ENHANCEMENT OF GERMINATION, SEEDLING GROWTH, WATER RELATION BEHAVIOR OF WHEAT THROUGH OSMO AND HYDRO PRIMING UNDER DROUGHT STRESS CONDITION

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CERTIFICATE

*This is to certify that the thesis entitled, "***Enhancement of Germination, Seedling Growth, Water Relation Behavior of Wheat Through Osmo and Hydro Priming Under Drought Condition***" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of* ***Master of Science in Agronomy****, embodies the result of a piece of bona fide research work carried out by* ***Most. Sadia Afrin*** *bearing Registration No.14-06132 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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Dedicated To

My Beloved Parents

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ENHANCEMENT OF GERMINATION, SEEDLING GROWTH, WATER RELATION BEHAVIOR OF WHEAT THROUGH OSMO AND HYDRO PRIMING UNDER DROUGHT STRESS CONDITION

ABSTRACT

A laboratory experiment was carried out at the central laboratory of Sher-e-Bangla Agricultural University Dhaka-1207 during the period from December 2019 to February 2020 to find out the effect of various Mannitol concentrations on the germination, seedling growth and water relation behavior of wheat under drought stress condition. This study consisted of two different experiments. Mannitol was used as priming agent and PEG (Polyethylene glycol) was used for inducing drought stress. Ethanol was used as surface sterilizer of wheat seeds. The data on different parameters were recorded for analysis the result of this experiment. For the first experiment four levels of mannitol such as 1%, 2%, 3% and 4% were used for osmopriming and water was used for hydropriming. Seeds primed with 2% mannitol gave the best results on studied parameters. Results revealed that the seed primed with 2% Mannitol for 9 hours showed the highest germination rate (98%), shoot length (162.3 mm), root length (183.3mm), shoot dry weight (12.2 mg), root dry weight (13.5 mg), fresh weight (354.0 mg), water saturation deficit (29.75), relative water content (70.25%) and vigor index (338.69). Seeds without osmo priming and hydropriming showed lowest results in studied parameters. In the second experiment, 2% mannitol primed (9 hours) seeds of wheat with and without drought (PEG) stress condition was evaluated. The drought stress level was induced by 0%, 5%, 10%, 15% and 20% PEG solution. It was observed that the genotype BARI Gom 31 primed with 2% mannitol for 9 hours seeds placed without drought (control) gave the best performance on studied parameters but under drought stress, the highest germination rate, shoot length, root length, shoot dry weight and root dry weight, fresh weight, relative water content and vigor index were achieved from primed seeds placed on 5% PEG where primed seeds placed on 20% PEG induced drought condition showed lowest results in respected parameters. From the result of the study, it was revealed that seeds primed with 2% mannitol for 9 hours showed the best results compared with water primed and non-primed seed under drought stress condition.

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ABBREVIATIONS AND ACRONYMS

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

CHAPTER I

INTRODUCTION

Drought is one of the most critical environmental stresses that reduce crop productivity (Khan *et al*., 2018).The agricultural production areas face a continuous decrease in irrigation water (Hafez, Ragab and Kobata, 2014). Crop productivity is reduced by 50% - 73% under limited water supply (Mehrdad, Sepideh & Hamed, 2017). By 2050, about 50% of arable lands are expected to be under drought stress (Kasim *et al*., 2013). Drought can occur at all growth stages, but the first and foremost effect is on seed germination (Kaya *et al*., 2006; Fahad *et al*., 2017). Where water entrance into the seed decreases due to hydraulic reduction; and thereby, all the physiological and metabolic germination processes are affected (Barekee*t al*., 2018).

Wheat is one of the major cereals in the world, being a main source of calories and protin in most parts of the world. In 2019, world production of wheat was 731.45 million tons (FAO, 2020), making it the third most produced cereal after maize and rice. In Bangladesh, wheat production in 2019 was 8, 57,610 M. Tons (BBS, 2019).

However, its production is severely threatened by different abiotic factors, including drought. Shortage of fresh water affects various aspects of plants, including morphological factors, as well as several physiological and biochemical mechanisms, resulting in reduced final production (Akram *et al*., 2018, Cao *et al*., 2018, Reza *et al*.,2018).Among different physiological mechanisms, water-stress-induced excessive production of reactive oxygen species (ROS), such as singlet oxygen (O_2), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radical (OH^-) are the important ones that damage the lipids, proteins, photosynthetic pigments, and nucleic acid (Richards *et al*.,2015). Under severe stress, these damages eventually lead to death of cells, and finally of the plant (Shan *et al*., 2015). At cellular levels, chloroplasts, vacuoles, micro bodies, and mitochondria are the organelles or sites for production of ROS (Ashraf, 2009).

In order to overcome the damaging impacts of drought stress, plants have also developed the mechanism of osmotic adjustment through increased synthesis of osmolytes (glycine betaine (GB) and proline (Pro), secondary metabolites, and carbohydrates (Shafiq *et al*., 2018; Khan *et al*., 2018). Osmotic homeostasis plays an important role in maintaining plant growth and cell targer by reducing osmotic potential, resulting in better growth (Mafakheri *et al*., 2010). (Gou *et al*., 2015) found that in maize plants under drought stress, accumulation of choline and GB was found to be effective in plant water states as a result of better growth. In parallel, drought-induced higher accumulation of osmoprotectants, such as total soluble proteins and proline, was effective for osmoragulation in cotton plants (Wu *et al*., 2010). However, the stress tolerance mechanism in plants is species and crop cultivar specific. Some high-yielding crop genotypes are sensitive to different abiotic stresses. Different methods or techniques are being used to improve the crop stress tolerance for better production (Ali *et al*., 2013; Noman *et al*., 2018; Akram *et al*., 2018). The response of drought-treated *Brassica napus* plants was found to be positively associated with osmotic regulation (Hatzig *et al*., 2014).

Pre-sowing seed treatment with different inorganic and organic chemicals is considered an important way to increase the plant tolerance to stressful conditions (Wang *et al*., 2014; Ali *et al*., 2011). Of the different seed priming agents, mannitol is one such compound. Mannitol is a six-carbon sugar alcohol and wide distribution in nature (Stoop *et al*., 1996). Also found in *Fraxinus ornus* (Schwarz*l*, 1994). As a naturally occurring polyol (sugar alcohol), mannitol is widely used in the food, pharmaceutical, medical, and chemical industries (Scetaert *et al*., 1995; Saha *et al*., 2011). Mannitol also named as mannite or manna sugar is a white, crystalline solid and chemical formula is $C_6H_{14}O_6$, it occurs in small quantities in most fruit and vegetables such as onions, celery, olives, beets and pumpkins.

Seed priming is a pre-sowing treatment which leads to a physiological state that enables seed to germinate more efficiently. Seed priming ensures increased and uniform germination by reducing the imbibition time (Brocklehurst *et al*., 2008), increasing the pre-germinative enzyme activation, increasing metabolite production (Hussain *et al*., 2016). There are many reports on seed priming toward improving seed germination, seedling emergence, stand establishment, crop growth, nodulation, and productivity in various crop species viz., rice (Jisha *et al*., 2016; Samota *et al*., 2017; Kavitha *et al*., 2018), wheat (Tabassum *et al*., 2018; Bagheri *et al*., 2019), pulses (Ali *et al*., 2005; Sajjan *et al*., 2005; Bhowimik*et al*., 2020; Shariatmadari *et al*., 2020), okra (Puthiyotti *et al*., 2015), Chinese cabbage (Yan *et al*., 2015), sunflower (Moghanibashi *et al*.,2013) melons (Castanares and Bouzo, 2018). Seed priming induces antioxidant activity and storage protein solubilization and minimizes lipid peroxidation (Iseri *et al*., 2014). Priming significantly increases the quantity of mitochondria and regulation of proteins for cell division (α- and β-tubulin). Rehydration through seed priming brings major cellular changes in seeds such as de novo synthesis of nucleic acids and proteins ATP (adenosine tri phosphate) production activation of sterols and phospholipids and repairing DNA damaged during threshing. However, the priming-induced molecular mechanisms are not studied as compared to transcriptome and proteome (omics) mechanisms behind the drought stress. Exploring the molecular mechanisms in the field of seed science may not only satisfy the seed traders but can also be useful for small and marginal farmers toward managing climate risk crop husbandry in a cost-effective manner (Padgham, 2009). Thus, the present review is intended to discuss (i) the impact of drought stress on seed germination and establishment, (ii) seed priming methods and their molecular mechanism of drought tolerance, (iii) challenges and opportunities, with the aim to promote the seed priming strategy as a future, cost-effective research tool to increase yield and productivity under drought stress.

In Bangladesh a little is known about hydro priming but osmotic priming induced drought tolerant capacity in Wheat is not well established. Therefore, the present study on seed priming of Wheat was conducted with following objectives:

i. To evaluate the effects of mannitol on changes of germination behavior, growth and morpho-physiological feature of BARI Gom - 31.

ii. To investigate the effects of drought and mannitol on changes of germination behavior, growth and morpho-physiological parameters of BARI Gom - 31.

CHAPTER II

REVIEW OF LITERATURE

Drought stress is a great problem in the northern region of Bangladesh. Wheat is an important cereal crop in Bangladesh and it is a great source of carbohydrate and protein. The scientists of Bangladesh are conducting different experiments to adopt different crops in the drought prone area; wheat is one of them. Different treatments were applied before at different locations to overcome drought stress. External use of mannitol is one of them. Very limited research works have been conducted to adapt wheat in the drought prone area of Bangladesh. An attempt has been made to find out the performance of wheat at different levels of drought. To facilitate the research works different literature have been reviewed in this chapter under the following headings.

2.1 Seed priming

Since right from the beginning of agriculture, man realized that most seeds do not germinate easily and uniformly. Seed priming was an age-old technique practiced by the Greek farmers. Theophrastus (372–287 BC) focused on seed physiology and suggested that germination process may be temporarily interrupted. He recommended the pre-soaking of cucumber seeds in milk or water to germinate earlier and vigorously (Evenari, 1984). Research reports also revealed that pre-hydration of legume seeds was done by Roman farmers in order to increase the germination rate and synchronize germination. In 1664, Evelyn mentioned that temperature prior to sowing may have an impact on further germination. During 1779, In green house studied the impact of light on seedling emergence. Amici during 1830 and Sachs during 1859 described the morphological process associated with seed germination (Amici, 1830; Sachs, 1859). The role of plant hormones in seed desiccation tolerance, reserve mobilization, cell division and cell elongation were discovered in 1920 (Lutts *et al*., 2016). The word seed priming was coined by Heydecker in 1973 and he successfully adopted seed priming to improve seed germination and emergence under stressful conditions (Sivasubramaniam *et al*., 2011).

Seed priming is partial hydration of seeds and metabolic activity is attained in a desirable manner thereby allowing important pre-germination steps to be initiated within the seeds. Repair of membranes, repair and synthesis of DNA and RNA, development of immature embryo, alterations of endosperm tissues surrounding the embryo, dormancy breakage and pre -germination metabolism enrichment are the major changes takes place during seed priming. Since seeds are physiologically closer to germination, primed seeds have increased germination rate, early germination, uniformity in germination, better growth attributes, faster emergence and better stand establishment (Farooq *et al*., 2007).

2.2 Effect of seed priming

2.2.1 Effect of seed priming on germination and growth parameters

A lab experiment was done by Dhakal and Subedi (2020) with maize seed to observe the drought stress on seed germination and growth. Maize seed was primed using Mannitol @ 0%, 2%, 4%, 6% and 8% (w/v) concentrations subjected to germination under induced drought of 0 M pa, 0.15 M pa, 0.5 M Pa, 1.0 M Pa and 1.7 M Pa using NaCl. The experiment was laid in completely randomized design (CRD) with three replications. Priming with mannitol reduced the Mean Germination Time (MGT); the best result obtained in seeds primed with 2% mannitol. However, the final germination count, Relative Water Content (RWC) and root and shoot length remained unaltered. Germination activities reduced with increasing moisture stress. The study indicated that priming with mannitol could improve the speed of germination in maize seeds.

A pot experiment was done by Hameed and Iqbal (2013) with different priming agents to find out growth of the seedling of wheat. Seed priming treatments were applied by soaking seeds in aerated solution of 1% mannose (56 m M) and 10 mM mannitol for 8 h while 100 µM H_2O_2 for 5 h. Seeds soaked in aerated water (hydropriming) and non-primed seed were used as controls. Drought stress significantly reduced the seedling fresh weight and leaf relative water content. Pre-sowing seed treatment with mannitol significantly increased the seedling, root and shoot fresh as well as dry weights under non-stress condition. Moreover, H_2O_2 increased the root length; seedling and root dry weights while mannose increased the shoot dry weight under drought stress. Leaf relative water content (RWC) improved after mannitol and H_2O_2 priming under drought and non-stressed conditions. Hydropriming increased the root and shoot fresh weights, shoot dry weight and RWC under non-stress condition while seedling, root, shoot fresh weights and shoot dry weight along with raised TSP, MDA, reducing sugars under drought stress. Drought stress raised the total soluble protein (TSP), protease, APX and POD activities, MDA and reducing sugars in leaves. Mannitol and H_2O_2 confiscate the drought-induced increase in TSP while H_2O_2 significantly increase it under non-stress condition. Drought stress reduced the catalase activity in leaves while H_2O_2 and mannitol priming brought it back to control level. Drought stress elevated the MDA in leaves and H_2O_2 treatment prevented this increase. Only mannose priming rose the reducing sugars in leaves under non-stress condition. Under drought, mannose and mannitol priming raised the reducing sugars in the leaves as a tactic for osmotic adjustment. In conclusion, seed priming treatments ameliorated the drought tolerance in wheat by elevating the level of antioxidants, reducing oxidative damage of biomolecules and accumulating more reducing sugars for osmotic adjustments.

A laboratory experiment was conducted by Movaghatian and Khorsandi (2013) to investigate the effects of seed priming with salicylic acid (SA) on germination characteristics of wheat (Chamran cv.) under drought stress. The experimental design was completely randomized design with five SA concentrations (0, 0.00001, 0.001, 0.1 and 1 m M), four levels of stress (0, -3, -6 and -9 bar) with three replications. The seeds were soaked in SA solution for 12 hrs and after drying, were placed in Petri dishes containing 7 ml of polyethylene glycol solution. The results showed that drought stress inhibited all of the germination characteristics measured. Primed seeds with 0.00001 mM SA had the highest germination percentage and rate, and radicle and plumule lengths. The 0.00001 m M SA concentration had the highest germination percentage under high stress (-9 bar) and the highest germination rate under medium stress (-6 bar). Low concentrations of SA had more positive effects on wheat germination than high concentrations.

Xiao *et al.* (2016) worked with plants of spring wheat (*Triticum aestivum L.* cv. Vinjett). They exposed the plant to moderate water deficit at the vegetative growth stages six-leaf and/or stem elongation to investigate drought priming effects on tolerance to drought and heat stress events occurring during the grain filling stage. Compared with the non-primed plants, drought priming could alleviate photo-inhibition in flag leaves caused by drought and heat stress episodes during grain filling. In the primed plants, drought stress inhibited photosynthesis mainly through decrease of maximum photosynthetic electron transport rate, while decrease of the carboxylation efficiency limited photosynthesis under heat stress. The higher saturated net photosynthetic rate of flag leaves coincided with the lowered non photochemical quenching rates in the twice-primed plants under drought stress and in the primed plants during stem elongation under heat stress. Compared to the non-priming treatment, drought priming either applied once or twice alleviated the grain yield reduction by drought stress during grain filling, and priming during the stem elongation stage alleviated yield loss by heat stress at grain filling. The higher concentration of abscisic acid in primed plants under drought stress could contribute to higher grain yield compared to the non-primed plants.

Nasir *et al.* (2019) conducted a pot trial to study the effect of various priming agents to mitigate drought stress in cotton. Two water levels i.e. 100% field capacity (control) and 70% field capacity (drought stress) were maintained. Sowing materials (seeds) were primed with water, benzyl amino purine (BAP) moringa leaf extract (MLE), calcium chloride ($CaCl_2$) and their performance was compared with non primed seeds (control). Bi factorial randomized complete block design was used. The results revealed that all parameters under observation were significantly higher in well watered pots than in water stressed pots. All priming agents produced better results than control, however, BAP priming proved to be the most promising. Under drought condition; highest emergence index (0.85) was computed for MLE primed seeds. $CaCl_2$ primed seeds took least time to germinate (MGT) (13 days). Maximum no. of bolls per plant under control (8.50) and drought condition (4), highest bolls weight per plant under control (22.1 g), lint weight (9.56 g) and seed weight (11.21 g) were observed for BAP priming.

An experiment was done by Qi Zhang and Kevin (2014). Glycinebetaine (GB) seed priming enhances stress tolerance in various plants during the germination and seedling growth stage. In this study, GB at 5 to 50 m M was used to prime seeds of six turfgrass species to evaluate the potential of GB priming in enhancing tolerance to drought, salinity, and sub-optimal temperature during germination. Stress tolerance was determined as relative final germination percentage (FGP) and daily germination percentage (DGP), expressed as percentage of germination under stress conditions compared with the control treatment (i.e. unprimed seeds germinated under non-stress condition) for each species. Daily germination percentage was more sensitive to stress than FGP. Perennial ryegrass (*Lolium perenne* L.) showed high tolerance to drought, salinity, and chilling temperatures (5 and 10 8C below optimal germination temperature) followed by tall fescue (*Festuca arundinacea* Schreb.) and creeping bentgrass (*Agrostis palustris* L.), whereas kentucky bluegrass (*Poa pratensis* L.), bermudagrass [Cynodondactylon var. dactylon (L.) Pers.], and zoysiagrass (*Zoysia japonica* Steud.) were stress sensitive. Kentucky bluegrass and bermudagrass showed higher germination at 10 mM GB under temperature stress and drought and temperature stresses, respectively; however, other grasses showed limited responses to seed priming.

An experiment was done by Jira-Anunkul and Pattanagul (2020) in greenhouse to find out the effect of priming in rice. This study investigated the effects of seed priming with H_2O_2 on growth, some physiological characteristics and antioxidant enzyme activities in rice seedling under drought stress. Rice (*Oryza sativa* L.) cv. 'Khao Dawk Mali 105 seeds were primed with 0 (distilled water), 1, 5, 10, and 15 mM H_2O_2 and grown for 21 days. The results showed that priming with low concentrations of H_2O_2 improved plant growth and biomass as well as relative water content, malondialdehyde content, electrolyte leakage. Priming with H_2O_2, however, had no beneficial effect on chlorophyll content, proline and leaf total soluble sugar. Seed priming with appropriate levels of H_2O_2 also enhanced antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX).

Laghari *et al*. (2016) reported that seed priming is a controlled hydration method in which seeds are soaked in water or low osmotic potential solution for a point where germination related metabolic exercises start in the seeds, however radical development does not happen. During seed priming, it was found effective for legumes that is, yields of legume harvest were increased impressively by priming seeds before sowing. The maximum mean seed germination (86.78%) was recorded at Hydro-priming period 4 hours, whereas the lower seed germination (68.88%) no priming in mungbean.

Kumar *et al*. (2017) reviewed that osmo-priming treated seed showed significantly higher germination percentage in PEG at 20% followed by mannitol 4% in chick pea.

It was informed by Kaya *et al*. (2006) seed priming had an important result on increasing of germination percent; germination speed and seedling dry weight of sunflower. Priming also decreased abnormal seedling in drought stress.

Seed priming boosts rapidity and uniformity of germination (Khalil *et al*., 2010; Khan *et al*., 2008; Heydecker *et al*., 1975) through inducing several chemical alterations in the seed. That alterations are obligatory to begin the germination, such as breaking of dormancy, hydrolysis or mobilization of inhibitors, imbibition and enzyme activation. Some or all of these ways that lead the germination are faster by priming and continue following the re-desiccation of the seeds (Asgedomand Becker, 2001).

Hydropriming and osmo-priming treatments on shoot length provide significant variation. 20% Polyethylene glycol (PEG) for 24 hr (13.14cm) shows better effect on rest of the treatments except at 100 ml distilled water for 12 hr (12.11 cm) and 20% Polyethylene glycol (PEG) for 12 hr (12.77 cm) on pea (*Pisum sativum*) experimented by (Singh *et al*.,2017).

Chitosan treatment of wheat seeds induced resistance to certain disease and improved seed quality (Reddy *et al*., 1999). Seed soaked with chitosan increased the energy of germination, germination percentage, lipase activity, and gibberellic acid (GA_3) and indole acetic acid (IAA) levels in peanut (Zhou *et al*., 2002). The results showed that the chitosan priming increased the chilling tolerance of maize seedlings demonstrated by improving germination speed and shoot and root growth and maintaining membrane integrity and higher activities of anti-oxidative enzymes. The 0.50% chitosan seems to be a suitable concentration for seed priming it significantly increased seedling growth, root dry weight and root length as compared to control.

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

2.2.2 Effect of priming on yield and yield contributing parameters

Laboratory tests and a field experiment were carried out by Kazem and Golezani (2011) to evaluate the effects of priming methods on seed invigoration and field performance of soybean (cv. 'Zan'). The field experiment was arranged as split plot based on RCB design with three replications. Irrigation treatments (I_1, I_2 and I_3: irrigation after 70, 110 and 150 mm evaporation from class A pan) and priming methods (water, 3% KH_2PO_4 and 3% KNO_3 for 8 h at 15±1°C) were allocated to main and sub-plots, respectively. Germination percentage, seedling dry weight and field emergence percentage decreased, but mean emergence time increased, due to seed priming. Grain yield under severe water deficit was 29.32% less than that under normal irrigation. Pods per plant, grains per plant and grain yield per plant were significantly enhanced as a result of low stand establishment caused by seed priming. Consequently, biological and grain yields per unit area and also harvest index were statistically similar for plants from primed and unprimed seeds.

A glass house experiment was done by Kareem (2019) with the use of solutions of osmotic salts and plant hormones in rice variety MR219. Data on germination percentages, height, number of tillers and productive tillers, tiller efficiency and yield were taken. In both osmopriming and hormonal priming treatments, the highest number of tillers and productive tillers were from pre-germination. The tallest plants from osmopriming were from 150mM treatment, while 50 ppm GA3 had the tallest in hormonal priming. The highest tiller efficiency for osmopriming was from 150mM and and 200mM sodium chloride, while in hormonal priming it was 200 ppm salicylic acid. For yield per panicle in osmopriming, it was 50mM and 100mM magnesium chloride that had the highest, while in hormonal priming it was 200 ppm methyl jasmonate. Finally, the highest grain yield per hill was produced by 200 ppm methyl jasmonate in hormonal priming, while 50Mm magnesium chloride had the highest yield in osmotic priming. So, it is concluded that the use of 200 ppm methyl jasmonate and 50 Mm magnesium chloride could be used as potential hormonal priming and osmopriming, respectively, for yield improvement of MR219 rice in Malaysia.

An investigation was carried out at experimental farm by Shete (2018). Different priming treatments were given before one day of sowing with seven treatments and three replications such as hydropriming, osmopriming, halopriming and control. Results indicated relatively higher mean performance of hydropriming for one hour in yield and yield contributing trades such as days to field emergence, number of pods per plant, seed yield per plant, seed yield per ha, test weight and harvest index.

A field experiment was conducted by Ali (2018) to measure the impact of seed priming and nitrogen levels on maize yield and nitrogen uptake. Nitrogen at the rate of 0, 75 and 150 kg ha-1 were applied at three split doses, such as at sowing, knee and tasseling stage along with dry seed, water soaked and seed primed with potassium di-hydrogen phosphate (KH_2PO_4) at the rate of 0.2% Phosphorous concentration. Seeds were soaked for 12 hours and then air dried for 30 minutes. Maize biological yield, grain yield, stover yield, grains per cob, thousand grain weights, harvest index and total N uptake were significantly ($P \leq 0.05$) affected by seed priming and nitrogen levels. Maximum values of mentioned parameter were recorded at 150 kg N ha^{-1} in seeds primed with 0.2% phosphorous solution. Minimum values of all parameters were noted for control. P priming enhanced total N uptake by 34% than the dry seed. . Thus it is concluded that use of nitrogen at 150 kg ha^{-1} along with 0.2% P priming gives maximum maize yield and high N uptake.

The study was conducted by Hafieez (2010) to evaluate the on-farm assessment of direct seeded rice by employing different priming techniques such as on-farm priming, hydropriming, hardening, and osmohardening with $CaCl_2$ and KCl. Untreated seeds were taken as control. Among all the seed priming techniques, osmohardening with $CaCl_2$ improved the stand establishment, allometric response, agronomic traits, yield, and quality of harvested paddy compared with other priming techniques, and non-primed control in direct seeded culture. Improved crop stand as indicated by lower values of time to emergence and higher values for emergence index and final emergence, higher crop growth rate, and improved plant height, tiller numbers, and straw and kernel yield with high harvest index were recorded from osmohardening with $CaCl_2$. In addition, seed priming treatments also improved the kernel quality. Osmohardening with $CaCl_2$ was the best way to reduce sterile spikelets, abortive and chalky kernels, and improve kernel length. However, none of the seed priming techniques could improve the number of kernels per branch, 1000-kernel weight, kernel width, and kernel water absorption ratio. Moreover, improved phosphorus, calcium, and potassium contents were also observed from osmohardening with $CaCl_2$ followed by KCl. Osmohardening with $CaCl_2$ can therefore be employed for better crop stand, growth, yield, and quality in direct seeded rice.

Three experiments were carried out by Hamza (2018). First experiment was conducted in laboratory according to Complete Randomized Design (CRD) with four replicates. Second and third experiments were conducted at field according to Randomized Complete Block Design (RCBD) with four replicates for each one. Same two factors were studied at each experiment. First factor was seed priming by seed soaking for 10 hours in distilled water, solvents of GA_3, KCl and thiamine (300, 40 and 30 mgL^{-1}, respectively) and non-primed seed. Second factor was three cultivars of sorghum (Inqath, Kafier and Rabeh). The aim was to improve field emergence and grain yield of sorghum under wide range of environmental conditions by using seed priming. Data were analyzed at $P<0.05$. The results showed significant effects of seed priming, cultivars of sorghum and their interaction on most traits under this study. A significant superiority of interaction was found between thiamine and Rabeh on length of plumule and dry weight of seedling at laboratory experiment. A significant superiority of interaction was found between thiamine and Rabeh on percentage of field emergence and total grain yield during spring season. A significant superiority of interaction was found between KCl and Inqath on percentage of field emergence and total grain yield during fall season. It can be concluded that technique of seed priming is effective to improve field emergence and grain yield of sorghum under wide range of environmental conditions

Two drought stress treatments were applied on *Vicia faba* (cv. Giza 716) seedlings (14-days-old) by Wadad and Kasim (2019). Watering was not done for 14 days (D_1) or 22 days (D_2) and left to grow on clay-sandy soil (2:1 w/w) till yield stage. The impact of seed priming by presoaking in the extract of carrot root (Cr), garlic cloves (G) or ascorbic acid (AA) on the alleviation of the hurtful influence of drought stress was studied. Results showed that drought stress (D_1 and D_2) caused reduction in glucose, sucrose and starch contents of the increased seeds and in the yield also, while the total soluble protein content was increased.

Mangena (2020) Carried out an experiment. In this study, seeds of soybean cultivar LS678 and TGx1835-10E were pretreated with an optimum level of benzyladenine

($4.87 mgL^{-1}$) before sowing into pots containing pasteurised mixture of vermiculite and sand. Plants were grown up to V3 stage before exposure to moderate and severe drought stress. According to the results, germination was rapid in hydroprimed seeds than BA primed seeds, which took longer to emerge. However, growth, yield and biomass of BA primed plants were increased (number of branches per plant- 7.32, flowering-87.6%, 100 seed weight- 22.6 g, overall biomass fraction- >40.5%) compared to plants developed from hydroprimed seeds (number of branches- 3.61, seed weight- 19.2 g, biomass-under similar growth conditions. This study indicated that, hormonal seed priming with BA reasonably enhanced soybean growth, particularly root biomass, flowering and fruiting. These effects further suggest that BA may play a significant role in improving drought tolerance in soybean.

2.2 Effect of priming on different crop genotype in drought condition

A pot experiment was conducted by Saha *et al*. (2019) to study the effect of drought stress on growth and accumulation of proline in five rice varieties namely BRRI dhan 30, BRRI dhan 32, BRRI dhan 34, BRRI dhan 38 and BRRI dhan 56 and to characterize them on the basis of their behavior of drought tolerance. Drought stress caused the decrease of growth like root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, Shoot dry weight, the ratio of root-shoot length. Among the rice varieties, BRRI dhan-56 showed the lowest decrease of growth of plant. BRRI dhan-56 showed the least decrease of water content in both root and shoot. On the other hand, the accumulation of proline was increased in five rice varieties under stress. BRRI dhan-56 showed the highest (3.7- folds) increase in the accumulation of proline in leaf under stress.

Cokkizgin *et al.* (2019) carried out an experiment to find out the effect of Mannitol ($C_6H_{14}O_6$) on the germination of *Vicia faba* L seed was investigated. Broad bean (*Vicia faba* L.) genotypes, Sevilla and Emiralem seeds were treated in various levels of Mannitol ($C_6H_{14}O_6$) (1%, 2.5% and 5%) and distilled water (2.5µs/cm) at 20°C. The experiment was arranged under completely randomized design (CRD) with three replicates in Petri dishes. In the research Seedling Length (SL), Germination Percentage (GP %), Seed Vigor Index (SVI) and Angular Transformation Value (Arcsin) was used. Accordingly, Sevilla broad bean cultivar has higher values for SL, SVI and Arcsin parameters however has a lower value for GP parameter. It's observed that 1% mannitol ($C_6H_{14}O_6$) application has the highest value for both parameters.

Buzdar *et al.* (2019) conducted an experiment to find out Priming effect of silicon sources (silica gel and sodium silicate) on the seeds of four wheat cultivars of Balochistan was studied to determine their effectiveness in increasing relative salt tolerance. The study depicts that all priming treatments of silicon sources (except sodium silicate 2%) enhanced germination and reduced mean germination time (MGT) of seeds in comparison to the control i.e. hydropriming. After germination, the young seedlings were grown in hydroponics in Hoagland's culture solution under controlled conditions in non-saline and saline (100 mM NaCl) environments. All the growth parameters (root and shoot length, fresh and dry weights of plants and chlorophyll content of leaves) severely reduced in hydroprimed seeds under saline environments, although increased by priming seeds with silicon sources. Thus silica compounds have potential effects to break the seed dormancy and improve the growth of wheat under salinity stress.

Langeroodi and Noora (2017) conducted a study, This study, consisting of two seprate experiments was conducted to evaluate the role of various seed priming in mitigating the adverse effect of water deficit on germination, biochemical and yield parameters of two soybean cultivars viz. DPX, drought tolerant, and Wiliams, drought sensitive. Seeds either subjected to hormonal priming, osmopriming, halopriming and hydropriming; dry seed being as control. Crops were subjected to 2 and 3 different moisture regimes in growth chamber and field conditions, respectively. Under water deficit, the germination and field performance of tested soybean cultivars was hampered. Seed priming treatments improved the physiological, biochemical, yield and yield parameters under both the optimal and water deficit. Hydropriming for 12 h and hormonal priming with gibberlic acid for 14 h with cultivar DPX was best in this regard.

Samota *et al*. (2017) conducted an experiment, rice varieties in this study showed differential responses for proline accumulation and enzymatic activities measured. The scavenging system in drought tolerant variety nagina-22 exhibited higher CAT, POD and SOD activities, than in the drought susceptible variety (pusa sugandh-5) drought-susceptible variety, PS-5 was markedly affected even at the lowest drought level used. The activity of antioxidant enzymes CAT, POD and SOD in the drought tolerant and drought susceptible varieties increased markedly during drought stress. Drought tolerance of the rice varieties associated with buildup of antioxidant enzymes and proline. Among the biotic elicitors, MJ was found to be the most effective priming reagent, followed by PBZ.

A field experiment was conducted during the dry by Senapati *et al.* (2019).Seed priming was employed to enhance the anaerobic germination potential in two near isogenic rice lines (IR64 and IR64-Sub1). Under natural field condition, oxygen concentration in floodwater never dropped to zero. It varied from 3.0 to 5.2 mg L^{-1}. Seed priming with calcium peroxide and seed soaking with water followed by sun drying showed greater beneficial effects as compared to potassium nitrate. Seed priming with sodium peroxide found deleterious. Cultivar IR64-Sub1 responded well due to seed priming as compared to cultivar IR64 under submergence. To improve anaerobic germination potential in rice, seed soaking with water followed by sun drying found economicalas compared to other methods of seed priming as this technique was not linked with additional investment in chemicals and was more environments friendly.

Anny *et al.* (2015) tested combinations of seed priming (unprimed control, water priming, P-priming), and inherent seed P concentrations in contrasting rice genotypes (DJ123, Sadri Tor Misri), and two near isogenic sister lines of IR74 with (+Pup1) and without (−Pup1) the Pup1 QTL. Treatment effects on germination were studied in Petri dishes, while seedling growth and P accumulation were assessed using pots with P deficient soil. Germination was less than 75% in seeds with low seed P content. Seed priming with both water and P enhanced germination and seedling growth. In plants growing from high P seeds, water priming outperformed P-priming. In Sadri Tor Misri with low seed P, we observed a tendency for better performance in some parameters when P-primed. While the presence of the Pup1 QTL in IR74 increased shoot biomass and total root length, these effects could be further enhanced by water priming.

Wedad *et al.* (2012) conducted an experiment, aimed to improve wheat growth under drought stress conditions through priming with beneficial bacteria considered as plant-growth promoting bacteria (PGPB). Two bacterial strains, Bacillus amyloliquefaciens 5113 and Azospirillumbrasilense NO_40, were used to prime the wheat cv. Sids1. Bacteria-treated plants showed attenuated transcript levels suggesting improved homeostatic mechanisms due to priming. The present study reports on the ability of certain PGPB to attenuate several stress consequences in plants which strongly supports the potential of such an approach to control drought stress in wheat.

Nowsherwan *et al*. (2018) done an experiment, they designed to evaluate the changes in different physiological traits such as proline content, cell membrane stability, relative water content and chlorophyll content under drought stress in sixteen wheat genotypes. Wheat genotypes (99FJ-03, Marvi-2000, WC13, WC-24, WC-19, Faisalabad-85, Kaghan, Bahawalpur, Zarlashta, Punjab-96, Shafaq, Maxi-pak, WC-20, Chenab-70, AUR-0809, Chakwal) were sown during rabiseason of 2013-14 following randomized complete block design with three replications. Among tested wheat genotypes, Maxi-Pak was found to be potential variety for relative water content, cell membrane stability, chlorophyll content and yield.

An experiment was done by Aneela *et al*. (2017) and the objective of this study was to explore the process of better wheat growth and development under premises of drought. Five wheat cultivars were used to study the effect of drought and to copedrought with hormonal priming. Wheat seeds were primed in 10-4 M concentration of Salicylic acid (SA) and Gibberellic acid (GA), control was also used. The highest yield reductions were found in CHAKWAL-50 under normal condition. Hormonal priming improved yield under normal condition and overcome the effect of stress under drought. Priming increased the grain yield in CHAKWAL-50 and other genotypes. PAKISTAN-13 had the highest harvest index under normal condition and also under stress condition. PAKISTAN-13 and FSD-08 had the highest stress tolerance index while SA priming increased the stress tolerance index in CHAKWAL-50. Total sugar and protein contents were increased under drought. Seed priming was able to overcome stress and increased yield. Stress tolerance index was also improved by using hormonal priming. Genotype response was different under normal and drought stress.

Alireza *et al.* (2020) conducted an experiment. Present research was performed to determine the effect of drought stress on several physiological and agro-morphological traits in 17 durum wheat genotypes under two conditions (control and drought) over two years. The results of analysis of variance indicated that the various durum wheat genotypes responded differently to drought stress. Drought stress significantly reduced the grain filling period, plant height, peduncle length, number of spikes per plot, number of grains per spike, thousand grains weight, grain yield, biomass, and harvest index in all genotypes compared to the control condition. The heatmap-based correlation analysis indicated that grain yield was positively and significantly associated with phenological characters (days to heading, days to physiological maturity, and grain filling period), as well as number of spikes per plant, biomass, and harvest index under drought conditions. The yield-based drought and susceptible indices revealed that stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), and harmonic mean (HM) were positively and significantly correlated with grain yields in both conditions. Based on the average of the sum of ranks across all indices and a three-dimensional plot, two genotypes (G9 and G12) along with the control variety (G1) were identified as the most tolerant genotypes. Among the investigated genotypes, the new breeding genotype G12 showed a high drought tolerance and yield performance under both conditions.

Somasundaram and Bhaskaran (2017) conducted an experiment and they take three rice genotypes each in low (AC 35024, AC 39021, Gangavati Sona) and high (Jaya, PS 267 and AC 39004) longevity were primed with water (hydropriming), 4% Pseudomonas fluorescens, 20% Azospirillumsp, 50 mM NaCl, 20% PEG 8000, 75% Coconut water, 2% Pulse sprout extract and 0.5% Nutrigold for 12 hours. In general, high longevity rice seeds responded well to the priming treatments compared to low longevity seeds. The seeds of PS 267 primed with 4% P. Fluorescens recorded highest germination (99%), root length (20.5 cm), shoot length (8.5 cm), dry matter production (10.5 mg/seedling), vigour index I (2868) and II (1037) compared to all other genotypes. Among the priming treatments, seeds primed with 4% P. Fluorescens for 12 h recorded higher seed germination and vigour followed by 2% pulse sprout extract, irrespective of seed longevity nature and genotypes.

2.3 Effect of priming duration on different crop

Laboratory and field trials were carried by Christos (2019) for two years to study the effect of hydro-priming on faba bean germination and field performance in spring sowing. In laboratory trials, the effects of hydro-priming for 0, 8, 16, 24, 36, and 48 h on final germination percentage, germination speed, Timson's germination index, mean germination time, mean daily germination, synchronization index, and seedling vigor index were studied. All hydro-priming treatments improved germination parameters of faba bean seeds, except for final germination percentage and mean daily germination compared with non-primed seeds. Averaged over priming duration treatments, hydro-priming improved germination speed by 16.2%, germination synchrony by 20.7%, and seedling vigor index by 13.4%. All hydro-priming durations improved germination synchrony, while hydro-priming for 8, 16, and 24 h provided the highest values of germination speed (2.56, 2.58, and 2.37 seeds day^{-1}, respectively). Hydro-priming for 8 and 16 h provided the lowest values of mean germination time (5.81 and 5.96 days, respectively). In field trials, hydro-priming periods of 0, 8, 16, and 24 h were compared. On average, seed hydro-priming did not affect significantly seedling emergence 14 days after sowing in the first year, but significantly improved seedling emergence by 34.4% in the second year. No significant effect of seed hydro-priming was noted in the number of plants at 28 and 35 days after sowing. Seed priming for 8 h resulted in higher fresh weight at anthesis by 22.3% and 8.6% in the first and the second year, respectively, than the non-primed control. Similarly, seed priming for 8 h provided higher seed yield by 12.0% in the first year and by 5.9% in the second year compared with non-primed control.

Laboratory and field studies were carried out by Singh (2014) to study the effect of seed osmopriming duration on the germination, emergence, and growth of cowpea seeds. Treatments consisted of three osmopriming duration (soaking in 1% KNO3 salt for 6, 8, and 10 hrs), one hydroprimed control (10 hr), and an unprimed control. These five treatments were laid out in a completely randomized design (CRD) replicated four times. The results showed that osmopriming with KNO_3 for different durations was at par but was superior to unprimed treatments in terms of seed germination, emergence, plant height, and dry matter accumulation at 3 weeks after sowing. From this study, it can therefore be concluded that seeds of cowpea could be primed (both hydro and osmopriming) for increased performance. However, osmopriming with

KNO_3 salt (soaked in 1% KNO_3 salt solution and dried before sowing) for 6 hours could result in greater seed germination and seedling height than hydropriming.

The research work was carried out by hammad *et al*. (2014) to find out the effect of hydropriming methods on maize seedling emergence. The hydropriming methods were- T1 (Non-priming), T2 (14 hours soaking + drying + storing), T3 (18 hours soaking + drying + storing), T4 (22 hours soaking + drying + storing), T5 (14 hours soaking + surface drying), T6 (18 hours soaking + surface drying), and T7 (22 hours soaking + surface drying). Effect of different hydropriming methods on seedling emergence performance of maize was evaluated at two moisture levels viz., 30% and 60% moisture of saturated sand in the experiment. Germination percentage, germination index and mean germination time were influenced significantly by hydropriming methods. The highest germination percentage, germination index, and lowest mean germination time were recorded with T6 (18 hours soaking + surface drying).

A field experiment was conducted by Pandit *et al.* (2016) on medium black soils to know the effect of seed priming on growth and productivity of chickpea. The results revealed that, seed soaking in vermi wash for 8 hours resulted in early emergence (33.33 plants m-2), higher plant height (36.8 cm), total dry matter production (24.8 g plant^{-1}), required minimum duration for fist (32.2 days) and 50% flowering (45.2 days). This treatment also exhibited maximum number of pods plant^{-1} (37.3), pod weight plant-1 (83.93 g), seed weight plant^{-1} (10.48 g), 100 seed weight (19.87 g), seed yield (1341 kg ha-1), haulm yield (3353 kg ha^{-1}) and harvest index (28.57). It may be inferred from the present investigation that, seed soaking in vermiwash for 8 hours may be recommended under dry land conditions as it resulted in early emergence, required minimum duration for 1st and 50% flowering, maximum number of pods plant^{-1}, pod weight plant^{-1}, seed weight plant^{-1}, 100 seed weight, seed yield and haulm yield.

The experiments were conducted by Vaibhav Pradhan (2017) to study Influence of halopriming and organic priming on germination and seed vigor in Black gram (*Vigna mungo* L.) in the post-graduation experiment laboratory of Seed Science at the Department of Genetic and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar

Pradesh. The seeds were treated with un-soaked seed (control), Hydro-priming (soaked with distill water for 12 hrs), Organic priming (Cow urine, Coconut water), Halopriming with KNO_3, KC_l, and $CaSO_4$ (1% solution) soaked for 12 hrs, on seed of Blackgram. KCl @1% primed seed recorded higher germination per cent (83.25%),energy of emergence (78.75), seedling length (40.30 cm), seedling dry weight (0.452 gm/10 seedlings), vigor index I (3358.93) and vigor index II (37.66). The treatment interactions were significant & the seeds treated with KC_l followed by KNO_3 recorded numerically higher values compared to control.

A lab investigation was carried out by Faijunnahar *et al.* (2017) to find out the suitable pre-sowing priming time on the germination, seedling growth and water relation behavior of four wheat genotypes viz., BARI Gom 28, ESWYT-5, ESWYT - 6 and ESWYT-7. The seeds of wheat genotypes were primed with 10% PEG solution for 3 h, 6 h, 9 h, 12 h and 15 h. The results of the experiment revealed that, among 4 wheat genotypes ESWYT-5 wheat genotype performed the best in most of the germination, seedling growth and water relation behaviors of wheat under all priming times followed by ESWYT-6 and BARI Gom 28 and ESWYT- 7 showed consistently poor performance. The germination, seedling growth and water relation behaviors' value of wheat genotypes increased with increasing priming time up to 9h and then gradually decreased. She also concluded that priming time might help to increased enzymatic activities of seed which trigger the vigorous plant growth and in consequence increased the shoot length of wheat; on the other hand over priming time might facilitate the ageing of seed which resulted loose the potentiality for better germination, growth and development of seedling. Similar findings were observed by Ajirloo *et al*. (2013); Dastanpoor *et al.* (2013); Moradi *et al.* (2012); Sadeghi *et al.* (2011);Yari *et al.* (2010) and Ahammad *et al.* (2014) who observed that the highest germination percentage in cv. Azar-2 was recorded when the seeds primed with 20% PEG solution for 12 h.

Afzal *et al.* (2006) carried out an experiment to find out the influence of hormonal priming with abscisic acid (ABA), salicylic acid (SA), or ascorbic acid on wheat (*Triticum aestivum* cv. Auqab-2000) where seeds are presoaked for 12 hours. The results showed that seeds of all hormonal priming treatments decreased the electrolyte leakage of steep water as compared to that of non-primed seeds even after 12 h of soaking. McDonald (1980) also observed that an increase in electrolyte leakage by 10

17 ppm ABA at all soaking periods, which was probably due to the loss of ability to reorganize cellular membranes rapidly and completely.

Osmo priming with PEG-6000 and solid matrix priming with press mud with 12hrs duration increase germination, final emergence, root and shoot length and enhanced the fresh and dry weight of roots and shoots of wheat (Abbas *et al*., 2018).

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from December 2019 to February 2020 to study the mannitol induced drought tolerant capacity of wheat under drought stress condition. The materials and methods describes a short description of the experimental site, climatic condition of the culture room, experimental materials, treatments and design, methods of the study, data collection procedure and data analysis. The detailed materials and methods which were used to conduct the study are presented below under the following headings:

Description of the experimental site

3.1 Location

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

3.1.1. Duration of the study

The experiment was conducted during the period from December 2019 to February 2020.

3.1.2. Laboratory condition

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital tharmo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study period of the culture room was 17^0C to 27^0C respectively and the average minimum and maximum relative humidity were recorded 54 % and 75 % respectively.

3.2 Test crops

BARI Gom - 31 was used for this experiment. Seeds were collected from Wheat Reasearch Cenre, Dinajpur Bangladesh. The collected wheat genotype was free from any visible defects, disease symptoms and insect infestation.

3.3 Experimental materials

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

3.4 Chemicals for seed priming

Mannitol ($C_6H_{14}O_6$) and distilled water were used for priming wheat genotype. Polyethylene Glycol (PEG) 6000 was used for inducing drought stress.75% alcohol was used for seed treating.

3.5 Experimental design

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

3.6 Experimental treatments

The experiment comprised of

i) Six levels of priming agent concentrations viz. 0 (control), water, 1%, 2%, 3% and 4%, mannitol solution

ii) Five levels of drought stress viz. 0 %, 5%, 10%, 15% and 20% PEG.

3.7 Steps of the experiment

This experiment was completed in two steps. In the 1st step, the best mannitol concentration was investigated through the response of genotype and in 2^{nd} step the best result under drought stress condition.

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

3.7.1.1 Treatments:

Single factor experiment considering one wheat genotype with six levels of seeds priming (Control, water, 1%, 2%, 3% and 4% mannitol) solutions for 9 hours based on the findings of Faijunnahar *et al*. (2017).

i) P_0 = Seeds without priming
ii) P_1 = Seeds primed with distilled water
iii) P_2 = Seeds primed with 1% mannitol solution
iv) P_3 = Seeds primed with 2% mannitol solution
v) P_4 = Seeds primed with 3% Mannitol solution
vi) P_5= Seeds primed with 4% mannitol solution
The wheat genotype was BARI Gom 31

3.7.1.2 Surface treatment

Seeds were firstly treated with 75% alcohol solution for 5 min for surface sterilization. These sterilized seeds were rinsed with distilled water for 3 times to remove the residual effect of alcohol from the seed surface. After that seeds were dried in room temperature to regain the normal weight

3.7.1.3 Priming solutions

0 (control), distilled water (DW), and 1%, 2%, 3%, 4% mannitol solutions were used as priming solutions.

3.7.1.4 Preparation of priming agents

a) Solutions Mannitol solutions (1%, 2%, 3%, 4%): For preparing 1% mannitol solution, 2.5g of mannitol was dissolved in 250 ml of DW. Similarly, 5g, 10g and 15g mannitol were dissolved in 250 ml of DW to prepare 2%, 3%, and 4% of mannitol respectively.

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

3.7.1.5 Priming technique

Both hydro and osmopriming techniques without mannitol and with mannitol were applied to our experiments. The surface sterilized seeds were sub-sampled into two groups. One of the subsample was considered as control (unprimed) and also used for hydropriming with distilled water and the other sub-samples were used for priming with priming chemicals (osmopriming). For osmopriming seeds were divided into four sub-samples and treated with mannitol of 1% 2%, 3%, and 4%, concentrations for 9 hours (Faijunnahar *et al*., 2017).

Priming was done in different plastic and glass containers covered with lid stop revent evaporation loss. All priming seeds were taken from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for several times and taken it to blotting paper for drying lightly and finally air dried near to gain original weight (Umair *et al*., 2011) in room temperature for 24 hours for back to the original level of moisture.

3.7.1.6 Germination of seeds

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

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

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

3.7.2.1 Treatments: Single factor experiment with primed seeds of one wheat genotype under five levels of PEG concentration (0%, 5%, 10%, 15%, and 20% PEG) was done.

3.7.2.2 Surface treatment

Seeds were firstly treated with 75% alcohol solution for 5 min for surface sterilization. These sterilized seeds were rinsed with distilled water for 3 times. After that seeds were dried in room temperature to regain the normal weight.

3.7.2.3 Priming solutions and time

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

3.7.2.4 Preparation of priming solutions

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

b) Preparation of stress solutions with PEG

In 250 ml of distilled water, 12.5g of polyethylene glycol (PEG) was dissolved for preparing 5% solution of PEG. Similarly, 25 g, 37.5 g, 50 g polyethylene glycol(PEG) was dissolved in 250 ml of distilled water for preparing 10%, 15%, 20%solution of PEG, respectively.

3.7.2.5 Priming technique

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

3.7.2.6 Germination of seeds

The germination test was done by placing randomly selected 30 seeds in 90-mm-diameter petri dishes on what man No.1. filter paper. Petri dishes containing primed and control seeds were watered with salt solutions as mentioned. In that case, whatman No.1 filter paper were used as growth media for germination. Experiment units (90 Petri dishes) were designed in a completely randomized design with five replications. During the test, filter papers in the Petri dishes were kept under saturated condition. Seeds were kept at room temperature 25±1°C under normal light to help germination for 10 days. Germination was occurred to have considered when radicles were 2 mm long (Akbari, Sanavy and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and this process continued up to 10 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were discarded during counting. At the end of germination test (10 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and length was measured and transferred to brown paper. Then these shoots and roots were dried in an oven at 75±2°C for 72hours.

3.8 Data collection

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

The following data were measured and weighted:

1. Rate of germination (%)
2. Shoot length (mm)
3. Root length (mm)
4. Shoot dry weight (mg)
5. Root dry weight (mg)
6. Fresh weight (mg)
7. Water saturation deficit
8. Relative water content (%)

9. Water retention capacity
10. Vigor index

3.9 Procedure of recording data

3.9.1 Rate of germination (%)

The number of germinated seeds was counted every day. Germination was recorded at 24 hrs interval and continued up to 10th days. More than 2 mm long plumule and radicle was considered as germinated seed.
The germination rate was calculated by using following formula of Othman *et al*. (2006).

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

3.9.2 Shoot length (mm)

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

3.9.3 Root length (mm)

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

3.9.4 Dry weight of shoot and root (mg)

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

3.9.5 Water saturation deficit

Water saturation deficit was recorded using following formula; (Baque *et al.,* 2002)
Water saturation deficit (WSD) = 100- Relative water content.

3.9.6 Relative water content (%)

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

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

3.9.7 Water retention capacity

Water retention capacity was measured by using following formula

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

3.9.8 Vigor index

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

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

3.10 Statistical Analysis

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

CHAPTER IV

RESULTS AND DISCUSSION

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

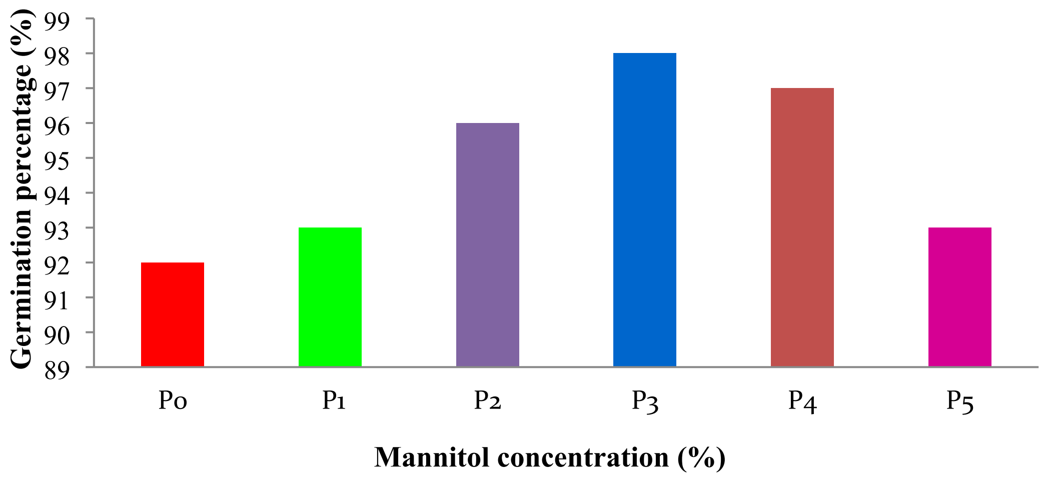
4.1 First experiment: Study on the effect of different concentrations of mannitol on the germination and growth behavior of wheat.

In this chapter, the results found from the first experiment regarding the effects of different concentrations of mannitol on the germination rate of the wheat variety have been presented.

4.1.1 Rate of germination

There was significant variation in terms of germination rate due to seed priming with mannitol at different concentration (Figure 1). Result shows that among the tested sample, the highest germination rate (98%) was found from seeds pretreated with 2% mannitol solution for 9 hours. The lowest germination rate (92%) was observed from without priming treatment followed by hydropriming and 4% mannitol solution for 9 hours. Dhakal and subedi (2020) found that priming with 2% mannitol inprove the seed germination in poaceae family (Maize). Preveously Elkoca (2014) studied that, osmopriming treatments, seeds treated with -0.5 bar solution of PEG and 1% solution of mannitol and also hyropriming increase the germination rate. Anoshah and Seyedeh (2020) reported that different types of seed priming have positive effects on germination, emergence, growth, yield as well as biochemical traits and quality of plants, it seems that seed priming could be promising approach for both saline and non saline condition. Seed priming facilitates in plant growth promotion and influences yield (Sandesh, 2020). It was reported that, the DBD plasma treatment could alleviate the adverse effects of drought stress on wheat seed germination and seedling growth; the germination potential and germination rate increased by 27.2%

and 27.6%, and the root length and shoot length of the wheat seedlings also increased (Qiao *et al.,* 2017).

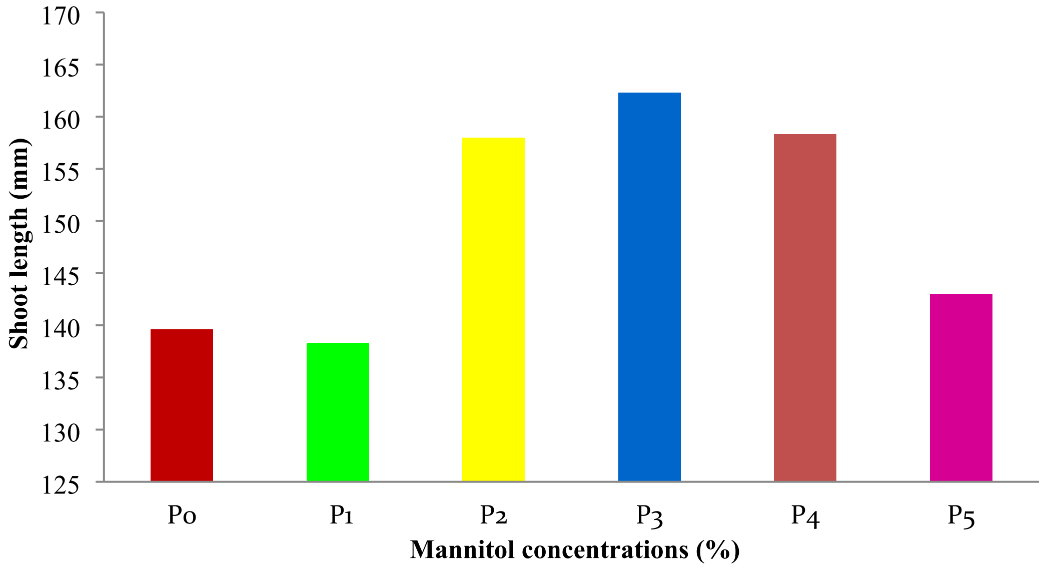


P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 1: Effect of different mannitol concentrations on germination percentages of wheat (BARI Gom - 31)

4.1.2 Shoot length (mm)

Significant variation was observed on shoot length among different concentration of mannitol including control treatment (Figure 2 and Appendix I). Results found thatthe maximum shoot length (162.30 mm) was recorded for primed seeds with 2% mannitol solution for 9 hours followed by (158.30 mm) primed with 3% mannitol. The lowest shoot length (138.30 mm) was recorded from primed with distilled water.The result is similar to (Afzal, 2008). Maximum root length and fresh and dry weights were obtained in Wheat plants raised from seeds primed with $CaSO_4$ followed by $CaCl_2$. It was reported that, all the growth parameters (root and shoot length, fresh and dry weights of plants and chlorophyll content of leaves) increased by priming seeds with silicon sources (Buzdar *et al*., 2019). Experimental results showed that the DBD plasma treatment could alleviate the adverse effects of drought stress on wheat seed germination and seedling growth; the germination potential and germination rate increased by 27.2 % and 27.6 % and the root length and shoot length of the wheat seedlings also increased (Qiao *et al*., 2017). preveous result showed that, seed priming with salicylic acid helps to improve root length, shoot length in wheat (Mahnoor, 2019).



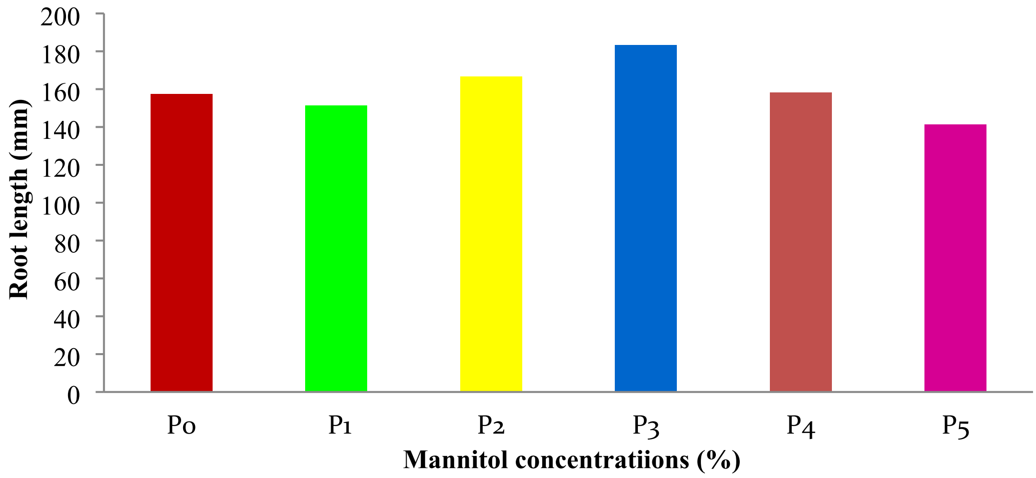
P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 2: Effect of mannitol concentrations on shoot length (mm) of wheat (BARI Gom - 31)

4.1.3 Root length (mm)

The wheat plant has two types of roots, the seminal (seed) roots and roots that initiate after germination, the nodal (crown or adventitious) roots. About six root primordia are present in the embryo. At germination, the primary root bursts through the coleorhiza, followed by the emergence of four or five lateral seminal roots. These form the seminal root system, which may grow to 2 m in depth and support the plant until the nodal roots appear. Nodal roots are associated with tiller development and are usually first seen when the fourth leaf emerges and tillering starts. Compared with the seminal roots, they are thicker and emerge more or less horizontally; when they first appear they are white and shiny (the 'white root' stage). Root length was significantly varied among the test genotypes primed with different concentration of mannitol including control (Figure 3). It was found that the maximum root length

(183.30 mm) was recorded for primed seeds with 2% mannitol solution for 9 hours followed by seeds primed with 3% mannitol (158.30 mm). The lowest root length (141.30 mm) was observed primed with 4% mannitol. Buzdar *et al.* (2019) also found similar results who observed. All the growth parameters (root and shoot length, fresh and dry weights of plants and chlorophyll content of leaves increased by priming seeds with silicon sources. Experimental results showed that the DBD plasma treatment could alleviate the adverse effects of drought stress on wheat seed germination and seedling growth; the germination potential and germination rate increased by 27.2 % and 27.6%, and the root length and shoot length of the wheat seedlings also increased (Qiao *et al*., 2017). It was also reported that, seed priming increased germination percentage, germination speed, seedling length, root-shoot ratio and decreased mean germination time wheat grass (Ali *et al*., 2012).

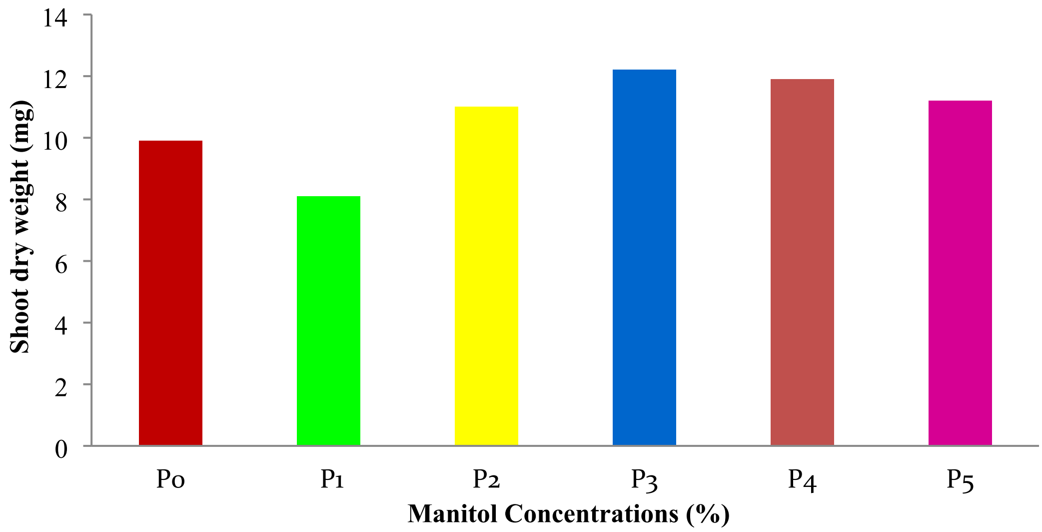


P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 3: Effect of Mannitol concentrations on root length (mm) of wheat (BARI Gom - 31)

4.1.4 Shoot dry weight (mg)

Statistically significant variation was found in case of shoot dry weight due to priming with different mannitol concentrations including control treatment (Figure 4). Dry weight of shoot was highest with 2% mannitol primed seeds for 9 hours priming time, it was observed that shoot dry weight decreased with the increasing mannitol concentration and also with control (without priming) and primed with distilled water. Results revealed that the highest shoot dry weight (12.20 mg) was recorded primed seeds with 2% mannitol solution for 9 hours priming treatment. The lowest shoot dry weight (8.10 mg) was observed from primed with distilled water. Previously reported that, Seed priming with ascorbic acid resulted in maximum final germination and emergence percentage (FGP and FEP), radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root dry weight, shoot dry weight and seedling dry weight compared to control (untreated seeds) in wheat (Khan *et al*., 2011). Hasan *et al*. (2016) reported that, Priming with 5% KCl for 54 hours showed the highest root length while 5% of the same solution for 24 hours showed the highest root dry mass in poaceae family (Rice).

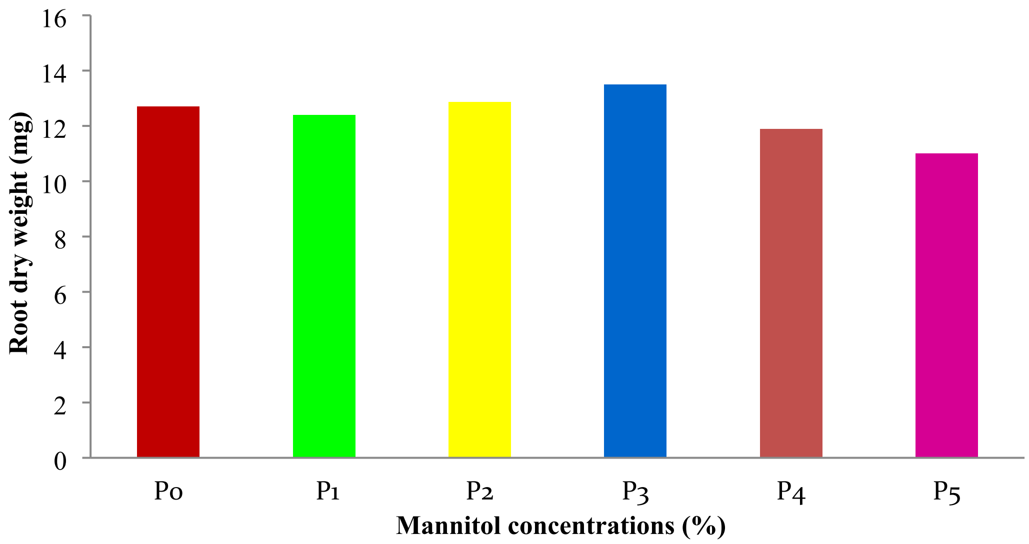


P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 4: Effect of mannitol concentrations on shoot dry weight (mg) of wheat (BARI Gom 31)

4.1.5 Root dry weight (mg)

Significant variation was found in terms of root dry weight due to priming with different mannitol concentrations including control (Figure 5). Results revealed that the highest root dry weight (13.50mg) was recorded primed with 2% mannitol solution for 9 hours followed by Seeds primed with 1% mannitol solution for 9 hours. This result is similar with (Afzal, 2008),Seed priming with ascorbic acid resulted in maximum final germination and emergence percentage (FGP and FEP), radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root dry weight, shoot dry weight and seedling dry weight compared to control (untreated seeds) in wheat. Hasan *et al.* (2016) reported that, priming with 5% KCl for 54 hours showed the highest root length while 5% of the same solution for 24 hours showed the highest root dry mass in poaceae family (Rice).

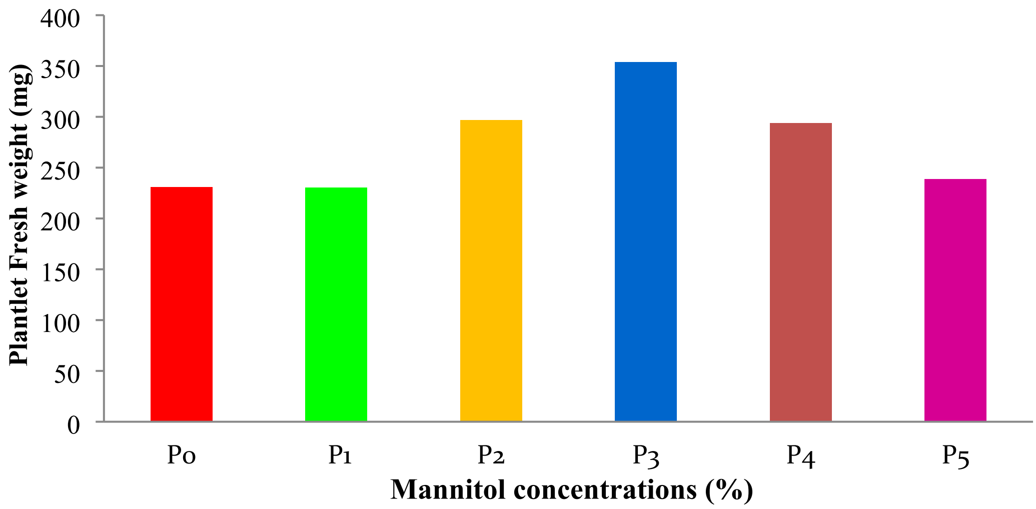


P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 5: Effect of mannitol concentrations on root dry weight (mg) of wheat (BARI Gom - 31)

4.1.6 Fresh weight (mg)

Significant variation was observed on fresh weight priming with different concentration of mannitol including control treatment (Figure 6). It was found that the maximum fresh weight (354 mg) was recorded primed seeds with 2% mannitol solution for 9 hours followed by 1% mannitol solution for 9 hours (296.80 mm). The lowest fresh weight (230.40 mg) was recorded for primed with distilled water. Preveous result shows, osmo priming with PEG-6000 and solid matrix priming with press mud with 12hrs duration increase germination, final emergence, root and shoot length and enhanced the fresh and dry weight of roots and shoots of wheat (Abbas *et al.,* 2018).

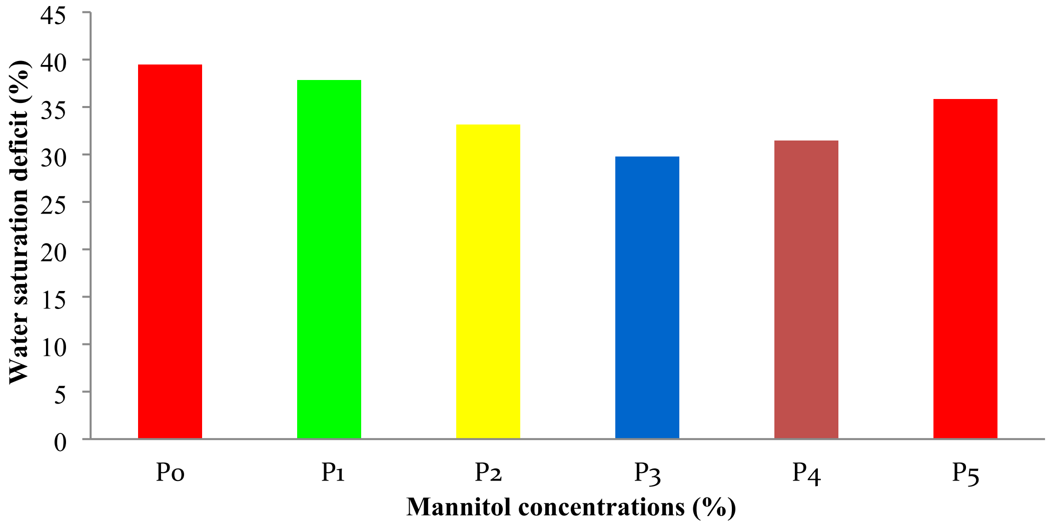


P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 6: Effect of mannitol concentrations on Plant Fresh weight (mg) of wheat (BARI Gom - 31)

4.1.7 Water saturation deficit

Significant variation was observed on water saturation deficit priming with different concentration of mannitol including control treatment (Figure 7). It was observed that the maximum water saturation deficit (39.490) was recorded for unprimed seed. The lowest water saturation deficit (29.750) was recorded for primed seed with 2% mannitol. This result was supported by previous findings of Faijunnahar *et al*. (2018) Priming helps to increase the germination, seedling growth and water relation behaviors of wheat genotypes.

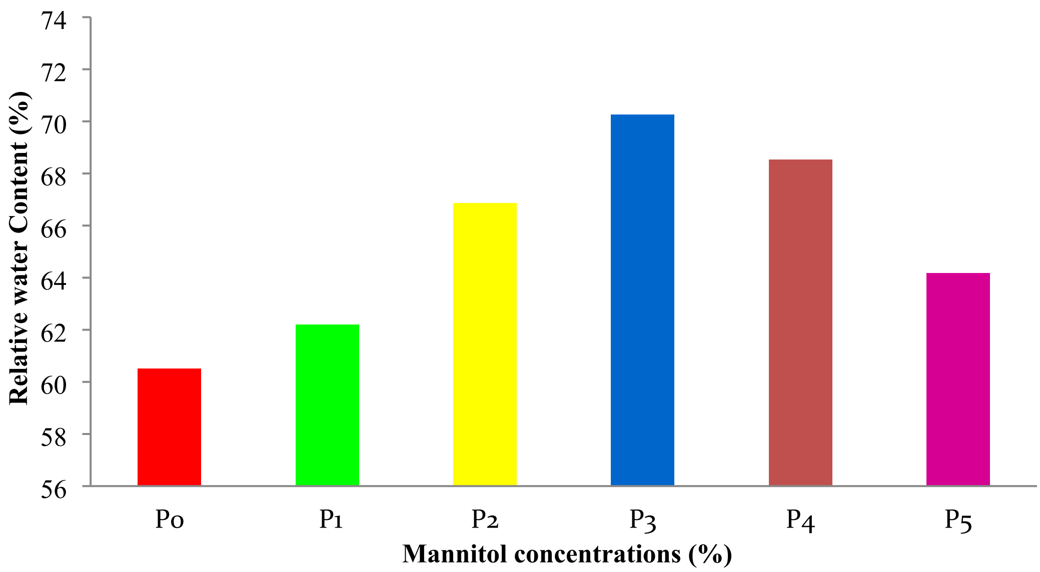


P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 7: Effect of mannitol concentrations on water saturation deficit (%) of wheat (BARI Gom - 31).

4.1.8 Relative water content (%)

Relative water content (RWC) is probably the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. Hence RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA. In this experiment relative water content of different genotypes of wheat showed statistically significant variation due to different concentrations of mannitol solutions including control (Figure 8).Results indicated that the highest relative water content (70.257 %) was recorded primed with 2% mannitol solution for 9 hours followed by seeds primed with 3% mannitol solution for 9 hours where the lowest relative water content (60.510%) was observed without priming followed by Seeds primed with distilled water. It was reported that, priming helps to increase the germination, seedling growth and water relation behaviors of wheat genotypes (Faijunnahar *et al*., 2018).

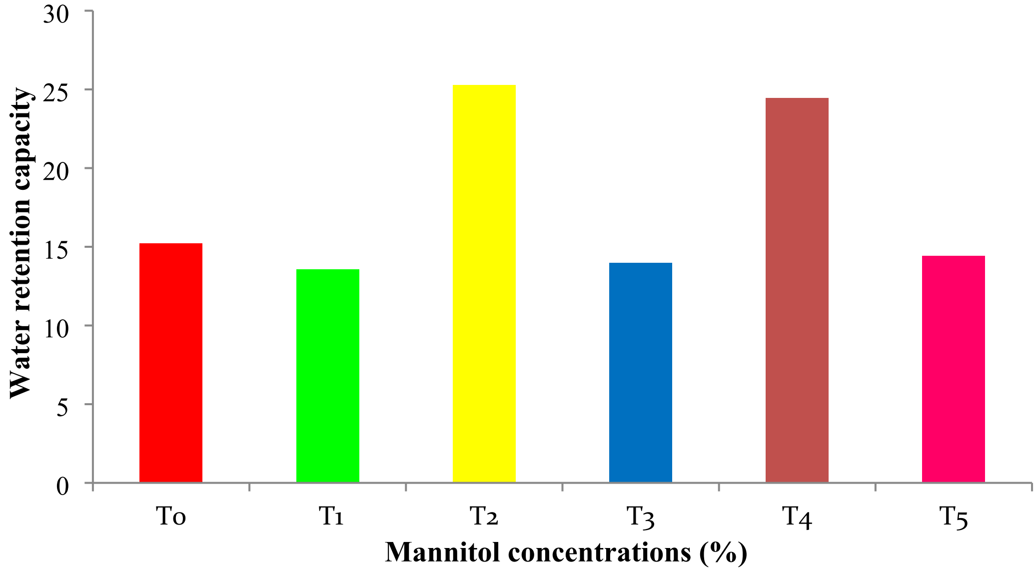


P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 8: Effect of mannitol concentrations on relative water content (%) of wheat (BARI Gom - 31)

4.1.9 Water retention capacity

The results showed that the highest water retention capacity (25.17) was obtained from seeds primed with 1% mannitol solution for 9 hours where the lowest water retention capacity (13.561) was observed primed with distilled water (Ali *et al.*, 2013) reported that seed priming improves irrigation water use efficiency which helps to increase higher water retention capacity. Previous finding shows that, Priming helps to increase the germination, seedling growth and water relation behaviors of wheat genotypes (Faijunnahar *et al*., 2018).

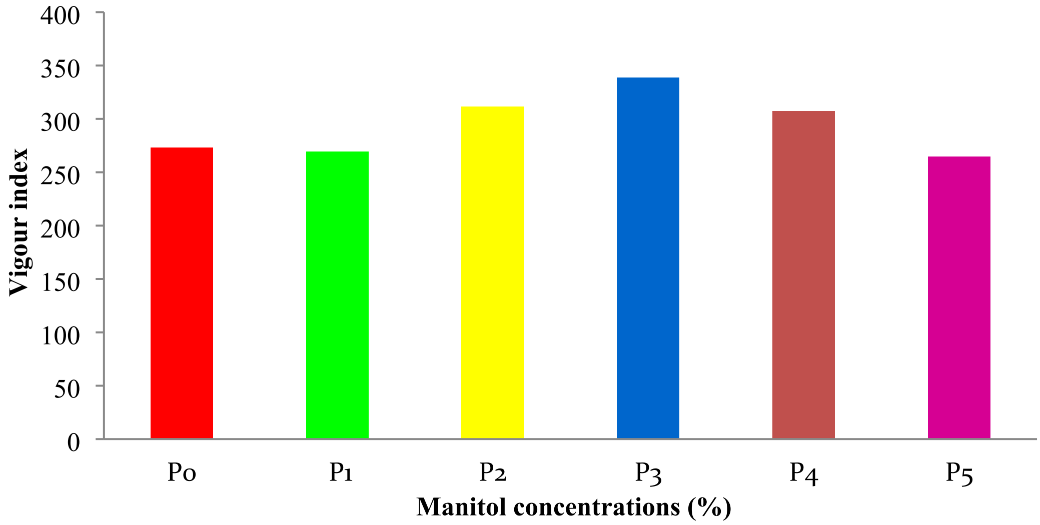


P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 9: Effect of mannitol concentrations on water retention capacity of wheat (BARI Gom - 31)

4.1.10 Vigor index

Seedling vigor (seedling size, health and growth rate) is the product of several factors related to genetics and environmental influences, and can also be manipulated through management. Significant influence was found in terms of vigor index due to different priming solution of mannitol and control treatment (Figure 10). Results revealed that the highest vigor index (338.69) was recorded from the treatment primed seed with 2% mannitol. Where the lowest vigor index (264.40) was observed primed with with 4% mannitol. It was reported that, PEG 20% give higher germination percent shoot length, root length, seedling length, fresh weight, dry weight, vigor index in wheat (Kumar singh, 2018). It's observed that 1% mannitol application has the highest value for vigor index and seedling length (Cokkizgin *et al*., 2019). Previous result shows, priming with mannitol increase the vigor index and germination in cotton seed. (Toselli and Casenave, 2014).



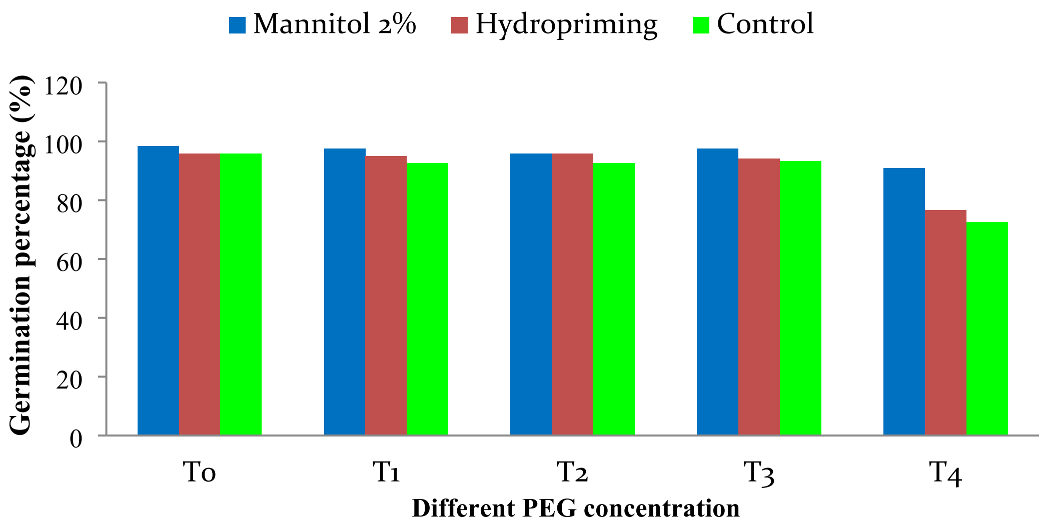
P_0=Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 1% mannitol soluion, P_3=Seeds primed with 2% mannitol solution, P_4=Seeds primed with 3% mannitol solution, P_5=Seeds primed with 4% mannitol solution.

Figure 10: Effect of mannitol concentrations on vigor index of wheat (BARI Gom- 31)

4.2 Second experiment: Germination and growth behavior of mannitol primed seeds (wheat) under drought (PEG) stress condition. This experiment was conducted under laboratory condition. BARI Gom- 31was primed with 2 % mannitol solution and water for 9 hours. Dry seed used as control and was exposed to 0 (water), 5, 10, 15 and 20% PEG induced drought stress conditions in petri dishes. The results have been presented and discussed under the following headings:

4.2.1 Rate of germination (%)

Different drought levels revealed significant variation in respect of germination rate (Figure 11). Result revealed that the germination from primed seeds decreased significantly with increasing drought level. But under drought condition the highest germination rate was found in primed seeds placed in 5% PEG and thereafter gradually decreased germination rate was found with increased drought levels. It was observed that the highest germination rate (98.33%) was under primed seeds placed without PEG and after that the second highest germination rate (97.50%) was in Primed seeds placed with 5% PEG, where the lowest germination rate (90.83%) was obtained primed seeds placed in 20% PEG.This result was supported by previous findings of Zheng et al.(2015) drought stress severely hampered the germination rate, seedling growth, and starch metabolism, but increased the antioxidant enzymes activity and lipid peroxidation in rice. It had been discovered that increasing PEG concentrations up to 30% significantly decrease germination criteria and seedling growth traits and that priming treatments in most cases significantly increased all germination and seedling parameter (Khafagy *et al.*, 2017). This result was in agreement with the previous findings of (Baque *et al.*, 2016; Yagmur and Kaydan, 2008; Afzal, 2005; Afzal *et al.*, 2005; Demir and Ermis, 2003 and Roy and Srivastava, 2000).

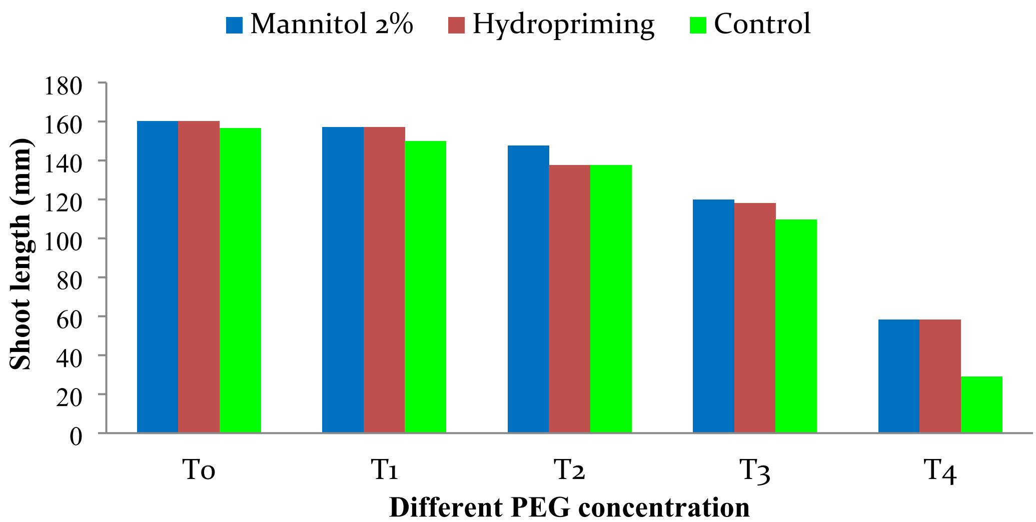


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 11: Effect of different drought levels on Germination percentage of BARI Gom -31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol (2%)

4.2.2 Shoot length (mm)

Shoot length of different wheat genotypes was significantly influenced by different salinity levels (Figure 12). Result exposed that the shoot length from primed seeds decreased significantly with increasing drought level. Results revealed that the highest shoot length (160.30 mm) was observed under primed seeds placed without PEG. But under drought stress highest shoot length (157.00 mm) was primed seeds placed with 5% PEG, where the lowest shoot length (58.30 mm) was observed in Primed seeds placed with 20% PEG. Mannitol treatment reduced the root length under drought stressed condition. This result was agreement with, Pre-sowing seed treatment with mannose, mannitol and H_2O_2 increased the shoot length under non-stress condition (Hammed and Iqbal, 2013). Previous result shows, 300 μmol L^{-1} of melatonin alleviated the negative effect of water stress on germination and increased radicle length, radicle number, and plumule length of the germinated seeds (Li *et al.*, 2020).

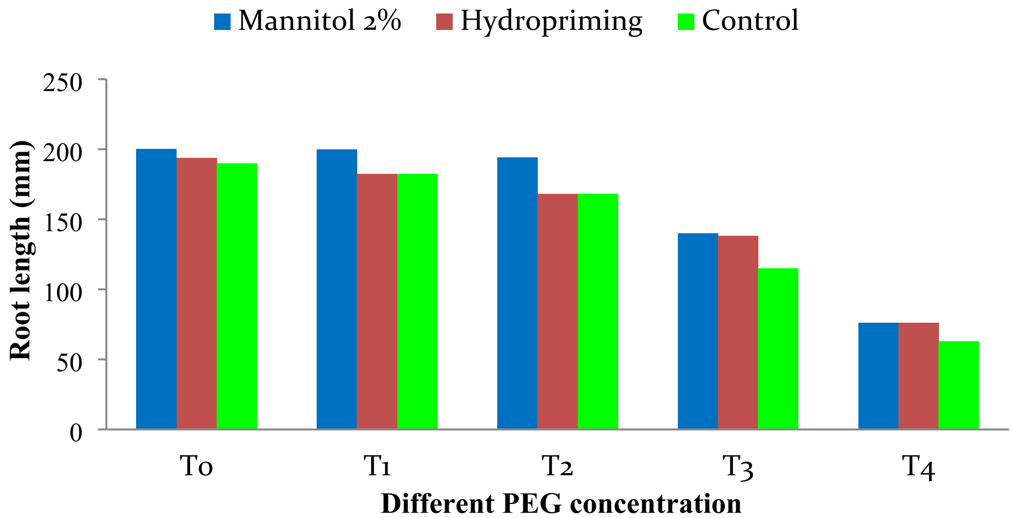


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG. (Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 12: Effect of different drought levels on shoot length of BARI Gom -31 seeds treated with different priming agents (Control, Hydro- Priming and Mannitol (2%)

4.2.3 Root length (mm)

Root length of different wheat genotypes was significantly influenced by different drought levels (Figure 13). Result exposed that the root length from primed seeds decreased significantly with increasing drought level where no drought stress gave highest root length. Results indicated that the highest root length (200.30 mm) was observed in primed seeds placed without PEG. Under drought stress, the highest root length (200.0 mm) was found primed seeds placed with 5% PEG solution and thereafter decreasing trend was observed with increasing drought level. The lowest root length (76.0 mm) was observed in primed seeds placed with 20% PEG solution. This result is similar to, Water stress reduced seed germination percentage, root length, and seedling WC in lentil cultivars to different extent(Muscolo *et al*., 2014). Previously reported that, 300 µmol L^{-1} of melatonin alleviated the negative effect of water stress on germination and increased radicle length, radicle number and plumule length of the germinated seeds (Dongxiao *et al*., 2020).

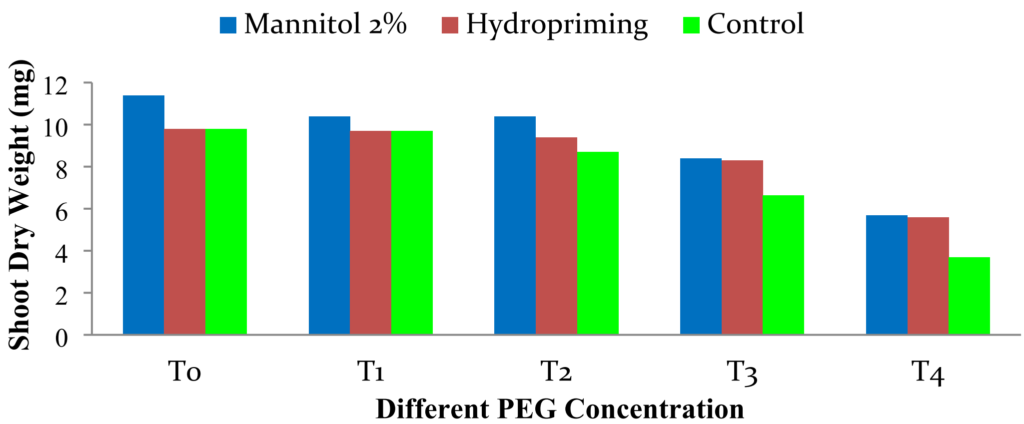


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 13: Effect of different drought levels on root length of BARI Gom - 31 seeds treated with different priming agents (Control, Hydro-Priming And mannitol 2%)

4.2.4 Shoot dry weight (mg)

Significant variation was found for shoot dry weight of wheat genotype affected by different drought levels (Figure 14). Decreased shoot dry weight was observed with increased drought level where no drought level gave highest shoot dry weight. The results showed that the highest shoot dry weight (11.40 mg) was observed in primed seeds placed without PEG but under drought stress, highest shoot dry weight (10.40 mg) under primed seeds placed with 5% PEG solution where the lowest shoot dry weight (5.70 mg) was observed in primed seeds placed with 20% PEG solution. Previous result shows, Treatment with mannose was able to increase the shoot dry weight under stress condition in wheat (Hameed and Iqbal, 2013). It was reported that, percentage of seed germination, germination index, speed of germination, seed vigor index, seedling shoot and root length, seedling root volume, seedling root and shoot dry weight and relative water content of castor were significantly higher with 2 per cent ZnSo4 primed seed, Hydropriming with 2% $ZnSO_4$ is the most promising priming technique for enhancing seedling characters and drought tolerance (Thiruppathi *et al*., 2018).

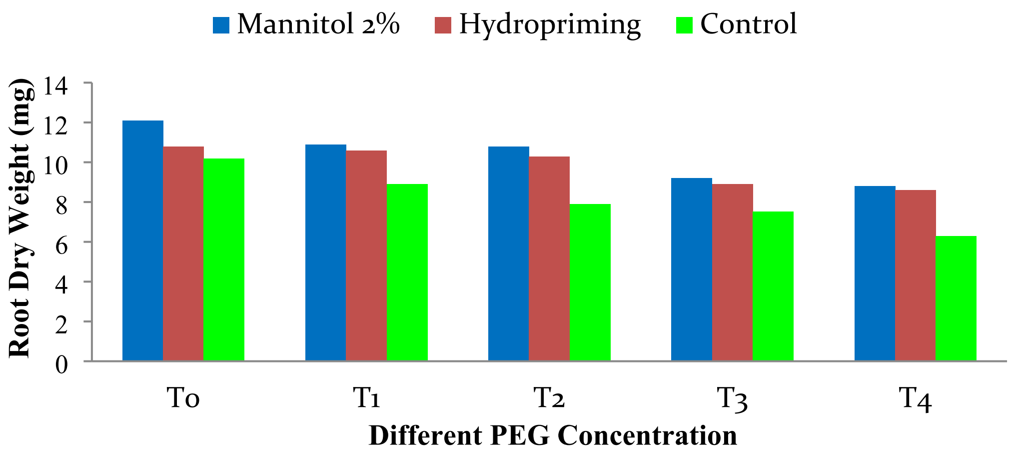


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 14: Effect of different drought levels on shoot dry weight of BARI Gom 31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.5 Root dry weight (mg)

Significant variation was also found for root dry weight of wheat genotype affected by different drought levels (Figure 15 and Appendix VII). Decreased root dry weight was observed with increased drought level where no drought level gave highest root dry weight. Results showed that the highest root dry weight (12.10 mg) was observed where primed seeds placed without PEG. Under drought stress, the highest root dry weight (10.89 mg) was found primed seeds placed with 5% PEG solution. The lowest root dry weight (8.80 mg) was found Primed seeds placed with 20% PEG. Habib *et al.* (2020) reported that seed priming with SNP and H_2O_2 alone, as well as in combination (SNP + H_2O_2), significantly improved the fresh and dry biomass of shoots of both wheat cultivars when grown under non-stressed and water-stressed conditions. Previously reported that, percentage of seed germination, germination index, speed of germination, seed vigor index, seedling shoot and root length, seedling root volume, seedling root and shoot dry weight and relative water content of castor were significantly higher with 2 per cent $ZnSO_4$ primed seed (Thiruppathi *et al.*, 2018).

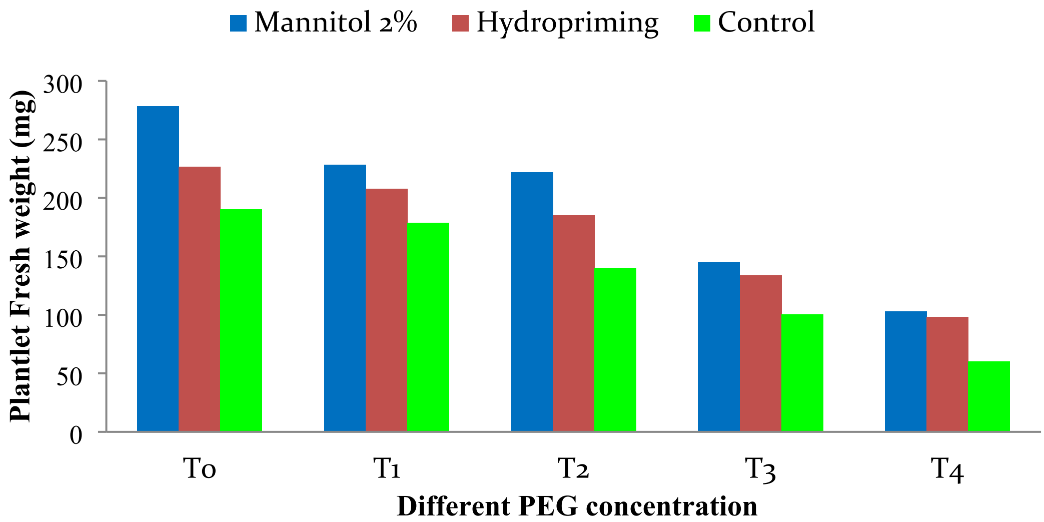


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 15: Effect of different drought levels on root dry weight of BARI Gom 31 treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.6. Fresh weight (mg)

Significant variation was also found for fresh weight of wheat genotype affected by different drought levels (Figure 16). Decreased fresh weight was observed with increased drought level where no drought level gave highest fresh weight. Results showed that that the highest fresh weight (278.43 mg) was observed without drought stress. Under drought stress, the highest fresh weight (228.20 mg) was found in Primed seeds placed with 5% PEG solution. The lowest fresh weight (103.00 mg) was found Primed seeds placed with 20% PEG solution. It was reported that, Drought stress caused the decrease of growth like root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, the ratio of root-shoot length (Saha *et al.*, 2019). It was found that, under drought stresses, seed priming with H_2O_2 increased the root fresh weigh (Hameed and Iqbal, 2013).

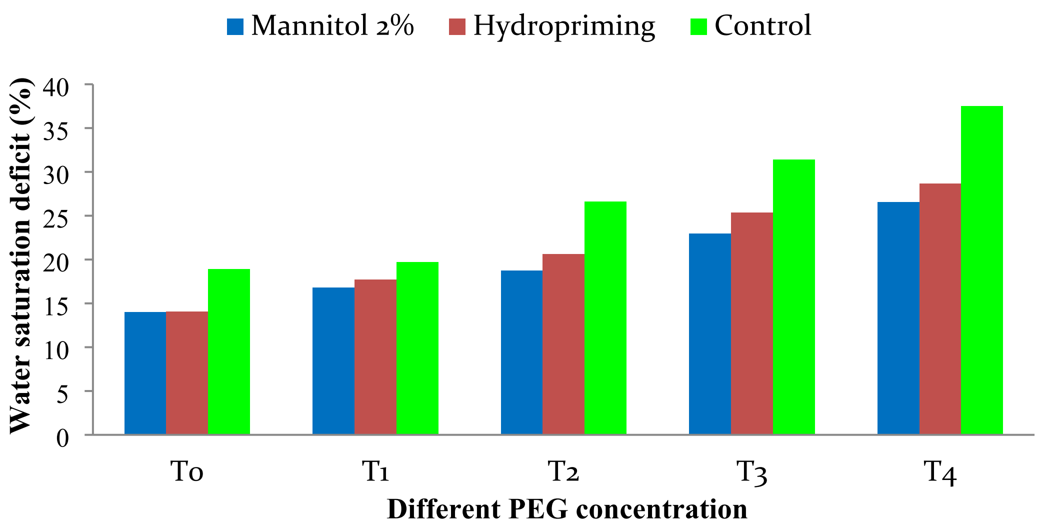


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 16: Effect of different drought levels on fresh weight of BARI Gom 31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.7 Water saturation deficit

Water saturation deficit of wheat genotype was significantly influenced by different drought levels (Figure 17). Result exposed that the water saturation deficit from primed seeds increased significantly with increasing PEG level. Results revealed that the lowest water saturation deficit (14.02%) was observed in primed seeds placed without drought. But under drought lowest water saturation deficit (16.81%) with primed seeds placed with 5% PEG solution. Where the height water saturation deficit (26.56%) was observed primed seeds placed with 20% PEG solution. The water saturation deficit of primed wheat genotypes was gradually increased with increasing stress condition (Baque *et al.*, 2018). But osmopriming might help to recover this physiological damage and minimize the water saturation deficit over priming time accelerate ageing process and produced weak and lean seedling which were failed to uptake enough water and provided more water saturation deficit value (Faijunnahar *et al.*, 2017).

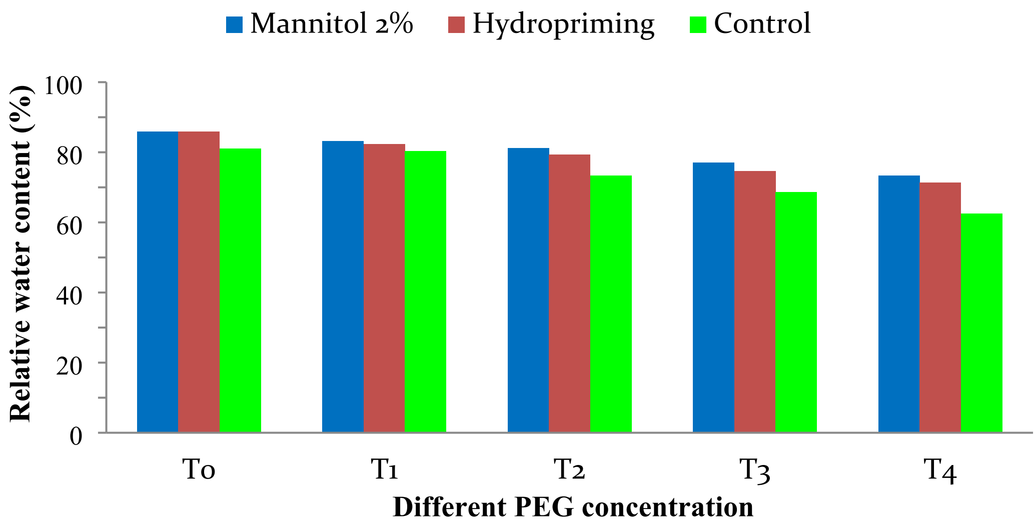


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 17: Effect of different drought levels on water saturation deficit of BARI Gom 31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.8 Relative water content (%)

Relative water content of wheat genotype was significantly influenced by different drought levels (Figure 18). Result exposed that the relative water content from primed seeds decreased significantly with increasing drought level. Results indicated that the highest relative water content (85.98%) was observed in primed seeds without PEG. Under drought stress, the highest relative water content (83.18%) was found in primed seeds placed with 5% PEG solution. The lowest relative water content (73.44%) was observed in Primed seeds placed with 20% PEG solution. Plants treated with SA and BF together under drought stress had significantly increased relative water status by 238% of wheat plants as compared to the non-inoculated control under non-stressed conditions (Azmat *et al*., 2020). Relative water content (RWC) improved after mannitol and H_2O_2 priming under drought and non-stressed conditions (Hameed and Iqbal, 2013).

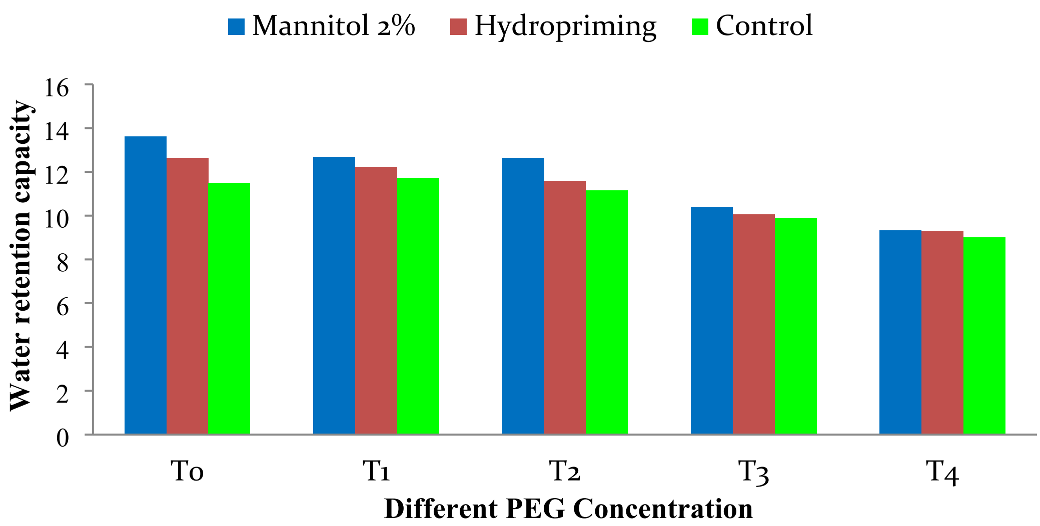


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 18: Effect of different drought levels on relative water content of BARI Gom 31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.9 Water retention capacity

Significant influence was not found for water retention capacity of wheat genotype affected by different drought levels (Figure 19). But the results showed that the highest water retention capacity (13.62) was observed where primed seeds placed without PEG but under drought stress, the highest water retention capacity (12.68) was found in Primed seeds placed with 5%PEG solution. The lowest water retention capacity (9.31) was observed primed seeds placed with 20% PEG solution. The water retention capacity of primed wheat genotypes was decreased gradually with increasing the salinity stress (Baque *et al*., 2018).Vigorous seedling can uptake enough water than the weaker seedling which ensured maximize the turgid weight of seedling so the water retention capacity might be higher than the lower and over priming time (Faijunnahar *et al*., 2017).

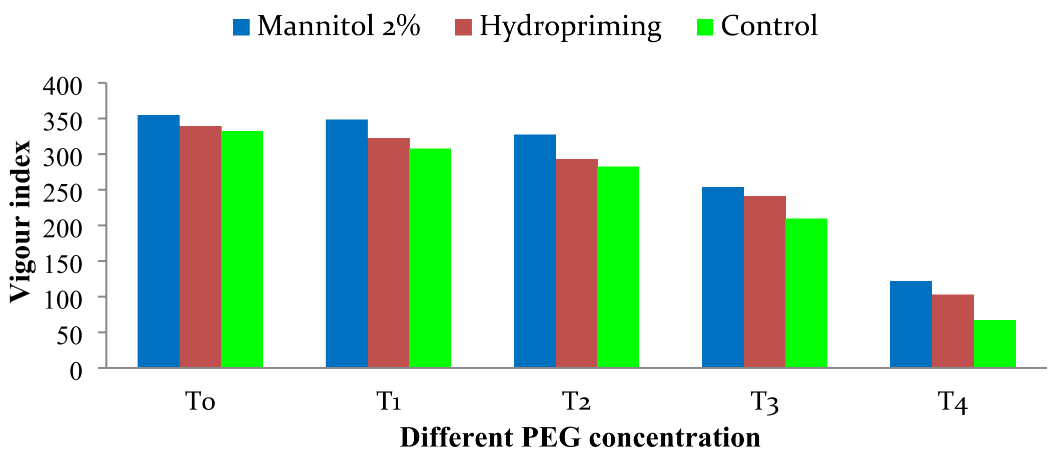


T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG. (Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 19: Effect of different drought levels on water retention capacity of BARI Gom 31 seeds treated with different priming agents (Control, Hydro-Priming and Mannitol 2%)

4.2.10 Vigor index

Significant influence was found for vigor index of wheat genotype affected by different drought levels (Figure 20). Result exposed that the vigor index from primed seeds decreased significantly with increasing drought level and no drought stress gave highest vigor index. It was found that the highest vigor index (354.58) was observed in primed seeds without drought stress. Under drought stress, the highest vigor index (348.08) was found 5% PEG solution. The lowest vigor index (121.98) was observed with 20% PEG solution. Priming with acetylsalicylic acid 200 mg/L increases germination rate, germination percent, root length, seedling length, shoot length and seed vigor index under drought in stress Caper (*Capparis Spinosa*) (Heydariyan *et al*., 2014). With increase in drought stress, germination percentage, shoot length, root length, seedling fresh weight, seedling dry weight and vigor index significantly decreased whereas catalase and peroxidase activity increased as compared to control with enhancement of drought stress. In general, priming with ascorbic acid significantly relived the harsh effects of drought stress (Azadeh Razaji *et al*., 2012).



T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed on 5% level of PEG, T_2= Primed seeds placed on 10% level of PEG, T_3= Primed seeds placed on 15% level of PEG, T_4= Primed seeds placed on 20% PEG.(Polyethylene Glycol (PEG) solutions (0%, 5%, 10%, 15% and 20%).

Figure 20: Effect of different drought levels on Vigor index of BARI Gom 31 treated seeds with different priming agents (Control, Hydro-Priming and Mannitol 2%)

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at Central Laboratory of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from December 2019 to February 2020. Two experiments were conducted to study the mannitol induced drought tolerant capacity of wheat under drought stress condition. The experiment was laid out in a Completely Randomized Design (CRD) with five replications.

BARI Gom- 31was used as test crop. Different priming chemicals such as mannitol and water were utilized for osmopriming and hydropriming respectively. Alcohol was used to sterilize surface of seed. PEG (polyethylene glycol) was used as drought stress inducing chemical.

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

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

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

5.1 First experiment

The first experiment was carried out to find the effect of different concentrations of mannitol on the germination and growth behavior of wheat genotype (BARI Gom-31) without any stress condition. Four levels of mannitol such as 1%, 2%, 3%, 4%, were used for osmopriming and water as hydropriming agent for 9 hours. Seeds without priming (control) also took as treatment.

Results revealed that the highest germination rate (98.0%), shoot length (162.30 mm), root length (183.30 mm), shoot dry weight (12.20 mg), root dry weight (13.5mg), fresh weight (354.00 mg),lowest water saturation deficit (29.75), relative water content (70.25%) and vigor index (338.69) was observed in seeds primed with 2% mannitol solution for 9 hours where seeds without priming showed lowest. Seeds soaked with water also gave better result than without priming but lower result than 2% mannitol primed seed.

5.2 Second experiment

In the second experiment germination and growth behavior of primed seeds of wheat genotype (BARIGom31) with and without drought (PEG) stress condition was evaluated. Mannitol solution 2% were used as priming solutions and 9 hours as priming time and drought stress levels; without PEG but primed (control), T_0= 0 %, T_1 = 5%, T_2 = 10%, T_3 = 15%, T_4 = 20 % were used in this experiment for inducing drought stress.

Results revealed that primed seeds placed without drought stress; control gave the highest germination rate (98.33%), shoot length (160.30 mm), root length (200.30 mm), shoot dry weight (11.40 mg), root dry weight (12.10 mg), fresh weight (278.43 mg), vigor index (354.58), highest relative water content (85.98%), water retention capacity(13.62) and lowest water saturation deficit(14.02). But under drought stress condition, the highest germination rate (97.5%), shoot length (157.00 mm), root length (200.0 mm), shoot dry weight (10.40 mg) and root dry weight (10.89 mg), fresh weight (228.28 mg), relative water content (83.18%) and vigor index (348.08) water retention capacity(12.68) and lowest water saturation deficit(16.81) were achieved from primed seeds placed with 5% PEG where primed seeds placed with 20% PEG showed lowest results in respected parameters.

From the result of above study arrived at a judgment that the performance of wheat genotype BARI Gom-31 which was 2% mannitol primed gave better response in respect of germination and growth parameter. Wheat seeds primed with 2% mannitol and distilled water promoted germination behavior and seedling growth up to 5% induced drought stress condition, after that significantly decreased with increasing drought stress. Reduction of germination and seedling growth was more profound in control seeds than primed seeds under drought stress condition. Thus the priming may be effective method to meet the demands of farmer during the setting of culture in the field under drought stress condition. For this reason, further studies are needed to assess the efficacy of seed priming during the later stage of the culture.

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APPENDICES

Appendix I: Effect of priming concentrations on the seedling germination percentage (%) of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	123.33	24.66	4.27**
Error	18	104.0	5.77	
Total	23	227.33		

**Significant at 1% level of significance

Appendix II: Effect of priming concentrations on the fresh weight of BARI Gom- 31

Source	DF	SS	MS	F
Treatment	5	49178.2	9835.65	98.36**
Error	18	1800.0	100.00	
Total	23	50978.2		

**Significant at 1% level of significance

Appendix III: Effect of priming concentrations on the root length of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	4085.63	817.12	14.71
Error	18	1000.00	55.55	
Total	23	5085.63		

**Significant at 1% level of significance

Appendix IV: Effect of priming concentrations on the sooth length of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	2312.75	462.55	20.71**
Error	18	402.00	22.33	
Total	23	2714.75		

**Significant at 1% level of significance

Appendix IV: Effect of priming concentrations on the root dry weight of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	14.88	2.977	41.22**
Error	18	1.30	0.072	
Total	23	16.18		

**Significant at 1% level of significance

Appendix VI: Effect of priming concentrations on the sooth dry weight of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	45.71	9.14	121.01**
Error	18	1.36	0.07	
Total	23	47.07		

**Significant at 1% level of significance

Appendix VII: Effect of priming concentrations on the relative water content of BARI Gom- 31

Source	DF	SS	MS	F
Treatment	5	284.97	56.9	28.50**
Error	18	36.0	2.00	
Total	23	320.97		

**Significant at 1% level of significance

Appendix VIII: Effect of priming concentrations on the water saturation deficit of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	284.97	56.99	128.24**
Error	18	8.00	0.44	
Total	23	292.97		

**Significant at 1% level of significance

Appendix IX: Effect of priming concentrations on the water retention capacity of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	603.47	120.69	543.12**
Error	18	4.00	0.22	
Total	23	607.47		

**Significant at 1% level of significance

Appendix X: Effect of priming concentrations on the water vigor index of BARI Gom - 31

Source	DF	SS	MS	F
Treatment	5	17594.9	3518.98	35.19**
Error	18	1800.0	100.00	
Total	23	19394.9		

**Significant at 1% level of significance

Appendix XI. Effect of different drought levels on germination percentages of BARI Gom- 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of germination percentages on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	8.33**	25.00**	14.78**	19.48**	370.19**
Error	9	0.22	0.50	0.05	0.11	0.88

**Significant at 1% level of significance

Appendix XII. Effect of different drought levels on shoot length of BARI Gom 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of shoot length on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	18.25**	65.33**	133.33**	121.81**	1144.65**
Error	9	0.22	0.88	0.56	1.17	0.57

**Significant at 1% level of significance

Appendix XIII. Effect of different drought levels on root length of BARI Gom 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of root length on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	109.29**	417.72**	901.33**	772.0**	225.33**
Error	9	4.10	5.55	10.88	8.22	2.0

**Significant at 1% level of significance

Appendix XIV. Effect of different drought levels on shoot dry weight of BARI Gom- 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of shoot dry weight on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	3.41**	0.65**	2.92**	3.95**	5.08**
Error	9	0.03	0.05	0.08	0.01	0.02

**Significant at 1% level of significance

Appendix XV. Effect of different drought levels on root dry weight of BARI Gom - 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degree sof freedo m	Mean square of root dry weight on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatme nt	2	3.77**	4.62**	9.72**	3.17**	7.72**
Error	9	0.03	0.00	0.03	0.00	0.00

**Significant at 1% level of significant

Appendix XVI. Effect of different drought levels on plant fresh weight of BARI Gom - 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of plant fresh weight on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatme nt	2	7851.49 **	2463.88 **	6679.89 **	2134.33 **	2220.85 **
Error	9	3.56	3.92	5.56	2.94	0.89

**Significant at 1% level of significant

Appendix XVII. Effect of different drought levels on relative water content (%) of BARI Gom - 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of relative water content (%) on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatmen t	2	31.68**	8.45**	67.53**	75.28 **	134.55 **
Error	9	0.01	0.00	0.01	0.22	0.46

**Significant at 1% level of significant

Appendix XVIII. Effect of different drought levels on water saturation deficit (%) of BARI Gom - 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of water saturation deficit (%) on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	31.75 **	8.89**	67.53**	75.21**	134.55**
Error	9	0.00	0.10	0.11	0.87	0.54

**Significant at 1% level of significant

Appendix XIX. Effect of different drought levels on water retention capacity of BARI Gom - 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of water retention capacity on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	4.49**	0.92 **	2.36**	0.26 **	0.12 **
Error	9	0.08	0.03	0.09	0.00	0.00

**Significant at 1% level of significant

Appendix XX. Effect of different drought levels on vigour index of BARI Gom 31 seeds treated with different primed agents. (Control, Hydro-Priming and Manitol (2%)

Source of variation	Degrees of freedom	Mean square of vigour index on different drought level				
		PEG 0%	PEG 5%	PEG 10%	PEG 15%	PEG 20%
Treatment	2	526.91**	1695.23**	2193.46**	2045.53**	3152.89**
Error	9	14.22	8.21	10.89	5.56	14.15

**Significant at 1% level of significant

PLATES

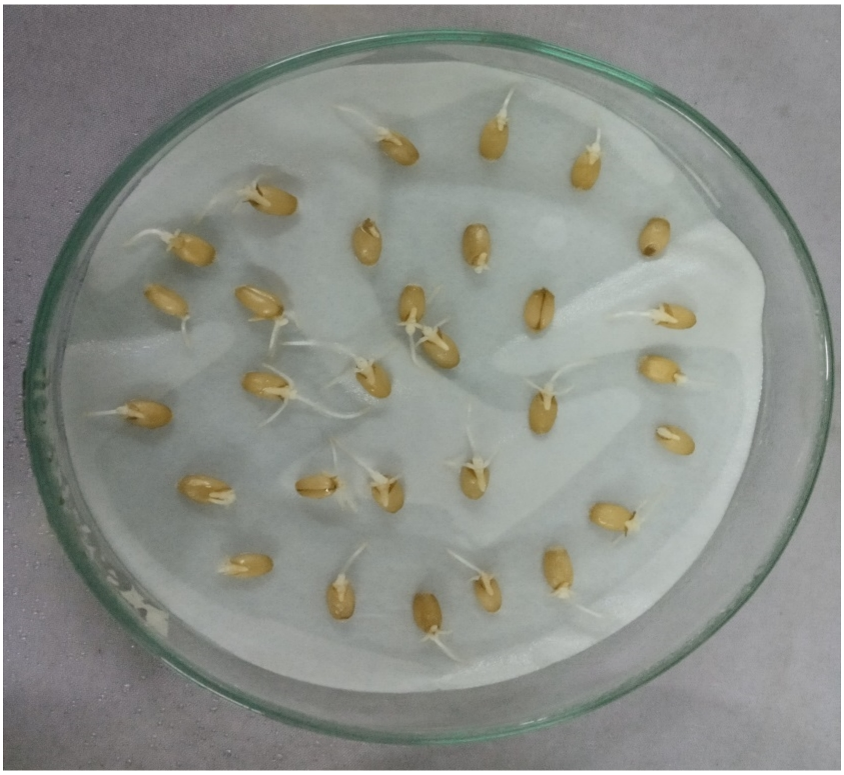

Plate 1: Germinated seed after 2+ days

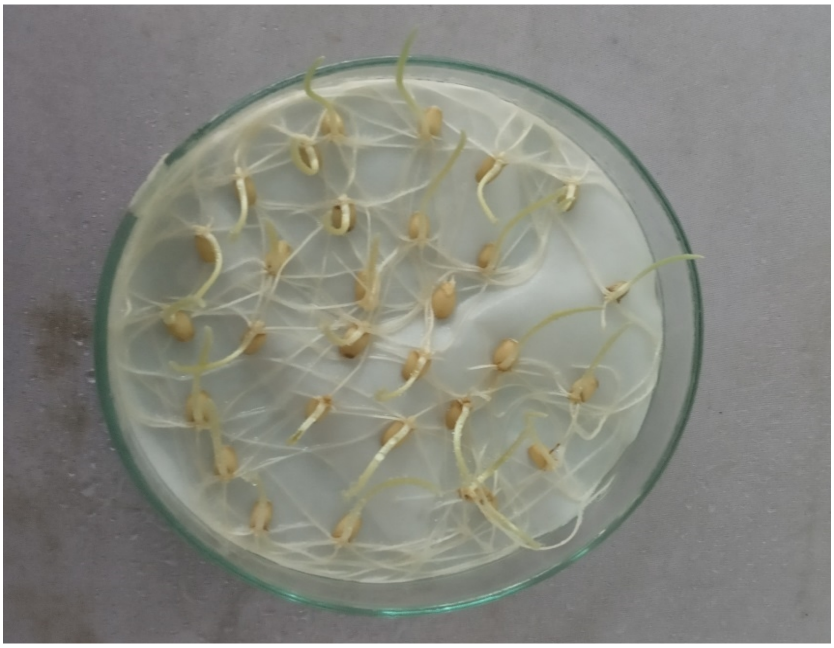

Plate 2: Germinated seed after 3 days

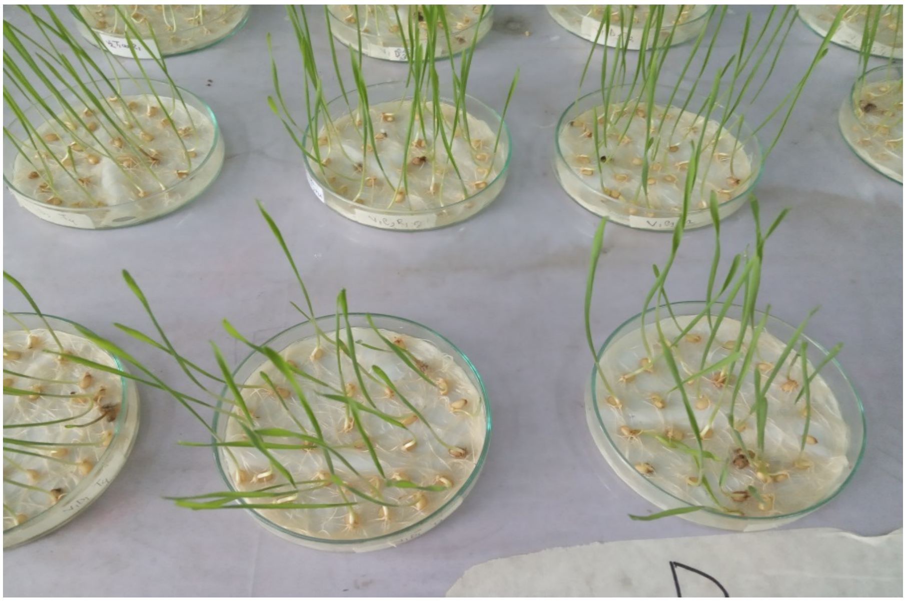

Plate 3: Seedling after 10 days of placement in petri deshes

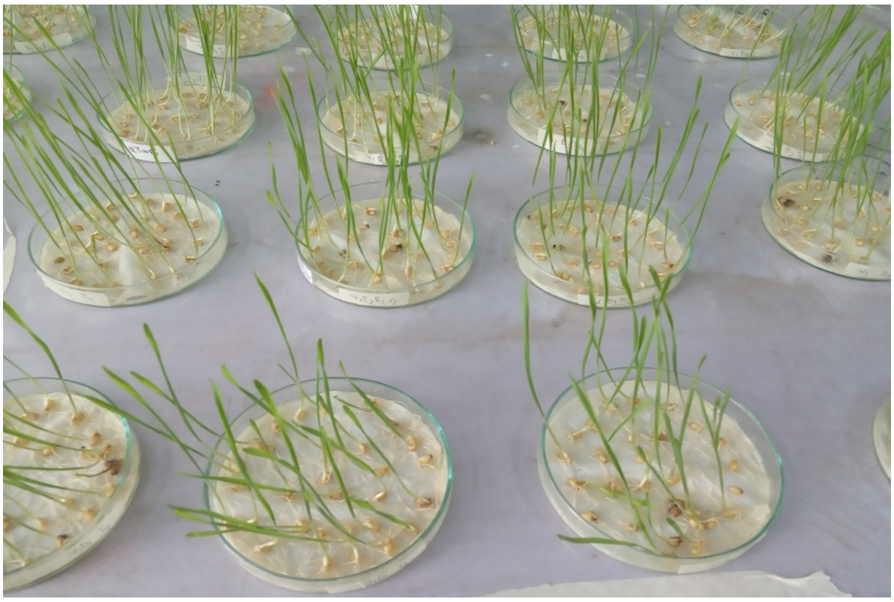

Plate 4: Seedling after 10 Days of placement in petri deshes