ENHANCEMENT OF DROUGHT TOLERANCE IN MUNGBEAN THROUGH OSMO AND HYDRO PRIMING

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ENHANCEMENT OF DROUGHT TOLERANCE IN MUNGBEAN THROUGH OSMO AND HYDRO PRIMING

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CERTIFICATE

This is to certify that the thesis entitled " ***ENHANCEMENT OF DROUGHT TOLERANCE IN MUNGBEAN THROUGH OSMO AND HYDRO PRIMING"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS)*** *in* ***AGRONOMY,*** *embodies the results of a piece of bona fide research work carried out by* ***MD ANISUR RAHMAN,*** *Registration. No.* ***09-03328*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

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

Dated:
Dhaka, Bangladesh

(Prof. Dr. Md. Abdullahil Baque)
Supervisor

DEDICATED TO

MY

BELOVED PARENTS

LIST OF ACCRONYMS AND ABBREVIATIONS

Agric.	Agriculture	*LSD*	Least significant difference
Agril.	Agricultural	Mg	Milligram
Anon.	Anonymous	MGT	Mean germination time
AOSA	Association of Official Seed Analysis	Min	Methyl jasm-onate
BARI	Bangladesh Agricultural Research Institute	mL	Milliliter
BBS	Bangladesh Bureau of Statistics	Mm	Millimeter
^{o}C	Degree centigrade	mM	Millimole
Cm	Centi-meter	MoA	Ministry of Agriculture
CRD	Completely Randomized Design	M.S	Master of Science
CV	Coefficient of velocity	No.	Number
Dev.	Development	PEG	Polyethylene Glycol
dSm^{-1}	Deci-Siemens m^{-1}	*Res.*	Research
EC	Electrical conductivity	ROS	Reactive Oxygen Species
ed.	Edition	RWC	Relative water content
EG	Energy of emergence	SAU	Sher-e-Bangla Agricultural University
Environ.	Environmental	*Sci.*	Science
et al.	And others	Technol.	Technology
Expt.	Experimental	TG	Total germination
GI	Germination index	VI	Vigour Index
Hr	Hour	Viz	Namely
i.e.	idest (L), that is Inst. Institute	WRC	Water retention capacity
Int.	International	WSD	Water saturation deficit
ISTA	International Seed Testing Association	Wt.	Weight
j.	Journal	%	Percentage

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The Author

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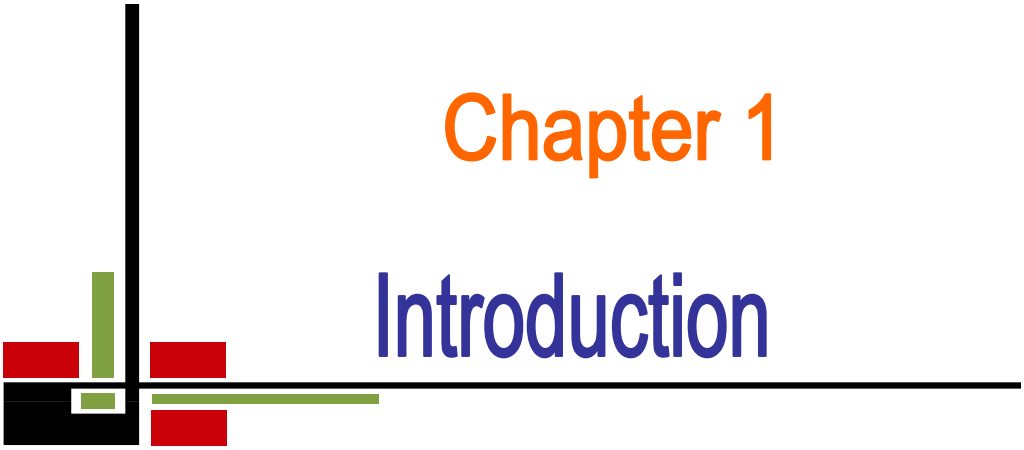
ENHANCEMENT OF DROUGHT TOLERANCE IN MUNGBEAN THROUGH OSMO AND HYDRO PRIMING

ABSTRACT

In order to evaluate the effect of pre-sowing seed treatment with mannitol on germination behavior of mungbean (*Vigna radiata* L.) (BARI Mung 3 and BARI Mung 6) in relation to drought tolerance and to optimize the priming time of the best priming solution concentration on germination behavior of mungbean an experiment was conducted under the laboratory conditions of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka from 13 May, 2014 to 15 July, 2014. The whole experiment was divided into three experiments. In first experiment, BARI Mung 3 and BARI Mung 6 were primed in 3, 6, 9, 12, 15, and 18 hours under 2%, 4%, 6%, and 8% mannitol solution and distilled water, respectively. After priming the electrical conductivity (EC) of seed, leachates from 3, 6, 9, 12, 15, 18 hours primed seeds, hydro primed seeds and non primed seeds were measured after 24 hours of soaking in distill water. 9 hours priming time showed the best result for its lowest EC value. In second experiment, two mungbean varieties were surface sterilized with 75% alcohol solution, soaked in water and mannitol (2%, 4%, 6%, and 8%) for 9 hours and dry seed used as control. The highest total germination percentage (98.38%), germination index (87.78), vigor index (186.9), relative water content (65.64%), water retention capacity (21.22) and lowest water saturation deficit (34.36%) were obtained from seeds primed in 2% mannitol for BARI Mung 3 compare to total germination percentage (93.09%), germination index (85.41), energy of emergence (97.31%), vigor index (136.5), relative water content (77.22%), water retention capacity (15.94) and lowest water saturation deficit (22.78%) were obtained from seeds primed in 6% mannitol for BARI Mung 6. In the final experiment, seeds were primed with distilled water and 2% mannitol for BARI Mung 3 and 6% mannitol for BARI Mung 6 by 9 hours; dry seed used as control and were exposed to 0%, 5%, 10%, 15%, and 20% Polyethylene Glycol (PEG) induced drought stress conditions in Petri dishes. Priming with mannitol followed by water were more effective than the control seed in inducing drought tolerance of mungbean cultivars owing to enhanced germination and growth parameters under drought stress condition. From the results of the study, it was observed that seeds primed with 2% mannitol for BARI Mung 3 and 6% mannitol for BARI Mung 6 by 9 hours showed the best result in comparison to water primed seed and dry seed.



Chapter 1

Introduction

CHAPTER I

INTRODUCTION

Mungbean (*Vigna radiata* L.) is an important grain legume in Bangladesh belonging to the family Fabaceae. As an excellent source of vegetable protein. Its edible grain is characterized by good digestibility, flavor, high protein content and absence of any flatulence effects (Ahmed *et al.*, 2008). It holds the 3rd position in protein content and 4th position in both acreage and production in Bangladesh (MoA, 2014). It is extensively grown in the tropical and sub-tropical region. In Bangladesh, it is used as whole or split seed as Dal (soup) but in many countries sprouted seeds are widely used as vegetable. The whole seeds of the crop are rich in 348 kcal energy, 24.5 mg protein, 1.2 mg fat, 59.9 mg carbohydrate, 75 mg calcium, 8.5 mg minerals, 0.72 mg thiamin, .0.15 µg riboflavin, 49 µg beta-carotene (BARI, 2008). The lysine content makes mungbean a good complementary food for rich-based diets because lysine is usually the first limiting amino acid.

Besides, the crops have the capability to enrich soils through nitrogen fixation (Sharma and Behera, 2009). According to FAO (2013) recommendation, a minimum intake of pulse by a human should be 80 gm/day, whereas it is 7.92 g in Bangladesh (BBS, 2012). This is because of fact that national production of the pulses is not adequate to meet our national demand. In Bangladesh, total production of pulses is only 0.65 million ton against 2.7 million tons requirement. This means the shortage is almost 80% of the total requirement (Rahman and Ali, 2007). This is mostly due to low yield (MoA, 2013).

At present, the area under pulse crop is 0.406 million hectare with a production of 0.322 million tons (BBS, 2013), where mungbean is cultivated in the area of 0.108 million ha with production of 0.03 million tons (BBS, 2014). Plant growth and productivity affected by nature’s wrath in the form of various abiotic stress factors. Plants are frequently exposed to the plethora of stress

conditions such as salt, drought, oxidative stress and others. All these stress factors are a means for plants and prevent them from reaching their full genetic potential and limit the crop productivity worldwide. Lack of adequate soil moisture in the seedbed is a major obstacle to the establishment of the crop, because inadequate soil moisture can reduce germination, slow down seedling growth and decrease yield.

There are many strategies which have been adopted to overcome the negative effects of drought. A good strategy is the selection of cultivars and species tolerant to drought condition (Pavlousek, 2011). However, an alternative strategy for the possibilities to overcome drought stress is by seed pre-sowing treatments (Ghiyasi *et al.,* 2008). Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows the seed to imbibe water to proceed to the first stage of germination, but prevents radical protrusion through the seed coat (Yari *et al*., 2012). Seed priming techniques such as hydro priming, hardening, osmo priming, osmo hardening, hormonal priming have been used to accelerate emergence more vigorous plants and better drought tolerance in many field crop like wheat (Iqbal and Ashraf, 2007), chickpea, sunflower (Kaya *et al*., 2006), cotton (Casenave and Toselli, 2007) triticale (Yagmur and Kaydan, 2008).

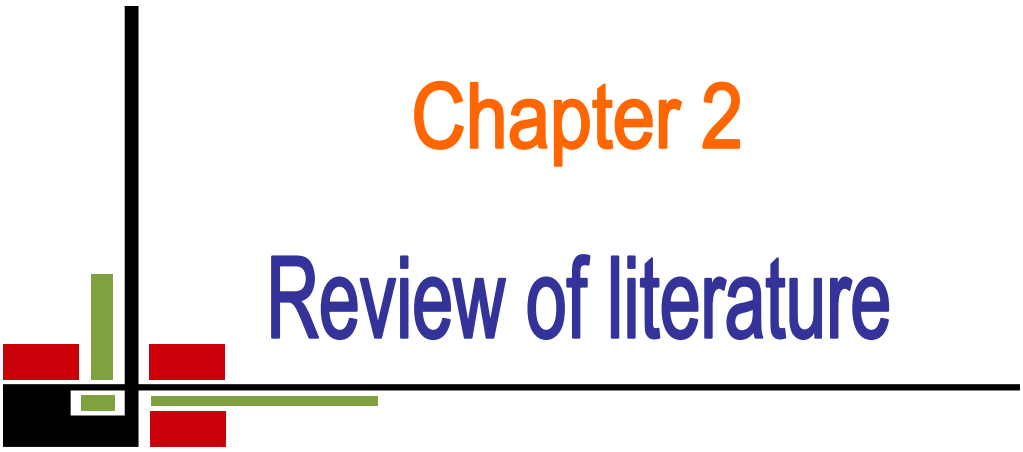
Improvement in germination of primed seeds may be attributed due to the fact that priming induced quantitative changes in biochemical activities including greater α-amylase activity, increasing free sugars and DNA during seed germination (Sung and Chang, 1993). In mungbean, 4 hours and 8 hours primed seeds showed significant difference in germination percentage and seed moisture percentage over non-primed seeds (Saha *et al.*, 2006).

Primed seeds usually to exhibit an increased germination rate, greater germination uniformity and greater total germination percentage. Increased germination rate and uniformity have been attributed to metabolic repair during

imbibitions build up of germination enhancing metabolites (Abbasdokht, 2011).

In Bangladesh little is known about hydro priming and information regarding seed priming with osmotic priming agent for inducing drought tolerant capability in mungbean or other crops in Bangladesh is scarce. Therefore, the present study will be undertaken with the following objectives:

i) To evaluate the effect of pre-sowing seed treatment with mannitol on germination behavior of mungbean in relation to drought tolerance.
ii) To optimize the priming time of the best priming chemical on germination behavior of mungbean.
iii) To evaluate the effect of seed priming on germination and vigor of mungbean under drought stress.



Chapter 2

Review of literature

CHAPTER II

REVIEW OF LITERATURE

Mungbean is one of the most important pulse crop in Bangladesh and productivity of mungbean is greatly influenced by drought stress. Preplant treatment of seeds can be applied to improve germination under adverse conditions. Seed priming is one of important pretreatment which can be used to escape the adverse conditions. Available literatures, pertinent to this study, on different legumes as well as other crops and priming agents are, therefore, presented below:

2.1 Seed priming and electrical conductivity

Electrical conductivity is related to deterioration processes of seeds as degradation of cell membrane and electrolyte leakage out of seeds (Delouche and Baskin, 1973). Thus many researchers have been used electrical conductivity test to indicate seed vigor (Normash and Chin., 1991). Electrical conductivity is a measurement of electrolyte leakage which is negatively correlated with germination and field emergence in a number of crop species (Baki and Anderson, 1970). The relationship between germination percentage and electrical conductivity was negatively correlated ($p<0.05$) indicating that electrical conductivity can be used to evaluate germination ability of sudangrass (Fu *et al.,* 2000). However it is reported that priming can reduce electrolyte leakage and maintain better seed quality over a considerable period. Chang and Sung (1998) found reduced electrolyte leakage from primed seeds. Priming might have enhanced the repair of cell membranes that were disrupted during ageing (Senaratna *et al*., 1988). The repair of membrane could initiate there-activation or re-synthesis of membrane-bound enzymes and enhanced germination (Rao *et al.,* 1987). Similarly in canola seeds, electrical conductivity from the leachate of osmoprimed and hydro-primed seeds were lower than that of non-primed seeds. Seed priming was effective in decreasing

electrical conductivity of seed leachates, which show membrane stability. Decreased leakage of solute from primed seed may be because of better membrane repair during hydration (Fu *et al.,* 1988). Sadeghi *et al.* (2011) found that lowest electrical conductivity (EC) value indicate the best priming time. Greater membrane integrity in primed seed was also reported by Rudrapal and Naukamura (1998) for egg plant and radish, and Afzal *et al.* (2002) for hybrid maize.

2.2 Effect on germination parameters

2.2.1 Total Germination (%)

Seed priming, a controlled hydration process followed by re-drying is pragmatic approach to counteract the salinity effects in many crops because of its simplicity, low cost and effectiveness (Wahid *et al*., 2007; Afzal *et al*., 2011). It improved the germination percentage and uniformity of growth following reduced emergence time and increased yields are reported in many field crops including rice (Farooq *et al*., 2006b; Afzal *et al*., 2006; Afzal *et al*., 2011). Sun *et al. (*2010) concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination. It was reported seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition (Demir Kaya *et al*., 2006). Aerated hydration treatment of pepper at 250 ^{0}C followed by drying increased germination percentage were reported by Demir and Okcu, 2004. The final germination percentage of *Melilotus officinalis* was much higher than that of *M. sativa* and *A. adsurgens* at 300 mM NaCl (Wang *et al*., 2009b), and the germination rate in six alfalfa cultivars was also differentially affected by treatments with 200 mM NaCl and 35% PEG (Wang *et al*., 2009a). Seed primed with potassium hydrophosphate (KH_2PO_4) and water improved germination percentage compared to untreated seed treatments. Similarly Korkmaz and Pill (2003) reported that priming with KH_2PO_4 improved the

germination synchrony of low vigour cultivar in lettuce. According to Ghana and Schillinger (2003) seed primed with KH_2PO_4 and water treatments enhanced germination in wheat under normal condition compared to untreated seed. Basra *et al*. (2003) and Salinas (1996) reported improvement in germination percent, emergence and seedling stand by using seed priming techniques. In fact priming induces a range of biochemical changes in the seed that required initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation (Ajouri *et al*., 2004). Some previous researcher indicated that some or all process that precede the germination are triggered by priming and persist following the re-desiccation of the seed (Asgedom and Becker, 2001). Primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). According to McDonald (2000) primed seeds acquire the potential to rapidly imbibe and revive the seed metabolism thus enhancing the germination rate. In soybean too, seed priming with PEG was successfully carried out by Khalil *et al*. (2001).

Osmo priming with PEG results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach (Chen and Arora, 2011). Osmo conditioning of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions (Hur, 1991). According to Posmyk and Janas (2007), hydropriming and hydro priming along with proline can be used as a safe priming method for improving seed germination and growth of *Vigna radiata* seedlings at low temperature and also allowing fast repair of injuries caused by stress.

More uniform germination and emergence were observed in primed seeds on canola (*Brassica compestris*) (Zheng *et al*., 1994), wheat (*Triticum aestivum*)

(Nayyar *et al*., 1995) and rice (*Oryza sativa*) (Lee and Kim, 2000; Basra *et al*., 2003) who described improved germination rate and percentage in seeds subjected to hydro priming and seed hardening for 24 hr (Farooq *et al*., 2006b). Coolbear and Grierson (1979) who reported that higher germination rate was a result of higher levels of nucleic acid in primed seeds of tomato cultivars. They indicated that increase in nucleic acid content in primed seeds was due to an enhanced ribonucleic acid (RNA) synthesis during and after priming treatment.

Ascorbic acid, another important vitamin is also used for priming due to its antioxidant nature. It has already been proved that a high level of endogenous acrobat is essential to maintain the antioxidant capacity that protects plants from oxidative stress (Zhou *et al*., 2009). ABA priming showed increased rate of germination as compared to non-primed seeds in Indian mustard (Srivastava *et al*., 2010 a, b). Salicylic acid priming in fennel seeds also showed better germination under low water potential (Farahbakhsh, 2012). Moreover, in *Salicornia utahensis*, which is a halophyte, priming with growth regulators like fusicoccin, thiourea, kinetin, and ethephon alleviated the inhibitory effects of salinity on the germination, whereas GA_3, proline, betaine and nitrate had little effect on germination at all salinities (Gul and Khan, 2003). 3% KNO_3 supplemented with 3 lM methyl jasm-onate (MeJA) could promote germination and emergence of dormant *Amaranthus cruentus* L. seeds (Tiryaki *et al*., 2005). More recently, seeds of *Agropyron elongatum* primed with gibberellin (GA) and abscisic acid (ABA) exhibited induced CAT and SOD activities under drought conditions when compared to unprimed seeds (Eisvand *et al*., 2010).

In many crops, seed germination and early seedling growth are the most sensitive stages of water limitation and the water deficit may delay the onset and reduce the rate and uniformity of germination, leading to poor crop per dormance and yield (Demir *et al*., 2006).

Therefore, the beneficial effects of priming may be more evident under unfavorable rather than favorable conditions (Parera and Cantliffe, 1994). In

mungbean, 4 hours and 8 hours primed seeds showed significant difference in germination percentage and seed moisture percentage over non-primed seeds (Saha *et al.,* 2006). Hardening (150 gm seeds soaked in 500 mL water for 18 and 24 hours) of aged rice seeds increase germination rate by *10-15%* through increasing total sugar content and α-amylase activity in aged rice seeds (Lee *et al.,* 2000). Primed seeds usually exhibit an increased germination rate, greater germination uniformity, and at times, greater total germination percentage (Basra *et al.,* 2005). These attributes have practical agronomic implications, notably under adverse germination conditions (McDonald, 2000). Therefore, there is a strong interest in the seed industry to find suitable priming agent(s) that might be used to increase the tolerance of plants under adverse field conditions (Job *et al*., 2000).

2.2.2 Mean germination time (days)

Priming treatments are being used to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such earlier and synchronized emergence often leads to uniform stands and improved yield (Farooq *et al*., 2006b; Afzal *et al*., 2006; Afzal *et al*., 2011). Like germination percentage, prime seeds had lower mean emergence time (MET) compared with non-primed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassanpouraghdam *et al*., 2009; Sivritepe *et al*., 2003). Improved seed invigoration techniques were known to reduce emergence time, accomplish uniform emergence and give better crop stand in many horticultural and field crops (Ashraf and Foolad, 2005). Priming decreased the temperature optimum and ceiling temperature for germination and also helped in advancing the germination time and did not decrease the final percentage emergence (Finch-Savage *et al*., 2004). ‘‘On-farm’’ seed priming (soaking seeds in water prior to sowing) has been shown to be effective in producing early germination, better establishment and increased yields in a wide range of crops in diverse

environments (Rashid *et al*., 2006). It had been a common pretreatment that reduces the time between seed sowing until emergence and synchronizes seedling emergence (Parera and Cantliffe, 1994). According to Basra *et al.* (1989) priming of corn seed using polyethylene glycol or potassium salt (K_2HPO_4 or KNO_3) resulted in accelerated germination.

Janmohammadi *et al*. (2008) presented hydro priming as a suitable, cheap and easy seed invigoration treatment for inbreed lines of maize, especially when germination is affected by salinity and drought stress. Hydropriming has been shown to result in the earlier germination of desert cacti (Dubrovsky, 1996), *Allium porrum* (Ashraf and Bray, 1993), pyrethrum (*Tanacetum cinerariifolium*) (Li *et al*., 2011), and coriander (Rithichai *et al*., 2009). MoradiDezfuli *et al.* (2008) revealed hydro primed seeds for 36 h had lowest values (T50 and MGT).

Osmotic seed priming of maize caryopses resulted in more homogenous and faster seed germination as compared to the control was reported by Fotia *et al.* (2008). According to Gray *et al*. (1990) (-0.5 MPa) lowered the mean germination time of seeds of lettuce, carrot and onion. Goobkin (1989) and Ozbingol *et al*. (1999) also reported that PEG 6000 solution treated tomato seeds germinate faster than untreated seeds and this is due to more rapid water uptake. The probable reason for early emergence of the primed seed may be due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005). Yamauchi and Winn (1996) indicated that seed priming may help in dormancy breakdown possibly by embryo development and leaching of emergence inhibitors which resulted in an earlier start of emergence.

2.2.3 Germination index

Seed performance under drought or salt stress is affected by the concentration of priming materials. Sun *et al*. (2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than

hydro priming, while higher concentrations of PEG had negative effects on seed germination.

In addition to better establishment, primed crops grew more vigorously, flowered earlier and yielded higher (Farooq *et al*., 2008). Ruan *et al*. (2002a) had observed that KCl and $CaCl_2$ seed priming had improved germination index of rice. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops particularly seeds of vegetables and small seeded grasses (Dell Aquila and Tritto, 1991; Donaldson *et al*., 2001). Rashid *et al*. (2006) reported that priming enhanced germination, better establishment and increased yields in many diverse environments for a number of crops (Khan *et al*., 2008). Arif *et al*. (2005) who reported that seed priming enhanced germination which may be attributed to repair processes, a buildup of germination metabolites or osmotic adjustments during priming treatment. Maiti *et al.* (2006) also reported that osmotic seed priming of maize caryopses in copersulphate, zinc sulphate, manganese sulphate, or boric acid induced high levels of seed germination. Hydro priming was found to be the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 hr compared to 48 hr (Caseiro *et al*., 2004). It improved germination and later growth of different crops species such as in maize, rice, chickpea (Harris *et al*., 1999).

2.2.4 Coefficient of velocity

Improving germination and coefficient of velocity in treated fenugreek seeds may be explained by an increase of cell division in the seeds (Bose and Mishra, 1992).

2.2.5 Energy of emergence (%)

Seed priming enhances speed and uniformity of germination (Khalil *et al*., 2010; Khan *et al*., 2008; Heydecker *et al*., 1975) and induces several biochemical changes in the seed that are required to start the germination process such as breaking of dormancy, hydrolysis or mobilization of inhibitors,

imbibition and enzyme activation. Some or all of these processes that precede the germination are trigged by priming and persist following the re-desiccation of the seeds (Asgedom & Becker, 2001). Thus upon seeding, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). The resulting improved stand established can reportedly increase the drought tolerance, reduce pest damage and increase crop yield in cereals and legumes (Harris *et al*., 1999; Mussa *et al*., 1999; Harris *et al*., 2000; Khan *et al*., 2005). Seed priming stimulates many of the metabolic processes involved in the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously and perform better in adverse conditions (Cramer, 2002). It has also been reported that seed priming improves emergence, stand establishment, tillering, allometry, grain and straw yields, and harvest index (Farooq *et al*., 2008).

Seed priming has been found a double technology to enhance rapid and uniform emergence, and to achieve high vigor and better yields in vegetables and floriculture (Dear man *et al*., 1987; Parera and Cantliffe, 1994; Bruggink *et al*., 1999) and some field crops (Hartz and Caprile 1995; Chiu *et al*., 2002; Giri and Schillinger, 2003; Murungu *et al*., 2004; Basra *et al*., 2005; 2006; Kaur *et al*., 2005; Farooq *et al*., 2006 a, b; 2007 a, b). The enhanced phenology in mungbean due to primed seed is associated with faster emergence and reduced germination imbibition periods (Harris *et al*., 1999). It has been declared that priming had been resulted in more germination speed especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Sivritepe *et al*., 2003; Demir Kaya *et al*., 2006; Foti *et al*., 2002). Soybean seed priming are made better seedling emergence and yield improvement (Arif *et al*., 2008).

Seed priming techniques such as hydro priming, hardening, osmo priming, osmo hardening, hormonal priming and hydro priming have been used to

accelerate emergence more vigorous plants and better drought tolerance in many field crop like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006), cotton (Casenave and Toselli, 2007) triticale (Yagmur and Kaydan, 2008). Potassium hydro phosphate (K_2HPO_4), polyethylene glycol (PEG 6000) (Dell Aquila and Taranto, 1986) and potassium chloride (KCl) (Misra and Dwibedi, 1980) have been introduced as the osmoticum which have shown good potential to enhance germination, emergence, growth, and/or grain yield of wheat. Water has also been used successfully as a seed priming medium for wheat (Harris *et al*., 2001). Ghiyasi *et al*. (2008) declared osmo priming of maize (*Zea mays* L.) seeds with polyethylene glycol 8000 (PEG 8000) at -0.5 MPa osmotic potential had improved emergence, grain and biological yields compared with other treatments. The probable reason for early emergence of the primed seed may be due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005). Halo priming with $CaCl_2$ significantly improved emergence and seedling growth in Shaheen Basmati whereas as $CaCl_2$ and KCl proved better in case of Basmati-2000 which could be related to dormancy breakdown of rice seeds due to enhanced seed K and Ca concentration and amylase activity (Farooq *et al*., 2006b).

Zheng *et al*. (2002) reported earlier and uniform emergence in rice *(Oryza sativa)* seeds osmoprimed with KCl and $CaCl_2$ and mixed salts under flooded conditions. However, Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon (*Cucumis melo*) seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale *et al*., 2003; Giri and Schillinger, 2003).

2.3 Effect on growth parameters

2.3.1 Shoot length (mm)

Priming with KNO_3 can be used to increase watermelon germination (Demir and Mavi, 2004) and in tomato, seed priming with KNO_3 increased germination percentage, germination index, root length, shoot length and seedling fresh weight (Nawaz *et al*., 2011). It was reported that osmo and hydro priming of chickpea seeds with mannitol and water alleviated the adverse effects of water deficiency and salt stress on seedling growth. The treatment of seeds with water, 2% and 4 % mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions (Kaur *et al*., 2002, 2005).

Recently, auxin is also used for priming (Akbari *et al*., 2007). In wheat seed germination, auxin treatments increased the hypocotyl length, seedling fresh and dry weight and hypocotyl dry weight (Akbari *et al*., 2007). Hydro priming has resulted in 3 to 4-fold increases in root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition (Kaur *et al*., 2002).

2.3.2 Root length (mm)

Seed priming techniques such as hydro priming, hardening, osmo-conditioning, osmo-hardening and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007). ABA-primed seeds of *Brassica napus* exhibited earlier (2–7 days) germination and higher final percent radicle protrusion than non-primed control seeds, under salt (100 mM NaCl) or water stress (20 % PEG 8000) and at a low temperature (8 LC) (Gao *et al*., 2002). Kulkarni and Eshanna (1988) stated that pre-sowing seed treatment with IAA at 10 ppm improved root length, rate of germination, and seedling vigor. Kathiresan *et al*. (1984) also found similar findings and

reported maximum root and shoot growth; seedling height and field emergence in sunflower seeds in response to priming with $CaCl_2$. Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells Kaya *et al*. (2006).

Osmo priming and hydro priming of wheat seeds may improve germination and emergence (Ashraf and Abu-Shakra, 1978) and may promote vigorous root growth (Carceller and Soriano, 1972). Hydro primed seeds produced the largest roots, compared to other seed treatments Kathiresan and Gnanarethinam (1985) in sunflower. This means that during priming, seeds would be simultaneously subjected to processes of repair and deterioration and force between the two determined the success or failure of the treatment (McDonald, 2000). Also, important to consider is the toxic effect reported for PEG (Grzesik and Nowek, 1998) and the decrease in oxygen solubility (Welbaum, 1998; Toselli and Casenave, 2002, 2003) that could be responsible for the anoxia damages suggested by Sung and Chang (1993).

2.3.3 Shoot dry weight (mg) and root dry weight (mg)

Harris *et al*. (2004) reported that higher plant dry weight and seed yield following seed priming. The increase in the dry matter and grain yield of mungbean was due to better emergence and better performance per plant (Parera and Cantliffe, 1994). Increased plumule dry weight due to osmo priming was reported by Harris *et al*. (2004).

2.3.4 Vigor index

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

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

Primed crops grew more vigorously, flowered earlier and yielded higher (Farooq *et al*., 2008). This technique used for improvement of germination speed, germination vigour, seedling establishment and yield (Talebian *et al*., 2008; Bodsworth and Bewley, 1981). Harris *et al*. (1999) demonstrated that on-farm seed priming (soaking seeds overnight in water) markedly improved establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. Similarly, vigorous early growth is often associated with better yields (Okonwo and Vanderlip, 1985; Austin, 1989; Carter *et al*., 1992). Seed-priming technology has twofold benefits: enhanced, rapid and uniform emergence, with high vigour and better yields in vegetables and floriculture (Bruggink *et al*., 1999) and some field crops (Basra *et al*., 2005; Kaur *et al*., 2005). It has been reported that primed seeds showed better germination pattern and higher vigor level than non- primed (Ruan *et al*., 2002a). It has been also reported invigorated seeds had higher vigour levels (Ruan *et al*., 2002b), which resulted in earlier start of emergence as high vigour seed lots performed better than low vigor ones (Hampton and Tekrony, 1995).

Seed priming techniques such as hydro priming, hardening, osmo conditioning, osmo hardening and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007). Various works have shown that hydro priming of seeds have many advantages as compared to non-primed seeds. This phenomenon was explained to be due to faster emergence of roots and shoots, more vigorous plants, better drought tolerance under adverse conditions (Amzallag *et al*., 1990; Passam and Kakouriotis, 1994; Cayuela *et al*., 1996; Lee-suskoon *et al*., 1998). Fujikura *et al*. (1993) presented hydropriming as a simple and inexpensive method of seed

priming and according to Abebe and Modi (2009) it is a very important seed treatment technique for rapid germination and uniform seedling establishment in various grain crops.

Priming of seeds with water promoted seedling vigor, yield and crop establishment of chickpea, maize and rice in India (Harris *et al.*, 1999). Harris *et al*. (1999) also found that hydro priming enhanced seedling establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. The resulting improved stand establishment can reportedly increase drought tolerance, reduce pest damage and increase crop yield (Harris *et al*., 1999). Similarly, vigorous early growth is often associated with better yields (Okonwo and Vanderlip, 1985; Austin, 1989; Carter *et al*., 1992). Priming of tomato *(Lycopersicon lycopersicum)* seeds with NaCl had been reported to improve seedling growth.

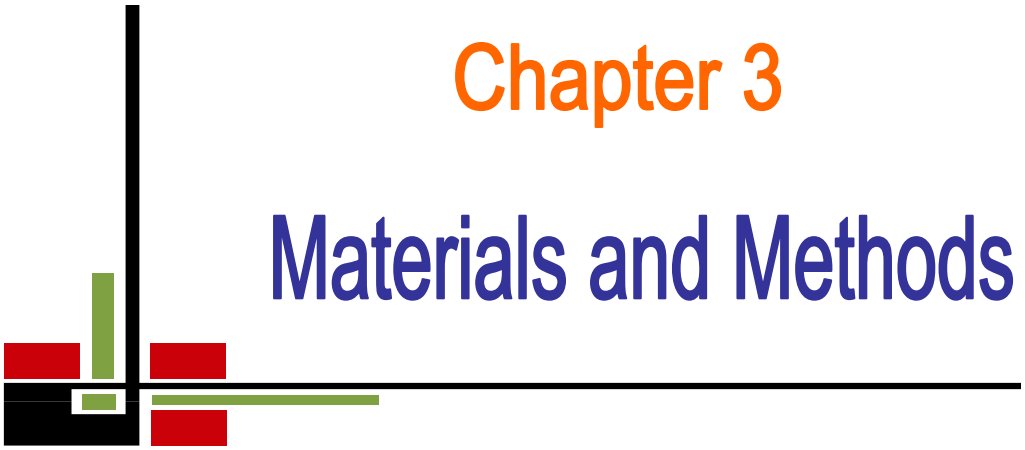
Osmopriming with KNO_3 improved the rate and generally improved the uniformity of seedling emergence in leek (Brocklehurst *et al*., 1984), sorghum (Moradi and Younesi, 2009) and tomato (Heydecker *et al*., 1973; Ozbingol *et al*., 1998). Chiu *et al.* (2006) reported that KNO_3 effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower varieties. Salt priming with KNO_3, is an effective way to improve seed and seedling vigour of sunflower and cucumber (Singh and Rao, 1993; Ghassemi-Golezani and Esmaeilpour, 2008).

Hydro priming improved the early and vigorous crop establishment in maize (Nagar *et al.,* 1998) and *Heiichrysum bracteatum* L. (Grzesik and Nowak, 1998). However, other studies resulted in poor emergence from hydro primed Kentucky bluegrass seeds under field conditions (Pill and Necker, 2001). However Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research

workers (Mwale *et al.,* 2003; Giri and Schillinger, 2003).

2.4 Relative water content (%), water saturation deficit (%) and water retention capacity

Baque *et al.* (2002) observed that higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium however, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions. Sangakkara *et al.* (1996) observed that when *phasiolous vulgaris* L. plants were subjected to moisture stress, the WRC increase with the increasing potassium concentrations.



Chapter 3

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from 13 May, 2014 to 15 July, 2014 to study the effect of mannitol induced seed priming for enhancing drought tolerance capability in mungbean (*Vigna radiata*) under drought stress. 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 that were used to conduct the study are presented below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

The experiment was conducted at the Agronomy Laboratory, Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207. It was located in $23^0 41'$ N latitude and $90^0 22'$ E longitude at a height of 8.6 m above the sea level.

3.1.2 Conditions of laboratory room

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study months of the culture room was 17.4^0 C to 38.2^0 C, respectively and average minimum and maximum relative humidity was 40% and 89.20%, respectively (Appendix I).

3.2 Test crops

Two mungbean varieties namely BARI Mung 3 and BARI Mung 6 were used for this experiment. Both mungbean varieties were collected from Bangladesh Agricultural Research Institute (BARI). The collected mungbean varieties were

free from any visible defects, disease symptoms and insect infestations and transported to the laboratory of the Department of Agronomy, SAU, Dhaka with careful handling to avoid disease and injury.

3.3 Experimental materials

Different equipments such as electric balance, EC (Electrical conductivity) meter, Petri dish, filter paper, micro pipette, forcep, oven etc. were used for this study.

3.4 Chemicals for seed priming

Different priming chemicals such as Mannitol ($C_6H_{14}O_6$) and distilled water were utilized for osmo and hydro priming. Polyethylene Glycol (PEG) was utilized for induce drought stress. 75% alcohol was used as seed treating chemical.

3.5 Experimental treatments and design

The experiment comprises of

(a) Six levels of priming time viz. 3, 6, 9, 12, 15, 18 hours

(b) Six levels of priming agent concentration viz. water, 0, 2%, 4%, 6% and 8% mannitol ($C_6H_{14}O_6$) and

(c) Five levels of drought stress viz. 0, 5%, 10%, 15%, 20% Polyethylene Glycol (PEG) 6000.

The experiment was laid out in a Completely Randomized Design (CRD) with 5 replications.

3.6 Experimental details

The whole experiment was conducted under three different experiments.

3.6.1 1st Experiment

Optimization of pre-sowing priming time through the electrical conductivity (EC) value by the leakage of mungbean (BARI Mung 3 and BARI Mung 6).

3.6.1.1 Weight of seeds

200g seeds were weighted from the total seed from each of two mungbean varieties BARI Mung 3 and BARI Mung 6 to reduce the unnecessary loss of seeds. Remaining seeds are taken in poly bag and preserved in refrigerator.

3.6.1.2 Surface treatment

75% alcohol solution initially used to treat the seeds for 5 min for surface sterilization. The sterilized seeds were rinsed 2 min with distilled water for 3 times to reduce the residual alcohol from the seed surface. Seeds are then dried in room temperature for 24 hr to regain the normal weight.

3.6.1.3 Treatments and design

Mungbean variety (02):

1. BARI Mung 3
2. BARI Mung 6

Thirty one types of priming times are used as treatment to measure the EC. They are as follows:

1. T_0=Seeds without priming (control)
2. T_1= Seeds primed with water for 3 hours
3. T_2= Seeds primed with water for 6 hours
4. T_3= Seeds primed with water for 9 hours
5. T_4= Seeds primed with water for 12 hours
6. T_5= Seeds primed with water for 15 hours
7. T_6= Seeds primed with water for 18 hours
8. T_7= Seeds primed with 2% mannitol solution for 3 hours
9. T_8= Seeds primed with 2% mannitol solution for 6 hours
10. T_9= Seeds primed with 2% mannitol solution for 9 hours
11. T_{10}= Seeds primed with 2% mannitol solution for 12 hours
12. T_{11} = Seeds primed with 2% mannitol solution for 15 hours
13. T_{12}= Seeds primed with 2% mannitol solution for 18 hours
14. T_{13}= Seeds primed with 4% mannitol solution for 3 hours

15. T_{14}= Seeds primed with 4% mannitol solution for 6 hours
16. T_{15}=Seeds primed with 4% mannitol solution for 9 hours
17. T_{16}=Seeds primed with 4% mannitol solution for 12 hours
18. T_{17}=Seeds primed with 4% mannitol solution for 15 hours
19. T_{18}=Seeds primed with 4% mannitol solution for 18 hours
20. T_{19}=Seeds primed with 6% mannitol solution for 3 hours
21. T_{20}= Seeds primed with 6% mannitol solution for 6 hours
22. T_{21}=Seeds primed with 6% mannitol solution for 9 hours
23. T_{22}=Seeds primed with 6% mannitol solution for 12 hours
24. T_{23}=Seeds primed with 6% mannitol solution for 15 hours
25. T_{24}=Seeds primed with 6% mannitol solution for 18 hours
26. T_{25}=Seeds primed with 8% mannitol solution for 3 hours
27. T_{26}=Seeds primed with 8% mannitol solution for 6 hours
28. T_{27}=Seeds primed with 8% mannitol solution for 9 hours
29. T_{28}=Seeds primed with 8% mannitol solution for 12 hours
30. T_{29}=Seeds primed with 8% mannitol solution for 15hours
31. T_{30}=Seeds primed with 8% mannitol solution for 18 hours

3.6.1.4 Priming solutions

2%, 4%, 6%, and 8% of mannitol solution and distilled water were used as priming solutions.

3.6.1.5 Preparation of priming solutions

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

5 g of mannitol was dissolved in 250 mL of water to prepare 2% solution of mannitol. Similarly, 10g, 15g, 20g mannitol was dissolved in 250 mL of water to prepare 4%, 6%, and 8% solution of mannitol, respectively.

b) Distilled water

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

3.6.1.6 Priming technique

The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed). Seeds of a sub-sample were divided into six sub-sample soaked in distilled water for six different priming times such as 3, 6, 9, 12, 15, and 18 hours for hydro priming. For osmo priming the remaining sample of seeds were divided into more six sub-sample and presoaked with mannitol for 3, 6, 9, 12, 15, and 18 hours. Priming is done in different plastic containers covered with lids to prevent evaporation loss. Seeds were removed from the priming solution at the required time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.6.1.7 Conductivity test

Conductivity test is used to quantify the leakage of electrolytes from the seed coat with respect to age, storage life and other factors i.e. temperature, humidity, soil and water stress. A sample of 50 seeds was taken from each treatment, placed in a 250 mL flask with 200 mL of distilled water. Experimental units (155 Petri dishes for each variety) were arranged in a completely randomized design with five replications. The flasks were stirred to remove air bubbles and floating seed, covered with aluminum foil and were kept at room temperature for 24 hr. After soaking, seeds were gently swirled and the conductivity of the soaked water was measured with a dip type cell (Cell Constant of 1.0) conductivity meter. Conductivity was expressed on a weight basis in deci-Siemens $m^{-1}(dSm^{-1})$ g^{-1} of seed (ISTA, 1993).

3.6.2 2nd Experiment

Study on the effect of different concentrations of Mannitol on the germination behavior of mungbean.

3.6.2.1.1Weight of seeds

200 g seeds were weighted from the total seed from each of two mungbean varieties BARI Mung 3 and BARI Mung 6 to reduce the unnecessary loss of seeds.

3.6.2.1.2 Surface treatment

Seeds were initially treated with 75% solution of alcohol for 5 min for surface sterilization. The sterilized seeds were rinsed 2 min with distilled water for 3 times to reduce the residual alcohol from the seed surface. Seeds were then dried in room temperature to regain the normal weight.

3.6.2.1.3 Treatments

Six different treatments were applied separately in case of two Varieties

BARI Mung 3	BARI Mung 6
T_0= Seeds without priming (control)	T_0= Seeds without priming (control)
T_1= Seeds primed with distilled water	T_1= Seeds primed with distilled water
T_2= Seeds primed with 2% mannitol solution	T_2= Seeds primed with 2% mannitol solution
T_3= Seeds primed with 4% mannitol solution	T_3= Seeds primed with 4% mannitol solution
T_4= Seeds primed with 6% mannitol solution	T_4= Seeds primed with 6% mannitol solution
T_5= Seeds primed with 8% mannitol solution	T_5= Seeds primed with 8% mannitol solution

3.6.2.1.4 Priming solutions

2%, 4%, 6%, and 8% of mannitol solution and distilled water were used as priming solutions.

3.6.2.1.5 Preparation of priming solutions

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

5 g of mannitol was dissolved in 250 mL of water to prepare 2% solution of mannitol. Similarly 10g, 15g, 20g mannitol was dissolved in 250 mL of water to prepare 4%, 6% and 8% solution of mannitol, respectively.

b) Distilled water

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

3.6.2.1.6 Priming technique

Two priming techniques viz., osmo priming and hydro priming were applied on both the mungbean varieties. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were primed with priming chemicals. For hydro priming seeds of a sub-sample were soaked in distilled water and for osmo priming seeds of another sub-sample were divided into another four sub-sample and pretreated with mannitol at a four levels of concentration of 2%, 4%, 6%, and 8% for 9 hours. Priming was done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al.,* 2011) in room temperature for 24 hours back to the original moisture level.

3.6.2.1.7 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 120 mm diameter Petri dishes on whatman No.1 filter paper moist with 8 mL of distilled water. Here, whatman No.1 filter paper were used as growth media

for germination. Experimental units (30 Petri dishes for each variety) were arranged in completely randomized design with five replications. During the test filter papers in the Petri dishes were kept saturated condition with water. Seeds were kept at room temperature 25°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari *et al.,* 2007). Germination progress was inspected and data were collected at every 24hr intervals and continued up to 8 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 excluded during counting. At the end of germination test (8 days), 6 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75°C for 48 hours.

3.6.2.2 Relative water content (%), water saturation deficit (%) and water retention capacity of shoot

Two normal seedlings from each of 5 replication per treatment were carefully collected. Fresh weight was measured immediately after removing the redicles. Thereafter, the shoots were immersed in distilled water for 24 hr at room temperature in the dark. These shoots were weighted after removing excess water by gently wiping with paper towel to determine their turgid weight. The shoots were then dried in an oven for 72 hr at 70^0C to determine their dry weights. The fresh, turgid and dry weights of shoots were used to calculate relative water content (%), water saturation deficit (%) and water retention capacity (Baque *et al.,* 2002).

3.6.3 3rd experiment

Germination behavior of primed Seed (mungbean) under drought stress condition induce by PEG (Polyethylene Glycol)

3.6.3.1.1 Weight of seeds

Seeds were weighted 200g from each of the total seed of BARI Mung 3 and BARI Mung 6 for this experiment to reduce the unnecessary loss of seeds.

3.6.3.1.2 Surface treatment

Seeds were initially treated with 75% solution of alcohol for 5 minutes in surface sterilization. The sterilized seeds were rinsed 2 minute with distilled water for 3 times to reduce the residual alcohol from the seed surface. Seeds were then dried in room temperature to regain the normal weight.

3.6.3.1.3 Treatments

Five different treatments were applied separately in case of two Varieties

BARI Mung 3	BARI Mung 6
T_0= Primed (mannitol and water) and non-primed (control) seeds placed without PEG (control)	T_0= Primed (mannitol and water) and non-primed (control) seeds placed without PEG (control)
T_1= Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG concentration	T_1= Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG concentration
T_2= Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG concentration	T_2= Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG concentration
T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG concentration and	T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG concentration and
T_4= Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG concentration.	T_4= Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG concentration.

3.6.3.1.4 Priming solutions and time

2% mannitol solution for BARI Mung 3, 6% mannitol solution for BARI Mung 6 and distilled water were used as priming solutions and 9 hours as priming time.

3.6.3.1.5 Preparation of priming solutions

a) **Mannitol solutions (2% and 6%)**

   5g and 15 g of mannitol were dissolved in 250 mL of water to prepare 2% and 6% solution of mannitol.

b) **Distilled water**

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

3.6.3.1.6 Preparation of stress solutions

Drought (PEG) solutions (5%, 10%, 15% and 20%)

12.5 g of Polyethylene Glycol (PEG) was dissolved in 250 mL of water to prepare 5% solution of PEG. Similarly, 25g, 37.5g, 50g Polyethylene Glycol (PEG) was dissolved in 250 mL of water to prepare 10%, 15% and 20% solution of PEG (6000) respectively.

3.6.3.1.7 Priming technique

Two priming techniques viz., osmo priming and hydro priming were applied on BARI Mung 3 and BARI Mung 6. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were primed with priming chemicals. Seeds of a sub-sample were soaked in distilled water for hydro priming for 9 hours and seeds of another sub-sample were pretreated with mannitol for osmo priming at a concentration of 2% for BARI Mung 3 and 6% for BARI Mung 6 by maintaining 9 hours priming duration, respectively. Priming is done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for

three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.6.3.1.8 Germination of seeds

The standard germination test was performed by placing randomly selected 30 seeds in 120 mm diameter Petri dishes on whatman No.1. Petri dishes containing primed and control seeds were irrigated with solutions of 8 mL drought stress levels. Here whatman No.1 filter paper were used as growth media for germination. Experimental units (75 Petri dishes for each varieties) were arranged in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept in saturated state with respected solution. Seeds were kept at room temperature 25°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari *et al.,* 2007). Germination progress was inspected and data were collected at every 24 hr intervals and continued up to 8 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 excluded during counting. At the end of germination test (8 days), 6 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75°C for 48 hours.

3.6.3.2 Relative water content (%), water saturation deficit (%) and water retention capacity of shoot

Two normal seedlings from each of 5 replication per treatment were carefully collected. Fresh weight was measured immediately after removing the redicles. Thereafter, the shoots were immersed in distilled water for 24hr at room temperature in the dark. These shoots were weighted after removing excess water by gently wiping with paper towel to determine their turgid weight. The

shoots were then dried in an oven for 72hr at 70^0C to determine their dry weights. The fresh, turgid and dry weights of shoots were used to calculate relative water content (%), water saturation deficit (%) and water retention capacity (Baque *et al.,* 2002).

3.7 Data recording

Parameters that are measured as follows:

3.7.1 Total germination (TG %)

Total germination (TG) was calculated as the number of seeds which was germinated within total days as a proportion of number of seeds shown in each treatment expressed as a percentage (Othman *et al.,* 2006).

$$TG\ (\%) = \frac{\text{Number of germinated seed}}{\text{Total number of seed set for germination}} \quad X\ 100$$

3.7.2 Mean germination time (MGT)

Mean germination time (MGT) was calculated according to the equation of Moradi Dezfuli *et al.* (2008).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where,
n = number of seeds germinated on day D, and
D = number of days counted from the beginning of germination.

3.7.3 Germination index (GI)

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae:

$$\text{Germination index} = \frac{Gt}{Tt}$$

Where,

Gt = number of seeds germinated on day t and

Tt = the number of germinated seeds at time Ti.

3.7.4 Coefficient of velocity (CV)

Coefficient of velocity (CV) = (number of germinated seeds per day) is measured according to the method described by Scott *et al.* (1998).

$$CV = 100 \times (\sum Ni / \sum Ti\ Ni)$$

Where,

Ti= number of days after sowing and

Ni = number of seeds germinated on ith day.

3.7.5 Energy of emergence (EG %)

Energy of emergence (EG) was recorded on the 4th day after placement of seeds. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al.*, 2002a). Energy of emergence expressed in percentage.

3.7.6 Shoot length (mm) and root length (mm)

Randomly selected 6 seedlings from each treatment were collected and cotyledons were removed from them. Shoot and root length were measured with a ruler and accuracy of measurement was 1 mm.

3.7.7 Shoot dry weight (mg) and root dry weight (mg)

The dried radicles and shoots were weighted to the nearest gram (g) and converted to milligram. The mean radicle and shoot dry weight were determined with a electric balance.

3.7.8 Vigour Index (VI)

Vigour Index (VI) was calculated from total germination and seedlings length by using the formula of Abdul- Baki and Anderson (1970).

$$VI = \frac{TG(\%) \times \text{seedling length(mm)}}{100}$$ Here, TG = total germination.

3.7.9 Relative Water Content (RWC %)

Relative water content was calculated from the fresh, turgid and dry weights of shoots by using the following formula used by Baque *et al*. (2002). Relative water content expressed in percentage.

$$\text{Relative Water Content (RWC)} = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

3.7.10 Water Saturation Deficit (%)

Water saturation deficit was calculated from RWC by using the following formula used by Baque *et al.* (2002).

Water Saturation Deficit (WSD) = 100- RWC

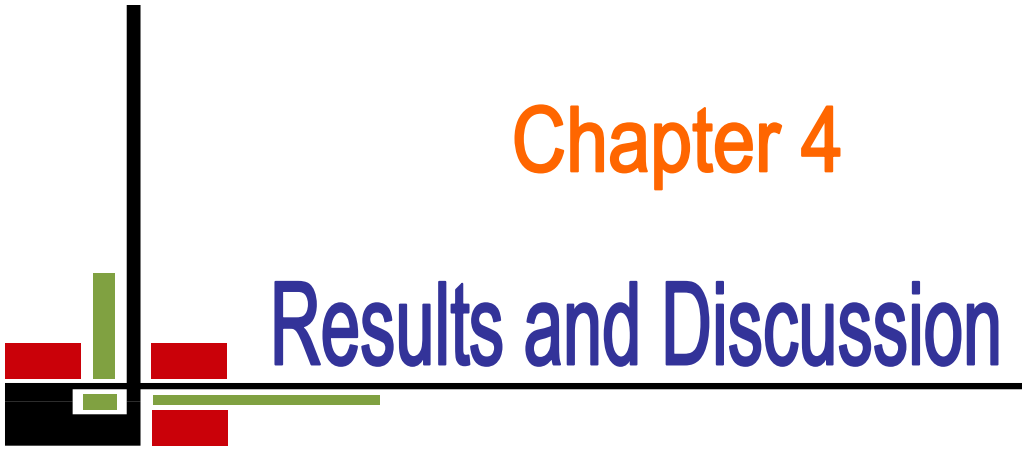
3.7.11 Water Retention Capacity (WRC)

Water retention capacity was calculated from the turgid and dry weights of shoots by using the following formula used by Baque *et al.* (2002). Water retention capacity expressed in percentage.

$$\text{Water Retention Capacity (RWC)} = \frac{\text{Turgid weight}}{\text{Dry weight}}$$

3.8 Statistical analysis

The data obtained for different parameters were statistically analyzed to observe the significant difference among the treatments. The mean value of all the parameters was calculated and analysis of variance was performed. The significance of difference among the treatments means was estimated by the least significant difference (LSD) test at 5% level of significance. A computer software MSTAT-C was used to carry out the statistical analysis. Drawings were made using Excel software.



Chapter 4

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprises presentation and discussion of the results obtained from the experiment enhancement of drought tolerance in mungbean (*vigna radiata* L.) through osmo and hydro priming in two mungbean varieties cv. BARI Mung 3 and BARI Mung 6. The results of the germination and growth parameters of mungbean as influenced by different concentrations of priming agent (mannitol) and priming time in drought stress condition have been presented and discussed in this chapter.

4.1 Experiment 1: Optimization of pre-soaking priming time on electrical conductivity (EC) and germination behavior of mungbean (BARI Mung 3 and BARI Mung 6)

Results obtained from the present study regarding the effects of different priming time of different mannitol concentrations and water on the electrical conductivity (EC) and germination behavior of mungbean (BRRI mung-3 and BARI Mung 6) has been presented, discussed and compared in this chapter. The analytical results have been presented in Table 1 and Appendices II.

4.1.1 Effect on electrical conductivity (EC) value (dSm^{-1})

The electrical conductivity (EC) of seed leachates from 3, 6, 9, 12, 15, 18 hours primed seeds, hydro primed seeds and non primed seed were measured after 24 hours of soaking in distill water. The electrical conductivity (EC) (dSm^{-1}) was significantly influenced by priming (water and mannitol) time (Appendix II and Table 1). EC gradually decrease up to 9 hours in both water and mannitol priming seeds and gradually increases after 9 hours for both water and mannitol priming seeds. Results revealed that amon the varieties the lowest EC 0.0660 dSm^{-1} was recorded from T_9 treatment at 9hr for BARI Mung 3, on the other hand the lowest EC 0.1110 dSm^{-1} was observed from T_{21} treatment at 9hr for BARI Mung 6. The highest EC 0.3636 dSm^{-1} & 0.5953 dSm^{-1} was recorded in

control treatment for both verieties. The probable reason for the reduction of EC value of the hydro primed and mannitol primed seed may be priming might have enhanced the repair of cell membranes that were disrupted during ageing (Senaratna *et at*., 1988). The repair of membrane could initiate there-activation or re-synthesis of membrane-bound enzymes and enhanced germination (Rao *et al.,* 1987). Similarly in canola seeds, electrical conductivity from the leachate of osmo primed and hydro-primed seeds were lower than that of non-primed seeds. Seed priming was effective in decreasing electrical conductivity of seed leachates, which show membrane stability. Decreased leakage of solute from primed seed may be because of better membrane repair during hydration (Fu *et al.*, 1988). Sadeghi *et al.* (2011) found that lowest electrical conductivity (EC) value indicate the best priming time.

Table 1. Effect of different priming time on electrical conductivity (EC) of primed (mannitol and water) and non-prime (control) seeds

Treatments	EC (dSm^{-1})	
	BARI Mung 3	BARI Mung 6
T_0	0.3636 a	0.5953 a
T_1	0.1010 m	0.2220 kl
T_2	0.0909 n	0.1816 m
T_3	0.0707 p	0.1514 no
T_4	0.1111 l	0.2321 k
T_5	0.1212 k	0.2725 i
T_6	0.1313 j	0.2926 h
T_7	0.0909 n	0.2119 l
T_8	0.0808 o	0.1614 n
T_9	0.0606 q	0.1413 o
T_{10}	0.1010 m	0.2321 k
T_{11}	0.1111 l	0.2725 i
T_{12}	0.1313 j	0.2926 h
T_{13}	0.1010 m	0.1816 m
T_{14}	0.0909 n	0.1614 n
T_{15}	0.0757 op	0.1413 o
T_{16}	0.1111 l	0.2220 kl
T_{17}	0.1212 k	0.2523 j
T_{18}	0.1414 i	0.2825 hi
T_{19}	0.1515 h	0.1816 m
T_{20}	0.1414 i	0.1614 n
T_{21}	0.1515 h	0.1110 p
T_{22}	0.1717 g	0.2220 kl
T_{23}	0.1717 g	0.2321 k
T_{24}	0.1717 g	0.2725 i
T_{25}	0.1818 f	0.3229 g
T_{26}	0.1717 g	0.3635 f
T_{27}	0.2121 e	0.3834 e
T_{28}	0.2424 d	0.4238 d
T_{29}	0.2525 c	0.4843 c
T_{30}	0.2626 b	0.5054 b
LSD(0.05)	0.007917	0.01252
CV(%)	4.54	4.07

T_0=Seeds without priming (control), T_1= Seeds primed with water for 3 hours, T_2= Seeds primed with water for 6 hours, T_3= Seeds primed with water for 9 hours, T_4= Seeds primed with water for 12 hours, T_5= Seeds primed with water for 15 hours, T_6= Seeds primed with water for 18 hours, T_7= Seeds primed with 2% mannitol solution for 3 hours, T_8= Seeds primed with 2% mannitol solution for 6 hours, T_9= Seeds primed with 2% mannitol solution for 9 hours, T_{10}= Seeds primed with 2% mannitol solution for 12 hours, T_{11} = Seeds primed with 2% mannitol solution for 15 hours, T_{12}= Seeds primed with 2% mannitol solution for 18 hours, T_{13}= Seeds primed with 4% mannitol solution for 3 hours, T_{14}= Seeds primed with 4% mannitol solution for 6 hours, T_{15}=Seeds primed with 4% mannitol solution for 9 hours, T_{16}=Seeds primed with 4% mannitol solution for 12 hours, T_{17}=Seeds primed with 4%

mannitol solution for 15 hours, T_{18}=Seeds primed with 4% mannitol solution for 18 hours, T_{19}=Seeds primed with 6% mannitol solution for 3 hours, T_{20}= Seeds primed with 6% mannitol solution for 6 hours, T_{21}=Seeds primed with 6% mannitol solution for 9 hours, T_{22}=Seeds primed with 6% mannitol solution for 12 hours, T_{23}=Seeds primed with 6% mannitol solution for 15 hours, T_{24}=Seeds primed with 6% mannitol solution for 18 hours, T_{25}=Seeds primed with 8% mannitol solution for 3 hours, T_{26}=Seeds primed with 8% mannitol solution for 6 hours, T_{27}=Seeds primed with 8% mannitol solution for 9 hours, T_{28}=Seeds primed with 8% mannitol solution for 12 hours, T_{29}=Seeds primed with 8% mannitol solution for 15hours and T_{30}=Seeds primed with 8% mannitol solution for 18 hours and all were soaked for 24 hr. EC of distill water 0.00 dSm^{-1}.

4.2 Experiment 2: Study on the effect of different concentrations of mannitol on the germination behavior of mungbean

Results obtained from the present study regarding the effects of different concentrations of mannitol on the germination behavior of mungbean varieties cv. BRRI Mung 3 and BARI Mung 6 have been presented, discussed and compared in this chapter. The analytical results have been presented in Figures 1 to 7; Tables 2 to 7 and Appendices III to XV.

4.2.1 Effect on total germination (%)

Osmo priming in comparison with hydro priming can preserve plasma membrane structure and cause seeds to have better responses to germination traits because of controlled long hydration in seeds. In BARI Mung 3, significant variation was observed among the treatment (Figure 1 and Appendix III) priming with different concentrations of mannitol, water and control, statistically similar result was found in T_3, T_4 and T_5 treatments. On the other hand in BARI Mung 6, significant variation was observed among the different treatments (Figure 1 and Appendix II) priming with different concentrations of mannitol, water and control, statistically similar result was observed among T_1, T_2, T_3. Total germination percentage increased with mannitol concentration up to 2% and 6% for BARI Mung 3 and BARI Mung 6, respectively thereafter decreased due to increasing concentration of mannitol. The highest total germination 98.38 % of BARI Mung 3 was observed from T_2 treatment compare to total germination 93.09 % of BARI Mung 6 was observed in T_4 treatment. The lowest germination percentage 87.45 % and 78.83 % for BARI

Mung 3 and BARI Mung 6, respectively was found in T_0 treatment. Total germination of BARI Mung 3 was higher than BARI Mung 6. These findings are consistent of the results of Ghana and Schillinger (2003) seed primed with KH_2PO_4 and water treatments enhanced germination in wheat under normal condition compared to untreated seed. Basra *et al*. (2003) and Salinas (1996) reported improvement in germination percent, emergence and seedling stand by using seed priming techniques.

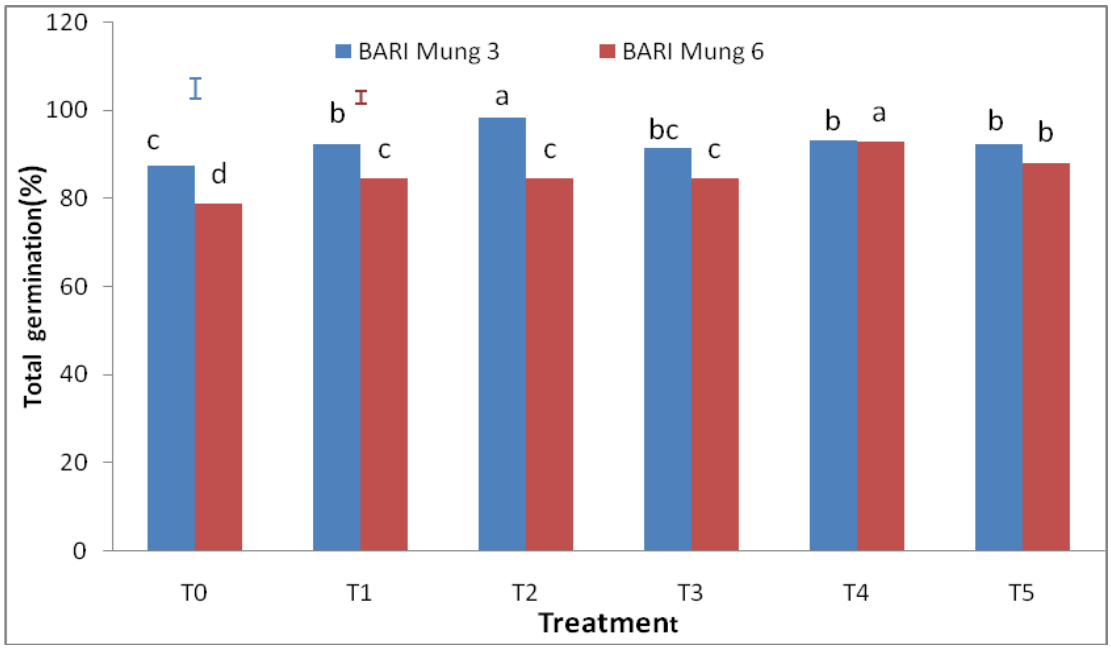


Figure 1. Effect of different concentrations of priming solution on total germination percentage of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours; T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours.

4.2.2 Effect on mean germination time (days)

Mean germination time for primed seeds is less than non-primed seeds. Such these positive effects is probably due to stimulatory effects of seed priming on biochemical activities and meiosis during primary stages of germination (Sivritepe *et al.*, 2003). There was no significant variation observed on mean germination time at different treatments (Table 2 and Appendix IV) for BARI

Mung 3 and BARI Mung 6. Mean germination time was affected by water priming and different mannitol concentration. With increasing mannitol concentration mean germination time decrease gradually up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter increased with increasing mannitol concentration. The longest mean germination time 5.529 days was observed for BARI Mung 3 from T_0 treatment compare to mean germination time 5.565 days of BARI Mung 6 was found at T_0 treatment. The shortest mean germination time 5.461 days was found in T_2 treatment compare to mean germination time 5.428 days was foundin T_4 treatment of BARI Mung 3 and BARI Mung 6, respectively. In corn seed Mohseni *et al*. (2010) shows that the most germination time is observed for treatments with 10% PEG and 2% KCl, and the least time is observed for treatment with 2% KNO_3. However Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions.

Table 2. Effect of different concentrations of priming solution on mean germination time (days) of primed (mannitol and water) and non-primed (control) seeds

Treatments	Mean germination time (days)	
	BARI Mung 3	BARI Mung 6
T_0	5.529	5.565
T_1	5.474	5.507
T_2	5.461	5.495
T_3	5.469	5.433
T_4	5.481	5.428
T_5	5.508	5.475
LSD(0.05)	NS	NS
CV(%)	2.66%	3.24%

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours; T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours.

4.2.3 Effect on germination index

Analysis of variance showed highly significant difference for germination index in BARI Mung 3 and BARI Mung 6 due to 9 hr seed priming with different priming concentration (Figure 2). The variation of germination index in BARI Mung 3 and BARI Mung 6 over different priming concentration, hydro priming and control treatment also confirmed by highly significant mean squares for treatment (Appendix V). Asaduzzaman (2014) also found significant difference in germination index for control and different priming treatments in BARI Mung 6 and BU 4. Results revealed that germination index increased up to 2% mannitol for variety BARI Mung 3 and up to 6% for variety BARI Mung 6 in mannitol concentration and then decreased slightly. Highest germination index 87.78 was recorded in T_2 treatment (statistically similar with T_1 & T_3 treatment) compare to germination index 85.41 was recorded from T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively. The lowest germination index 81.25 for BARI Mung 3 and 71.74 for BARI Mung 6 were found in T_0 treatment. Germination index of BARI Mung 3 was higher than BARI Mung 6.

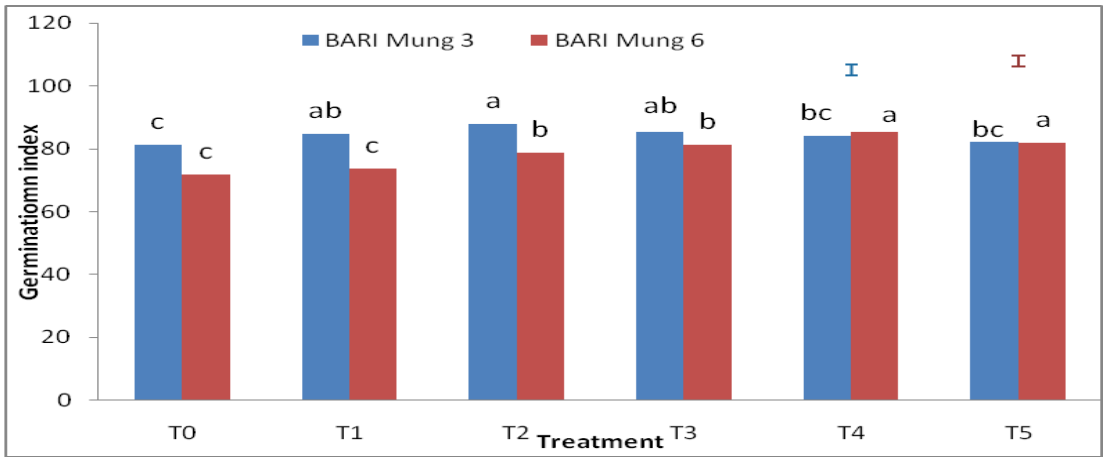


Figure 2. Effect of different concentrations of priming solution on germination index of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.4 Effect on coefficient of velocity

Higher Coefficient of velocity of primed seed than non primed seed was observed due to the increase of cell division inside the primed seed. There was no significant difference was observed among the different treatments (T_0, T_1, T_2, T_3, T_4, T_5) (Table 3) on the variable coefficient of velocity for BARI Mung 3 and BARI Mung 6. Coefficient of velocity increased with increasing mannitol concentration upto T_2 treatment and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter decreased gradually. The maximum coefficient of velocity (18.54) of BARI Mung 3 was observed from T_2 treatment compare to coefficient of velocity (18.71) of BARI Mung 6 at T_4 treatment. The minimum coefficient of velocity 18.31 was found for BARI Mung 3 and 18.25 for BARI Mung 6 were found in T_0 treatment, respectively. Improving germination and coefficient of velocity in treated fenugreek seeds may be explained by an increase of cell division in the seeds (Gallais *et al.*, 2000).

Table 3. Effect of different concentrations of priming solution on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds

Treatments	Coefficient of velocity	
	BARI Mung 3	BARI Mung 6
T_0	18.31	18.25
T_1	18.49	18.44
T_2	18.54	18.49
T_3	18.51	18.55
T_4	18.47	18.71
T_5	18.38	18.69
LSD(0.05)	NS	NS
CV(%)	2.58%	3.22%

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours; T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.5 Effect on energy of emergence (%)

As a result of faster cell division in the root tips is the possible reason for higher energy of emergence in minnitol and water primed seed than non primed seed. Energy of emergence showed significant variation for BARI Mung 6 and there was no significant variation observed in case of BARI Mung 3 on different concentrations of mannitol, water and control treatment (Appendix VII and Figure 3). Result showed that energy of emergence increase with increasing mannitol concentration up to 2 % and 6 % for BARI Mung 3 and BARI Mung 6, respectively and therefore decreased slightly. The highest energy of emergence 99.17 % was recorded for BARI Mung 3 in T_2 treatment compare to energy of emergence 97.31 % of BARI Mung 6 was recorded in T_4 treatment. The lowest energy of emergence 91.67% and 80.53% was recorded for BARI Mung 3 and BARI Mung 6 in T_0 treatment. Faster emergence rate after priming may be explained by an increased rate of cell division in the root tips as previously found for wheat (*Triticum aestivum*) (Bose and Mishra, 1992; Basra *et al*., 2002) and fine rice (*Oryza sativa*) (Basra *et al*., 2003).

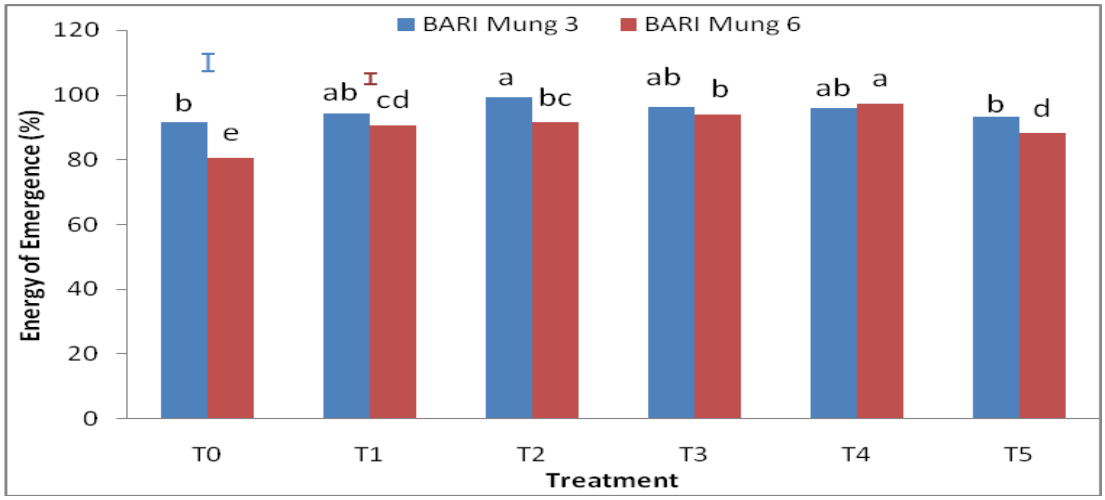


Figure 3. Effect of different concentrations of priming solution on energy of emergence (%) of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.6 Effect on shoot length (mm)

Seed priming technique helps to increase the embryo growth in seed that's why earlier germination occurs ultimately the shoot length of primed seed is higher than that of non primed seed. Significant variation was observed on shoot length among the two varieties (BARI Mung 3 and BARI Mung 6) priming with different concentrations of mannitol and water (Appendix VIII and Table 4). Shoot length increase upto T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and therefore decrease with the increasing mannitol concentrations. The maximum shoot length 143.7 mm was recorded for BARI Mung 3 in T_2 treatment compare to shoot length of BARI Mung 6 (110 mm) was recorded in T_4 treatment (Statistically similar with T_3 and T_5 treatment). The minimum shoot length 117.40 mm was found in T_0 treatment for BARI Mung 3 and shoot length 95.68 mm was found in T_0 treatment for BARI Mung 6. Shoot length of BARI Mung 3 was higher than BARI Mung 6. Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds.

Table 4. Effect of different concentrations of priming solution on shoot length (mm) of primed (mannitol and water) and non-primed (control) seeds of mungbean (BARI Mung 3 and BARI Mung 6)

Treatments	**Shoot length (mm))**	
	BARI Mung 3	BARI Mung 6
T_0	117.4 d	95.68 c
T_1	134.4 b	103.8 b
T_2	143.7 a	104.7 b
T_3	128.2 c	106.6 ab
T_4	125.3 c	110 a
T_5	118.2 d	106.6 ab
LSD(0.05)	5.404	4.491
CV(%)	3.24%	3.29%

T_0= Dry seeds; T_1= Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.7 Effect on root length (mm)

Faster cell division in the root tips is the possible reason for longer root length in osmo primed seed than dry seed. Statistically significant variation was recorded in terms of root length of BARI Mung 3 and BARI Mung 6 due to priming with water and different mannitol concentrations (Appendix IX and Figure 4). Root length was affected by water priming and different mannitol concentration. Root length increase up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and therefore decrease with the increasing mannitol concentration. The maximum root length 46.09 mm was observed from T_2 treatment for BARI Mung 3 compare to root length 36.55 mm observed in T_4 treatment for BARI Mung 6. The minimum root length 22.69 mm was found in T_0 treatment for BARI Mung 3 and 19.80 mm for BARI Mung 6. From current findings; root length and dry root mass increase with mannitol in rice is confirming the previous results of many scientists practiced in different crops (Nighat *et al*., 2006; Nishimura *et al*., 2011; Hoekstra *et al*., 2001).

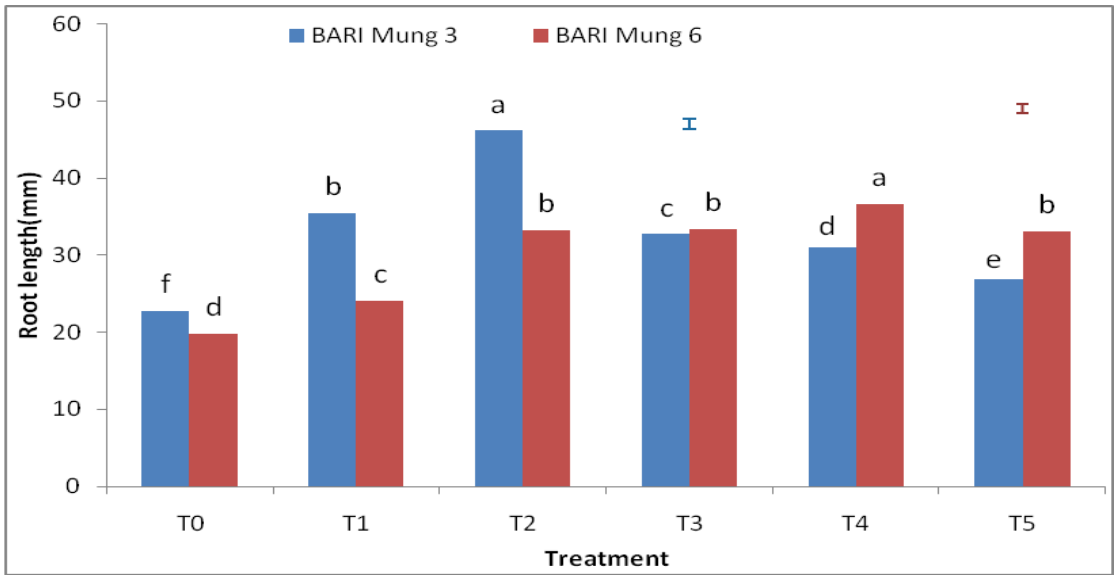


Figure 4. Effect of different concentrations of priming solution on root length (mm) of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.8 Effect on shoot dry weight (mg)

Seed priming favored shoot dry weight (mg) of mungbean differently depending on the differences in mannitol concentration, water and non-priming (control) (Appendix X and Table 5). There was significant variation among the treatments. Shoot dry weight of primed mungbean seeds was higher than those of unprimed seeds because of its higher germinability and faster emergence rate as observed in the laboratory test. Shoot dry weight increase up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter decrease with the increasing mannitol concentration. The maximum shoot dry weight 57.9 mg was observed from T_2 treatment for BARI Mung 3 compared to shoot dry weight 78.34 mg observed from T_4 treatment for BARI Mung 6. The minimum shoot dry weight 48.25 mg was found in T_0 treatment for BARI Mung 3 and 60.42 mg in T_0 treatment for BARI Mung 6. Shoot dry weight of BARI Mung 6 was higher than BARI Mung 3. In view of some earlier studies it is now evident that priming of seeds of different crops improved seedling shoot dry weight. These results are in agreement with Harris *et al*. (2004) who reported that higher plant dry weight and seed yield were observed were observed following seed priming. The increase in the dry matter and grain yield of mungbean was due to better emergence and better performance per plant (Parera and Cantliffe, 1994). Increased plumule dry weight due to osmo priming was reported by Harris *et al*. (2004).

Table 5. Effect of different concentrations of priming solution on shoot dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds of mungbean

Treatments	Shoot dry weight (mg)	
	BARI Mung 3	BARI Mung 6
T_0	48.25 d	60.42 d
T_1	55.15 b	62.13 d
T_2	57.90 a	65.65 c
T_3	54.94 b	69.28 b
T_4	53.38 bc	78.34 a
T_5	51.56 c	61.62 d
LSD(0.05)	2.729	2.497
CV(%)	3.91%	2.89%

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

4.2.9 Effect on root dry weight (mg)

Statistically significant variation was found in case of root dry weight of BARI Mung 3 and BARI Mung 6 due to priming with different mannitol concentrations and water (Appendix XI and Figure 5). Root dry weight was affected by water priming and different mannitol concentration. Dry weight increase up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter decrease with the increasing mannitol concentration. Results revealed that the highest seedling dry weight 19.96 mg was recorded in T_2 treatment for BARI Mung 3 compared to root dry weight 27.22 mg recorded in T_4 treatment for BARI Mung 6. The lowest root dry weight 13.33 mg & 18.85 mg was recorded in control treatment for both varieties. Root dry weight of BARI Mung 6 was higher than BARI Mung 3. These results are in agreement with those of Pill and Necker (2001) who reported that compared to non-primed seeds, seed priming resulted in greater seedling dry weights.

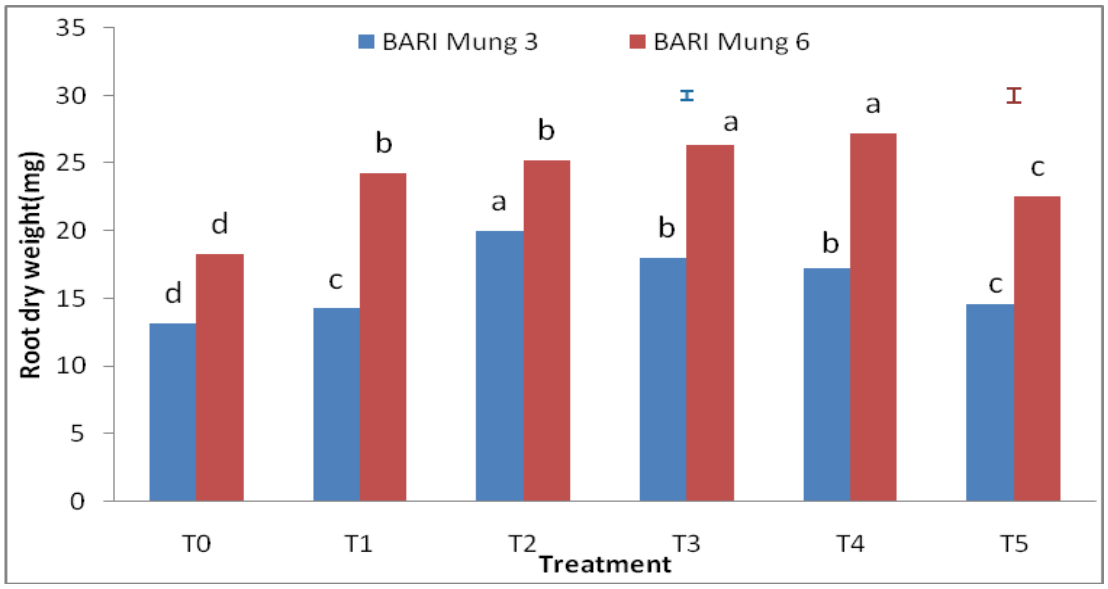


Figure 5. Effect of different concentrations of priming solution on root dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4 = Seeds socked with 6% mannitol solution for 9 hours and T_5 = Seeds socked with 8% mannitol solution for 9 hours

4.2.10 Effect on vigour index

The increase in seed vigor of primed seeds is due to reserve mobilization of food materials, activation and resynthesis of some enzymes and also due to the increased DNA and RNA synthesis. Priming with different concentrations of mannitol and water showed significant variation in vigour index of BARI Mung 3 and BARI Mung 6 (Appendix XII and Figure 6). Vigour index was affected by water priming and different mannitol concentration. Vigour index increased significantly up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter decreased gradually with the increasing mannitol concentration. The highest vigour index 186.9 of BARI Mung 3 was recorded from T_2 treatment compare to vigour index 136.5 recorded in T_4 treatment for BARI Mung 6. On the other hand, the minimum vigour index 122.6 and 91.09 was found in T_0 treatment for both varieties. Grandi *et al.* (1999) found that P enrichment by soaking seeds in 200mM KH_2PO_4 solution

improved the seedlings establishment. The increased vigour of P-enriched seed might be due to increased P content both inside the seeds and on the seed surfaces which leads to better establishment of seedlings (Bolland and Baker, 1988; Zhang *et al.*, 1990; Thomson and Bolger, 1993; Ros *et al.*, 1997). Similarly, the increase in seedling vigour due to salicylic acid may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis (Kathiresan *et al.*, 1984) and decreased catalase and peroxidase levels as recorded in pea seedlings (Srivastava and Dwivedi, 1998).

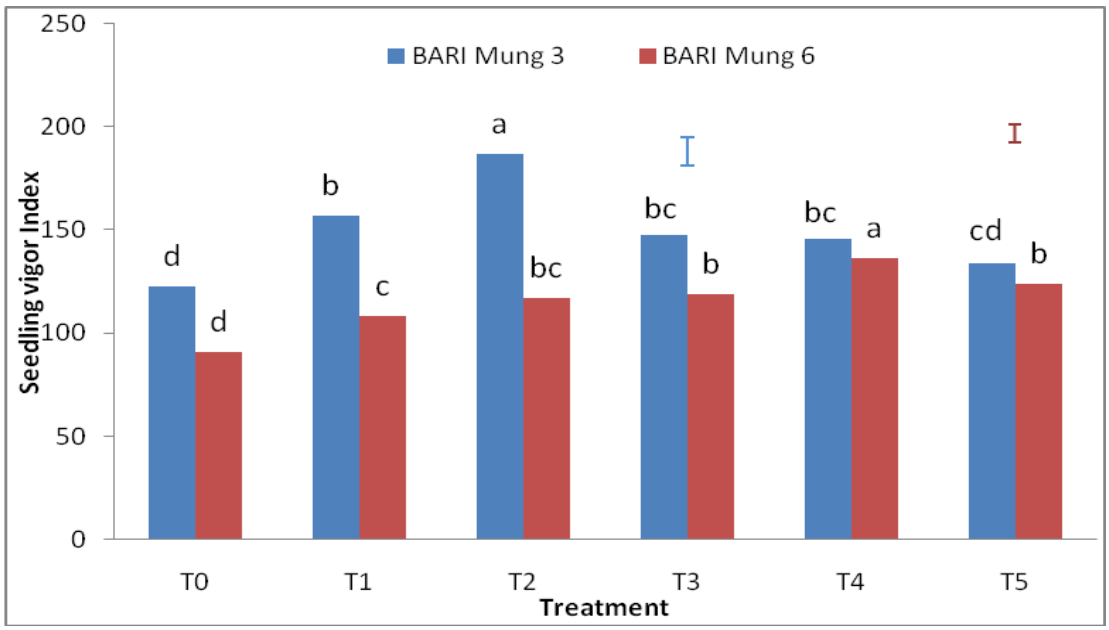


Figure 6. Effect of different concentrations of priming solution on seedling vigour index of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4 = Seeds socked with 6% mannitol solution for 9 hours and T_5 = Seeds socked with 8% mannitol solution for 9 hours

4.2.11 Effect on relative water content (%)

Relative water content (%) showed significant difference by priming with different concentrations of mannitol, water and control treatments to BARI Mung 3 & BARI Mung 6 (Appendix XIII and Table 6). Relative water content (%) increased significantly up to T_2 and T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively and thereafter decreased gradually with the increasing mannitol concentration. The highest Relative water content 65.64% of BARI Mung 3 was recorded from T_2 treatment (statistically similar with T_3 treatment) and the highest relative water content 77.22% was recorded in T_4 treatment for BARI Mung 6. On the other hand, the minimum relative water content 55.36% and 58.26% was found in T_0 treatment for both varieties. Higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium however, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions (Baque *et al.*, 2002).

Table 6. Effect of different concentrations of priming solution on relative water content (%) of primed (mannitol and water) and non-primed (control) seeds of mungbean

Treatments	Relative water content (%)	
	BARI Mung 3	BARI Mung 6
T_0	55.36 c	58.26 d
T_1	59.21 b	64.92 c
T_2	65.64 a	66.72 c
T_3	64.56 a	67.16 c
T_4	59.56 b	77.22 a
T_5	59.05 b	70.52 b
LSD(0.05)	2.701	2.949
CV(%)	3.42%	3.35%

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4 = Seeds socked with 6% mannitol solution for 9 hours and T_5 = Seeds socked with 8% mannitol solution for 9 hours

4.2.12 Effect on water saturation deficit (%)

Water saturation deficit of BARI Mung 3 and BARI Mung 6 was influenced by different priming agent (Figure 7) and variance analysis showed that there was significant difference between control (non-primed) and primed seed (Appendix XIV). Water saturation deficit was affected by water priming and different mannitol concentration. Results revealed that water saturation deficit decreased up to 2% for BARI Mung 3 and up to 6% for BARI Mung 6 in mannitol concentration and then increased slightly. Lowest Water saturation deficit 34.36% was recorded in T_2 treatment (statistically similar with T_3 treatment) compare to 22.78% in T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively. The highest water saturation deficit 44.64% for BARI Mung 3 and 41.74% for BARI Mung 6 was found in T_0 treatment. water saturation deficit of BARI Mung 3 was higher than BARI Mung 6. Higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium however, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions (Baque *et al.,* 2002).

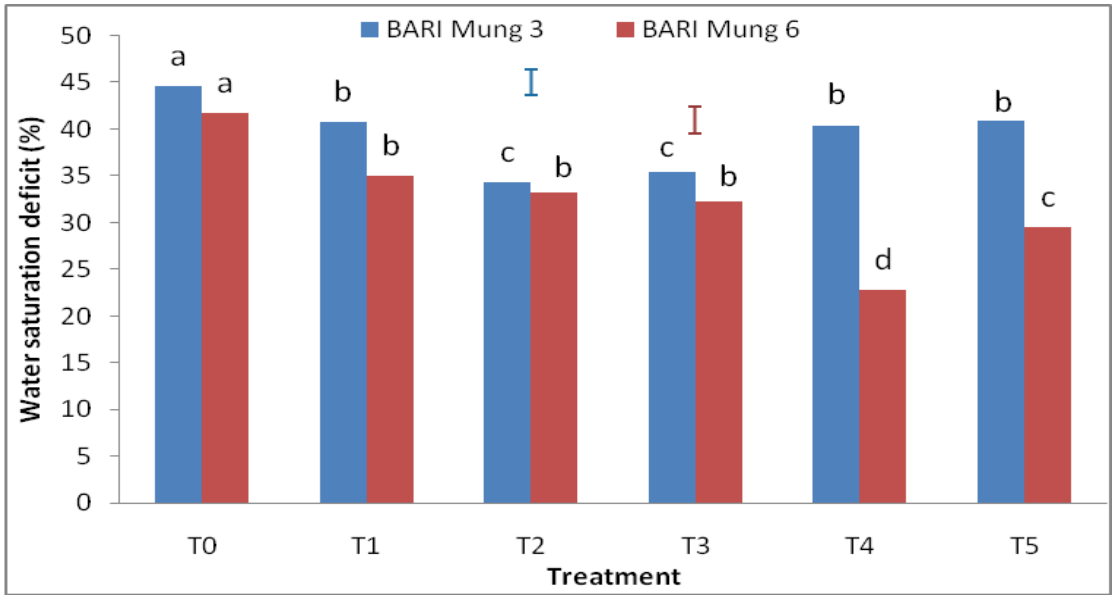


Figure 7. Effect of different concentrations of priming solution on water saturation deficit of primed (mannitol and water) and non-primed (control) seeds. Vertical bar represents the LSD value at p= 0.05 for BARI Mung 3 & BARI Mung 6

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours;T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4 = Seeds socked with 6% mannitol solution for 9 hours and T_5 = Seeds socked with 8% mannitol solution for 9 hours

4.2.13 Effect on water retention capacity

The water retention capacity (WRC) as represented by the ratio of turgid weight:dry weight (TW:DW) illustrates the water holding capacity of a shoot at a particular time. The TW:DW ratio is determined by the cell structures. Seed priming helps to repair damaged cell of seed. Water retention capacity showed significant variation for BARI Mung 3 and BARI Mung 6 on different concentrations of mannitol priming, water priming and control treatment (Appendix XV and Table 7). Result showed that water retention capacity increase with increasing mannitol concentration up to 2 % and 6 % for BARI Mung 3 and BARI Mung 6, respectively and thereafter decreased slightly. The highest water retention capacity 21.22 was recorded for BARI Mung 3 in T_2 treatment compare to water retention capacity 15.94 of BARI Mung 6 in T_4 treatment (statistically similar with T_3 treatment). The lowest water retention capacity 13.70 and 13.70 were recorded for BARI Mung 3 and BARI Mung 6

in T_0 treatment. Higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium however, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions (Baque *et al.*, 2002). Sangakkara *et al.* (1996) observed that when *phasiolous vulgaris* L. plant were subjected to moisture stress, the WRC increase with the increasing potassium concentrations.

Table 7. Effect of different concentrations of priming solution on water retention capacity of primed (mannitol and water) and non-primed (control) seeds of mungbean

Treatments	Water retention capacity	
	BARI Mung 3	BARI Mung 6
T_0	13.70 e	13.70 e
T_1	19.51 b	14.23 de
T_2	21.22 a	14.94 bc
T_3	18.81 c	15.50 ab
T_4	18.20 cd	15.94 a
T_5	18.06 d	14.78 cd
LSD(0.05)	0.6783	0.6368
CV(%)	2.85%	3.28%

T_0= Dry seeds; T_1 = Seeds socked with water for 9 hours; T_2 = Seeds socked with 2% mannitol solution for 9 hours; T_3 = Seeds socked with 4% mannitol solution for 9 hours; T_4= Seeds socked with 6% mannitol solution for 9 hours and T_5= Seeds socked with 8% mannitol solution for 9 hours

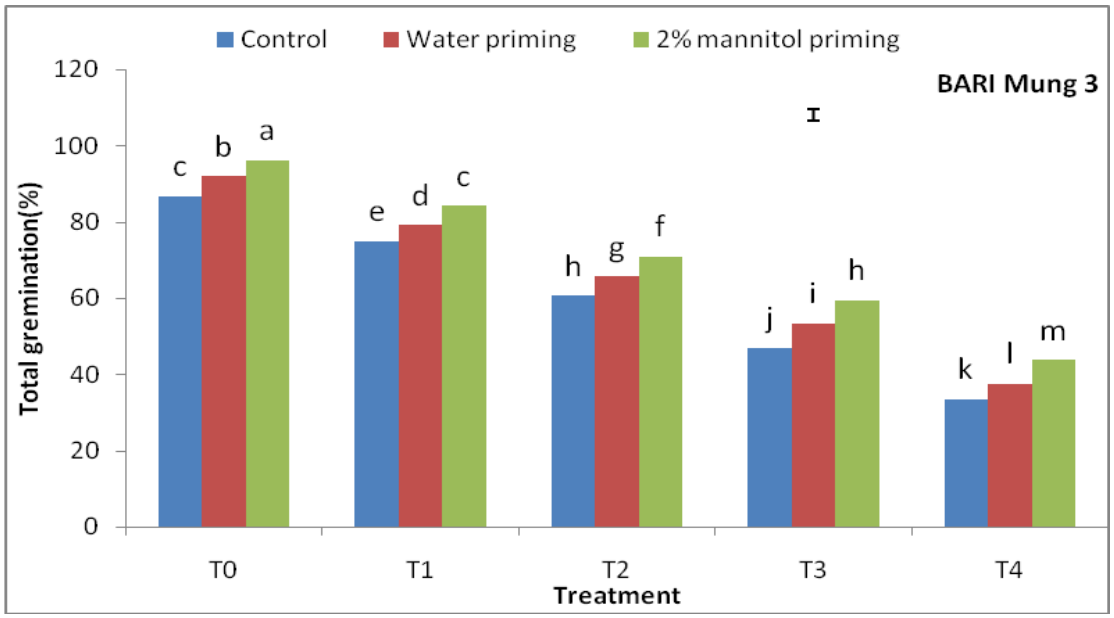
4.3 Experiment 3: Germination behavior of primed Seed (mungbean) under drought stress condition of mungbean

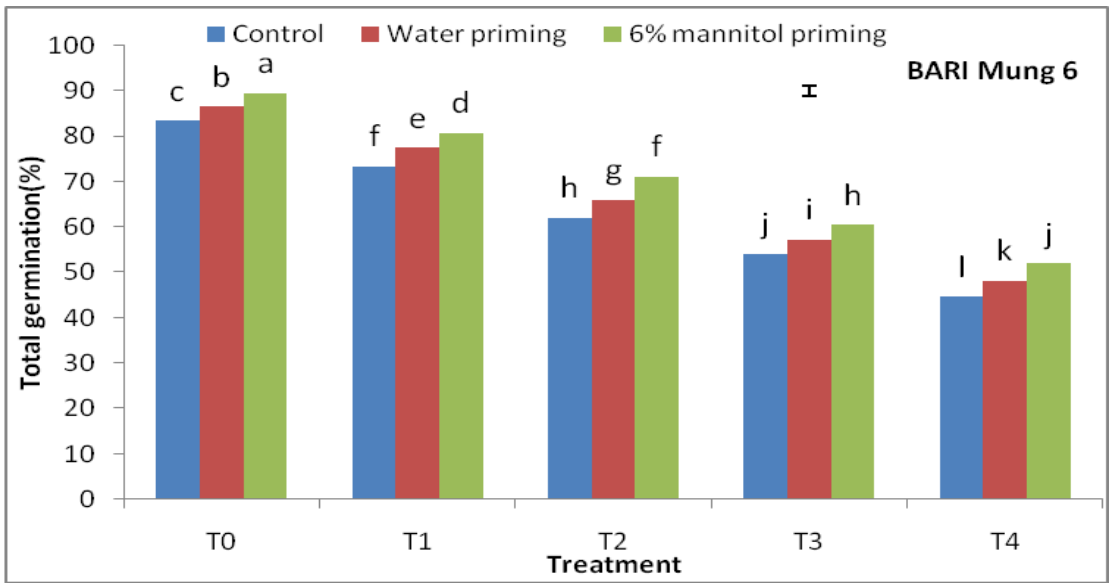
According to the results, all most all of the traits were affected by the experimental factors and there was completely significant difference between control (non primed seeds) and primed seeds. Mannitol and water priming increased the germination parameters (total germination percentage, germination index, coefficient of velocity, energy of emergence) and growth parameters (shoot length, root length, dry weight and vigour index) of mungbean, as compared with non-primed seeds up to a certain stage, under drought condition. The increase in drought stress in culture medium causes a significant decrease in germination percentage, mean germination time, germination index, coefficient of velocity, energy of emergence, shoot length, root length, dry weight and vigour index, as well as for non primed seed after the certain period. However, the decrease was more significant for non-primed seeds than mannitol and water seed priming.

4.3.1 Effect on total germination (%)

Priming improved seed germination performance, especially under sub-optimal environmental condition such as drought and salinity. Seed priming results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds. There are some factors affecting the response of seed to priming in terms of resistance to drought suggesting that seeds harvested at appropriate time and suitable material with precise concentration used for priming, the performance of seed germination will be improved under drought conditions. Total germination percentage was significantly decreased by water stress (Figure 8 and Appendix XVI). Total germination percentages were gradually lower in stressed condition than the controlled one where only water was used. Irrespective of water stress, the highest total germination was found in mannitol primed seeds than water primed and unprimed seeds. The highest total germination percentage 96.15 % was obtained in BARI Mung 3 and 89.35 %

was obtaining in BARI Mung 6 under control condition in osmo primed seed. The lowest total germination 33.39% and 44.5% was recorded in BARI Mung 3 and BARI Mung 6, respectively in non primed seed in 20% PEG concentration. Although the total germination percentage was decreased with increase the drought level but in every case osmo primed seed give highest result in every drought level than hydro primed and nonprime seeds. Razaji *et al.* (2014) concluded that priming resulted improvement in germination components and enzymes activity of rapeseed on drought stress condition and boost the resistance of rapeseed to drought stress condition. Compared to hydro-priming, priming with PEG in a proper concentration had a better effect on seed germination under drought stress although such effects had limited capability and severe drought stress inhibited germination (Sun *et al.*, 2010).



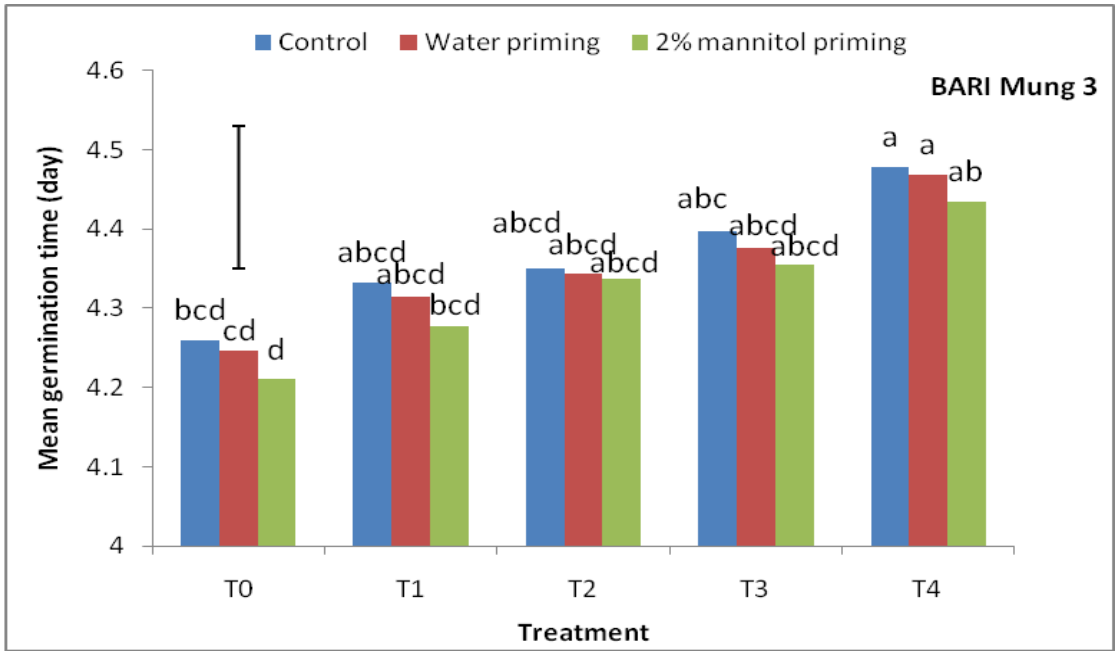


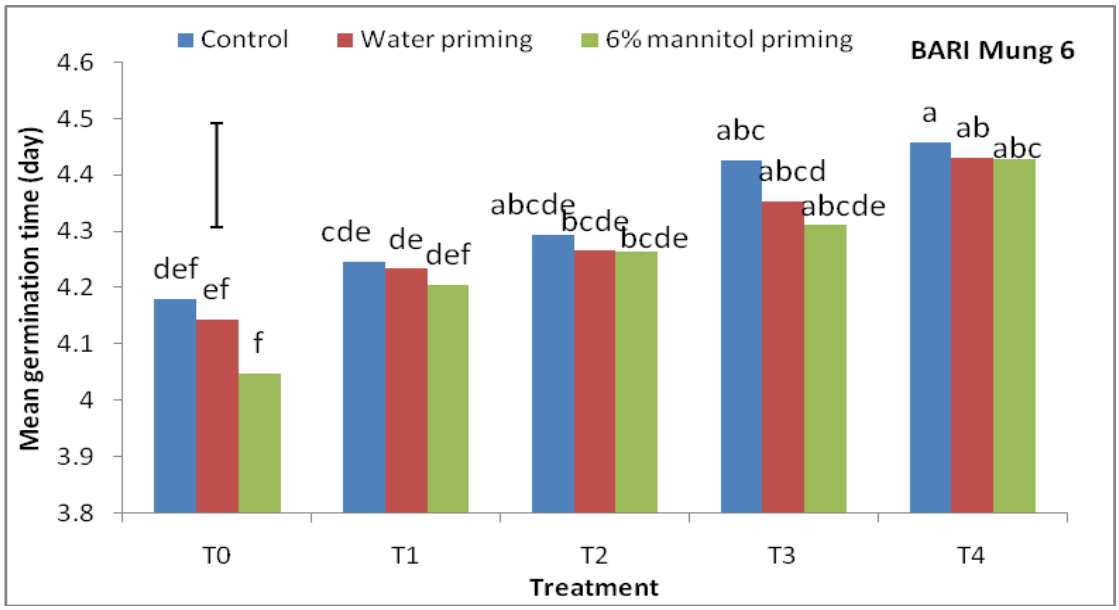
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 8.Effect of different drought levels on total germination percentage of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.2 Effect on mean germination time (days)

Significant variation was observed among the treatments on mean germination time only for BARI Mung 6. Mean germination time of mungbean was gradually decreased with increasing different drought (PEG %) levels (Appendix XVII and Figure 9). Results revealed that mean germination time increased for both primed (mannitol and water) and non-primed seed due to increasing drought level by PEG concentration. But mean germination time of mannitol and water primed seeds were lower compared to non-primed seeds at 0% PEG concentration and different levels of drought stress whereas mannitol primed seed gave the best result. However, mannitol primed seeds have lower mean germination time 4.21 days and 4.048 days at 0% PEG concentration for BARI Mung 3 and BARI Mung 6, respectively. The highest MGT 4.479 days and 4.457 days in control treatment was recorded from the 20% PEG concentration for BARI Mung 3 and BARI Mung 6, respectively. In case of BARI Mung 6 statistically similar MGT was found among several treatments and in BARI Mung 3 all treatments were statistically similar. Mannitol and water primed mungbean seeds germinated earlier than unprimed ones as it has been reported by Ashraf and Rauf (2001) working with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA.



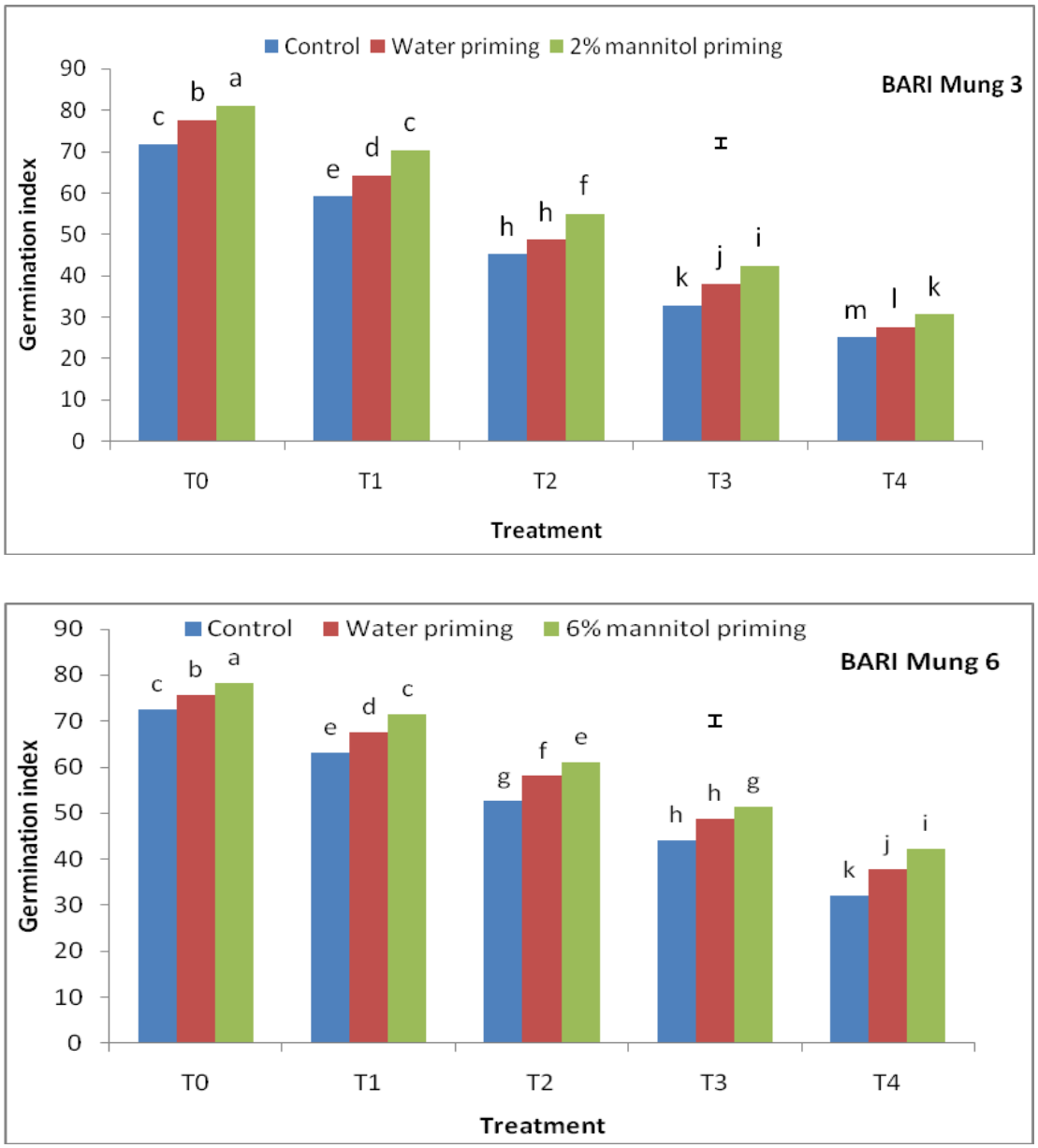


T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 9. Effect of different drought levels on mean germination time (day) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.3 Effect on germination index

At different drought level germination index of mungbean showed significant difference in case of BARI Mung 3 and BARI Mung 6 (Appendix XVIII and Figure 10). With increasing drought level germination index from both primed and non-primed seeds decreased significantly for BARI Mung 3 and BARI Mung 6. At 0% PEG concentration germination index of mannitol and water primed seeds was higher compare to nonprime seeds, in the mean time mannitol primed seed at different level of PEG concentration gave the best result for both BARI Mung 3 and BARI Mung 6. Highest germination index was recorded 81.11 and 78.33 from mannitol priming seed in 0% PEG concentration compared to (77.51 and 75.51) and (71.71 and 72.48) for BARI Mung 3 and BARI Mung 6 at water priming and control treatment, respectively. Lowest germination index 25.02 and 32.07 was found in control at 20% PEG concentration for BARI Mung 3 and BARI Mung 6, respectively. The results under the present study was in agreement with the findings of Ruan *et al*. (2002b) who demonstrated that priming the rice seed with KCl and $CaCl_2$ had improved germination index. Moghanibashi *et al.* (2012) reported that as salinity and/or drought level increased, all of these parameters reduced under both conditions. Primed seeds produced higher GI under all salinity and drought levels as compared with non-primed seeds. Inhibition of germination index due to drought stress should be overcome by using osmopriming treatments in soybean (Ghiyasi and Tajbakhsh, 2013).

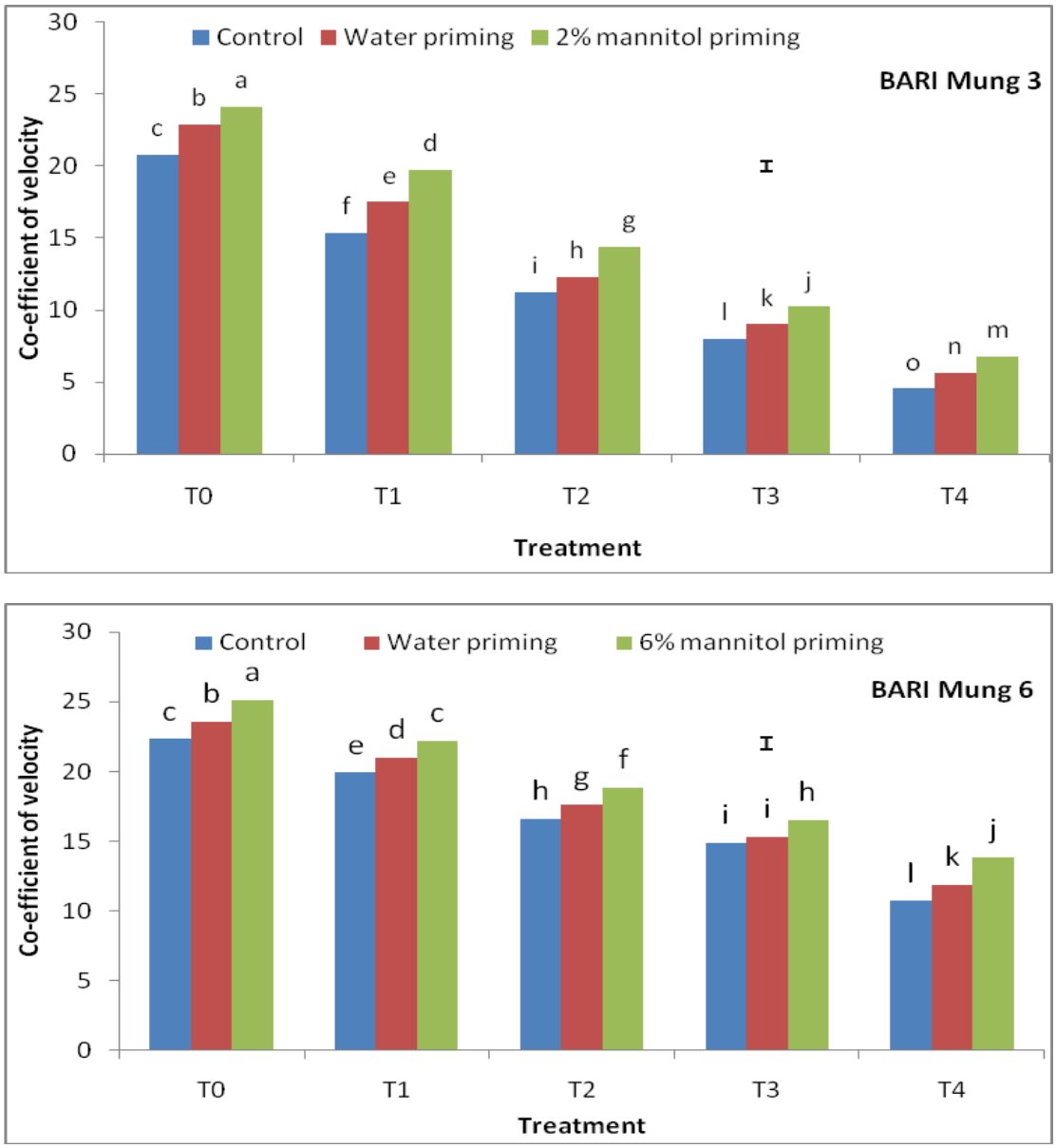


T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 10. Effect of different drought levels on germination index of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.4 Effect on coefficient of velocity

There was significant variation among the treatments for BARI Mung 3 and BARI Mung 6 in case of coefficient of velocity (Appendix XIX and Figure 11). With the increase of drought level significantly decreases values of coefficient of velocity. However, this decrease was more pronounced for nonprimed seeds than for primed seeds. Coefficient of velocity of mannitol and water primed seeds was higher compared to non-primed seeds at 0% PEG concentration and various drought stress levels. Where mannitol primed seed gave the best result. Coefficient of velocity was found the highest 24.14 & 25.13 with mannitol priming for BARI Mung 3 and BARI Mung 6 followed by water primed seeds 22.92 & 23.53 and control seed 20.77 & 22.32 in T_0 treatment. Lowest coefficient of velocity 4.538 & 10.71 was found in case of control seeds at 20% PEG concentration for BARI Mung 3 and BARI Mung 6. The maximum coefficient of velocity of germination were found in the low PEG level and decreased with increasing PEG concentation. The maximum coefficient of velocity of germination was found in low salinity treatment and decreased with increasing stress condition (Asaduzzaman, 2014).

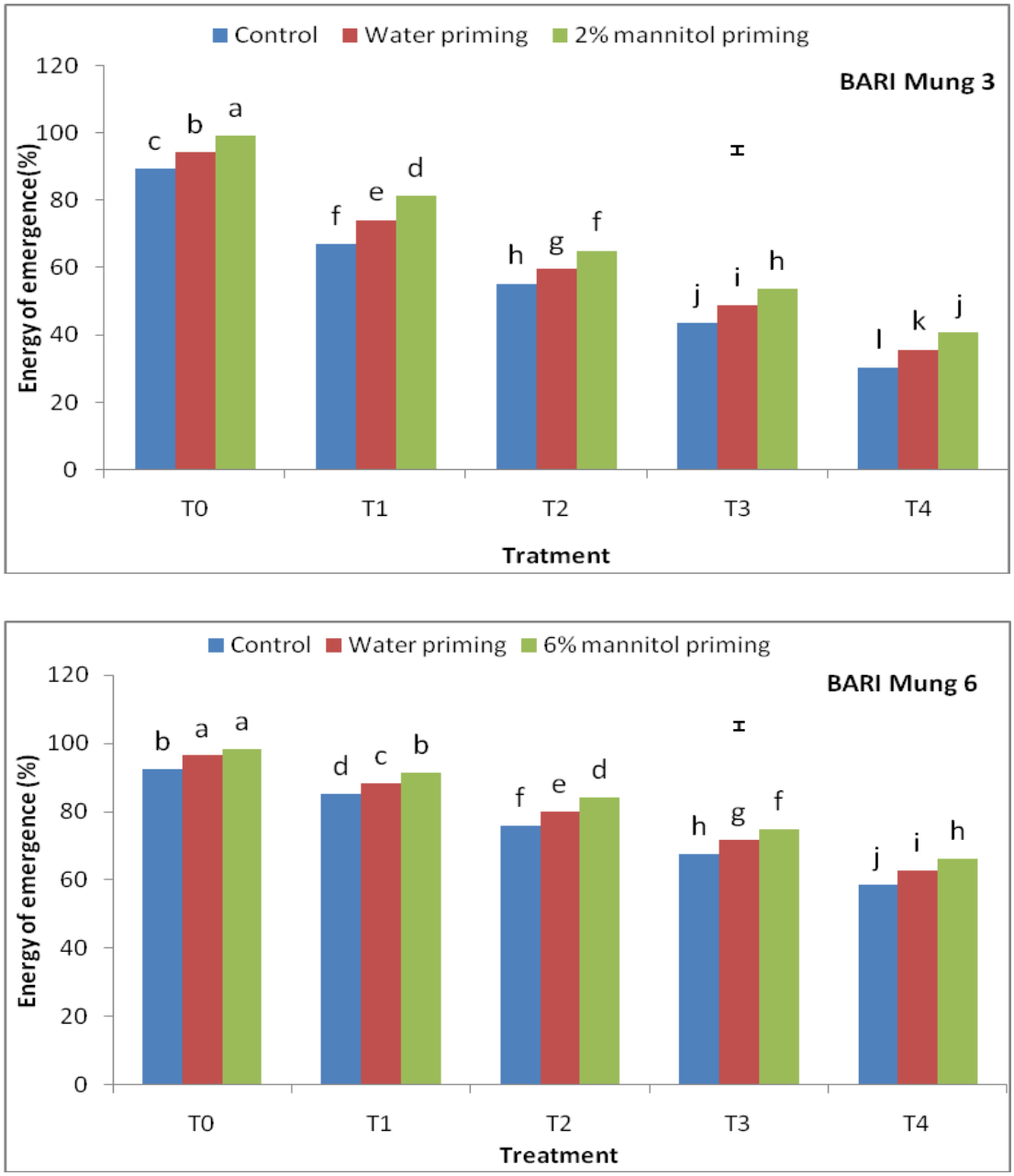


T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 11. Effect of different drought levels on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.5 Effect on energy of emergence (%)

Energy of emergence decreased in mannitol and water priming solution with increasing PEG concentration, the decreasing rate is lower in comparison to control seed. The energy of emergence parameter was significantly influenced by the different PEG levels (Appendix XX and Figure 12). With gradual increase of drought level significantly decreases values of energy of emergence. However, this decrease was more pronounced in non primed seeds than for primed seeds. Energy of emergence of mannitol and water primed seeds was higher than non-primed seeds at 0% PEG concentration and various levels of PEG % where mannitol primed seed gave the highest result. In this study, the maximum energy of emergence 99.36% and 98.42% was observed in mannitol primed seed of BARI Mung 3 & BARI Mung 6 at 0% PEG concentration, compare to (94.44% & 96.4%) and (89.4% & 92.27%) in water primed and control seeds of BARI Mung 3 and BARI Mung 6, respectively. Minimum energy of emergence 30.54% and 58.71 % in control treatment for BARI mng-3 and BARI Mung 6 was achieved from T_4 treatment. T_1, T_2 and T_3 showed intermediate results compared to maximum and minimum energy of emergence. It has been reported that priming had been resulted in more energy of emergence especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Demir Kaya *et al*., 2006; Foti *et al.*, 2002).

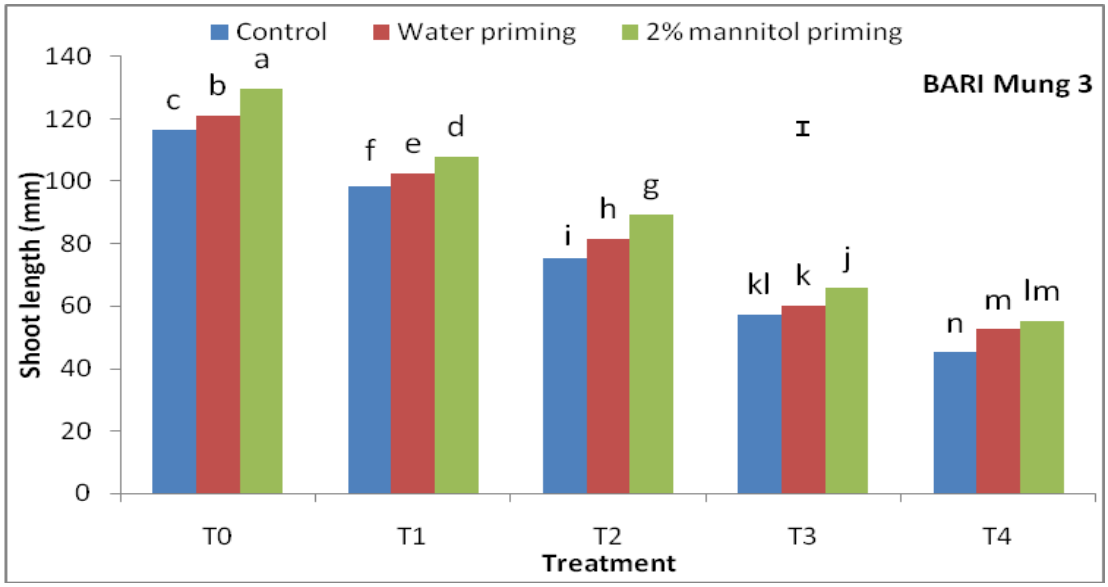


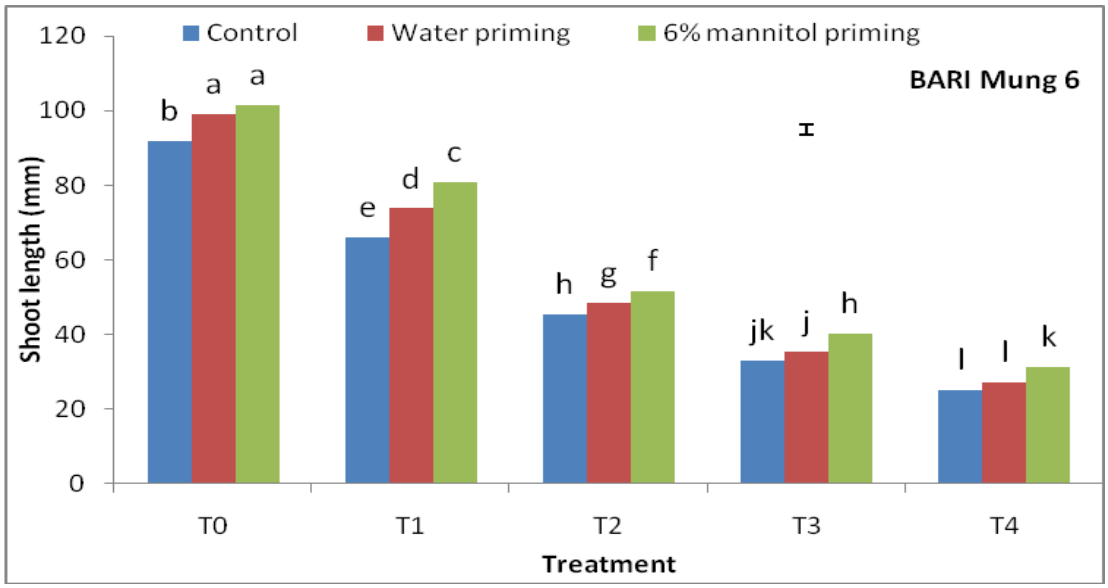
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 12. Effect of different drought levels on energy of emergence (%) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.6 Effect on shoot length (mm)

Primed seeds had better efficiency for water absorption from growing media, that's why metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance. Significant inhibitory effect was observed at different drought level on shoot length (Appendix XXI and Figure 13) for both primed and non-primed seeds. However, this effect was significantly higher in control seeds compare to seeds priming with mannitol and water. The highest shoot length 129.5 mm and 101.4 mm were observed with mannitol primed seeds of BARI Mung 3 and BARI Mung 6 at T_0 treatment compare to (121.2 mm & 99.11 mm) and (116.7 mm & 91.71 mm) primed with water and control seeds respectively. Lowest shoot length 45.11 mm and 25.21 mm from control seeds for BARI Mung 3 and BARI Mung 6 was found at 20% PEG concentration. The result of the present study corroborates with the study of previous researchers (Moghanibashi *et al.*, 2013) who reported that drought and/or salinity levels increased, shoot length reduces but the priming treatments clearly improved the parameter under drought and salinity conditions so can be used to improve seed performance of sunflower under normal and stress conditions.



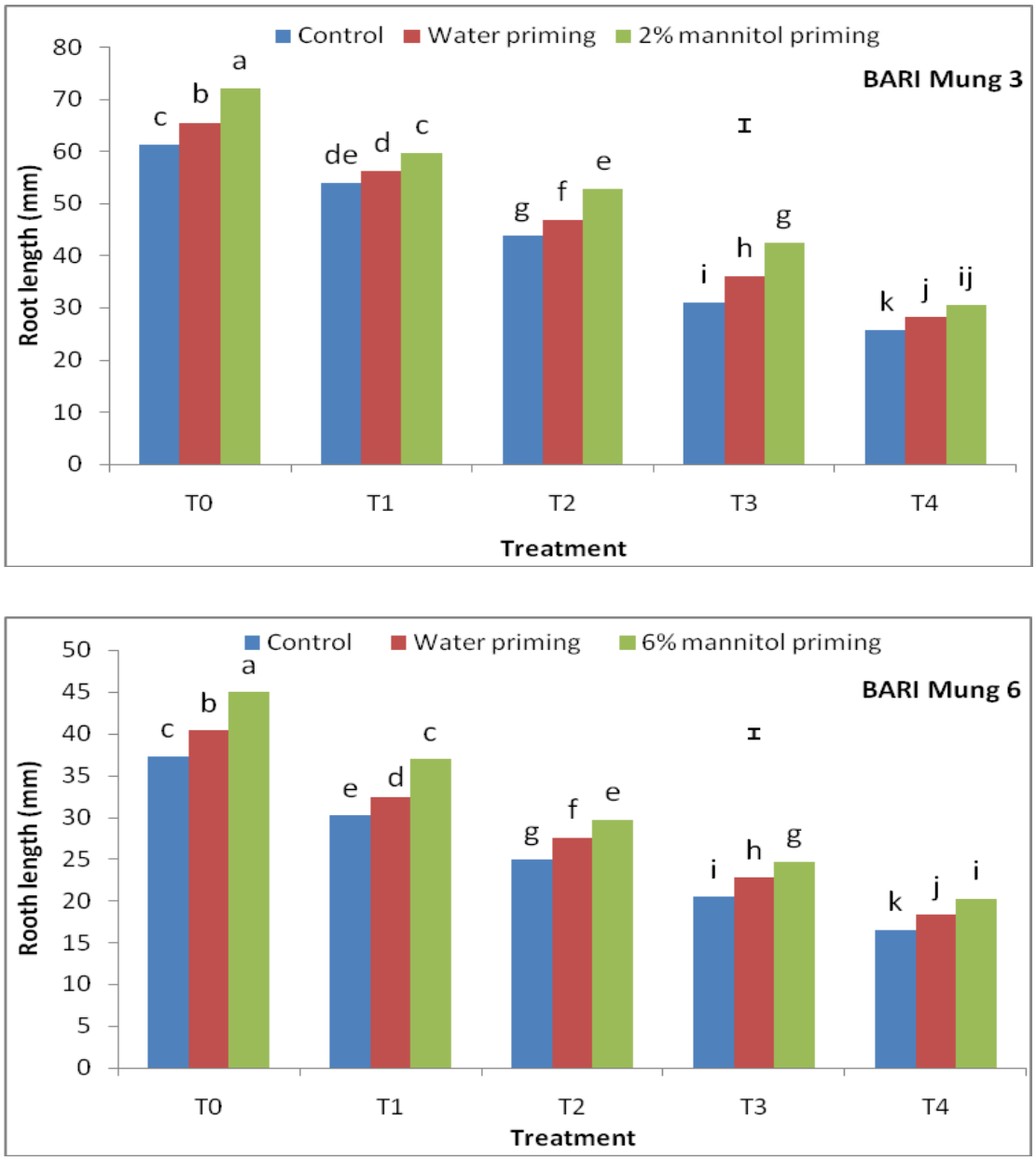


T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 13. Effect of different drought levels on shoot length (mm) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.7 Effect on root length (mm)

Primed seeds had better efficiency for water absorption from growing media, that's why metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance. Root length of mungbean significantly differs by the different drought levels (Appendix XXII and Figure 14). With the increase of drought level root length gradually decreased for both primed and non primed seed. But it was observed that root length increased with mannitol and water priming seeds in comparison to control seeds with increasing drought levels. The highest root length 72.2 mm & 44.92mm was found in BARI Mung 3 and BARI Mung 6 with mannitol priming compare to (65.58 mm & 40.38 mm) and (61.38 mm & 37.23 mm) with water priming and control seeds respectively, at 0 % PEG concentration. The lowest root length 25.79 mm &16.46 mm were found in control seeds at 20% PEG concentration (T_4 treatment). Significant improvement in root and shoot length may be attributed to earlier germination induced by primed over non-primed seeds (Farooq *et al*. 2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds. Present results confirm the findings of Stofella *et al*. (1992), who reported that priming of pepper seeds significantly improved radicle length.

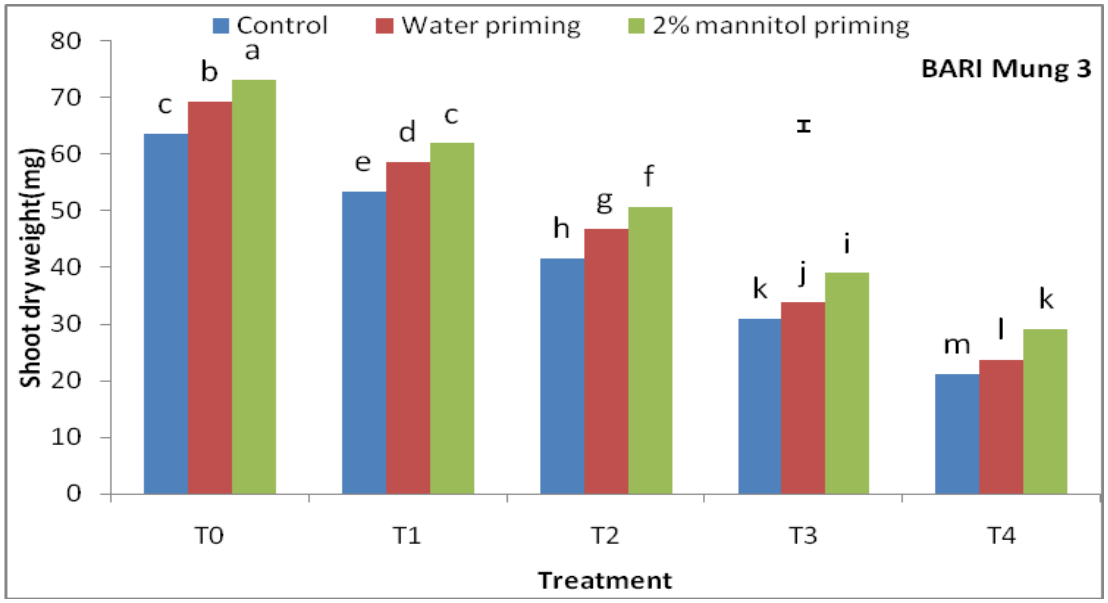


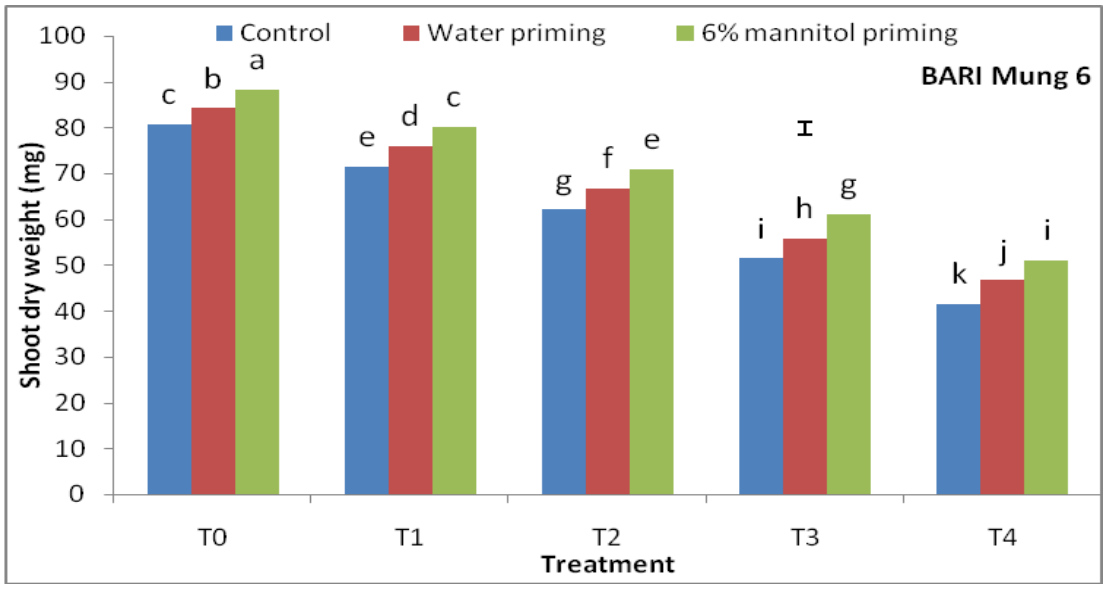
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 14. Effect of different drought levels on root length (mm) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.8 Effect on shoot dry weight (mg)

Seed priming is a process by which it is possible to increase the shoot dry weight at any condition than unprimed seed. Significant inhibitory effect was found in shoot dry weight of mungbean with increasing drought levels by PEG concentration (Appendix XXIII and Figure 15) for both primed and non-primed seeds. However, this effect was significantly more pronounced in control seeds in comparison with mannitol and water primed seeds. Lower the drought level higher the shoot dry weight. The highest shoot dry weight was obtained (73.27 mg and 88.22 mg) in mannitol priming seeds of BARI Mung 3 and BARI Mung 6, respectively in compare to water priming (69.34 mg and 84.19 mg) and (63.56 mg and 80.80 mg) in control seeds at 0 % PEG concentration. The minimum shoot dry weight 21.09 mg & 41.50 mg were obtained in case of control seeds for BARI Mung 3 and BARI Mung 6, respectively at 20% PEG level. Umair *et.al.* (2010) observed that priming treatments increased the dry matter yield of shoot as well as root as compare to control.



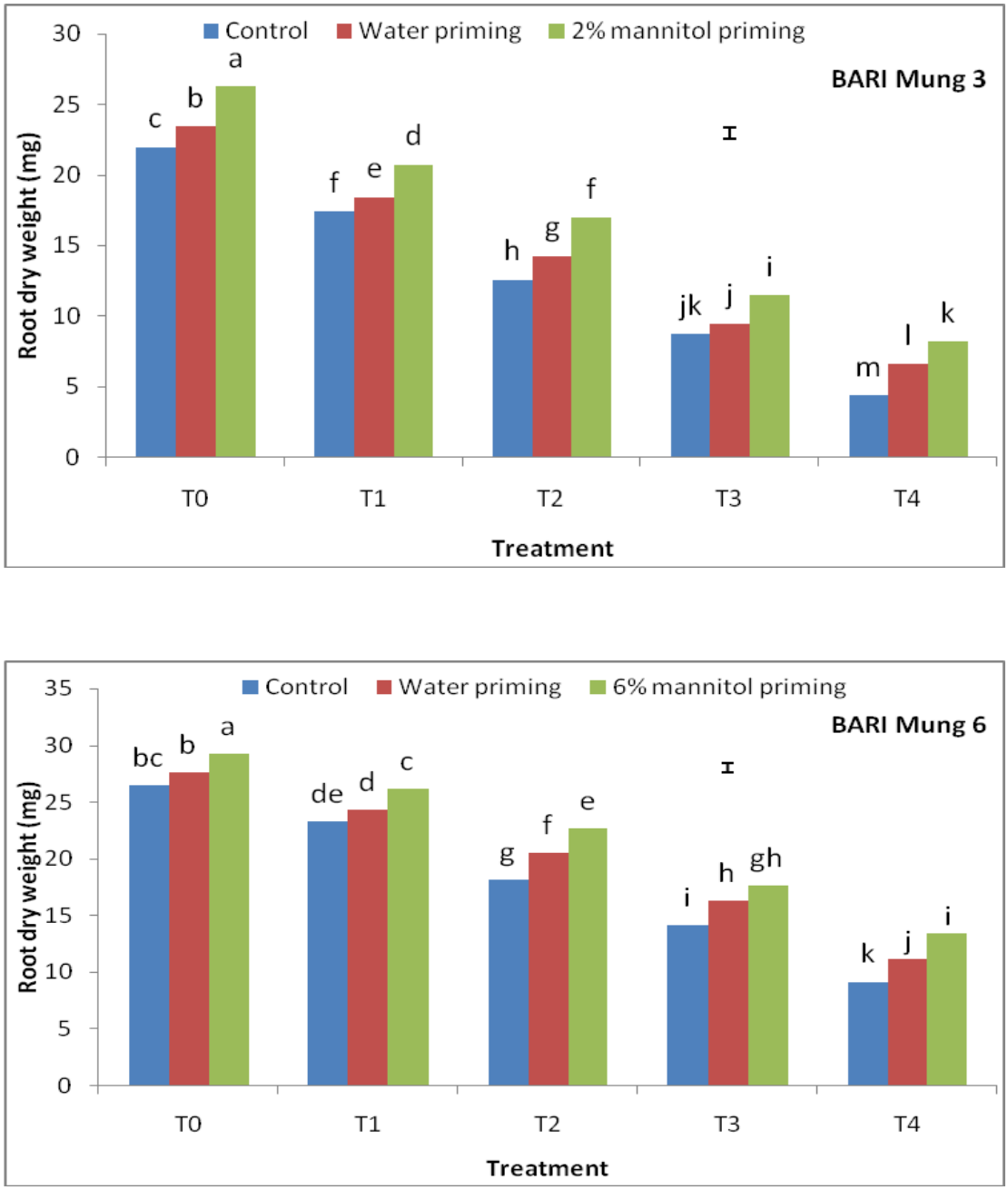


T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 15. Effect of different drought levels on shoot dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.9 Effect on root dry weight (mg)

Increasing PEG concentration significantly decreased mungbean root dry weight for both primed and un-primed seed (Appendix XXIV and Figure 16). Result reveled that root dry weight from both primed and non-primed seeds decreased significantly with increasing PEG concentration. But root dry weight of mannitol and water primed seeds was higher compared to non-primed seeds at 0% PEG concentration and various levels of drought stress where as mannitol primed seed gave the best result. Figure 16 shows that the highest root dry weight 26.25 mg and 29.22 mg followed by water primed root dry weight and control were recorded from mannitol primed BARI Mung 3 and BARI Mung 6, respectively at 0% PEG concentration at T_0 treatment. Lowest root dry weight 4.311 mg & 9.17 mg were obtained from control seeds of BARI Mung 3 and BARI Mung 6 at 20% PEG concentration. Root dry weight decreased linearly with increasing drought level. Umair *et.al.* (2010) observed that priming treatments increased the dry matter yield of shoot as well as root as compare to control.

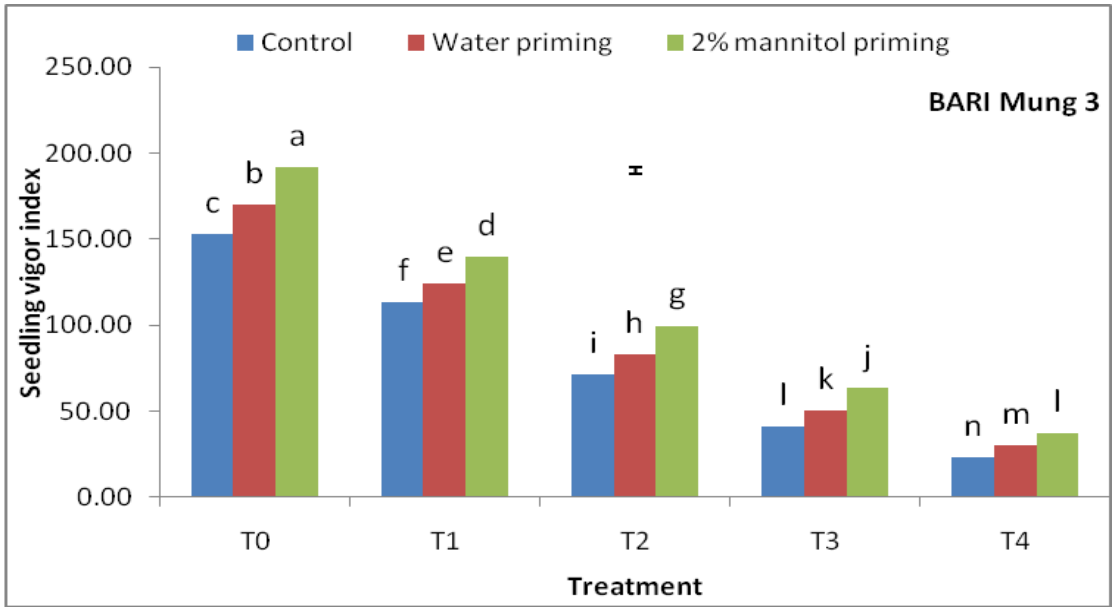


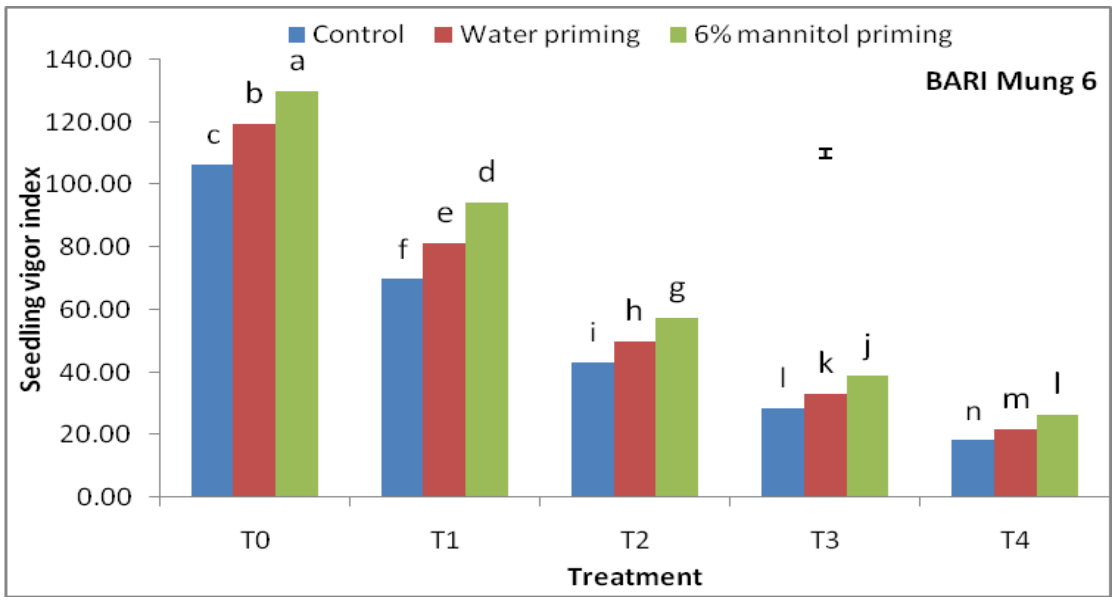
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 16. Effect of different drought levels on root dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.10 Effect on vigour index

Priming is capable of repairing some of the damages due to seed erosion which in turn results in increased vigor of primed seeds. The results regarding vigour index of mungbean are given in (Appendix XXV and Figure 17), which showed that vigour index differed significantly with increasing drought levels. However, this effect was significantly more pronounced in control seeds in comparison with mannitol and water primed seeds. Vigour index of mannitol and water primed seeds was higher compared to non-primed seeds at 0 % PEG concentration and various levels of drought stress where mannitol primed seed gave the best result. Vigour index was found the highest 192.10 & 129.90 in mannitol priming seeds of BARI Mung 3 and BARI Mung 6, followed by water priming (170 & 119.60) and in control (152.80 & 106.60) at T_0 treatment. The least vigour index (23.44 & 18.42) for BARI Mung 3 and BARI Mung 6, respectively were found in control seeds at T_4 treatment. Seed priming improve mungbean vigour index under different stress condition. Similar results were also found by Ruan *et al.* (2002b) who reported that primed rice seeds showed higher vigour index than non-primed ones. As drought and/or salinity levels increased, vigour reduced but the priming treatments clearly improved the parameter under drought and salinity conditions so these treatments can be used to improve seed performance of sunflower under normal and stress conditions (Moghanibashi *et al.*, 2013).



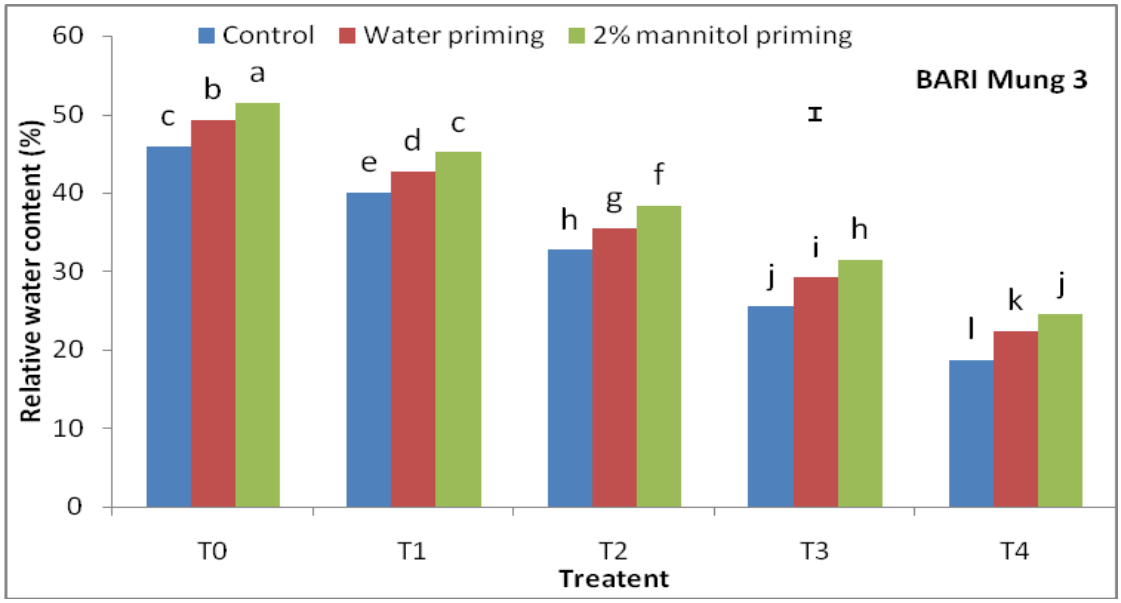


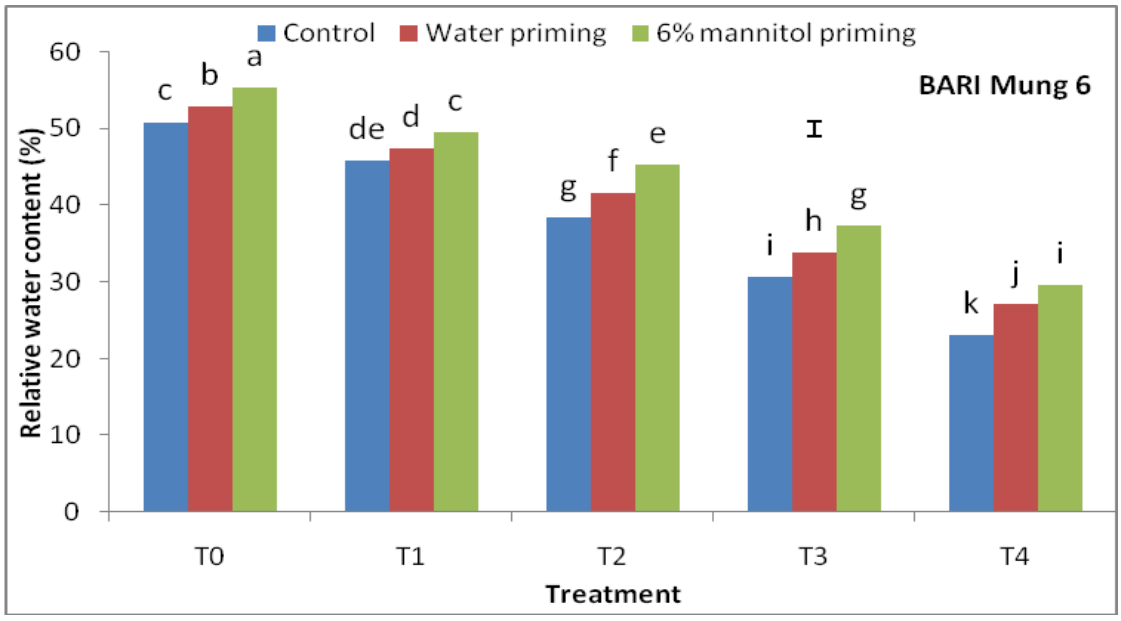
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 17. Effect of different drought levels on vigour index of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.11 Effect on relative water content (%)

The relative water content (%) of shoot signifies the water contents of plants. Plants grown under water deficit condition had lower relative water content (RWC). Among the all drought levels, RWC was greater in plants grown at control condition than the plants at the other drought level (Appendix XXVI and Figure 18). Plants with higher relative water content had the faster transpiration rate. There was significant variation observed among different drought treatments on relative water content of BARI Mung 3 and BARI Mung 6. Relative water content gradually decreases with the increase of drought level. Highest relative water content was observed in control condition for mannitol primed BARI Mung 3 and BARI Mung 6 followed by water priming than control (dry seed). In spite of decreasing relative water content with increasing drought level mannitlo primed seed gave highest result in every drought level than water primed seed and control seed. The highest relative water content was found 51.39% & 55.37% in mannitol priming seeds of BARI Mung 3 and BARI Mung 6 followed by water priming (49.28% & 52.92%) and in control (45.89% & 50.90%) at T_0 treatment. The least relative water content 18.73 & 23.18 for BARI Mung 3 and BARI Mung 6, respectively were found in control seeds at T_4 treatment. Baque *et al.*, (2002) observed that higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium However, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions.



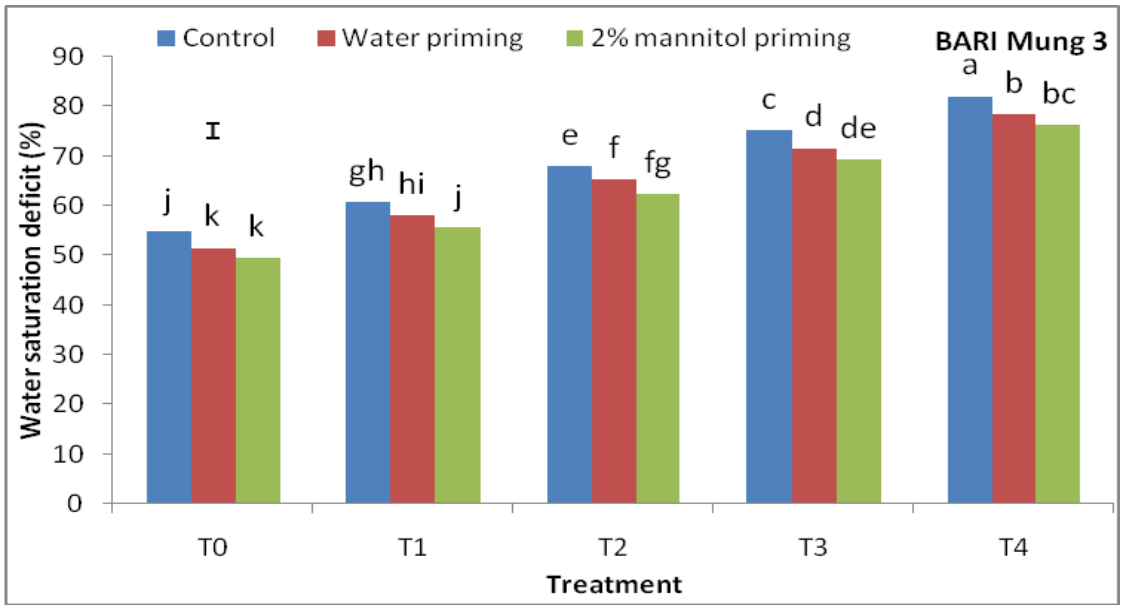


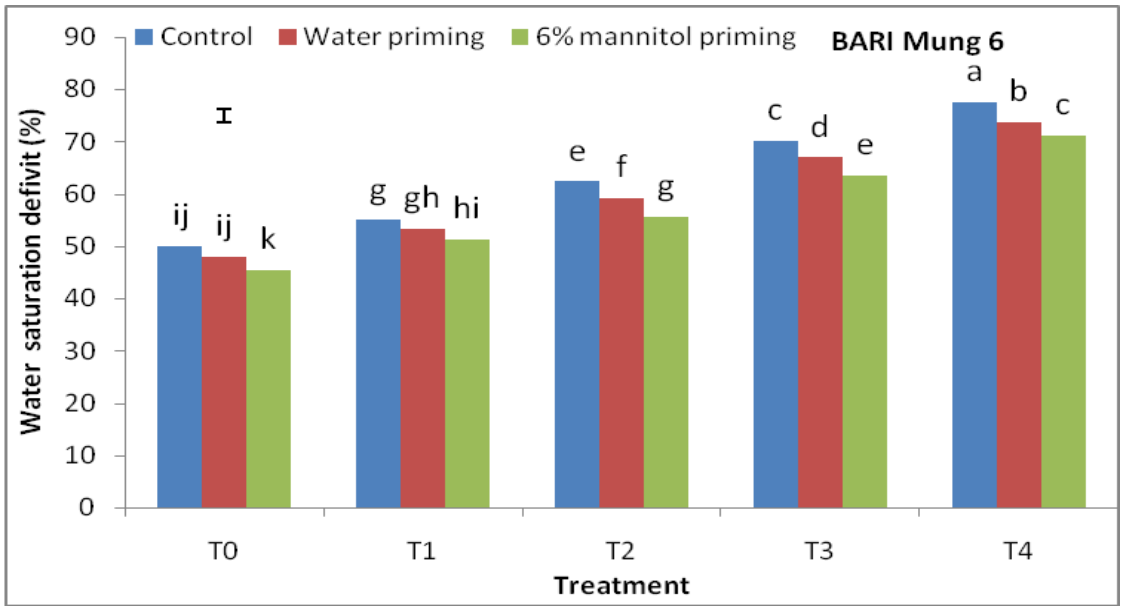
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 18. Effect of different drought levels on relative water content (%) of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.12 Effect on water saturation deficit (%)

The higher water saturation deficit (WSD) was observed in plants under severe stress condition. Seed priming improve this situation as a result water saturation deficit of plant. Figure 19 showed that water saturation deficit increase with the increase of drought level. But in every case mannitol primed seed gave lowest WSD value followed by water priming and dry seeds for both varieties. WSD of mannitol and water primed seeds was lower compared to non-primed seeds at 0% PEG concentration and different levels of drought stress whereas mannitol primed seed gave the best result. However, mannitol primed seeds have lower WSD (49.43% and 45.44%) at 0% PEG concentration for BARI Mung 3 and BARI Mung 6, respectively. The highest WSD (82.09% and 77.61%) in control treatment was recorded from the 20% PEG concentration for BARI Mung 3 and BARI Mung 6, respectively. Baque *et al.*, (2002) observed that higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate. Higher levels of K significantly reduced the water saturation deficit. Fertilizer potassium. However, made leaf water potential more negative. The beneficial effect of fertilizer potassium on water stress tolerance in wheat plants were more pronounced under water stressed conditions than under control conditions.



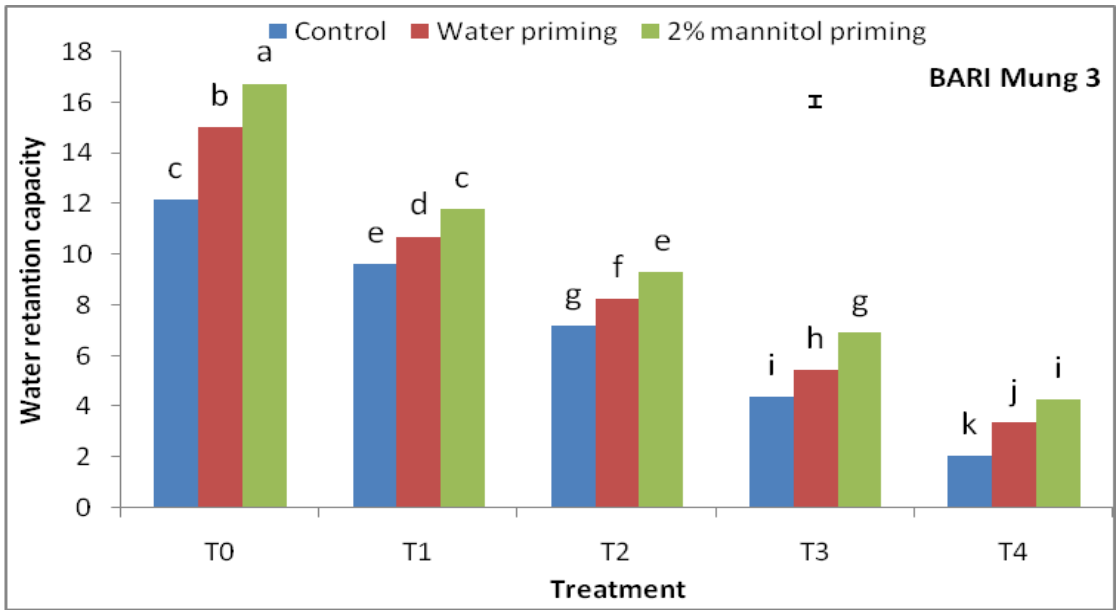


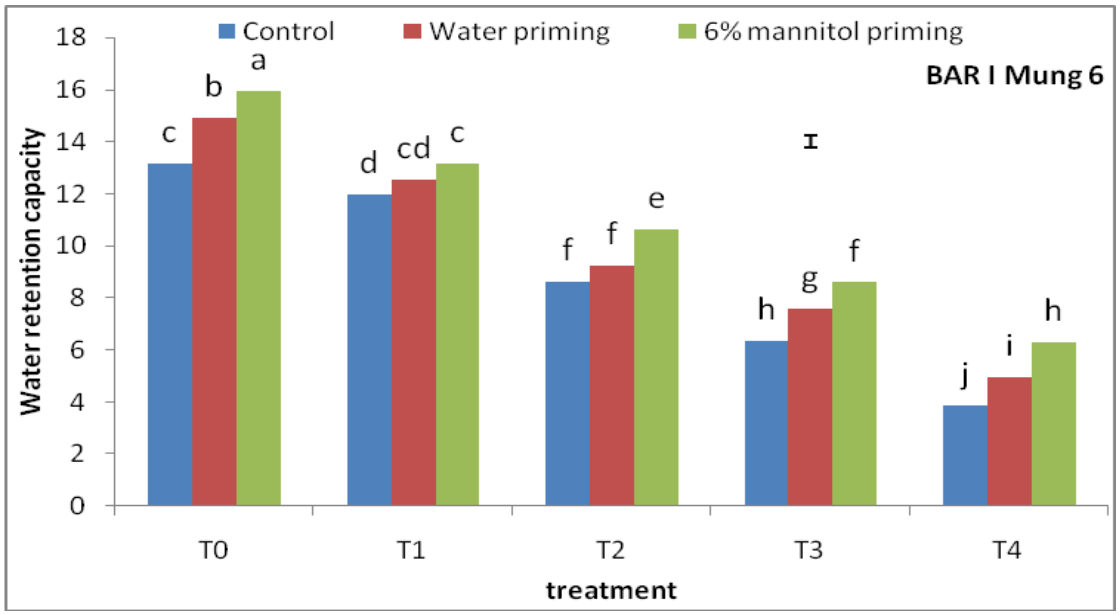
T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 19. Effect of different drought levels on water saturation deficit of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05

4.3.13 Effect on water retention capacity

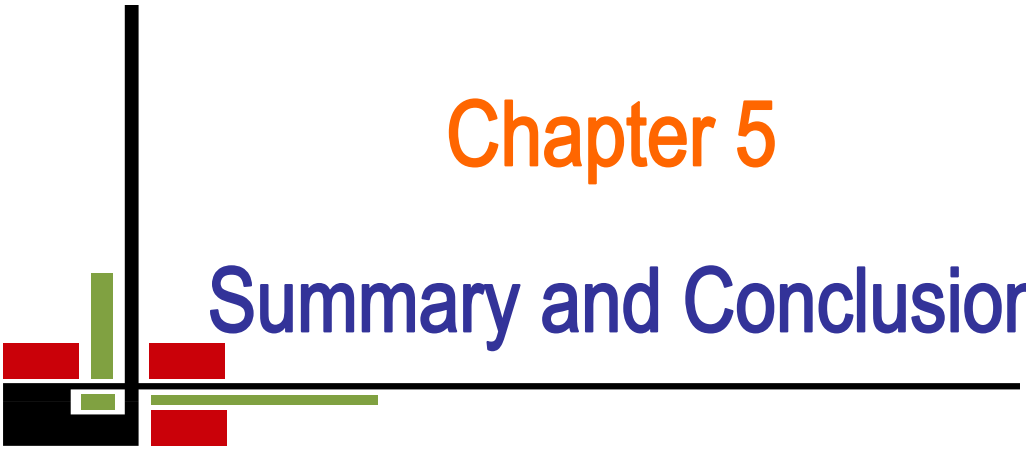
The water retention capacity (WRC) as represented by the ratio of turgid weight : dry weight (TW : DW) illustrates the water holding capacity of a shoot at a particular time. The TW : DW ratio is determined by the cell structures. Seedling grown under a high soil moisture regime had a higher ratio than that of the seedling grown under mild stress and severe stress conditions (Appendix XXVIII and Figure 20). The smallest ratio (2.068 and 3.892) was observed in seedling grown under severe stress (20% PEG concentration) condition which had non prime seed for BARI Mung 3 and BARI Mung 6, respectively. Using seed priming technique enhanced the WRC of seedling at all drought level. Higher ratios (16.74 & 15.96) were found in BARI Mung 3 and BARI Mung 6 seedling, respectively at 0% PEG concentration. Noticeably, the relative increment of WRC due to priming the seed with mannitol was greater in seedling grown under severe water stress condition than that in the plants grown under both control and mild stress condition. Perhaps the better maintenance of cell structure due to application of high level of potassium was partly responsible for the relatively high WRC at water stressed plants than the control plants (Sangakkara *et al.*, 1996).





T_0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T_1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T_4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG

Figure 20. Effect of different drought levels on water retention capacity of primed (mannitol and water) and non-primed (control) seeds of BARI Mung 3 & BARI Mung 6. Vertical bar represents the LSD value at p= 0.05



Chapter 5

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

This experiment was conducted at Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from 13 May, 2014 to 15 July, 2014 to study the mannitol induced seed priming on drought tolerance capability in mungbean varieties cv. BARI Mung 3 and BARI Mung 6 under drought stress condition.

The whole experiment was conducted in three different experiments. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Different priming chemicals such as 75% alcohol, mannitol ($C_6H_{14}O_6$), Polyethylene Glycol (PEG) and distilled water were utilized for osmo and hydro priming. Mannitol ($C_6H_{14}O_6$) used as seed priming chemical and alcohol used as a seed treating chemical. On the other hand PEG used as an artificial drought inducer. 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 120 mm diameter Petri dishes on whatman No.1 filter paper and filter paper was moistened with 8 mL of 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 continued up to 8 days for 2^{nd} experiment and 3^{rd} experiment. The abnormal or dead seedlings with short, thick and spiral formed hypocotyls and stunted primary root were excluded during counting. The data on germination parameters of mungbean like total germination percentage, mean germination time, germination index, coefficient of velocity, energy of emergence and growth parameters like plumule length, root length, dry weight and vigour index, Relative water content (RWC), water saturation deficit (WSD) and water retention capacity (WRC). Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the least significant difference (LSD) at 5% level of probability.

The first experiment was conducted to evaluate different pre-soaking priming time by electrical conductivity (EC) of seed. Seed leachates from 3, 6, 9, 12, 15, 18 hours primed seeds, hydro primed seeds and non primed seeds were measured by EC meter after 24 hours of soaking in distill water. Six different priming times such as 3, 6, 9, 12, 15, and 18 hours for hydropriming and osmopriming were used, respectively in this experiment. Dry seeds (control), water, 2%, 4%, 6%, 8% mannitol primed seeds for 3, 6, 9, 12, 15, and 18 hours were used as treatment which was soaked in water for 24 hr. The lowest electrical conductivity (EC) value 0.0606 dSm^{-1} was observed for BARI Mung 3 and 0.1110 dSm^{-1} for BARI Mung 6 at 9 hour. As 9 hour gave the lowest electrical conductivity (EC) value that's why 9hr is the best priming time for both varieties.

The second experiment was carried out to find the effect of different concentrations of priming solution (mannitol) on germination behavior of mungbean varieties cv. BARI Mung 3 and BARI Mung 6 without any stress condition. Four levels of mannitol such as 2%, 4%, 6%, and 8% were used for osmopriming and water used as hydropriming agent for 9 hour. The priming treatments were, seeds without priming (control) (T_0), seeds primed with distilled water for 9 hours (T_1), seeds primed with 2% mannitol solution for 9 hours (T_2), seeds primed with 4% mannitol solution for 9 hours (T_3), seeds primed with 6% mannitol solution for 9 hours (T_4) and seeds primed with 8% mannitol solution for 9 hours (T_5). For BARI Mung 3 and BARI Mung 6 maximum, total germination percentage 98.38% and 93.09%, germination index 87.78 and 85.41, energy of emergence (non sgnificant) and 97.31%, shoot length 143.7 mm and 110 mm, root length 46.09 mm and 36.55 mm, soot dry weight 57.9 mg and 78.34 mg, root dry weight 19.96 mg and 27.22 mg, vigor index 186.9 and 136.5, relative water content 65.64% and 77.22%, water retention capacity 21.22 and 15.94 were recorded at T_2 and T_4 treatment, respectively. Minimum water saturation deficit 34.36% and 22.78% in T_2 and T_4 treatment were observed for BARI Mung 3 and BARI Mung 6, respectively.

No significant effect was observed in case of mean germination time & coefficient of velocity in T_2 and T_4 treatment of BARI Mung 3 & BARI Mung 6

In the third experiment germination behavior of primed seed (mungbean) under different drought (PEG) stress condition was evaluated. Mannitol solution 2% for BARI Mung 3, 6% for BARI Mung 6 and distilled water were used as priming solutions, 9 hours as priming time and drought stress levels 5%, 10%, 15% and 20% were used in this experiment. Primed (mannitol and water) and non-primed (control) seeds placed with 0% PEG (T_0), 5% PEG (T_1), 10% PEG (T_2), 15% PEG (T_3) and 20% PEG (T_4) concentration were used as treatment. For BARI Mung 3 and BARI Mung 6 the maximum, total germination percentage 96.15% & 89.35%, germination index 81.11 & 78.33, coefficient of velocity 24.14 & 25.13, energy of emergence 99.36% & 98.42%, shoot length 129.5 mm & 101.4 mm, root length 72.2 mm & 44.92 mm, shoot dry weight 73.27 mg & 88.22 mg, root dry weight 26.25 mg & 29.22 mg, vigour index 192.10 & 129.90, relative water content 51.39% & 55.37% and water retention capacity 16.74 & 15.96 were found in mannitol primid seeds at T_0 treatment, respectively. The maximum mean germination time non significant & 4.457 days and water saturation deficit 82.09% & 77.61% were found in control seeds at T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively. The minimum total germination percentage 33.39% & 44.5%, germination index 25.02 & 32.07, coefficient of velocity 4.538 & 10.71, energy of emergence 30.54% & 58.71%, shoot length 45.11 mm & 25.21 mm, root length 25.79 mm & 16.46mm, shoot dry weight 21.09 mg & 41.50mg, root dry weight 4.311 mg & 9.17 mg, vigour index 23.44 & 18.42, relative water content 18.73% & 23.18% and water retention capacity 2.068 & 3.892 were found in non primid seeds at T_4 treatment for BARI Mung 3 and BARI Mung 6, respectively. The minimum mean germination time (non significant & 4.048 days) and water saturation drficit 49.43% & 45.44% was found in mannitol primed seeds at T_0 treatment for BARI Mung 3 and BARI Mung 6, respectively.

From the results of the study, it may be concluded that the performance of mannitol primed mungbean cv. BARI Mung 3 and BARI Mung 6 was better in respect of germination and growth parameters. Priming with 2 % mannitol concentration for BARI Mung 3 and 6% mannitol concentration for BARI Mung 6 with 9 hour priming time increase the germination behabiour of mungbean seeds. Reduction in germination parameters and seedling growth was more profound in control seeds than primed seeds under drought stress condition. Thus, the priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of drought stress. For this reason, further studies are needed to assess the efficacy of seed priming during the later stages of the culture.

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Appendices

APPENDICES

Appendix I: Monthly record of air temperature and relative humidity of the experimental site during the period from May, 2014 to July, 2014

Month	Air temperature (^{0}C)		Relative humidity (%)	
	Maximum	Minimum	Maximum	Minimum
May, 2014	38.2	19.3	89.2	40
June, 2014	37.2	17.4	88.4	46.3
July, 2014	35.6	18.2	88.2	55.4

Source: SAU Weather station

Appendix II: Mean square values on different priming time for electrical conductivity of leakage of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	30	0.663	0.022	510.095	<0.001
Error	124	0.005	0.000040		
Total	154	0.668			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	30	2.004	0.067	582.941	<0.001
Error	124	0.014	0.0001		
Total	154	2.018			

Appendix III: Mean square values on different concentrations of mannitol for total germination percentage of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	306.790	61.358	5.129	0.0024
Error	24	287.089	11.962		
Total	29	593.879			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	555.869	111.174	21.329	<0.001
Error	24	125.099	5.212		
Total	29	680.968			

Appendix IV: Mean square values on different concentrations of mannitol for mean germination time of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	0.017	0.003	0.162	
Error	24	0.511	0.021		
Total	29	0.529			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	0.065	0.013	0.412	
Error	24	0.757	0.032		
Total	29	0.822			

Appendix V: Mean square values on different concentrations of mannitol for germination index of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	135.577	27.115	3.941	0.0095
Error	24	165.148	6.881		
Total	29	300.725			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	676.630	135.326	18.760	<0.001
Error	24	173.127	7.214		
Total	29	849.757			

Appendix VI: Mean square values on different concentrations of mannitol for coefficient of velocity of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	0.193	0.039	0.171	
Error	24	5.431	0.226		
Total	29	5.624			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	0.742	0.148	0.417	
Error	24	8.546	0.356		
Total	29	9.288			

Appendix VII: Mean square values on different concentrations of mannitol for energy of emergence of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	169.288	33.858	1.849	0.1413
Error	24	439.536	18.314		
Total	29	608.824			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	820.990	164.198	26.668	<0.001
Error	24	147.770	6.157		
Total	29	968.760			

Appendix VIII: Mean square values on different concentrations of mannitol for shoot length of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	2518.268	503.654	29.383	<0.001
Error	24	411.381	17.141		
Total	29	2929.649			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	586.201	117.240	9.904	<0.001
Error	24	284.115	11.838		
Total	29	870.316			

Appendix IX: Mean square values on different concentrations of mannitol for root length of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	1616.775	323.355	311.076	<0.001
Error	24	24.947	1.039		
Total	29	1641.723			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	1114.790	222.958	249.675	<0.001
Error	24	21.432	0.893		
Total	29	1136.222			

Appendix X: Mean square values on different concentrations of mannitol for shoot dry weight of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	277.454	55.491	12.691	<0.001
Error	24	104.938	4.372		
Total	29	382.393			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	1140.464	228.093	62.357	<0.001
Error	24	87.789	3.658		
Total	29	1228.253			

Appendix XI: Mean square values on different concentrations of mannitol for root dry weight of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	173.070	34.614	111.958	<0.001
Error	24	7.420	0.309		
Total	29	180.491			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	261.730	52.346	79.393	<0.001
Error	24	15.824	0.659		
Total	29	277.554			

Appendix XII: Mean square values on different concentrations of mannitol for vigor index of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	12127.284	2425.457	22.609	<0.001
Error	24	2574.706	107.279		
Total	29	14701.990			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	5869.928	1173.986	24.983	<0.001
Error	24	1127.809	46.992		
Total	29	6997.737			

Appendix XIII: Mean square values on different concentrations of mannitol for relative water content of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	369.265	73.853	17.252	<0.001
Error	24	102.739	4.281		
Total	29	472.004			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	981.042	196.208	38.445	<0.001
Error	24	122.487	5.104		
Total	29	1103.529			

Appendix XIV: Mean square values on different concentrations of mannitol for water saturation deficit of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	369.265	73.853	17.252	<0.001
Error	24	102.739	4.281		
Total	29	472.004			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	981.042	196.208	38.445	<0.001
Error	24	122.487	5.104		
Total	29	1103.529			

Appendix XV: Mean square values on different concentrations of mannitol for water retention capacity of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	157.300	31.460	116.472	<0.001
Error	24	6.483	0.270		
Total	29	163.783			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	5	16.738	3.348	14.088	<0.001
Error	24	5.703	0.238		
Total	29	27.513			

Appendix XVI: Mean square values for total germination percentage under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	27872.423	1990.887	334.615	<0.001
Error	60	356.987	5.950		
Total	74	28229.410			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	14556.297	1039.735	314.000	<0.001
Error	60	198.676	3.311		
Total	74	14754.973			

Appendix XVII: Mean square values for mean germination time under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	0.430	0.031	1.550	0.1214
Error	60	1.188	0.020		
Total	74	1.618			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	0.976	0.070	3.277	0.0007
Error	60	1.276	0.021		
Total	74	2.253			

Appendix XVIII: Mean square values for germination index under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	24632.428	1759.459	540.755	<0.001
Error	60	195.223	3.254		
Total	74	24827.651			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	14560.141	1040.010	278.292	<0.001
Error	60	224.227	3.737		
Total	74	14784.368			

Appendix XIX: Mean square values for coefficient of velocity under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	2848.624	203.473	760.877	<0.001
Error	60	16.045	0.267		
Total	74	2864.669			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	1297.695	92.693	231.876	<0.001
Error	60	23.985	0.400		
Total	74	1321.680			

Appendix XX: Mean square values for energy of emergence under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	32453.607	2318.115	628.265	<0.001
Error	60	221.382	3.690		
Total	74	32674.989			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	11022.731	787.338	166.685	<0.001
Error	60	283.411	4.724		
Total	74	11306.142			

Appendix XXI: Mean square values for shoot length under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	53543.039	3824.503	378.510	<0.001
Error	60	606.247	10.104		
Total	74	54149.286			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	50052.434	3575.174	754.318	<0.001
Error	60	284.377	4.740		
Total	74	50336.810			

Appendix XXII: Mean square values for root length under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	14996.620	1071.187	317.677	<0.001
Error	60	202.316	3.372		
Total	74	15198.937			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	5094.492	363.892	377.779	<0.001
Error	60	57.794	0.963		
Total	74	5152.286			

Appendix XXIII: Mean square values for shoot dry weight under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	19656.604	1404.043	552.901	<0.001
Error	60	152.365	2.539		
Total	74	19808.969			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	14787.369	1056.241	216.488	<0.001
Error	60	292.739	4.879		
Total	74	15080.107			

Appendix XXIV: Mean square values for root dry weight under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	3099.600	221.400	556.229	<0.001
Error	60	23.882	0.398		
Total	74	3123.482			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	2775.684	198.263	438.659	<0.001
Error	60	27.119	0.452		
Total	74	2802.803			

Appendix XXV: Mean square values for vigor index under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	203815.425	14558.245	1323.231	<0.001
Error	60	660.123	11.002		
Total	74	204475.548			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	95906.836	6850.488	1234.253	<0.001
Error	60	333.019	5.550		
Total	74	96239.855			

Appendix XXVI: Mean square values for relative water content under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	7297.488	521.249	323.211	<0.001
Error	60	96.763	1.613		
Total	74	7394.251			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	7070.629	505.045	235.162	<0.001
Error	60	128.859	2.148		
Total	74	7199.488			

Appendix XXVII: Mean square values for water saturation deficit under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	7296.047	521.146	103.254	<0.001
Error	60	302.834	5.047		
Total	74	7598.881			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	7061.379	504.384	129.438	<0.001
Error	60	233.804	3.897		
Total	74	7295.183			

Appendix XXVIII: Mean square values for water retention capacity under drought stress condition of mungbean

BARI Mung 3	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	1284.150	91.725	807.845	<0.001
Error	60	6.813	0.114		
Total	74	1290.962			
BARI Mung 6	df	Sum of Squares	Mean Square	F value	Prob.
Treatment	14	952.645	68.046	531.916	<0.001
Error	60	7.676	0.128		
Total	74	960.320			

Plates

Plates

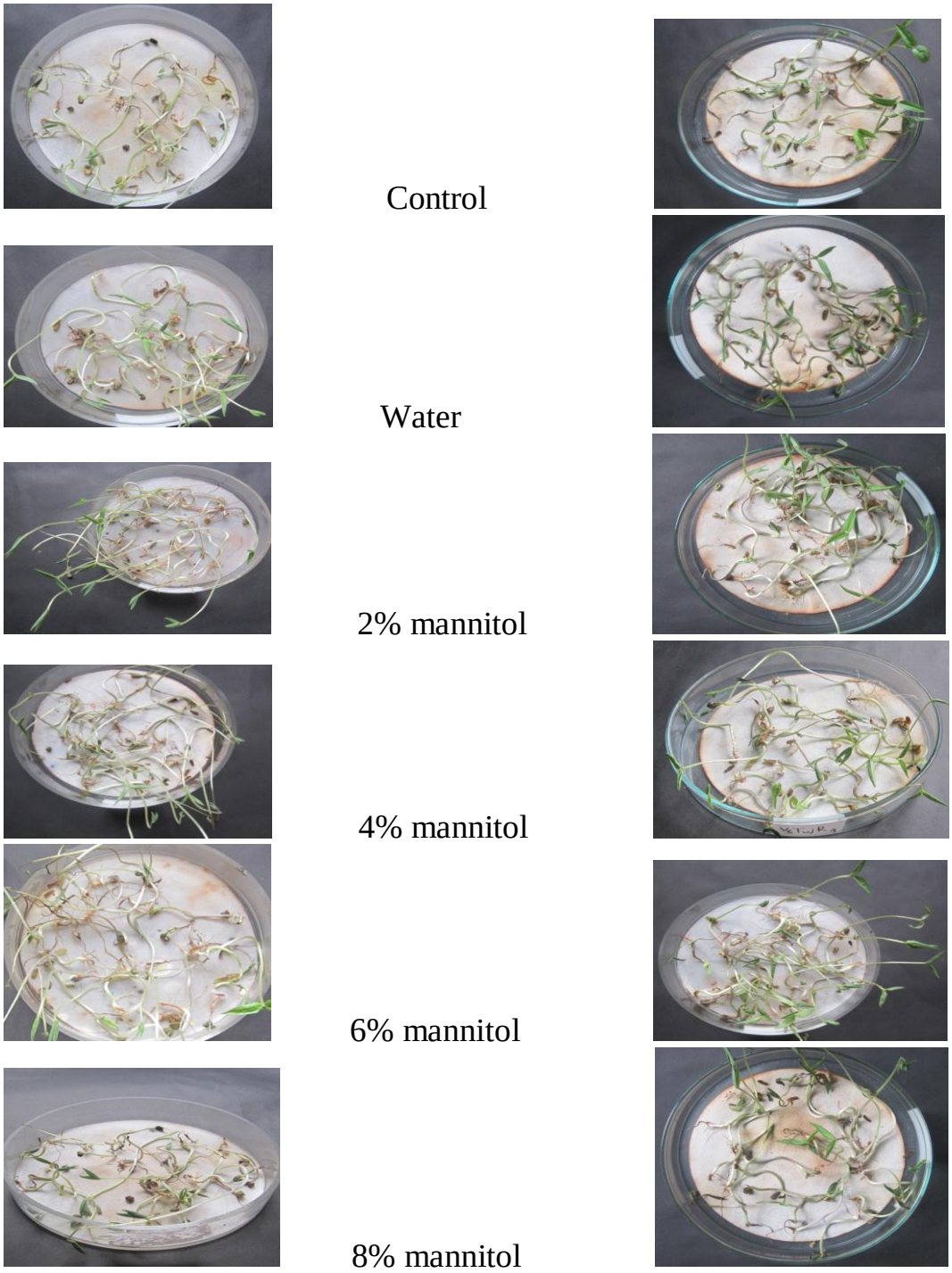

Plate 1: Effect of different concentration of priming solution on germination behabiour of mungbean varieties (BARI Mung 3 and BARI Mung 6)

Plate 2(i): Effect of different drought levels on (A) control, (B) water primed and (C) 2% mannitol primed seeds of BARI Mung 3. Here T_0 = without drought stress, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG and T_4 = 20% PEG induced drought.

A B C

Plate 2(ii): Effect of different drought levels on (A) control, (B) water primed and (C) 6% mannitol primed seeds of BARI Mung 6. Here T_0 = Without drought stress, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG and T_4 = 20% PEG induced drought.