among the treatments 24hours hydro priming duration found to improve the performance of growth indices measured whereas the 36 hours was the least effective. Ali *et al.* (2013) reported that seed priming improves irrigation water use efficiency, yield, and yield components of late-sown wheat under limited water condition. Seed priming treatments reduced the mean emergence time and promoted germination, early canopy development, and tillering in comparison to the untreated control. The number of fertile tillers, plant height, 1000-grain weight, and grain and biological yield were also increased by different priming techniques. On-farm priming and hydropriming for 12 h gave higher grain and biological yields and higher harvest index than other priming treatments. Seed priming increased the irrigation water use efficiency (IWUE) of all irrigation regimes. Grain yields were linearly increased at 100% ETo while maximum IWUE was achieved at 80% ETo.

Amoghein *et al.* (2013) conducted an experiment on the effect of osmopriming and hydropriming on the different index of germination & early growth of wheat under salty stress. They reported that the simple effect of priming for all the characteristics under study, except of shoot dry weight and simple effect of salinity for all the characteristics under study in the experiment at 1% level was significantly simple effect of seed soaking time (4 hours) only on hypocotyle length was significantly. Interaction of salinity on seed priming for root dry weight, longest root on the 5% level showed a statistical significant difference. Also shoot dry weight had a positive and significant correlation with the first and second leaf length, root number and root longest at the %1 level.

2.2.4 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 onNaCl injury during rice germination. Kinetin treatment increased germination except in cv. Dasanbyeo. However, some studies showed that ABA also accelerated germination.

Germination of *Lulium multiflorum* seeds under irrigation with saline water at concentrations of 2000, 4000 and 6000 mgL⁻¹ and presoaking in indloleacetic acid (IAA) (10 and 20 mgL⁻¹) and gebberellic acid (GA3) (100 and 300 mgL⁻¹) in addition to fresh water (control) were studied (El-Din. 1998). Germination reached 100% after 7 days from the presoaked grains in IAA (10 mgL⁻¹) and after 5 days from the presoaked grains in GA3 (300 mgL⁻¹). In pot trials, *Medicago varia* seeds soaked in 0-50 mgL⁻¹ BA were watered with 0-50% NaCl solution and *Medicago varia* seeds soaked in 0-20 mgL⁻¹ kinetin were watered with 0-3% NaCl solution (Kachel, 1993). BA slightly reduced germination of *Medicago varia* under non-saline conditions, but increased it from 55.33% to 62.67-73.67% when 5% NaCl was applied and reduced the inhibition of seedling growth caused by NaCl, *Medicago varia* failed to germinate when 1-3% NaCl was applied without growth regulators. Soaking in 0 or 10.0 mgL⁻¹ kinetin increased germination to 21.33-30.33% at 1% NaCl but had little or no effect at higher salinity levels.

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. Khan and Ungar (1985) determined the germination of

desert forb (*Zygophyllum simplex* L.) under various salinity, proline, betaine, GA and Kinetin treatments. Gebberellic acid (0.3 and 3 mM) and kinetin (0.05 and 0.5 mM) substantially alleviated both innate as well as salinity induced seed dormancy. At higher salinity (125 mM), low concentrations of kinetin (0.05 mM) and high concentrations of GA (3mM) were more effective. GA3 completely alleviated the effect of salinity at all concentrations used. Similarly, Rizvi *et al.* (2001) concluded that priming of *Hordeum vulgare* seeds in aqueous solutions (10⁻⁶*M*) of gibberellic acid and /or kinetin for 6, 12 and 24 h improved percent germination of the grains irrespective of the hormone and the soaking duration. However, the best response was generated in the grains soaked in gibberellic acid for 12h where germination increased by 156% over control.

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted during the period from October 2016 to December 2016. The materials and methods those were used and methods followed for conducting the experiment have been 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. It was located in 24.09° N latitude and 90.26° E longitudes.

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 13.2°C to 22.4°C, respectively and average minimum and maximum relative humidity was 56% and 84%, respectively.

3.4 Test crops

Four wheat genotypes namely- ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom28 were used for this experiment. Seeds were collected from Wheat Research Centre,Nashipur,dinajpurand 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 disease and injury.

3.5 Experimental materials

Different equipments such as 4-digit electric balance, Petri dish, filter paper, micro pipette, forcep, oven etc.were used for this study.

3.6 Chemicals for seed priming

Different priming chemicals such as PEG, salt (NaCl) and distilled water were utilized for osmo and hydro priming.

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

- Six levels of priming agent concentrationsviz. water, 5%, 10%, 15%, 20% and 0% (control) PEG 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 ds⁻¹NaCl

3.9 Steps of the experiment

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

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

3.9.1.1 Treatments: One factor experiment considering four wheat genotypes with six levels of seeds priming (distilled water, 5% PEG, 10% PEG, 15% PEG, 20% PEG and control) for 12 hours was done.

Four wheat genotypes; one wheat variety and three lineswere as follows:

- i) V₁ = ESWYT 5
 ii) V₂ = ESWYT 6
- iii) $V_3 = ESWYT7$
- iv) $V_4 = BARI \text{ gom}28$

3.9.1.2Priming solutions

0%, 5%, 10%, 15%, 20% PEG solution and distilled water were used as priming solutions.

3.9.1.3Preparation of priming solutions

a) PEG solutions (5%, 10%, 15%, 20%)

5 g of PEG was dissolved in 100 ml of distilled water to prepare 5% solution of PEG. Similarly, 10g, 15g, 20g PEG was dissolved in 100 ml of distilled water to prepare 10%, 15%, 20% solution of PEG, 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.*, osmopriming and hydropriming 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 osmopriming seeds of another sub-sample were divided into another sub-samples and pretreated with PEG a four levels of concentration of 5%, 10%, 15% and 20% for 12 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.

Achievement from the first experiment: From the first experiment, 10 ppm PEG solution gave the best result. So, 10% PEG solution was used for the next experiment to evaluate best priming time.

3.9.2 Second experiment: Optimization of pre-sowing priming time on the germination, seedling growth and water relation behavior of wheat

3.9.2.1 Treatments: One factor experiment considering four wheat genotypes with five levels of seeds priming time (3, 6, 9, 12 and 15 hours) by 10% PEG solution was done.

Four wheat genotypes; one wheat variety and three lines were as follows:

i) V₁ = ESWYT 6
ii) V₂ = ESWYT 5
iii) V₃ = ESWYT 7
iv) V₄ = BARI gom28

3.9.2.2 Priming solutions

10 % PEG solution was used for osmopriming.

3.9.2.3 Priming technique

For osmopriming the sample of seeds were divided into five sub-sample and pretreated with PEG for 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, 10 ppm PEG solution with 9 hours priming time gave the best result. So, 10% PEG solution with 9 hrs 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.

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

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

i) V₁ = ESWYT5
 ii) V₂ = ESWYT6
 iii) V₃ = BARI gom28

3.9.3.2Priming solutions and time

10 % PEG solution and 9 hours priming time were used to test salt stress.

3.9.3.3 Preparation of stress solutions - Salt (Nacl) solutions (5dsm⁻¹, 10dsm⁻¹, 15dsm⁻¹ and 20dsm⁻¹):

0.731 g of sodium chloride (Nacl) was dissolved in 250 ml of distilled water to prepare 5dsm⁻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 10dsm⁻¹, 15dsm⁻¹, 20dsm⁻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 PEG for osmopriming at a concentration of 10% for 9 hours, respectively. Priming is 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 weeks after placing of seeds. Seedling mortality was also counted upto 10 days after seed placing. The uprooted seedlings were washed with tap water and excess water was soaked with tissue paper.

The following data were taken:

- 1. Germination rate (%)
- 2. Shoot length (mm)
- 3. Root length (mm)
- 4. Shoot dry weight (mg)
- 5. Root dry weight (mg)
- 6. Relative water content (%)
- 7. Water saturation deficit(%)
- 8. Water retention capacity
- 9. Vigour 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 hrs interval and continued up to 10th. More than 2 mm long plumule and radicle was considered as germinated seed.

The germination rate was calculated using following formula:

Rate of germination (%) = $\frac{TotalNumberofgerminatedseeds}{Totalseedplacedforgermination} \times 100$

3.11.2 Shoot length

The shoot length of five seedlings from each Petridish was measured finally at 11 DAS. Measurement was done using the unit millimeter (mm) by a meter scale.

3.11.3 Root length

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

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 (gm).

3.11.5 Relative water content (%)

Relative water content was measured using following formula

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

3.11.6 Water saturation deficit

Water saturation deficit was recorded using following formula:

Water saturation deficit (WSD) = 100- Relative water content

3.11.7 Water retention capacity

Water retention capacity was measured following formula

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

3.11.8 Vigour index

Vigour index was calculated using following formula

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

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 program"MSTAT-C" and mean difference among the treatments were adjudged withLeast Significant Difference (LSD) as described by Gomez and Gomez (1984).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprises presentation and discussion of the results obtained from a study to investigate Polyethylene glycol (PEG) induces changes in growth and physiology of wheat (*Triticum aestivum*. *L*) under salt stress. The results of the germination and growth parameters of wheat genotypes as influenced by different concentrations of priming agent (PEG) and priming time in salt stress condition have been presented and discussed in this chapter.

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

Results obtained from the present study regarding the effects of different concentrations of PEG the germination rate of wheat varieties have been presented, discussed and compared in this chapter. The analytical results have been presented in Figure 1 to 9 and Appendices II to X.

4.1.1 Rate of germination

Significant variation was found in terms of germination rate due to varietal performance and seed priming with water and PEG at different concentration and also without priming solution (Fig. 1 and Appendix II). Results indicated that the genotype, V_1 (ESWYT 5) showed the highest germination rate at all seed priming concentration including control which was closely followed by V_2 (ESWYT 6) where genotype V_3 (ESWYT 7) showed the lowest germination rate at all seed priming concentration followed by V_4 (BARI gom28). Among all the test sample, the highest germination rate (95.30%) was found from V_1 (ESWYT 5) primed with 10% PEG solution for 12 hours which was statistically similar with V_2 (ESWYT 6) primed with 10% PEG solution for 12 hours.

The lowest germination rate (65.90%) was observed from V₃ (ESWYT 7) with without primingtreatment followed by Seeds primed with 20% PEG solution for 12 hours, 15% PEG solution for 12 hours and with distilled water for 12 hours with the same variety. These findings are consistent of the results of Mohseni*et al.* (2010) where they observed the highest germination percentage with water, and the least germination percentage with 4% KCL and 2% KNO₃. Sun *et al.*, (2010) also concluded that PEG priming with moderate concentration resulted in higher germination rate than hydro-priming, while higher concentrations of PEG had negative effects on seed germination. Ajirlo *et al.* (2013) reported that germination improves by seed priming technique. Ali *et al.* (2013) reported that seed priming treatments reduced the mean emergence time and promoted germination.

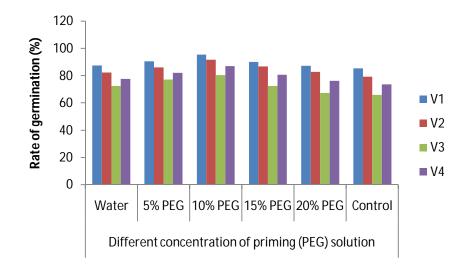


Fig. 1 Effect of different concentrations of priming solution with different wheat genotypes on germination rate of primed (PEG and water) and non-primed (control) seeds (LSD_{0.01} = 6.55, 6.83, 6.20, 7.21, 6.26, 7.91 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.2 Shoot length

Significant variation was observed on shoot length among the test genotypes priming with water and different concentration of PEG including control treatment (Fig. 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_3 (ESWYT7) showed the lowest shoot length. It was also observed that the maximum shoot length (168.20 mm) was recorded for V_1 (ESWYT 5) primed with 10% PEG solution for 12 hours followed by V₂ (ESWYT 6) with 10% PEG solution for 12 hours (155mm). The lowest shoot length (108.20 mm) was observed from V_3 (ESWYT 7) without primingfollowed by V_4 (BARI gom28) genotype (117.6 mm) without priming. Treated seeds of priming can changes enzyme concentration and formation which reduces lag time between imbibition and radicle emergence (Bradford et al., 1990). Earlier and faster synthesis of DNA, RNA and proteins are also observed which enhanced growth (Bray et al., 1989). Gray and Steckel (1983) also 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. Rafiq et al. (2006) reported that priming with CaCl₂ significantly enhanced shoot length under both saline and non-saline conditions.

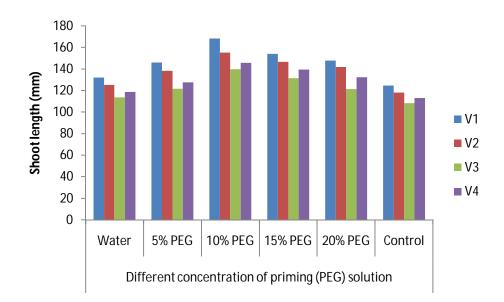


Fig. 2 Effect of different concentrations of priming solution on shoot length of primed (PEG and water) and non-primed (control) seeds (LSD_{0.01} = 8.96, 12.25, 13.21, 12.56, 13.51, 10.54 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.3 Root length

Significant variation was observed on root length among the test genotypes priming with water and different concentration of PEG (Fig. 3 and Appendix IV). It was found that the genotype, V_1 (ESWYT 5) showed the highest root length in all seed priming concentration where genotype V_3 (ESWYT 7) showed the lowest root length. It was also observed that the maximum root length (158.30 mm) was recorded for V_1 (ESWYT 5) primed with 10% PEG solution for 12 hoursfollowed by Seeds primed with 15% PEG solution for 12 hours with the same genotype. The lowest root length (98.46 mm) was observed from V_3 (ESWYT 7) without primingfollowed by V_4 (BARI gom28) genotype without priming. The root length of seedlings obtained from primed seeds was increased significantly compared to unprimed seeds. This improvement as affected by priming is supported by the findings of Basra *et al.* (2005) and Iqbal and Ashraf (2007) with wheat. Rafiq *et* *al.*(2006) reported that priming with $CaCl_2$ significantly enhanced root length under both saline and non-saline conditions.

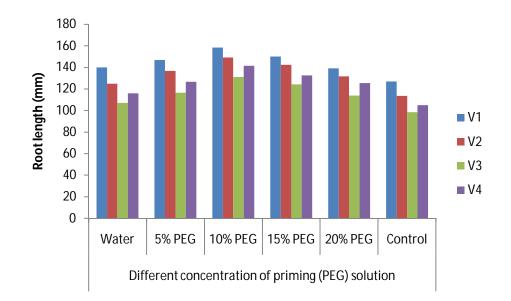


Fig. 3 Effect of different concentrations of priming solution on root length of primed (PEG and water) and non-primed (control) seeds ($LSD_{0.01} = 13.08$, 11.19, 13.28, 11.12, 11.27, 10.5 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.4 Shoot dry weight

Statistically significant variation was found in case of shoot dry weight of different genotypes of wheat due to priming with water and different PEG concentrations including control treatment (Fig. 4 and Appendix V). Dry weight of shoot increased with Seeds primed with 5% PEG solution for 12 hours, 10% PEG solution for 12 hours and 15% PEG solution for 12 hours for all the genotypes of wheat and therefore shoot dry weight decreased with the increasing PEG concentration and with water priming and also with control (without priming) treatment. Results revealed that the highest shoot dry weight (0.0522 mg) was recorded in V₁ (ESWYT 5)primed with 10% PEG solution for 12 hours treatment followed by V₂ (ESWYT 6) primed with 15% PEG solution for 12 hours. The lowest shoot dry weight (0.0212 mg) was observed from V₃ (ESWYT 7) without priming. These results were in agreement with those of Pill and Necker (2001) who reported that primed compared to non-primed plants resulted in greater seedling dry weights. Basra et al. (2005) and Iqbal and Ashraf (2007) also observed primed seeds gave increased shoot dry weight significantly compared to unprimed seeds in wheat.

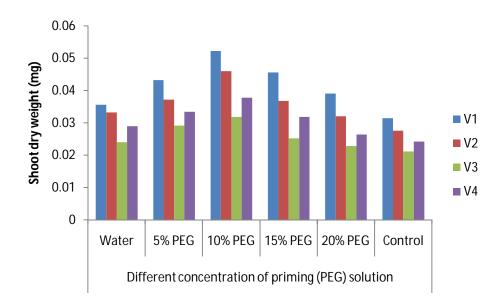


Fig. 4 Effect of different concentrations of priming solution on shoot dry weight of primed (PEG and water) and non-primed (control) seeds ($LSD_{0.01} = 0.01$, 0.01, 0.01, 0.01, 0.01, 0.01 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.5 Root dry weight

Statistically significant variation was found in case of root dry weight of different genotypes of wheat due to priming with water and different PEG concentrations including control (Fig. 5 and Appendix VI). Results revealed that the highest root dry weight (0.0432 mg) was recorded in V₁ (ESWYT 5)primed with 10% PEG solution for 12 hours followed by Seeds primed with 15% PEG solution for 12 hours with the same genotype. The lowest root dry weight (0.015 mg) was observed from V₃ (ESWYT 7) without priming. The root dry weight of seedlings obtained from primed seeds was increased significantly compared to unprimed seeds and this improvement as affected by priming is supported by the findings of Basra *et al.* (2005) and Iqbal and Ashraf (2007) in wheat.

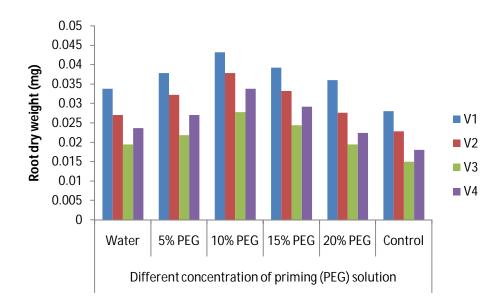


Fig. 5 Effect of different concentrations of priming solution on root dry weight of primed (PEG and water) and non-primed (control) seeds ($LSD_{0.01} = 0.01$, 0.01, 0.01, 0.01, 0.01, 0.01 respectively)

4.1.6 Relative water content

Relative water content of different genotypes of wheat showed statistically significant variation due to different concentrations of PEG solutions and water priming including control (Fig. 6 and Appendix VII). Among the different genotypes, V_1 (ESWYT 5) gave the best relative water content at all priming treatments where V_3 (ESWYT 7) gave the lowest relative water content with all priming treatments. Results revealed that the highest relative water content (95.05%) was recorded in V_1 (ESWYT 5) primed with 10% PEG solution for 12 hours followed by Seeds primed with 5% PEG solution for 12 hours with the same genotype where the lowest relative water content (61.63%) was observed from V_3 (ESWYT 7) without priming followed by Seeds primed with distilled water for 12 hours) with the same genotype.Hajer *et al.* (2006) found similar finding and they observed that relative water content (% RWC) decreased with the decrease in osmotic potential of PEG 6000 and NaCl.

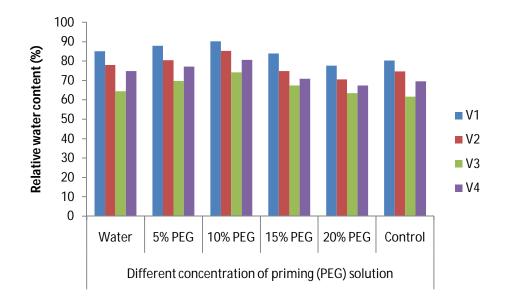


Fig. 6 Effect of different concentrations of priming solution on relative water content of primed (PEG and water) and non-primed (control) seeds $(LSD_{0.01} = 6.46, 6.31, 6.36, 4.23, 5.89, 6.69 respectively)$

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.7 Water saturation deficit

Different genotypes of wheat showed statistically significant variation on water saturation deficit due to different concentrations of PEG solutions and water priming including control (Fig. 7 and Appendix VIII). Among the different genotypes, V_1 (ESWYT 5) confirmed the lowest water saturation deficit at all priming treatments where V_3 (ESWYT 7) showed the highest water saturation deficit with all priming treatments. Results revealed that the lowest water saturation deficit (9.95%) was recorded in V_1 (ESWYT 5) primed with 10% PEG solution for 12 hours followed by Seeds primed with 5% PEG solution for 12

hourswith the same genotype where the highest water saturation deficit (38.37%) was observed from V_3 (ESWYT 7) without priming followed by Seeds primed with 20% PEG solution for 12 hourswith the same genotype.Ali *et al.* (2013) reported that seed priming improves irrigation water use efficiency resulted lower water saturation deficit.

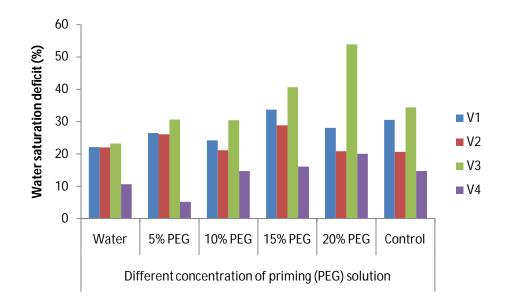
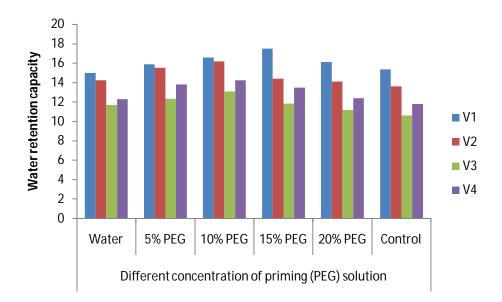


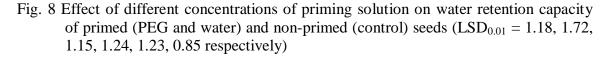
Fig. 7 Effect of different concentrations of priming solution on water saturation deficit of primed (PEG and water) and non-primed (control) seeds (LSD_{0.01} = 1.90, 2.11, 1.59, 2.73, 3.13, 2.04 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.8 Water retention capacity

Water retention capacity of different genotypes of wheat was significantly influenced by different priming solution of PEG and water priming including control (Fig. 8 and Appendix IX). Among the different genotypes, V₁ (ESWYT 5) gave the best performance on water retention capacity with all priming treatments where V₃ (ESWYT 7) gave the lowest performance on water retention capacity with all priming treatments. Results revealed that the highest water retention capacity (16.18) was recorded in V₁ (ESWYT 5)primed with 10% PEG solution for 12 hours where the lowest water retention capacity (8.98) was observed from V₃ (ESWYT 7) without priming treatment. Ali *et al.* (2013) reported that seed priming improves irrigation water use efficiency which helps to increase higher water retention capacity.





 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.1.9 Vigour index

Significant influence was found in terms of vigour index of different wheat genotypes due to different priming solution of PEG and water priming (Fig. 9 and Appendix X). Among the different genotypes, V_1 (ESWYT 5) gave the best performance on vigour index with all priming treatments where V_3 (ESWYT 7) gave the lowest performance on vigour index with all priming treatments. Results revealed that the highest vigour index (311.30) was recorded in V_1 (ESWYT 5)primed with 10% PEG solution for 12 hours where the lowest water vigour index (136.20) was observed from V_3 (ESWYT 7) without priming. Similar findings were also found by several authors. Afzal et al. (2008) observed that the priming-induced salt tolerance was associated with improved seedling vigor in wheat. Abdoli (2014) observed that unsoaked seed (control) and hydropriming treatments had the lowest vigor index where PEG and NaCl in all of traits were better than the water priming treatments, respectively. They also observed that PEG-6000 (1.6 MPa) is the best treatment for breaking seed dormancy. Ajirlo et al. (2013) reported that germination improves by seed priming technique. They found that all the priming treatments significantly affect the vigor index, time to start emergence and time to 50% emergence of forage maize.

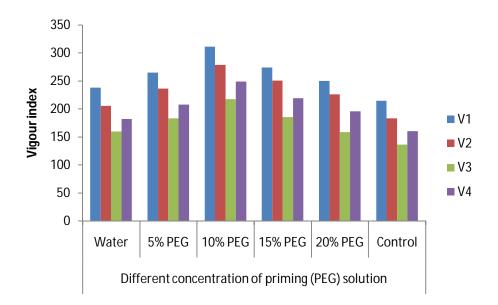


Fig. 9 Effect of different concentrations of priming solution on vigour index of primed (PEG and water) and non-primed (control) seeds ($LSD_{0.01} = 18.37$, 25.47, 17.28, 17.09, 16.09, 16.85 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.2 Second experiment: Optimization of pre-sowing 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 PEG 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 10 to 18 and Appendices XI to XX.

4.2.1 Rate of germination

The rate of germination of different wheat genotypes was significantly influenced by priming time with PEG (Fig.10 and Appendix XI). Among the genotypes, V_1 (ESWYT 5) showed the best performance in terms of germination rate where V_3 (ESWYT 7) showed the lowest germination rate at all priming time with PEG. It was also observed that all genotypes gave their best performance on germination rate under seeds primed with 10% PEG solution for 9 hours. Results revealed that, V_1 (ESWYT 5) gave the highest germination rate (83.33%) from 9 hours priming time with 10% PEG solution. The lowest germination rate (5.49%) was obtained by V_3 (ESWYT 7) genotype from 3 hours priming time with 10% PEG solution. The probable reason for enhancement of percentage and uniformity of germination of the PEG primed seed may be due to the completion of pregermination process such as repair and synthesis of nucleic acids (DNA and mRNA), protein, repair of membranes (Jowkar et al., 2012) and induction of a range of biochemical changes enzymes activation (Wattanakulpakin et al., 2012). Yari et al. (2010) observed thehighest germination percentage in wheat seeds when treated with PEG during 12 hours of priming.

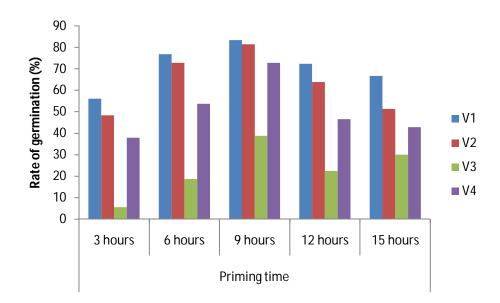


Fig.10 Effect of different priming time on germination rate of PEG primed seeds $(LSD_{0.01} = 3.28, 3.77, 7.04, 2.81, 4.78 \text{ respectively})$

4.2.2 Shoot length

The shoot length of different wheat genotypes was significantly influenced by priming time with PEG (Fig.11 and Appendix XII). Among the genotypes, V₁ (ESWYT 5) showed the best performance in terms of shoot length where V₃ (ESWYT 7) showed the lowest shoot length at all priming time with PEG. It was also observed that all genotypes gave their best performance on shoot length under Seeds primed with 10% PEG solution for 9 hours. Results revealed that the genotype, V₁ (ESWYT 5) gave the highest shoot length (181.1 mm) from 9 hours priming time with 10% PEG solution. The lowest shoot length (58.66mm) was obtained by V₃ (ESWYT 7) genotype from 3 hours priming time with 10% PEG solution. WunGuangand Khamiya (2009) reported that when pelleted seeds of tobacco were germinated under 25°C, the priming treatment of 100 mg/L GA3 under 25°C and 20°C for 36 hours were better than other treatments. Zareh *et al.*

(2006) indicated that priming of wheat seed with GA3 decreased germination rate but has a positive effect on shoot growth. Afzal *et al.* (2007) found that the maximum shoot length (18 cm) (28 cm) were recorded by hydropriming seeds followed by 24h of chilling.Elkoca *et al.* (2007) recodsm-1ended that hydropriming for 12 h or osmopriming (PEG -0.5 MPa) for 24 h for a better germination of chickpeas under cold soil conditions.

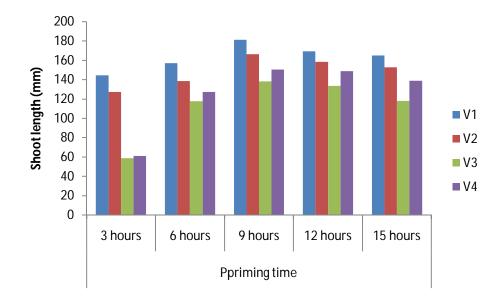


Fig.11 Effect of different priming time on shoot length of PEG primed seeds $(LSD_{0.01} = 10.18, 14.34, 14.90, 11.41, 14.12$ respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.2.3 Root length

Priming time with PEG showed significant influence on root length of different wheat genotypes (Fig.12 and Appendix XIII). It was observed that at all priming time treatment V_1 (ESWYT 5) gave the best performance on root length where V_3 (ESWYT 7) showed the lowest feat on root length. It was also observed that all

genotypes gave their best performance on root length under Seeds primed with 10% PEG solution for 9 hours. Among all the treatments combinations, the highest root length (145.0 mm) was found fromV₁ (ESWYT 5)treated by 9 hours priming time with 10% PEG solution. The genotype, V₃ (ESWYT 7) gave the lowest root length (42.30 dsm⁻¹) from Seeds primed with 10% PEG solution for 3 hours. The results of the present study are in agreement with observations of Yari *et al.* (2010) who reported that maximum radicle length of cultivar Sardari was obtained at 20% PEG-6000 solution primed for 24h. Afzal *et al.* (2007) found that the maximum root length (28 cm) were recorded by hydropriming seeds followed by 24 h of chilling.

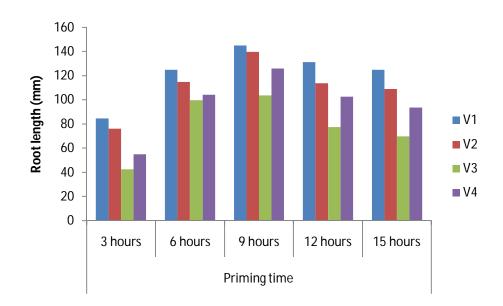


Fig.12 Effect of different priming time with on root length of PEG primed seeds $(LSD_{0.01} = 5.64, 10.45, 10.19, 10.90, 5.29 \text{ respectively})$

4.2.4 Shoot dry weight

Priming time with PEG showed significant influence on shoot dry weight of different wheat genotypes at 3, 6, 9, 12 and 15 hours priming time on shoot dry weight among the genotypes (Fig.13 and Appendix XIV). It was observed that at all priming time treatment, V_1 (ESWYT 5) gave the best performance on shoot dry weight where V_3 (ESWYT 7) showed the lowest results. It was also observed that all genotypes gave their best performance on shoot dry weight under Seeds primed with 10% PEG solution for 9 hours. The highest shoot dry weight (0.0536 mg) was found from V_1 (ESWYT 5) treated with 10% PEG solution for 9 hours where the genotype, V_3 (ESWYT 7) gave the lowest shoot dry weight (0.005 mg) under Seeds primed with 10% PEG solution for 3 hours. This result obtained from the present study was not supported by Moradi-Dezfuli *et al.* (2008) who indicated that PEG6000 soaked seeds did not act well from germination point of view, possibly due to low osmotic potential of the solution or long priming duration. Sharifzadeh *et al.* (2006) also found that osmopriming of wheat had no positive significant effect on germination characteristics.

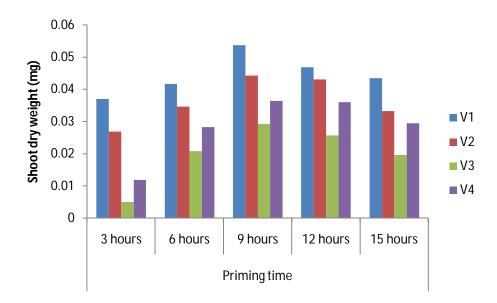


Fig.13 Effect of different priming time on shoot dry weight of PEG primed seeds $(LSD_{0.01} = 0.01, 0.01, 0.01, 0.01, 0.01respectively)$

4.2.5 Root dry weight

Priming time with PEG showed significant influence on root dry weight of different wheat genotypes at all priming time level with PEG (Fig.14 and Appendix XV). It was observed that among all priming time treatment, V_1 (ESWYT 5) gave the best performance on root dry weight where V_3 (ESWYT 7) showed the lowest results. It was also observed that all genotypes gave their best performance on root dry weight under priming treatment with 10% PEG solution for 9 hours. The highest root dry weight (0.0432 mg) was found from V_1 (ESWYT 5)under Seeds primed with 10% PEG solution for 9 hours where the genotype, V_3 (ESWYT 7) gave the lowest root dry weight (0.0044 mg) under Seeds primed with 10% PEG solution for 3 hours.

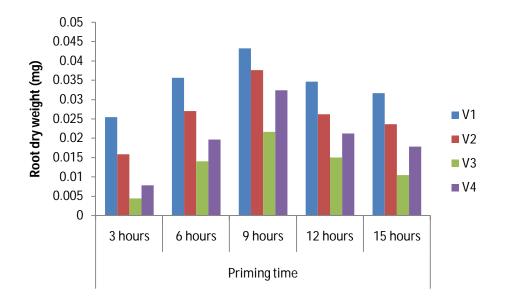


Fig.14 Effect of different priming time on root dry weight of PEG primed seeds $(LSD_{0.01} = 0.01, 0.01, 0.01, 0.01, 0.01respectively)$

4.2.6 Relative water content

The relative water content of different wheat genotypes was significantly influenced by priming time with PEG (Fig.15 and Appendix XVI). Among the genotypes, V_1 (ESWYT 5) showed the best performance in terms of relative water content where V_3 (ESWYT 7) showed the lowest relative water content at all priming time with PEG. It was also observed that all genotypes gave their best performance on relative water content under Seeds primed with 10% PEG solution for 9 hours. Results revealed that the genotype, V_1 (ESWYT 5) gave the highest relative water content (91.45%) from 9 hours priming time with 10% PEG solution. The lowest relative water content (30.00%) was obtained by V_3 (ESWYT 7) genotype from 3 hours priming time with 10% PEG solution.

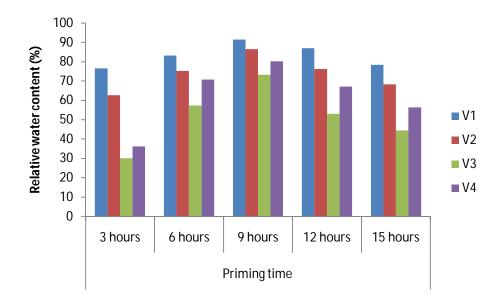


Fig.15 Effect of different priming time on relative water content of PEG primed seeds (LSD_{0.01} = 5.06, 7.52, 9.61, 4.31, 6.57 respectively)

4.2.7 Water saturation deficit

The water saturation deficit of different wheat genotypes was significantly influenced by priming time with PEG (Fig.16 and Appendix XVII). Among the genotypes, V_1 (ESWYT 5) showed the lowest performance on water saturation deficit where V_3 (ESWYT 7) showed the highest water saturation deficit at all priming time with PEG. It was also observed that all genotypes showed lowest value on water saturation deficit under Seeds primed with 10% PEG solution for 9 hours. Results revealed that, V_1 (ESWYT 5) gave the lowest water saturation deficit (8.55%) from 9 hours priming time with 10% PEG solution. The highest water saturation deficit (70.00%) was obtained by V_3 (ESWYT 7) genotype under Seeds primed with 10% PEG solution for 3 hours.

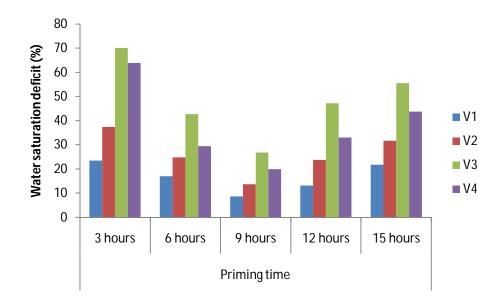


Fig.16 Effect of different priming time on water saturation deficit of PEG primed seeds (LSD_{0.01} = 3.29, 1.67, 1.20, 2.30, 2.63 respectively)

4.2.8 Water retention capacity

Priming time with PEG showed significant influence on water retention capacity of different wheat genotypes at all priming time with PEG (Fig.17 and Appendix XVIII). It was observed that at all priming time treatment, V_1 (ESWYT 5) gave the best performance on water retention capacity where V_3 (ESWYT 7) showed the lowest results under all priming time levels. It was also observed that all genotypes gave their best performance water retention capacity under Seeds primed with 10% PEG solution for 9 hours. The highest water retention capacity (19.94) was found from V_1 (ESWYT 5)under Seeds primed with 10% PEG solution for 9 hours where the genotype, V_3 (ESWYT 7) gave the lowest water retention capacity (4.51) under Seeds primed with 10% PEG solution for 3 hours.

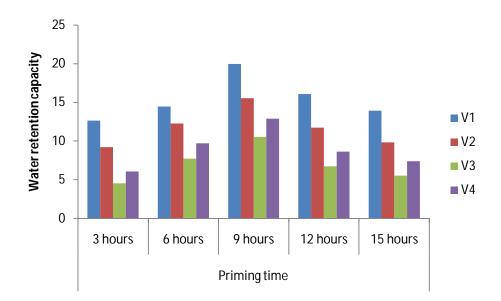


Fig.17 Effect of different priming time with different wheat genotypes on water retention capacity of PEG primed seeds (LSD_{0.01} = 0.93, 0.85, 1.08, 0.55, 0.96 respectively)

4.2.9 Vigour index

The vigour index of different wheat genotypes was significantly influenced by priming time with PEG (Fig.18 and Appendix XIX). Among the genotypes, V₁ (ESWYT 5) showed the best performance in terms of vigour index where V₃ (ESWYT 7) showed the lowest vigour index at all priming time with PEG. It was also observed that all genotypes gave their best performance on vigour index under Seeds primed with 10% PEG solution for 9 hours. Results revealed that the genotype, V₁ (ESWYT 5) gave the highest vigour index (272.3) from 9 hours priming time with 10% PEG solution. The lowest vigour index (4.54%) was obtained by V₃ (ESWYT 7) genotype from 3 hours priming time with 10% PEG

solution. The improvement in germination and vigourindex of normal/low-vigour seed might be due to reserve mobilization of food material, activation and resynthesis of some enzymes DNA and RNA synthesis start during osmotic priming. Rapid embryo growth resulted when the obstacle to germination was removed (Basra *et al.*, 2003). Harris *et al.* (1999) observed that hydro-priming technique has been employed to increase germination rate and seedling vigour in several vegetable crops.

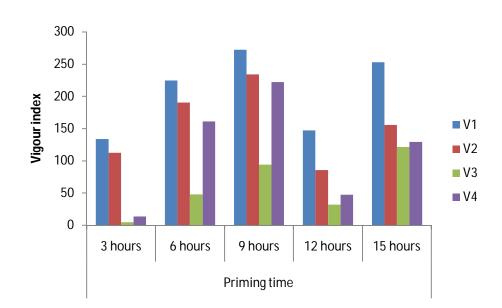


Fig.18 Effect of different priming time with different wheat genotypes on vigour index of PEG primed seeds (LSD_{0.01} = 5.61, 17.48, 18.75, 7.88, 13.12 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = ESWYT 7$, $V_4 = BARI \text{ gom}28$

4.3 Third experiment: Germination, seedling growth and water relation behavior of primed seed (wheat) under salt (NaCl) stress condition

This experiment was conducted under laboratory condition. Three wheat genotypes were primed in 10% PEG solution for 9 hours. Dry seed used as control and was exposed to 0, 5, 10, 15 and 20dsm⁻¹ NaCl induced salt stress conditions in Petridishes. The results have been presented separately in Figures 19 to 27 and Appendices XX to XVIII under the following headings:

4.3.1 Rate of germination

Different salinity levels revealed significant variation in respect of germination rate (Fig.19 and Appendix XX). Result exposed that the germination from primed seeds decreased significantly with increasing salinity level. It was found that the variety, V_1 (ESWYT 5) gave the promising result on seed germination at all salt concentration and germination rate was highest in no salinity level. But under salinity level the highest germination rate was found in Primed seeds placed with 5 dsm⁻¹ NaCl and thereafter gradually decreased germination rate was found with increased salinity levels. It was observed that the highest germination rate (93.81%) was in V₁ (ESWYT 5) under Primed seeds placed without salt and after that the second highest germination rate (87.62%) was in V_1 (ESWYT 5) with Primed seeds placed with 5 dsm⁻¹ where the lowest germination rate (61.49%) was obtained from V_3 (BARI gom28) with Primed seeds placed with 20dsm⁻¹ NaCl.Edalat-Pisheh et al. (2010) declared that total germination percentage in wheat seeds decreased when salinity of both primed and unprimed (control group) treatments increased. Kaya et al. (2006) and Khajeh-Hosseini et al. (2003) also find that reduction in total germination was significantly lower for non- primed seeds, compared to primed seeds and this may be due to the toxic effects of Na+ and Cl- in the process of germination.

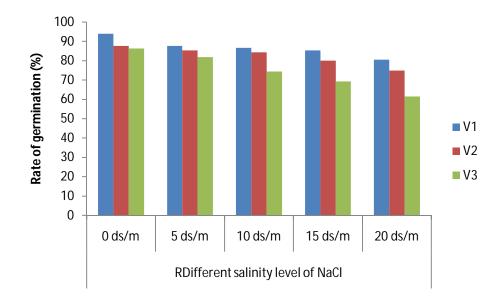


Fig.19 Effect of different salinity levels on germination rate of PEG primed wheat genotypes seeds (LSD_{0.01} = 6.96, 5.47, 4.61, 5.46, 5.92 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = BARI \text{ gom}28$

4.3.2 Shoot length

Shoot length of different wheat genotypes was significantly influenced by different salinity levels (Fig.20 and Appendix XXI).Result exposed that the shoot length from primed seeds decreased significantly with increasing salinity level regarding all tested genotypes.Results revealed that the highest shoot length (157.80 mm) was observed from V₁ (ESWYT 5)under Primed seeds placed without salt.But under salinity stressV₁ (ESWYT 5) gave highest shoot length (154.20 mm) with Primed seeds placed with 50 dsm⁻¹ NaCl where the lowest shoot length (100.20mm) was observed from V₃ (BARI gom28)Primed seeds placed with 20dsm⁻¹NaCl.Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita 2000). Similarly, toxic ion accumulation (Na⁺ and Cl⁻) negatively affect plant metabolism (Grieve and Fujiyama 1987). It has

also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim *et al.* 2008).

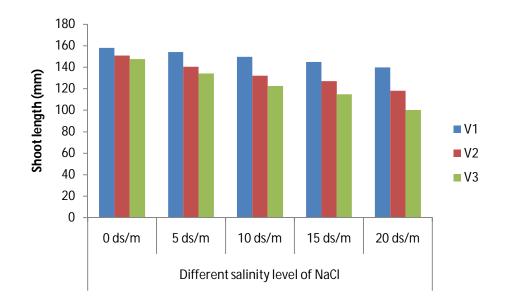


Fig.20 Effect of different salinity levels on shoot length of PEG primed wheat genotypes (LSD_{0.01} = 7.33, 8.19, 11.55, 8.33, 11.43 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = BARI \text{ gom}28$

4.3.3 Root length

Root length of different wheat genotypes was significantly influenced by different salinity levels (Fig.21 and Appendix XXII). Result exposed that the root length from primed seeds decreased significantly with increasing salinity level where no salinity stress gave highest root length for all the genotypes. Results revealed that the highest root length (124.6 mm)was observed inV₁ (ESWYT 5) Primed seeds placed without salt. Under salinity stress, the highest root length (118.50mm) was found from V₁ (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl and thereafter decreasing trend was observed with increasing salinity level. The lowest

root length (62.60mm) was observed from V_3 (BARI gom28) under Primed seeds placed with 20dsm⁻¹ NaCl. Significant improvement in root and shoot length may be attributed to earlier germination induced by primed over non-primed seeds (Farooq *et al.* 2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds.

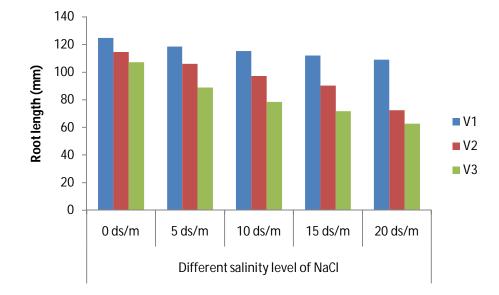


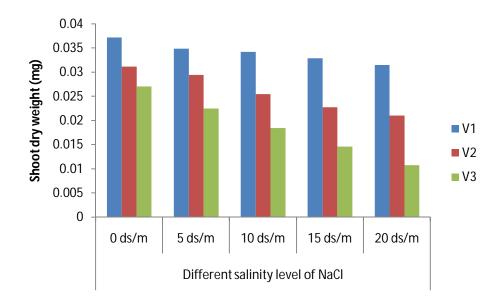
Fig.21 Effect of different salinity levels on root length fPEG primed wheat genotypes (LSD_{0.01} = 9.23, 5.13, 8.83, 7.79, 5.78 respectively)

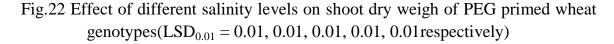
 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = BARI \text{ gom}28$

4.3.4 Shoot dry weight

Significant variation was found for shoot dry weight of different wheat genotypes affected by different salinity levels (Fig.22 and Appendix XXIII). Decreased shoot dry weight was observed with increased salinity level where no salinity level gave highest shoot dry weight. The results showed that the highest shoot dry weight (0.0371 mg) was observed from V_1 (ESWYT 5) Primed seeds placed without salt

but under salinity stress, V_1 (ESWYT 5) showed highest shoot dry weight (0.0349 dsm-1) under Primed seeds placed with 5 dsm⁻¹ NaCl where the lowest shoot dry weight (0.0107 mg) was observed from V_3 (BARI gom28)under Primed seeds placed with 20dsm⁻¹ NaCl.





 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = BARI \text{ gom}28$

4.3.5 Root dry weight

Significant variation was also found for root dry weight of different wheat genotypes affected by different salinity levels (Fig.23 and Appendix XIV). Decreased root dry weight was observed with increased salinity level where no salinity level gave highest root dry weight. Results showed that the highest root dry weight (0.0414 mg) was observed from V_1 (ESWYT 5)where Primed

seeds placed without salt.Under salinity stress, the highest root dry weight (0.0393 mg) was found from V_1 (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl. The lowest root dry weight (0.0124 mg) was found from V_3 (BARI gom28)under Primed seeds placed with 20 dsm⁻¹ Nacl.

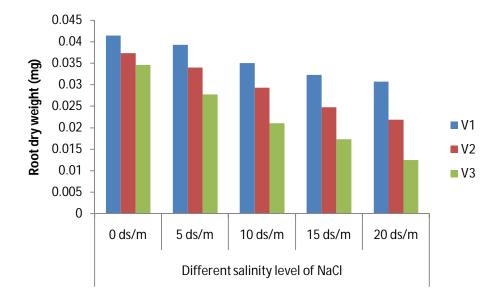


Fig.23 Effect of different salinity levels on root dry weight of different genotypes of PEG primed seeds (LSD_{0.01} = 0.01, 0.01, 0.01, 0.01, 0.01 respectively)

 $V_1 = ESWYT 5$, $V_2 = ESWYT 6$, $V_3 = BARI \text{ gom}28$

4.3.6 Relative water content

Relative water content of different wheat genotypes was significantly influenced by different salinity levels (Fig.24 and Appendix XV). Result exposed that the relative water content from primed seeds decreased significantly with increasing salinity level. Results indicated that the highest relative water content (89.13%) was observed from V₁ (ESWYT 5) where Primed seeds placed without salt. Under salinity stress, the highest relative water content (85.61 %) was found from V₁ (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl. The lowest relative water content (44.88%) was observed from V₃ (BARI gom28) under Primed seeds placed with 2dsm⁻¹ NaCl.

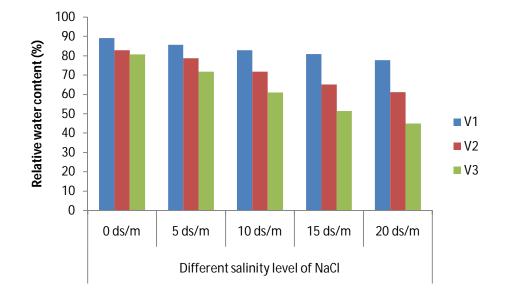
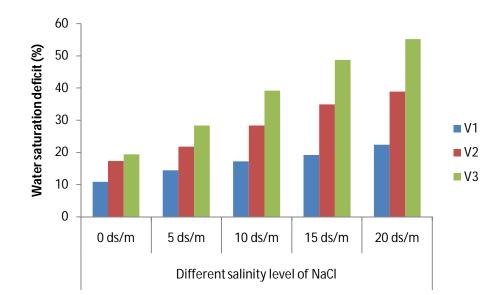
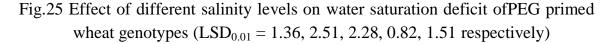


Fig.24 Effect of different salinity levels on relative water content of PEG primed wheat genotypes (LSD_{0.01} = 5.27, 4.61, 5.09, 7.54, 7.21 respectively)

4.3.7 Water saturation deficit

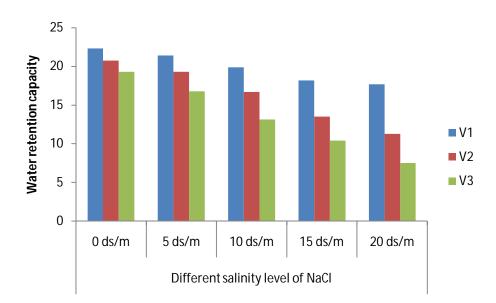
Significant influence was found in terms of water saturation deficit of different wheat genotypes affected by different salinity levels (Fig.25 and Appendix XVI). Increased water saturation deficit was observed with decreased salinity level where no salinity level gave lowest water saturation deficit.Results indicated that the highest water saturation deficit (55.12%) was observed from V₃ (BARI gom28)under Primed seeds placed with 20dsm⁻¹ NaClwhere the lowest water saturation deficit (10.87%) was observed from V₁ (ESWYT 5)where Primed seeds placed without salt. Under salinity stress, the lowest water saturation deficit (14.39%) was found from V₁ (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl.

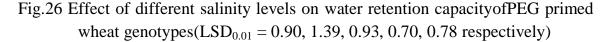




4.3.8 Water retention capacity

Significant influence was found for water retention capacity of different wheat genotypes affected by different salinity levels (Fig.26 and Appendix XVII). Decreased water retention capacity was observed with increased salinity level where no salinity level gave highest water retention capacity. Results indicated that the highest water retention capacity (22.32) was observed from V₁ (ESWYT 5)where Primed seeds placed without saltbut under salinity stress, the highest water retention capacity (21.40) was found from V₁ (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl. The lowest water retention capacity (7.510) was observed from V₃ (BARI gom28) where Primed seeds placed with 20dsm⁻¹ NaCl.





4.3.9 Vigour index

Significant influence was found for vigour index of different wheat genotypes affected by different salinity levels (Fig.27 and Appendix XVIII). Result exposed that the vigour index from primed seeds decreased significantly with increasing salinity level and no salinity stress gave highest vigour index. Results indicated that the highest vigour index (264.7) was observed from V₁ (ESWYT 5) with Primed seeds placed without salt.Under salinity stress, the highest vigour index (238.9) was found from V₁ (ESWYT 5) under Primed seeds placed with 5dsm⁻¹ NaCl. The lowest vigour index (100.1) was observed from V₃ (BARI gom28) under Primed seeds placed with 20dsm⁻¹ NaCl.Ruan *et al.* (2002b) also found similar results who observed that primed rice seeds showed higher vigour index than non-primed ones.

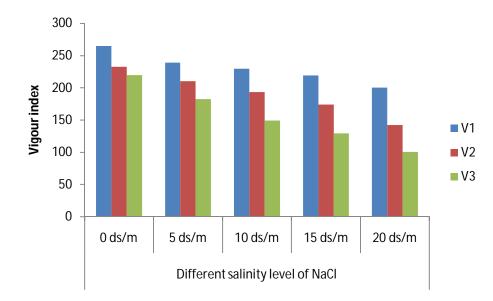


Fig.27 Effect of different salinity levels on vigour index of PEG primed wheat genotypes (LSD_{0.01} = 13.05, 13.56, 15.90, 9.93, 9.36 respectively)

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at Laboratory of Department of Agronomy, Shere-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207during the period from October 2016 to December 2016 to study the polyethyleneglycol (PEG) induce changes in growth and physiology of wheat (*Triticumaestivum.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.

Four wheat genotypes namely- ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom28 were used as test crop. Different priming chemicals such as PEG, salt (NaCl) and distilled water were utilized for osmo and hydro priming.

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 mmdiameter Petri dishes on whatman No.1filter 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 vigour index. Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the LSD at 1% level of probability.

5.1 First experiment

The first experiment was carried out to find the effect of different concentration of PEG on germination and growth behavior of four wheat genotypes (ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom 28) without any stress condition. Four levels of PEG such as 5%, 10%, 15% and 20% were used for osmopriming and distilled water used as hydropriming agent for 12 hours, respectively. Seeds without priming (control) also included as treatment.

Among the genotypes, V_1 (ESWYT 5) gave the best results on studied parameters. Results revealed that the variety V_1 (ESWYT 5) showed the highest germination rate (95.30%), shoot length (168.20 mm), root length (158.30 mm), shoot dry weight (0.0522 mg), root dry weight (0.0432 mg), relative water content (95.05%), water retention capacity (16.18) and vigour index (311.30) primed with 10% PEG solution for 12 hours where Seeds without priming showed lowest results in respected parameters with the genotype V_3 (ESWYT 7).

5.2 Second experiment

The second experiment was conducted to optimization of pre-sowing priming time on the germination and growth behavior of wheat genotypes. Four wheat genotypes (ESWYT 5, ESWYT 6, ESWYT 7 and BARI gom28) without any stress condition were considered. Five different priming times such as 3, 6, 9, 12 hours for osmopriming were used using 5% PEG solution.

The variety, V_1 (ESWYT 5) seeds primed with 5% PEG solution for 9 hours gave the highest germination rate (83.33%), shoot length (181.1 mm), root length (145.0 mm), shoot dry weight (0.0536 mg), root dry weight (0.0432 mg), relative water content (91.45%), water retention capacity (19.94) and vigour index (272.3). The lowest water saturation deficit (8.55%) was also obtained from V_1 (ESWYT 5) seeds primed with 5% PEG solution for 9 hours where seeds primed with 10% PEG solution for 3 hours showed lowest results in respected parameters with the genotype V_3 (ESWYT 7).

5.3 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. PEG solution 5% 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 (157.80%), shoot length (157.80 mm), root length (124.6 mm), shoot dry weight (0.037 mg), root dry weight (0.0414 mg), relative water content (89.13%), water retention capacity (22.32) and vigour index (264.7). But under salinity stress, the highest germination rate (154.20%), shoot length (154.20 mm), root length (118.50 mm), shoot dry weight (0.0349 mg) and root dry weight (0.0393 mg), relative water content (85.61 %), water retention capacity (21.40) and vigour index (238.9) were achieved from V_1 (ESWYT 5) primed seeds placed with 50 dsm⁻¹ NaCl where V_3 (BARI gom28) primed seeds placed with 20 dsm⁻¹ NaCl showed lowest results in respected parameters.

From the results of the study, it may be concluded that the performance of PEG primed wheat cv. ESWYT 5 was better in respect of germination and growth parameters. Priming with 10% PEG concentration and 9 hours priming time increase the germination and growth behaviour of wheat seeds. Reduction in germination parameters and seedling growth was more profound in control seeds than primed seeds under salt stress condition. Thus, the 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. For this reason, further studies are needed to assess the efficacy of seed priming during the later stages of the culture.

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APPENDICES

| | 1 | | | | | | |
|------|----------|-----------------------------------|---------|------|----------|----------|----------|
| Year | Month | Air Temperature (⁰ c) | | | Relative | Rainfall | Sunshine |
| | | Maximum | Minimum | Mean | humidity | (mm) | (hr) |
| | | | | | (%) | | |
| 2016 | November | 29.5 | 18.6 | 24.0 | 69.5 | 0.0 | 233.2 |
| 2016 | December | 26.4 | 15.6 | 21.0 | 68.6 | 00 | 230.4 |

Appendix I. Monthly records of Temperature, Rainfall, and Relative humidity of the experiment site during the period of November 2015

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

Appendix II. Mean square values of priming solution with different wheat genotypes on germination rate of primed (PEG and water) and non-primed (control) seeds

| Source of | 10 | Mean square of Rate of germination (%) at different priming solution | | | | | | |
|-----------|----|--|-------------------|-----------------------|-----------------------|-----------------------|------------------|--|
| variation | df | Water | 5% PEG | 10% | 15% | 20% | Control | |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (\mathbf{P}_6) | |
| Treatment | 3 | 118.12** | 116.71** | 127.38** | 125.61** | 122.49** | 136.24** | |
| Error | 6 | 7.59 | 11.82 | 10.08 | 9.40 | 6.12 | 5.36 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix III. Mean square values of priming solution with different wheat genotypes on shoot length of primed (PEG and water) and nonprimed (control) seeds

| | | Mean sq | uare of Sho | ot length (n | nm) at differ | ent priming | solution |
|---------------------|----|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-------------------|
| Source of variation | df | Water | 5% PEG | 10% | 15% | 20% | Control |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (P ₆) |
| Treatment | 3 | 52.344** | 44.389** | 62.641** | 48.322** | 39.642* | 38.399** |
| Error | 6 | 20.435 | 12.652 | 13.987 | 13.858 | 11.512 | 20.435 |

**Significant at 1% level of significance

Appendix IV. Mean square values of priming solution with different wheat genotypes on root length of primed (PEG and water) and non-primed (control) seeds

| G 6 | | Mean sq | uare of Roo | ot length (m | m) at differ | ent priming | solution |
|---------------------|----|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-------------------|
| Source of variation | df | Water | 5% PEG | 10% | 15% | 20% | Control |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (P ₆) |
| Treatment | 3 | 48.303** | 39.827** | 52.874** | 40.205** | 28.676* | 34.621** |
| Error | 6 | 9.309 | 8.536 | 12.811 | 11.144 | 12.273 | 13.711 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix V. Mean square values of priming solution with different wheat genotypes on shoot dry weight of primed (PEG and water) and non-primed (control) seeds s

| | | Mean | square of S | • | eight (mg) at ution | t different p | riming |
|-----------|----|---------------------------|-------------------|-------------------|------------------------|-----------------------|-------------------|
| Source of | df | Water | 5% PEG | 10% | 15% PEG | 20% | Control |
| variation | | (P ₁) | (P ₂) | PEG | (P ₄) | PEG (P ₅) | (P ₆) |
| | | | | (P ₃) | | | |
| Treatment | 3 | 5.077** | 6.666** | 4.265* | 8.705* | 9.312** | 9.047** |
| Error | 6 | 0.237 | 0.171 | 0.107 | 0.062 | 0.022 | 0.017 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix VI. Mean square values of priming solution with different wheat genotypes on root dry weight of primed (PEG and water) and non-primed (control) seeds

| G 6 | | Mean | Root dry v | veight (mg) | at different | priming sol | ution |
|---------------------|----|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-------------------|
| Source of variation | df | Water | 5% PEG | 10% | 15% | 20% | Control |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (P ₆) |
| Treatment | 3 | 2.661** | 4.311** | 5.204** | 4.312** | 3.751** | 4.226** |
| Error | 6 | 0.124 | 0.097 | 0.083 | 0.065 | 0.036 | 0.064 |

**Significant at 1% level of significance

Appendix VII. Mean square values of priming solution with different wheat genotypes on relative water content of primed (PEG and water) and non-primed (control) seeds

| Source of | 16 | Mean | square of Re | lative water solu | content (%) a tion | at different p | riming |
|-----------|----|-------------------|-------------------|----------------------|-----------------------|-------------------|-------------------|
| variation | df | Water | 5% PEG | 10% PEG | 15% PEG | 20% PEG | Control |
| | | (P ₁) | (P ₂) | (P ₃) | (P ₄) | (P ₅) | (P ₆) |
| Treatment | 3 | 48.439* | 90.128** | 74.961** | 53.897** | 79.958** | 62.361** |
| Error | 6 | 11.184 | 8.449 | 11.478 | 7.877 | 8.704 | 11.289 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix VIII. Mean square values of priming solution with different wheat genotypes on water saturation deficit of primed (PEG and water) and non-primed (control) seeds

| Source of | 10 | Mean squ | uare of Wat | | n deficit (%) |) at differen | t priming |
|-----------|----|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-------------------|
| variation | df | Water | 5% PEG | 10% | 15% | 20% | Control |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (P ₆) |
| Treatment | 3 | 29.311** | 32.466** | 27.814** | 39.532** | 42.149** | 36.522** |
| Error | 6 | 8.389 | 7.414 | 6.592 | 8.179 | 9.325 | 7.568 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix IX. Mean square values of priming solution with different wheat genotypes on water retention capacity of primed (PEG and water) and non-primed (control) seeds

| Source of | 16 | Mean square of Water retention capacity at different priming solution | | | | | | |
|-----------|----|---|-------------------|-----------------------|-----------------------|------------|------------------|--|
| variation | df | Water | 5% PEG | 10% | 15% | 20% | Control | |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | $PEG(P_5)$ | (\mathbf{P}_6) | |
| Treatment | 3 | 16.332* | 17.036** | 29.795* | 18.386* | 17.621** | 20.539** | |
| Error | 6 | 0.357 | 0.294 | 0.212 | 0.233 | 0.215 | 1.036 | |

**Significant at 1% level of significance

Appendix X. Mean square values of priming solution with different wheat genotypes on vigour index of primed (PEG and water) and non-primed (control) seeds

| G 6 | | Mean | square of V | vigour index | k at differen | t priming so | lution |
|---------------------|----|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-------------------|
| Source of variation | df | Water | 5% PEG | 10% | 15% | 20% | Control |
| | | (P ₁) | (P ₂) | PEG (P ₃) | PEG (P ₄) | PEG (P ₅) | (P ₆) |
| Treatment | 3 | 194.534** | 247.267** | 215.359** | 156.244** | 145.238* | 156.814** |
| Error | 6 | 12.455 | 8.637 | 4.219 | 3.662 | 5.217 | 6.221 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XI. Mean square values of different priming time with different wheat genotypes on germination rate of PEG primed seeds

| Source of | | Mean squ | Mean square of Rate of germination (%) with priming time | | | | | | |
|---------------------|----|-------------------|--|-------------------|-------------------|-------------------|--|--|--|
| Source of variation | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | | | |
| variation | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | | | |
| Treatment | 3 | 130.82** | 169.67** | 223.34** | 184.49** | 172.83** | | | |
| Error | 6 | 10.53 | 12.81 | 12.79 | 9.69 | 8.87 | | | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XII. Mean square values of different priming time with different wheat genotypes on shoot length of PEG primed seeds

| Source of | | Mean | Mean square of Shoot length (mm) with priming time | | | | | | | |
|-----------|----|-------------------|--|-------------------|-------------------|-------------------|--|--|--|--|
| variation | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | | | | |
| variation | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | | | | |
| Treatment | 3 | 4.071** | 4.621** | 6.429** | 8.766* | 10.520** | | | | |
| Error | 6 | 0.207 | 0.184 | 0.112 | 0.069 | 0.022 | | | | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XIII. Mean square values of different priming time with different wheat genotypes on root length of PEG primed seeds

| Source of variation | | Mean square of Root length (mm) with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 116.074** | 129.118** | 84.627** | 79.229** | 104.443* | |
| Error | 6 | 9.716 | 8.314 | 9.817 | 12.399 | 10.266 | |

**Significant at 1% level of significance

Appendix XIV. Mean square values of different priming time with different wheat genotypes on shoot dry weight of PEG primed seeds

| Source of variation | | Mean square of Shoot dry weight (mg) with priming time | | | | | |
|---------------------|----|--|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 6.071** | 7.341** | 7.841** | 6.542** | 8.319** | |
| Error | 6 | 0.166 | 0.271 | 0.158 | 0.074 | 0.036 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XV. Mean square values of different priming time with different wheat genotypes on root dry weight of PEG primed seeds

| Source of variation | | Mean square of Root dry weight (mg) with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 5.312** | 4.617** | 5.328** | 7.514** | 8.317** | |
| Error | 6 | 0.026 | 0.087 | 0.078 | 0.049 | 0.054 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XVI. Mean square values of different priming time with different wheat genotypes on relative water content of PEG primed seeds

| Source of variation | | Mean square of Relative water content (%) with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 52.113* | 114.056** | 86.271** | 68.389** | 62.445** | |
| Error | 6 | 9.184 | 7.449 | 11.861 | 7.144 | 9.032 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XVII. Mean square values of different priming time with different wheat genotypes on water saturation deficit of PEG primed seeds

| Source of variation | | Mean square of Water saturation deficit (%) with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 32.263** | 26.127** | 22.544** | 32.316** | 44.047** | |
| Error | 6 | 8.089 | 7.812 | 6.368 | 8.211 | 9.472 | |

**Significant at 1% level of significance

Appendix XVIII. Mean square values of different priming time with different wheat genotypes on water retention capacity of PEG primed seeds

| Source of variation | | Mean square of Water retention capacity with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 18.332* | 14.041** | 26.144* | 22.347* | 26.633** | |
| Error | 6 | 0.257 | 0.288 | 0.276 | 0.214 | 0.232 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XIX. Mean square values of different priming time with different wheat genotypes on vigour index of PEG primed seeds

| Source of variation | | Mean square of Vigour index with priming time | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 3 | 144.82** | 163.62** | 185.34** | 148.49** | 13783** | |
| Error | 6 | 11.36 | 9.82 | 12.56 | 9.88 | 10.87 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XX. Mean square values of different salinity levels on germination rate of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Rate of germination (%) on different salinity level of NaCl | | | | | |
|---------------------|----|--|------------------------------|------------------------------|-------------------------------|-------------------------------|--|
| | | 3 hours (T_1) | 6 hours (T ₂) | 9 hours (T ₃) | 12 hours (T ₄) | 15 hours (T ₅) | |
| Treatment | 2 | 112.84** | 136.60** | 168.36** | 149.42** | 172.59** | |
| Error | 4 | 7.56 | 9.741 | 12.72 | 9.69 | 8.81 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXI. Mean square values of different salinity levels on shoot length of PEG primed seeds of wheat genotypes

| Source of variation | | Mean square of Shoot length (mm) on different salinity level | | | | | |
|---------------------|----|--|-------------------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 2 | 4.088** | 5.052** | 6.433** | 8.711* | 6.215** | |
| Error | 4 | 0.2037 | 0.181 | 0.151 | 0.083 | 0.026 | |

**Significant at 1% level of significance

Appendix XXII. Mean square values of different salinity levels on root length of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Root length (mm) on different salinity level | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 2 | 111.073** | 109.134** | 92.227** | 79.381** | 104.178** | |
| Error | 4 | 6.712 | 6.311 | 7.838 | 192.354 | 8.261 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXIII. Mean square values of different salinity levels on shoot dry weight of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Shoot dry weight (mg) on different salinity level | | | | |
|---------------------|----|--|-----------------|------------------------------|------------------|------------------|
| | | 3 hours (T_1) | 6 hours (T_2) | 9 hours (T ₃) | 12 hours (T_4) | 15 hours (T_5) |
| The second second | 2 | (1) | < =/ | (=) | · · · | (=) |
| Treatment | 2 | 2.148** | 1.766** | 3.142**S | 3.074** | 4.053** |
| Error | 4 | 0.162 | 0.071 | 0.053 | 0.074 | 0.036 |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXIV. Mean square values of different salinity levels on root dry weight of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Root dry weight (mg) on different salinity level | | | | | |
|---------------------|----|---|-------------------|-------------------|-------------------|-------------------|--|
| | | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T ₂) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 2 | 3.661** | 2.718** | 1.517** | 4.228** | 3.108** | |
| Error | 4 | 0.028 | 0.083 | 0.071 | 0.059 | 0.051 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXV. Mean square values of different salinity levels on relative water content of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Relative water content (%) on different salinity level | | | | | |
|---------------------|----|--|-----------------|------------------------------|-------------------------------|------------------|--|
| | | 3 hours (T_1) | 6 hours (T_2) | 9 hours (T ₃) | 12 hours (T ₄) | 15 hours (T_5) | |
| Treatment | 2 | 47.117* | 62.054** | 68.228** | 48.317** | 39.433** | |
| Error | 4 | 9.182 | 7.488 | 7.822 | 7.314 | 9.039 | |

**Significant at 1% level of significance

Appendix XXVI. Mean square values of different salinity levels on water saturation deficit of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Water saturation deficit (%) on different salinity level | | | | | |
|---------------------|----|---|-----------------|-----------------|------------------|------------------|--|
| | | 3 hours (T_1) | 6 hours (T_2) | 9 hours (T_3) | 12 hours (T_4) | 15 hours (T_5) | |
| Treatment | 2 | 32.255** | 26.63** | 18.411** | 22.314** | 41.049** | |
| Error | 4 | 8.009 | 7.816 | 6.368 | 8.214 | 9.472 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXVII. Mean square values of different salinity levels on water retention capacity of different genotypes of PEG primed seeds

| Source of variation | df | Mean square of Water retention capacity on different salinity level | | | | | |
|---------------------|----|--|----------|-------------------|-------------------|----------|--|
| | | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T_2) | (T ₃) | (T ₄) | (T_5) | |
| Treatment | 2 | 11.144* | 16.367** | 21.524* | 25.312* | 22.153** | |
| Error | 4 | 0.207 | 0.234 | 0.268 | 0.219 | 0.259 | |

**Significant at 1% level of significance

^{NS} Non significant

Appendix XXVIII. Mean square values of different salinity levels on vigour index of different genotypes of PEG primed seeds

| Source of variation | | Mean square of Vigour index on different salinity level | | | | | |
|---------------------|----|---|---------|-------------------|-------------------|-------------------|--|
| | df | 3 hours | 6 hours | 9 hours | 12 hours | 15 hours | |
| | | (T ₁) | (T_2) | (T ₃) | (T ₄) | (T ₅) | |
| Treatment | 2 | 84.86** | 63.68** | 85.31** | 48.44** | 6788** | |
| Error | 4 | 9.36 | 10.83 | 11.31 | 9.80 | 8.89 | |

**Significant at 1% level of significance