MANNITOL INDUCED SEED PRIMING ENHANCES SALT TOLERANCE CAPABILITY IN MUNGBEAN (*VIGNA RADIATA*) UNDER SALT STRESS

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CERTIFICATE

This is to certify that the thesis entitled " ***MANNITOL INDUCED SEED PRIMING ENHANCES SALT TOLERANCE CAPABILITY IN MUNGBEAN (VIGNA RADIATA) UNDER SALT STRESS"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment 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. ASADUZZAMAN,*** *Registration. No.* ***08-03082*** *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:

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ABSTRACT

A laboratory experiment was conducted at the Agronomy lab, Sher-e-Bangla Agricultural University, during the period from August 2013 to February 2014 to investigate the potentiality of seed priming for induction of salt tolerance capability and the pre-sowing seed treatment with mannitol on germination behavior of mungbean (BARI Mung-6 and BU-4) under salt stress conditions. The whole experiment was divided intothree experiments.In first experiment, two mungbean varieties were surface sterilized with 5% Sodium hypochlorite (NaOCl) solution, soaked in water and mannitol (2%, 4%, 6%, and 8%) for 12 hours and dry seed used as control. The highest total germination(89.99%), germination index(48.64), coefficient of velocity(21.04), energy of emergence(94.99%) and vigor index(157.00) were obtain from seeds primed in 6% mannitol for BARI Mung-6 compare to total germination (85.21%), germination index (46.61),coefficient of velocity (20.21), energy of emergence (87.88 %) and vigor index (123.30), respectively was obtained in 2% mannitol solutionfor BU-4. In second experiment, BARI Mung-6 was primed in 0, 6, 9, 12, 15, and 18 hours under both 6% mannitol solution and distilled water, respectively. Priming time 6 hours with 6% mannitol showed the best result for increasing the effectiveness in inducing salt tolerance in comparison to 6 hours distilled water priming time. In the final experiment, seeds were primed with distilled water and 6% mannitol for 6 hours; dry seed used as control and were exposed to 0, 50, 100, 150, and 200 mMNaCl induced salt stress conditions in Petri dishes. Priming with mannitol followed by water were more effective than the control seed in inducing salt tolerance of mungbean cultivars owing to enhanced germination and growth parameters under salt stress condition. From the results of the study, it was observed that seeds primed with 6% mannitol for 6 hours showed the best result in comparison to water primed seed and dry seed.

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LIST OF ACCRONYMS AND ABBREVIATIONS

Agric.	Agriculture
Agril.	Agricultural
Anon.	Anonymous
AOSA	Association of Official Seed Analysis
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
^{o}C	Degree centigrate
cm	Centi-meter
CRD	Completely Randomized Design
CV	Coefficient of velocity
Dev.	Development
ed.	Edition
EG	Energy of emergence
Environ.	Environmental
et al.	And others
Expt.	Experimental
GI	Germination index
mg	Milligram
MGT	Mean germination time
ml	Milliliter
mm	Millimeter
mM	Millimole
No.	Number
Sci.	Science
i.e.	*idest* (L), that is
Inst.Institute	
Int.	International
ISTA	International Seed Testing Association
Res.	Research
ROS	Reactive Oxygen Species
j.	Journal
MeJA	Methyl jasm-onate
min	Minute
M.S	Master of Science
DMRT	Duncan's Multiple Range Test
SAU	Sher-e-Bangla Agricultural University
SE	Standard Error
Technol.	Technology
TG	Total germination
VI	Vigour Index
viz	Namely
%	Percentage

CHAPTER I

INTRODUCTION

Pulse crop belongs to grain legume. In Bangladesh various types of pulse crops aregrown. Among them lentil, cowpea, blackgram, mungbean, field pea and grass pea are important. Pulses constitute the main source of protein for the people, particularly the poor sections of Bangladesh. These are also the best source of protein for domestic animals. Besides, the crops have the capability to enrich soils through nitrogen fixation (Sharma and Behera, 2009). Pulse protein is rich in lysine that is deficient in rice. 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).

Mungbean (*Vignaradiata* L. Wilczek) is one of the most important pulse crops in Bangladeshbelonging to the family Fabaceae.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 in protein content and 4th in both acreage and production in Bangladesh(MoA, 2014). The agro-ecological condition of Bangladesh is favorable for growing this crop. Mungbean grain contains 51% carbohydrate, 26% protein, 10% moisture, 4% mineral and 3% vitamins (Khan, 1981; Kaul, 1982). On the nutritional point of view, mungbean is one of the best among pulses (Khan, 1981). It is widely used as "Dal" in the country like other pulses.

Poor crop establishment is a constraint for mungbean production (Naseem*et al*., 1997; Rahmianna*et al*., 2000) and high yields can be associated with early vigour (Kumar *et al*., 2002). Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects atphysiological, biochemical, and molecular levels, limiting crop productivity. The total world wide area of land affected by salinity is about 190 million ha (FAO, 2010). It has become a severe threat to ensure food security in the developing world. Increasing salinity had significant impact on food production and more agriculture lands are expected to become salt affected due to climate change effect (Rengasamy, 2006). Soil salinity affects germination by either an osmotic stress or ion toxic effect (Bewley and Black, 1982). Salinity causes a variety of biochemical, physiological and metabolic changes (Xiong and Zhu, 2002), which may result in oxidative stress and affect plant metabolism, performance and thereby the yield (Shafi*et al*., 2009). Salt and osmotic stresses are also responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri*et al*., 2001). Soil salinity may affect the germination of seeds either by creating a lower osmotic potential external to theseed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the germinating seed (Khajeh-Hosseini*et al*., 2003). The physiological mechanisms through which plants respond to salinity and drought show high similarity, suggesting that both stresses must be perceived by the plant cell as deprivation of water (Tavili*et al*., 2011). Other abiotic stress factors such as heat, cold, irradiation or light stress are also known to adversely affect the crops (Reyes and Cisneros- Zevallos, 2007).

Plant growth and development are regulated by a number of intrinsic and extrinsic factors, which can be modified in various ways. There are different approaches to mitigate the salt hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahloowalia*et al*., 2004), in vitro selection, use of plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H_2O_2) and osmoprotectants as foliar application and seed treatment (Senaratna*et al*., 2000; Farooq*et al*., 2009).

Since tolerance to salt in plants is a complex trait, conventional breeding techniques have had limited success in improving this trait in crops (Flowers, 2004). But these are not economically viable technology to facilitatecrop production under stress conditions.

Seed priming is considered as a promising approach to increase stress tolerance capacity of crop plants including salinity. It has been found a realizable technology to enhance rapid and uniform emergence, high vigor, andbetter yields for vegetable and field crops (Janmohammadi*et al*., 2009; Rouhi*et al*., 2011). In fact, this technique is a treatment that applied before germination in a specific environment that seeds are partially hydrated to a point where germination processes begin but radical emergence does not occur (Dell Aquila and Tritto, 1991; Giri and Schilinger, 2003; Kaur, 2002). On the other hand on seed priming the amount of water absorption is controlled so as necessary metabolic activities occurred for germination but radical emergence is prohibited. Seed priming can be accomplished through different methods such as hydropriming (soaking in DW), osmopriming (soaking in osmotic solutions such asmannitol, PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth inducers (CCC, Ethephon, IAA) (Capron *et al.*, 2000; Chiu *et al*., 2002; Harris *et al.*, 1999; Chivasa*et al.*, 1998).

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

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

i) To evaluate the effect of different concentrations of mannitol on the germination behavior of mungbean.

ii) To optimize the priming time of the best priming chemical on germination behavior of mungbean.

iii) To evaluate the effect of pre-sowing seed treatment with mannitol and Salt (NaCl)on germination behavior of mungbean in relation to salinity tolerance.

CHAPTER II

REVIEW OF LITERATURE

Mungbean is one of the most important pulse crop in Bangladesh and the growth are greatly influenced by salt stress. Literature on salt stress tolerance of mungbean is scarce. Moreover, information on the role of mannitol as priming agent on salt stress tolerance of this crop is unexpectedly low. Available literatures, pertinent to this study, on different legumes as well as other crops and priming agents are, therefore, presented below:

2.1 Effect on germination parameters

2.1.1Total 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). But such enhancements are often found under non-saline conditions (Farooq *et al*., 2006a; 2006b) and few studies are available for alleviation of adverse salinity effects in rice during germination and early seedling growth by seed priming (Xu *et al*., 2011). Patade *et al.* (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions.The higher germination percentage in seeds primed with $CaCl_2$ is according to

Ashraf and Rauf (2001) for wheat and Afzal *et al*.(2008b) for maize who reported an increase in germination percentage of plants raised from seeds primed with calcium salt under salinity stress.Short term seed priming with a low NaCl concentration also increases germination rate, field emergence and acquired stress tolerance (Nakaune *et al*., 2012). Sun *et al*.(2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination.It was reported seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition (Demir Kaya *et al*., 2006). 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). Vicente *et al*.(2009) have observed varying responses to saline solution of the seeds of three plant species (*Arthrocnemum macrostachyum*, *Juncus acutus*and *Schoenus nigricans*) and different germination recovery of the seeds after submersion in hypersaline solution of different salt types. 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.

Osmopriming with PEG was described as a good technique for improving seed germination of Bromus seeds under salt and drought stress (Tavili *et al*.,2011) and for increasing the germination percentage and seedling vigor of bersim (*Trifolium alexandrinum*) seeds (Rouhi *et al*.,2010). In soybean too, seed priming with PEG was successfully carried out by Khalil *et al*.(2001). Osmopriming with PEG results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach (Chen and Arora, 2011). Osmo conditioning of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions (Hur, 1991).According to Posmyk and Janas (2007), hydropriming and hydropriming along with proline can be used as a safe priming method for improving seed germination and growth of *Vigna radiata* seedlings at low temperature and also allowing fast repair of injuries caused by stress. More uniform germination and emergence were observed in primed seeds on canola

(*Brassica compestris*) (Zheng *et al*., 1994), wheat (*Triticum aestivum*) (Nayyar *et al*., 1995) and rice (*Oryza sativa*) (Lee and Kim, 2000; Basra *et al*., 2003) who described improved germination rate and percentage in seeds subjected to hydropriming and seed hardening for 24 h (Farooq *et al*., 2006b). 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). Ascorbic acid pretreatment results in improved germination properties of *Agropyron elongatum* under salt stress condition (Tavili *et al*.,2009).ABA priming showed increased rate of germination as compared to non-primed seeds in Indian mustard (Srivastava *et al*., 2010a,b).The growth regulators IAA and GA_3 were reported to improve germination of pyrethrum seeds under non-saline condition (Bisht *et al*.,2009).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). 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).

2.1.2Mean 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 hydropriming as a suitable, cheap and easy seed invigoration treatment for inbreedlines 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).Moradi Dezfuli 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 wasreported 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 maybe 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.

2.1.3 Germination index

Seed performance under drought or salt stress is also affected by the concentration of priming materials. It has been reported that, NaCl priming

generally requires long term treatment periods using solutions with relatively high concentrations of NaCl; however, short term seed priming with a low NaCl concentration also increases germination rate, field emergence and acquired stress tolerance (Nakaune *et al.*, 2012). Sun *et al*. (2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydropriming, while higher concentrations of PEG had negative effects on seed germination.

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). Seed priming could enhance sunflower seed germination under the stress conditions was found by Kaya *et al.*, (2006). Bray *et al*., (1989) and 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 coper sulphate, zinc sulphate, manganese sulphate, or boric acid induced high levels of seed germination. Hydropriming was found to be the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 h compared to 48 h (Caseiro *et al*., 2004).It improved germination and later growth of different crops species such as in maize, rice, chickpea (Harris *et al*., 1999).

2.1.4Coefficient 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).

2.1.5 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 hydropriming, hardening, osmopriming, 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 (casenve and Toselli, 2007) triticale (Yagmur and Kaydan, 2008). Potassium hydro phosphate (K_2HPO_4), polyethylene glycol (PEG6000) (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 osmopriming 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 maybe 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). Halopriming 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).

2.2 Effect on growth parameters

2.2.1Shoot 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 hydropriming of chickpea seeds with mannitol and water alleviated the adverse effects of water deficiency and salt stress on seedling growth. The treatment of seeds with water, 2 and 4 % mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions (Kaur *et al*.,2002, 2005).Priming of chickpea seeds with mannitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Previous studies on tomato (Cuartero *et al*., 2006) and melon(Sivritepe*et al*., 2003), showed that seed priming improves seed germination, seedling emergence and growth under saline conditions.Farhoudi and Sharifzadeh (2006) and Sarwar *et al*. (2006) while working with canola and chickpea, respectively, reported salt priming-induced improvement in seed germination, seedling emergence and growth under saline conditions.

Priming of seeds with water promoted seedling vigour, yield and crop establishment of chickpea, maize and rice in India (Harris *et.al*., 1999). It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity affects during early

seedling growth (Ashraf and Harris, 2004; Afzal *et al*., 2006).Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solution of KCl or potassium sulfate (K_2SO_4) significantly increased plant height, yield attributes, and grain yield in wheat. The beneficial effects of gibberellic acid (GA_3) on germination are well known (Angrish *et al*., 2001; Radi *et al*.,2001; Khan *et al*., 2002). GA_3 (100 mg l-1) applied as pre-sowing treatment resulted in the highest K^+ and Ca^{2+} content in the shoots of both faba beans (*Vicia faba*) and cotton (*Gossypium barbadense*) crops (Harb,1992). 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).

2.2.2 Root length (mm)

Seed priming techniques such as hydropriming, hardening, osmo-conditioning, osmo-hardening, and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007).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).

Osmopriming and hydropriming of wheat seeds may improve germination and emergence (Ashraf and Abu-Shakra, 1978) and may promote vigorous

rootgrowth (Carceller and Soriano, 1972). Hydroprimed 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).

2.2.3 Seedling length (mm)

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

Hydropriming method has also been used successfully in wheat (Harris *et al*., 2001), in sunflower (Kaya *et al*., 2006), chickpea (Kaur *et al*., 2002) and cotton (Casenave and Toselli, 2007). Moreover, hydropriming increased germination and seedling growth under salt and drought stresses (Kaur *et al*., 2002; Kaya *et al*., 2006; Casenave and Toselli, 2007). Emergence force and seedling growth were strengthened by hydropriming in watermelon seeds Sung and Chiu (1995).Elkoca *et al*. (2007), recommended that hydropriming for 12 h or osmopriming (PEG -0.5 MPa) for 24 h for a better germination of chickpeas

under cold soil conditions. Compared to hydropriming, priming with PEG in a proper concentration was found to have a better effect on seed germination and seedling growth under drought stress (Yuan-Yuan *et al*.,2010).

PEG is frequently used to simulate drought stress (Chen *et al*., 2010; Farahani *et al*., 2010; He *et al*., 2009; Khajeh-Hosseini *et al*., 2003; Tohidloo and Kruse, 2009; Zhu *et al*., 2006) as an inert osmoticum in germination tests (Dodd and Donovan, 1999) and is a non-penetrating solute (Almansouri *et al*., 2001), which results in osmotic stress that inhibits seed germination through the prevention of water uptake. However, it has been reported that the inhibitory effect of PEG on germination may not be solely related to water imbibition (Almansouri *et al*., 2001). Wang *et al*. (2009a) have observed that the fresh weight and the length of the roots and shoots of two alfalfa cultivars (Xinmu No.1 and Northstar) were significantly inhibited by 35% PEG treatment. For a potential medicinal plant, *Matricaria chamomilla*, both the seed germination rate and seedling growth have been found to be reduced with the PEG-mediated increasing osmotic potential of the growth medium (Afzali *et al*., 2006). Rouhi *et al*. (2011) also suggested that different priming techniques (hydro and osmo priming) had a varying effects on germination on each of the four grass species (*Bromus inermis*, *Festuca arundinacea*, *Agropyron elongatum* and *Festuca ovina*) and the result showed that, for most evaluated germination parameters, osmopriming treatment (with PEG) was more useful technique to reduce abiotic stress than hydropriming treatment.

Although priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions (Parera and Cantliffe, 1991), the effectiveness of different priming agents varies under different stresses and different crop species (Iqbal and Ashraf, 2005).Patade *et al.,* (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline

conditions. Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solutions with KCl or K_2SO_4 significantly increased plant height, grain yield and its components in wheat genotypes.Priming of chickpea seeds with manitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Seed treatment with water and mannitol is also useful under water deficit stress and primed chickpea seeds gave high yield as compared to non-primed seeds (Kaur *et al*., 2002). Musa *et al*. (1999) reported that overnight priming of chickpea seeds gave better crop production in Bangladesh. Priming with H_2O_2 failed to improve emergence and seedling growth in rice cultivars which is inconsistent with Wahid *et al*. (2007) who reported improved salt tolerance in wheat by alleviation of salt stress and oxidative damage by H_2O_2 pre-treatment.

2.2.4 Seedling 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). In basil (*Ocimum basilicum* L.) under saline conditions, the seedling vigor, germination percentage and seedling dry weight was found to increase due to hydropriming (Farahani and Maroufi, 2011).Increased plumule dry weight due to osmopriming was reported by Harris *et al*. (2004).

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

2.2.5 Vigour index

Post-harvest seed enhancement treatments improve germination and seedling vigour (Taylor, 1998). Maiti *et al*. (2009) studied the effect of priming on seedling vigour and productivity of tomato, 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 vigour of the mungbean seedlings (Umair *et al.,* 2010). It is also reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS) (Del Ryo *et al*., 2002). Afzal *et al*. (2008 a) observed that the priming-induced salt tolerance was associated with improved seedling vigour, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants. Seed priming is used for improvement of germination speed, germination vigour, seedling establishment and yield (Talebian*et al*., 2008). Afzal *et al*. (2005) also found that the priming-induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants.

Primed crops grew more vigorously, flowered earlier and yielded higher (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 vigour 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 vigour level than

non- primed (Ruan *et al*., 2002a). It has been also reported invigorated seeds had higher vigour levels (Ruan *et al*., 2002b), which resulted in earlier start of emergence as high vigour seed lots performed better than low vigour ones (Hampton and Tekrony, 1995).

Seed priming techniques such as hydropriming, hardening, osmo conditioning, osmo hardening and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007). Various works have shown that hydropriming of seeds have many advantages as compared to non-primed seeds. Hydropriming has resulted in 3 to 4-fold increases in root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition (Kaur *et al*.,2002). This phenomenon was explained to be due to faster emergence of roots and shoots, more vigorous plants, better drought tolerance under adverse conditions (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 hydropriming enhanced seedling establishment and early vigour 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 vigour index of the seeds of sunflower varieties. Salt priming with KNO_3, is an effective way to improve seed and seedling vigour of sunflower and cucumber (Singh and Rao, 1993; Ghassemi-Golezani and Esmaeilpour,2008).

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

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from August, 2013 to February, 2014to study the effect ofmannitol induced seed priming for enhancing salt tolerance capability in mungbean(*Vignaradiata*) under salt stress. The materials and methods describes a short descriptionof 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:

3.1Descriptionof 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 24.09^0 N latitude and 90.26^0 E longitudes.

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 period of the culture room was 26.2^0 C to 33.4^0 C, respectively and average minimum and maximum relative humidity was 56% and 84%, respectively.

3.2Test crops

Twomungbeanvarietiesnamely- BARI Mung-6 and BU-4 were used for this experiment. BARI Mung-6 was collected from Bangladesh Agricultural Research Institute (BARI) andBU-4 from Bangabandhu Sheikh MujiburRahman Agricultural University. The collected mungbeanvarieties

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.

3.3Experimental materials

Differentequipments such as electric balance, Petri dish, filter paper, micro pipette, forcep, ovenetc.were used for this study.

3.4Chemicals for seed priming

Different priming chemicals such as Mannitol($C_6H_{14}O_6$), salt (NaCl) and distilled water were utilized for osmo and hydro priming.Sodium hypochloride(NaOCl) used as seed treating chemical.

3.5 Experimental treatments and design

The experiment comprises of

(a) Five levels of salinity stress viz.0, 50, 100, 150, 200 mMNaCl,

(b) Six levels of priming time viz. 0, 6, 9, 12, 15, 18 hours and

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

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

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

3.6.1.1Weight of seeds

200 g seeds were weighted from the total seed from each of two mungbean variety BARI Mung-6 and BU-4 to reduce the unnecessary loss of seeds.

3.6.1.2 Surface treatment

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

3.6.1.3 Treatments

The experiment was comprised with two mungbean variety andsix types of priming solutions.

Mungbean variety (02)

1. Variety 1: BARI Mung-6
2. Variety 2: BU-4

Six types of treatments:

1. T_1 =Seeds without priming (control)
2. T_2= Seeds primed with distilled water
3. T_3=Seeds primed with 2% mannitol solution
4. T_4=Seeds primed with 4% mannitol solution
5. T_5=Seeds primed with 6% mannitol solution
6. T_6=Seeds primed with 8% mannitol solution

3.6.1.4 Priming solutions

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

3.6.1.5 Preparation of priming solutions

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

   5 g of mannitol wasdissolved 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

Two priming techniques viz., osmopriming and hydropriming 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 hydropriming seeds of a sub-sample were soaked in distilled water and for osmopriming seeds of another sub-sample were divided into another four sub-sample and pretreated with mannitolat a four levels of concentration of 2%, 4%, 6%, and 8% for 12 hours. Priming was done in different plastic containers covered with lids to prevent evaporation loss.All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hoursback to the original moisture level.

3.6.1.7Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 90 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 (60 Petri dishes) were arranged factorialy in a 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±1°C under normal lightto facilitate germination for 7 days. Germination was considered to have occurred when radicles were 2 mm long(Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 days.Theseedlings 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 wereexcluded during counting.At the end of germination test (7 days), 5 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±2°C for 48 hours.

3.6.2 2nd Experiment

Optimization of pre-sowing priming time on the germination behavior of mungbean.

3.6.2.1 Weight of seeds

Seeds were weighted 200gfrom the total seed of BARI Mung-6 for this experiment to reduce the unnecessary loss of seeds. Remaining seeds are taken in poly bag and preserved in refrigerator.

3.6.2.2 Surface treatment

Sodium hypochlorite(5.0 %) 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 chlorine from the seed surface. Seeds are then dried in room temperature to regain the normal weight.

3.6.2.3 Treatments and design

Mungbean variety:

1. Variety 1: BARI Mung-6

Eleven types of priming times are used as treatment. They are as follows:

1. W_0= Seeds without priming (control),
2. W_6= Seeds primed with water for 6 hours,
3. W_9= Seeds primed with water for 9 hours,
4. W_{12}= Seeds primed with water for 12 hours,
5. W_{15}= Seeds primed with water for 15 hours,
6. W_{18}= Seeds primed with water for 18 hours,
7. M_6= Seeds primed with 6% mannitol solution for 6 hours,
8. M_9= Seeds primed with 6% mannitol solution for 9 hours,
9. M_{12}= Seeds primed with 6% mannitol solution for 12 hours,
10. M_{15}= Seeds primed with 6% mannitol solution for 15 hours and
11. M_{18}= Seeds primed with 6% mannitol solution for 18 hours.

3.6.2.4 Priming solutions

Mannitol 6% are used as osmoprimingand distilled water were used as hydro priming solutions.

3.6.2.5 Preparation of priming solutions

a) Mannitol solutions (6%)

Fifteen g of mannitol was dissolved in 250 ml of water to prepare 6% solution of mannitol.

b) Distilled water

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

3.6.2.6 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 weredivided into five sub-sample soaked in distilled water for five different priming times such as 6, 9, 12, 15, and 18 hours for hydropriming.For osmoprimingthe remaining sample of seeds were divided into more five sub-sample and pretreated with mannitolfor 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.

3.6.2.7 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter petri dishes on whatman No.1 and filter paper was moistened with 8 ml of distilled water. Here, whatman No.1 filter paper were used as growth media for germination. Experimental units (55 Petri dishes) were arranged factorialy in a 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±1°C under normal light to facilitate germination for 7 days. Germination was considered to have occurred when radicles were 2 mm long(Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 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 (7 days), 5 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±2°C for 48 hours.

3.6.3 3rd experiment

Germination behavior of primed Seed (mungbean) under salt (NaCl) stress condition.

3.6.3.1Weight of seeds

Seeds were weighted 200gfrom the total seed of BARI Mung-6 for this experiment to reduce the unnecessary loss of seeds.

3.6.3.2 Surface treatment

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

3.6.3.3 Treatments

This experiment was comprises of osmopriming and hydropriming with four salt stress levels (50mM, 100mM, 150mM and 200mM). Salt stress was simulated by highly osmotic substance Nacl of molecular weight (MW) 58.5 g/L.

The treatments are as follows:

1. T_1= Primed (mannitol and water) and non-primed (control) seeds placed without salt (control),
2. T_2= Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt,
3. T_3= Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt,
4. T_4= Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt and

5. T_5= Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

3.6.3.4 Priming solutions and time

6% mannitol solution and distilled water were used as priming solutions and 6 hours as priming time.

3.6.3.5Preparation of priming solutions

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

   Fifteen g of mannitol was dissolved in 250 ml of water to prepare 6% solution of mannitol.

b) **Distilled water**

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

3.6.3.6 Preparation of stress solutions

Salt (Nacl) solutions (50mM, 100mM, 150mM and 200mM)

0.731 g of sodium chloride (Nacl) was dissolved in 250 ml of water to prepare 50mM solution of salt (Nacl). Similarly, 1.436 g, 2.18 g, 2.925 g sodium chloride (Nacl) was dissolved in 250 ml of water to prepare 100mM, 150mM and 200mM solution of mannitol, respectively.

3.6.3.7 Priming technique

Two priming techniques viz., osmopriming and hydropriming were applied on 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 hydropriming and seeds of another sub-sample were pretreated with mannitol for osmopriming at a concentration of 6% for 6 hours, respectively.Priming is done in different plastic containers covered with lids to prevent evaporation loss.All seeds were removed from the priming

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

3.6.3.8 Germination of seeds

The standard germination test was performed by placing randomly selected 30 seeds in 90-mm-diameter Petri disheson whatman No.1.Petri dishes containing primed and control seeds were irrigated with solutions of8 ml salt stress levels. Here whatman No.1 filter paper were used as growth media for germination.Experimental units (75 Petri dishes) were arranged factorialy in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept water saturated state. Seeds were kept at room temperature 25±1°C under normal light to facilitate germination for 7 days. Germination was considered to have occurred when radicles were 2 mm long(Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 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 (7 days), 5 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±2°C for 48 hours.

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 seedswhich was germinated within 7 daysas a proportion of number of seeds shown in each treatment,expressed as a percentage(Othman *et al*., 2006).

TG (%) = $\frac{number of germinated seeds}{total number of seeds set for germination}$ 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:

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 Kader and Jutzi (2004) formula.

CV= (∑Ni /100) x (∑Ti Ni)

Where,

Ti= number of days after sowing and

Ni = number of seeds germinated on ith day.

3.7.5Energy of emergence (EG %)

Energy of emergence (EG) was recorded on the 4th day after planting. 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 (mm), root (mm) and seedling length (mm)

Randomly selected 5 seedlings from each treatment were collected and cotyledons were removed from them. Shoot, root and seedling length was measured with a ruler and accuracy of measurement was 1 mm.

3.7.7Seedling dry weight(mg)

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

3.7.8 Vigour Index (VI)

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

$$VI = \frac{TG\ (\%)\ x\ seedlings\ \ length\ \ (mm)}{100}$$

Here,

TG = total germination.

3.8 Statistical analysis

The data obtained for different parameters were statistically analyzed to observed the significant difference among the treatment. 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 Duncan's Multiple Range Test (DMRT)according to Steel *et al*.(1997)at 5%

level of probability.A computer software SPSS 20 was used to carry out the statistical analysis. Drawings were made using Excel software.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprises presentation and discussion of the results obtained from a study to investigate the effect of seed priming with mannitolon the enhancement of satl tolerance capability in mungbean varieties cv. BRRI mung-6 and BU-4. The results of the germination and growth parameters of mungbean as influenced by different concentrationsof priming agent (mannitol) and priming time in salt stress condition have been presented and discussed in this chapter.

4.1 Experiment 1: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 mannitolon the germination behavior of mungbean varieties cv. BRRI mung-6 and BU-4 have been presented, discussed and compared in this chapter. The analytical results have been presented in Figures 1 to 10 and Appendices Ito X.

4.1.1 Effect on total germination (%)

There was no significant variation observed on total germination percentage at T_4, T_5 and T_6treatment for varietal variation but significant variation was observed at T_1, T_2 and T_3treatment(Figure 1 and Appendix I)priming with different concentrations of mannitol and water. Total germination percentage increased withmannitol concentration upto 6% and 2% forBARI Mung-6 and BU-4, respectively thereafter decreased due to increasing concentration of mannitol. The highesttotal germination(89.99 %) of BARI Mung-6 was observed from T_5 treatment compare tototal germination (85.21 %)of BU-4was observed in T_3 treatment. The lowestgerminationpercentage(58.88 %)forBU-4was found in T_1 treatment.Total germination ofBARI Mung-6 was higher than

BU-4. These findings are consistentof the results of Mohseni *et al*. (2010) where they observed the highest germination percentage with water, and the least germination percentage with 4% KCL and 2% KNO_3.

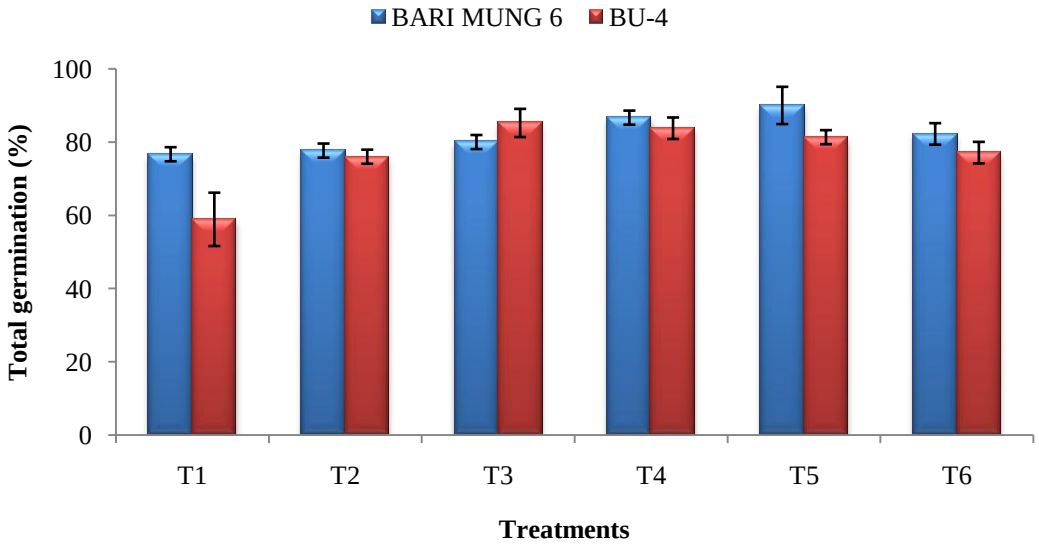

Figure 1. Effect of different concentrations of priming solution on total germination percentage of primed (mannitol and water) and non-primed (control) seeds. (SE=1.925, 7.285, 1.925, 1.925, 1.925, 3.848, 1.925, 2.939, 5.089, 1.922,2.939 and 2.939)

T_1= Dry seeds;T_2= Seeds socked with water for 12 hours;T_3=Seeds socked with 2% mannitol solution for 12 hours; T_4=Seeds socked with 4% mannitol solution for 12 hours; T_5=Seeds socked with 6% mannitol solution for 12 hours and T_6=Seeds socked with 8% mannitol solution for 12 hours.

4.1.2 Effect on mean germination time(days)

Different concentrations of mannitol solutions and waterpriming differed significantly in mean germination time of BARI Mung-6 andBU-4(Figure 2 and Appendix II). Mean germination time was affected by water priming and different mannitol concentration. With increasing mannitol concentrationmean germination timedecrease gradually upto T_5 andT_3treatmentforBARI Mung-6 and BU-4,respectively and thereafter increased with increasing mannitol concentration. The longestmean germination time(6.61days) was observed for BARI Mung-6 from T_6 treatment compare tomean germination time(5.41 days)of BU-4 was found at T_1treatment. The shortest meangermination time (4.46days) was found in T_5 treatmentcompare tomean germination time (4.52 days)was foundin T_3 treatment ofBARI Mung-6 and BU-4, respectively. Mean

germination time for primed seeds is less than non-primed seeds.Such these positive effects isprobably due to stimulatory effects of seed priming on biochemical activities and meiosis during primary stages of germination (Sirritepe*et al*., 2003). In corn seedMohseni*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.

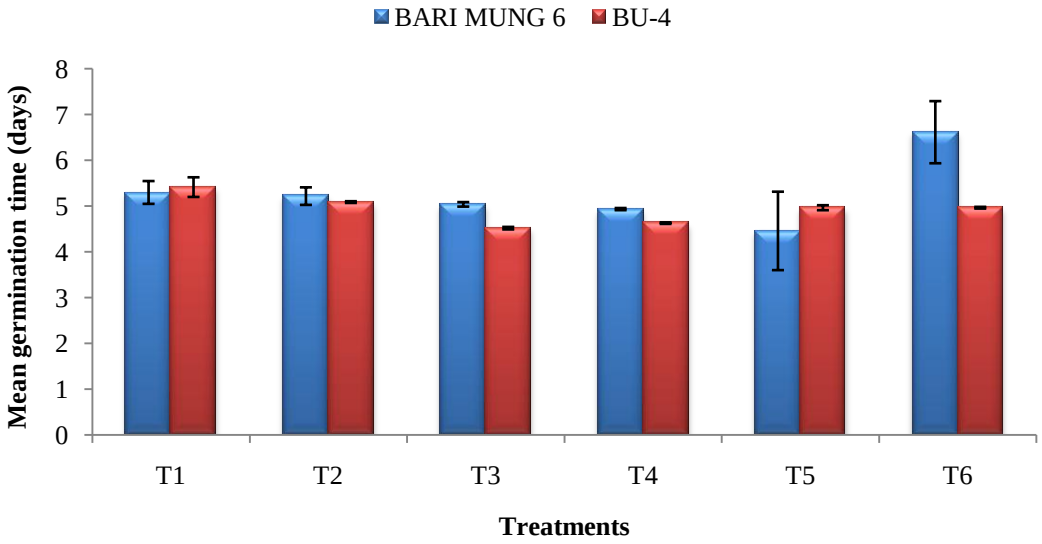

Figure 2. Effect of different concentrations of priming solution on mean germination time(days) of primed (mannitol and water) and non-primed (control) seeds. (SE= 0.249, 0.214, 0.191, 0.018, 0.049, 0.027, 0.023, 0.016, 0.856, 0.054, 0.678 and 0.018)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4= Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.3 Effect on germination index

Germination index of BARI Mung-6and BU-4 was influenced by different priming agent (Figure 3) and variance analysis showed that there was significant difference between control (non-primed) and primed seed (Appendix III).Germination index was affected by water priming and different mannitol concentration.Resultsreveled thatgermination index increasedupto 6

% for variety BARI Mung-6and upto 2 % for variety BU-4 in mannitolconcentration and then decreased slightly.Highest germination index (48.64) was recorded in T_5 treatment compare to germination index (46.61) was recorded from T_3 treatment forBARI Mung-6 and BU-4, respectively.The lowest germination index (25.14)forBU-4was found in T_1 treatment. Germination index of BARI Mung-6 was higher thanBU-4.Rashid *et al*. (2005) concluded that priming of seeds with water before implantation leads to early germination, better establishment and increase of function in some of the crops in unfavorable conditions.

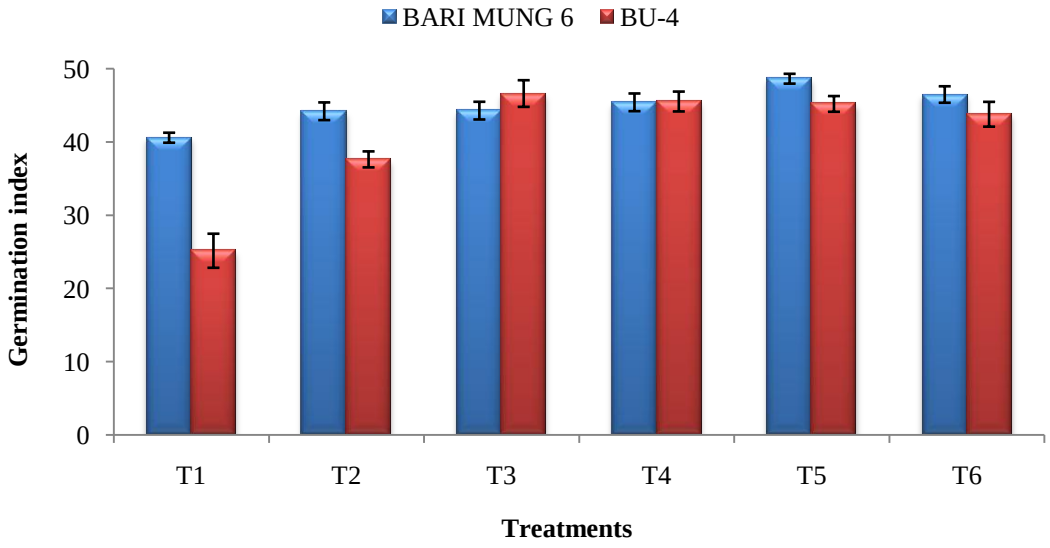

Figure 3. Effect of different concentrations of priming solution on germination index of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.682, 2.324, 1.210,1.092,1.210,1.820,1.210,1.356,0.670,1.074,1.124 and 1.69404)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4= Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.4 Effect on coefficient of velocity

Significant variation was observed in terms of coefficient of velocity for BARI Mung-6 and BU-4 due to priming with different mannitol concentrations and water except T_3, T_4 and T_5 treatment(Appendix IV and Figure 4). Coefficient of velocity increased with increasingmannitol concentration upto T_5 treatment and T_3treatment for BARI Mung-6 and BU-4, respectively and thereafter decreased

gradually. The maximum coefficient of velocity (21.04)of BARI Mung-6 was observed from T_5 treatment compare tocoefficient of velocity (20.21) of BU-4was observed from T_3 treatment. The minimumcoefficient of velocity (19.79) was found for BARI Mung-6and (19.17) forBU-4was found in T_1 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).

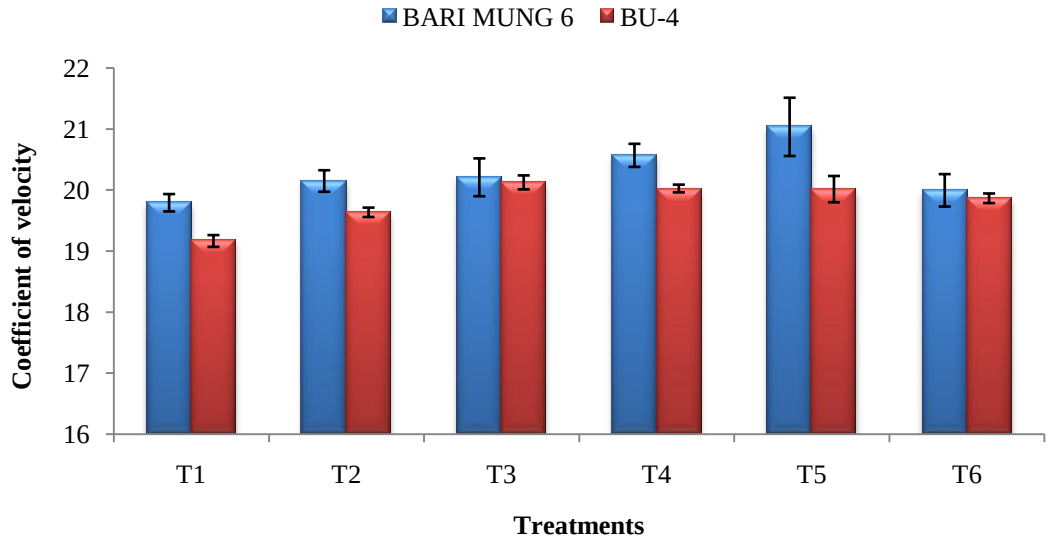

Figure 4. Effect of different concentrations of priming solution on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds. (SE= 0.142, 0.096840.176, 0.076, 0.310, 0.114, 0.189, 0.063, 0.477, 0.215,0.265 and 0.078)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4= Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.5 Effect on energy of emergence (%)

Energy of emergence showed no significant variation for BARI Mung-6 andBU-4 ondifferent concentrations of mannitol and water priming except T_1 and T_2 treatment (Appendix V and Figure 5). Result showed that energy of emergence increase with increasing mannitol concentration upto 6 % and 2 % for BARI Mung-6 and BU-4, respectively and therefore decreased slightly.The highest energy of emergence(94.99 %) was recorded for BARI Mung-6 in T_5 treatment compare to energy of emergence (87.88 %) of BU-4 was recorded in

T_3 treatment.The lowest energy of emergence (49.72 %) was recorded for BU-4 in T_1 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 (*Triticumaestivum*) (Bose and Mishra, 1992; Basra *et al*., 2002) and fine rice (*Oryza sativa*) (Basra *et al*., 2003).

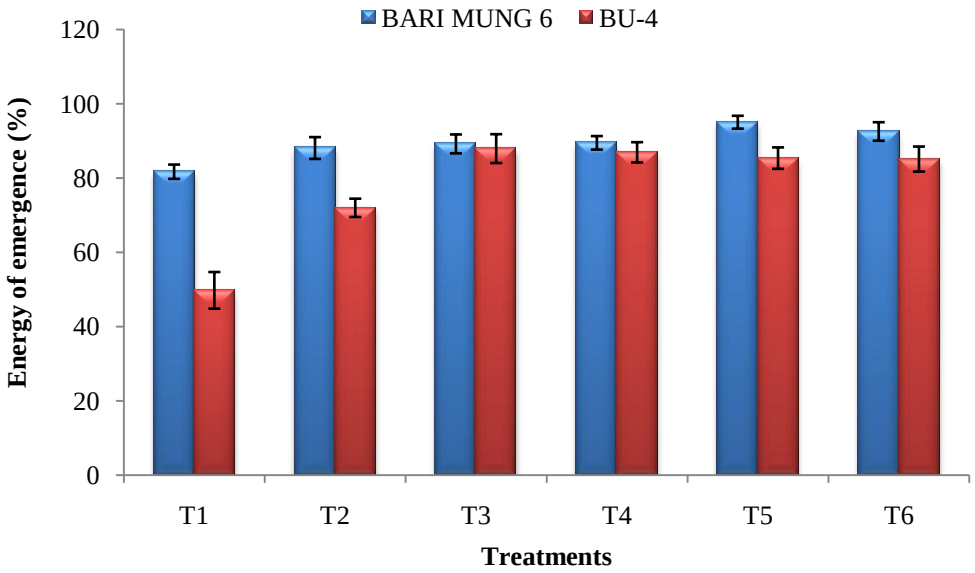

Figure 5. Effect of different concentrations of priming solution on energy of emergence (%) of primed (mannitol and water) and non-primed (control) seeds. (SE= 1.925, 4.935, 2.939, 2.467, 2.547, 3.887, 1.820, 2.733, 1.734, 2.886, 2.5 and 3.377)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.6 Effect on shoot length (mm)

Significant variation was observed on shoot length among the two varieties (BARI Mung-6 and BU-4) priming with different concentration of mannitol and water (Appendix VI and Figure 6). Shoot length increase with T_5 and T_3 treatmentfor BARI Mung-6 and BU-4, respectively and therefore decrease with the increasing mannitol concentration.The maximumshoot length (121.6 mm) was recorded for BARI Mung-6 in T_5 treatmentcompare toshoot length of BU-4 (94.93 mm)was recorded in T_3 treatment.Shoot length of BARI Mung-6 was higher thanBU-4. Treated seeds had high germination percentages and quicker

germination time. One hypothesis is that benefits of priming can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduces lag time between imbibition and radicle emergence (Bradford *et al*., 1990). Better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins are also some of the basis for enhanced growth (Bray *et al*., 1989). Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds.

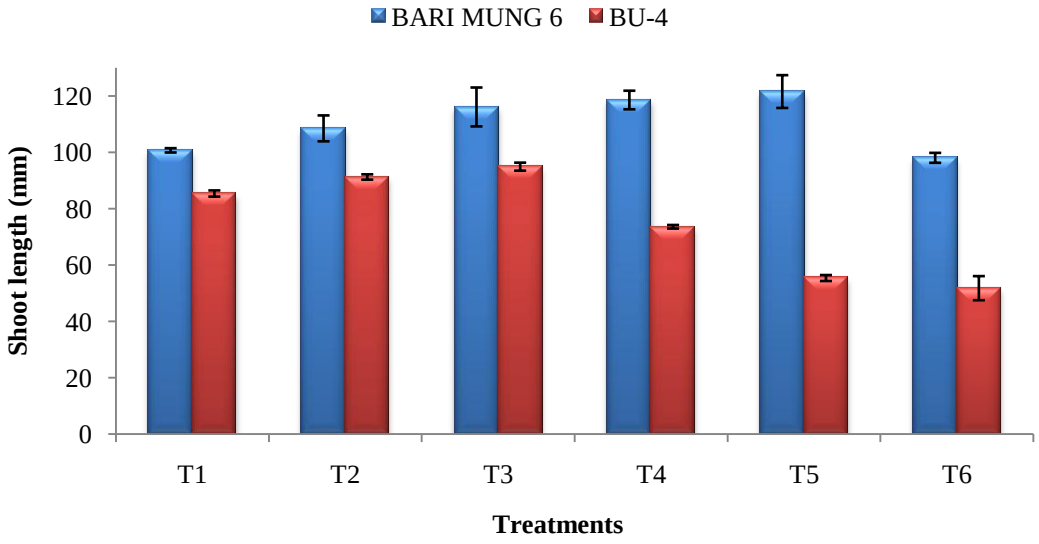

Figure 6. Effect of different concentrations of priming solution on shoot length (mm) of primed (mannitol and water) and non-primed (control) seeds of mungbean (BARI Mung-6 and BU-4) (SE= 0.768, 1.101, 4.596, 0.945 6.905, 1.425, 3.3,0.635, 5.820,1.058,1.763 and 4.277)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours; and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.7 Effect on root length (mm)

Statistically significant variation was recorded in terms of root length of BARI Mung-6 andBU-4 due to priming with water and different mannitol concentrations (Appendix VII and Figure 7).Root length was affected by water priming and different mannitol concentration.Root length increase with T_5 and T_3 treatment for BARI Mung-6 and BU-4, respectively and therefore decrease with the increasing mannitol concentration.The maximum root length(53.66

mm)was observed from T_3 treatment forBU-4 comparetoroot length (45.73 mm) was observed from T_5 treatment forBARI Mung-6. The minimumroot length (28.20 mm) was found in T_6 treatmentforBARI Mung-6 and root length (41.53 mm)was found in T_1treatmentforBU-4.Root length of BU-4 was higher than 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).

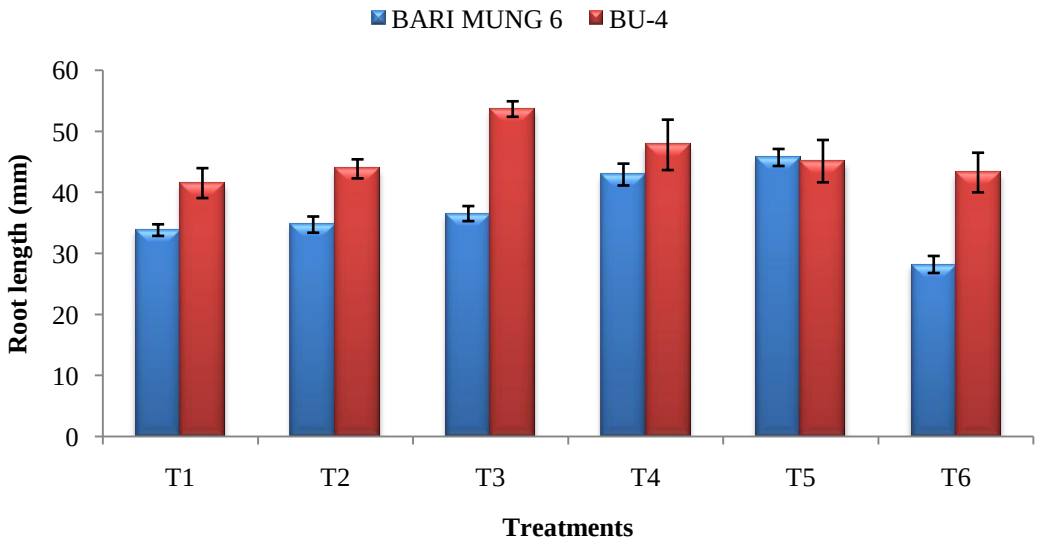

Figure 7. Effect of different concentrations of priming solution on root length (mm) of primed (mannitol and water) and non-primed (control) seeds. (SE= 0.952, 2.45 1.328, 1.559, 1.234 1.271, 1.79, 4.129, 1.396, 3.468,1.396 and 3.246)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.8Effect on seedling length (mm)

Seedling length of BARI Mung-6 and BU-4 showed significant variation due to priming with different mannitol concentrationsand water (Appendix VIII and Figure 8).Seedling length increase with T_5 and T_3 treatment for BARI Mung-6

and BU-4, respectively and therefore decrease with the increasing mannitol concentration. The maximum seedling length(174.73 mm) was observed forBARI Mung-6from T_5 treatmentcomparetoseedling length (137.00 mm) of BU-4 was recorded in T_3 treatment.Seedlings length of BARI Mung-6 was higher thanBU-4. Seedlings length was higher in seeds treated with water, mannitol and lower concentration of K_2HPO_4 and KNO_3 as compared to seedlings grown from no treated Chickpea seed (Nighat, Sumaira and Farhat, 2006).

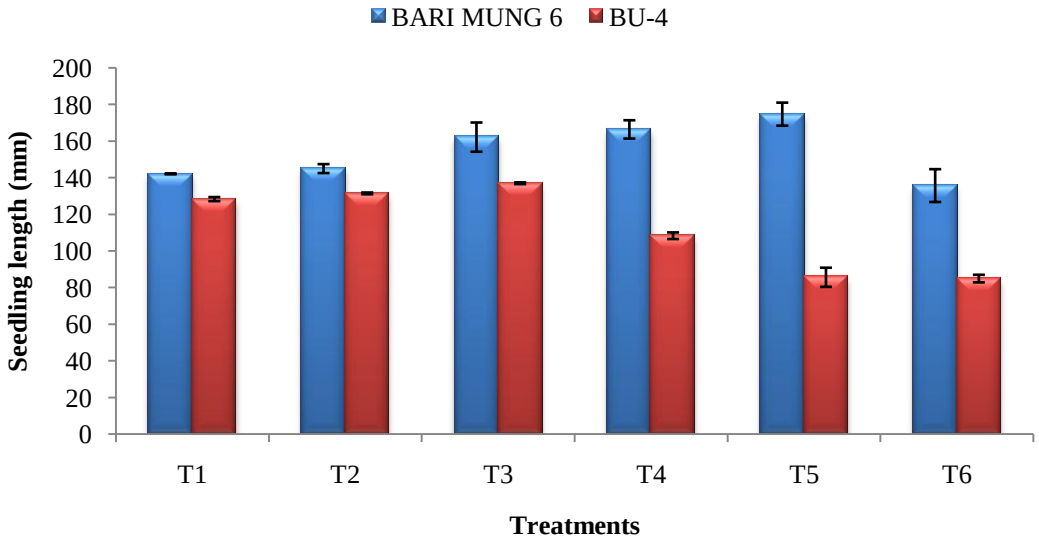

Figure 8. Effect of different concentrations of priming solution on seedling length (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.24, 1.109, 2.444,0.466, 7.975, 0.466, 5.011, 1.804, 6.293, 5.228, 8.959 and 2.066)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.9 Effect on seedling dry weight (mg)

Statistically significant variation was found in case of seedling dry weightof BARI Mung-6 andBU-4 due to priming with different mannitol concentrationsand water (Appendix IX and Figure 9). Dry weight was affected by water priming and different mannitol concentration. Dry weight increase with T_5 and T_3 treatment for BARI Mung-6 and BU-4, respectively and

therefore decrease with the increasing mannitol concentration. Results revealed that the highest seedling dry weight (124.9 mg) was recorded in T_5 treatment for BARI mung-6 compare toseedling dry weight (108.26 mg) was recorded in T_3 treatmentforBU-4.Seedling dry weight of BARI Mung-6 was higher thanBU-4.These results are in agreement with those of Pill and Necker (2001) who reported that primed compared to non-primed plants resulted in greater seedling dry weights.

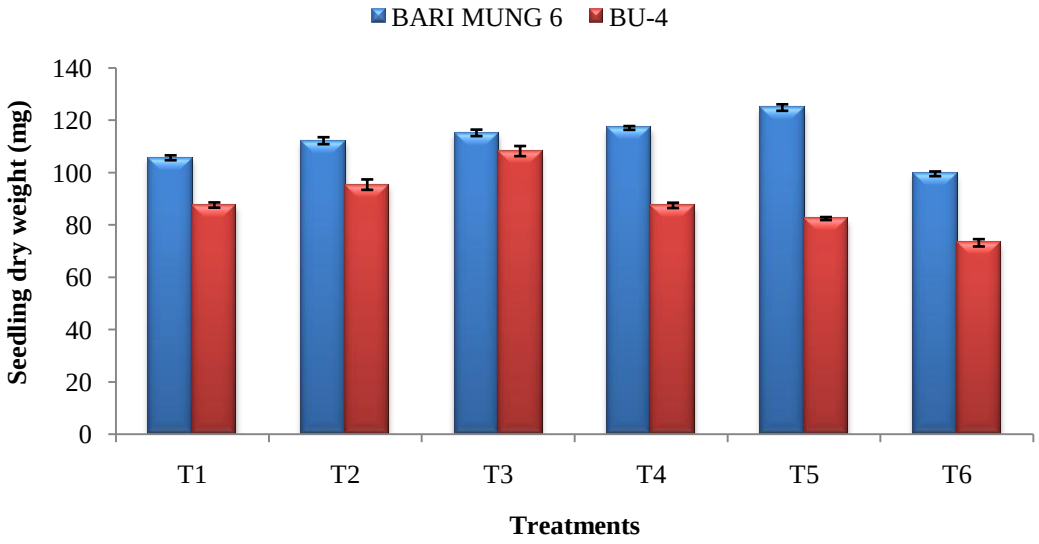

Figure 9. Effect of different concentrations of priming solution on seedling dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds. (SE= 0.929, 1.001, 1.338, 1.996, 1.246, 1.934, 0.723, 1.017, 1.246, 0.578, 0.902 and 1.401)

T_1 = Dry seeds;T_2 = Seeds socked with water for 12 hours;T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.1.10Effect on vigour index

Priming with different concentrations of mannitol and watershowed significant variation in vigor index of BARI Mung-6 andBU-4 (Appendix X and Figure 10). Vigor index was affected by water priming and different mannitol concentration. Vigor index increased significantly upto T_5 and T_3 treatment for BARI Mung-6 and BU-4, respectively and thereafter decreased drastically with the increasing mannitol concentration. The highest vigor index (157.00) of

BARI Mung-6 was recorded from T_5 treatmentcompare to vigour index (123.30) was recorded in T_3 treatment for BU-4. On the other hand, the minimum vigourindex (67.31) was found in T_1 treatmentfor BU-4which was statistically similar with T_6 (69.92)treatment.Grandi*et al*. (1999) found that P enrichment by soaking seeds in 200 mM 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 (Karthiresan*et al*., 1984) and decreased catalase and peroxidase levels as recorded in pea seedlings (Srivastava and Dwivedi, 1998).

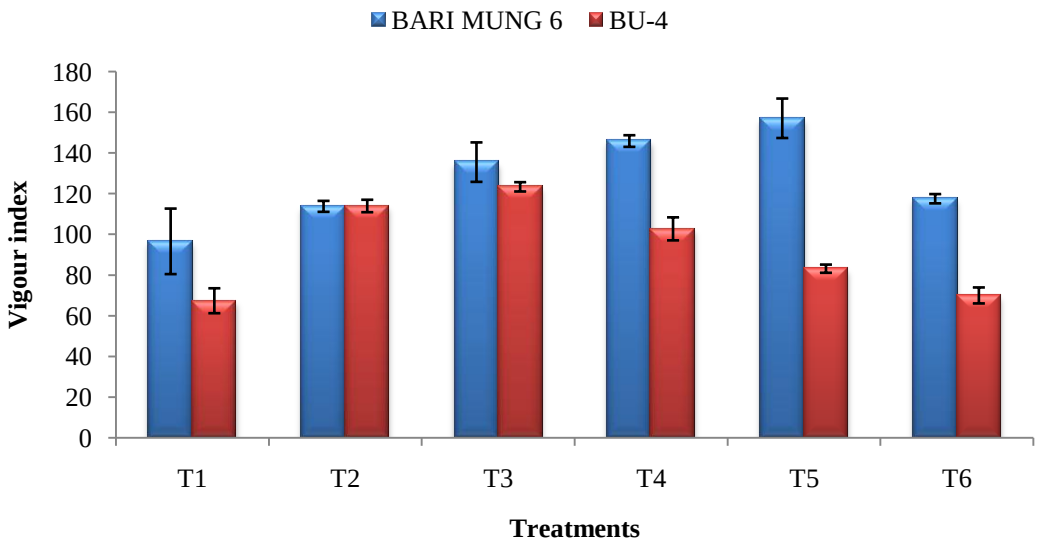

Figure 10. Effect of different concentrations of priming solution on vigour index of primed (mannitol and water) and non-primed (control) seeds.(SE= 16.098, 6.133, 2.694 3.065, 9.699, 2.286, 2.858, 5.657, 9.699, 2.022, 2.276and 3.927)

T_1 = Dry seeds; T_2 = Seeds socked with water for 12 hours; T_3 = Seeds socked with 2% mannitol solution for 12 hours; T_4 = Seeds socked with 4% mannitol solution for 12 hours; T_5 = Seeds socked with 6% mannitol solution for 12 hours and T_6 = Seeds socked with 8% mannitol solution for 12 hours.

4.2 Experiment 2:Optimization of pre-sowing priming time on the germination behavior of mungbean.

Results obtained from the present study regarding the effects of different priming time of mannitol and wateron the germination behavior of mungbean(BRRI mung-6) have been presented, discussed and compared in this chapter. The analytical results have been presented in Figures 11 to 20 and Appendices IIto XX.

4.2.1 Effect on total germination (%)

The total germination percentagewas significantly influenced by priming (water and mannitol) time (Appendix XI and Figure 11). Totalgermination percentage gradually decrease after 6 hours in both water and mannitol priming. Results revealed, highest total germination percentage (81.11 %) was recorded from M_6 treatment which was statistically similar with W_6(71.1 %) and M_9 (75.55 %)treatment, on the other hand the lowest total germination percentage (47.77 %) was observed from W_{18} treatment.The probable reason for enhancement of percentage and uniformity of germination of the hydroprimed andmannitol primed seed may be due to the completion of pre-germination process such as repair and synthesis of nucleic acids (DNA and mRNA), protein, repair of membranes (Bewley, 1997; McDonald, 2000; Jowkar*et al*., 2012) and induction of a range of biochemical changes enzymes activation (Wattanakulpakin*et al*., 2012).Yari*et al*. (2010a) observed thehighestgermination percentage in wheat seeds when treated with PEG during 12 hours of priming.

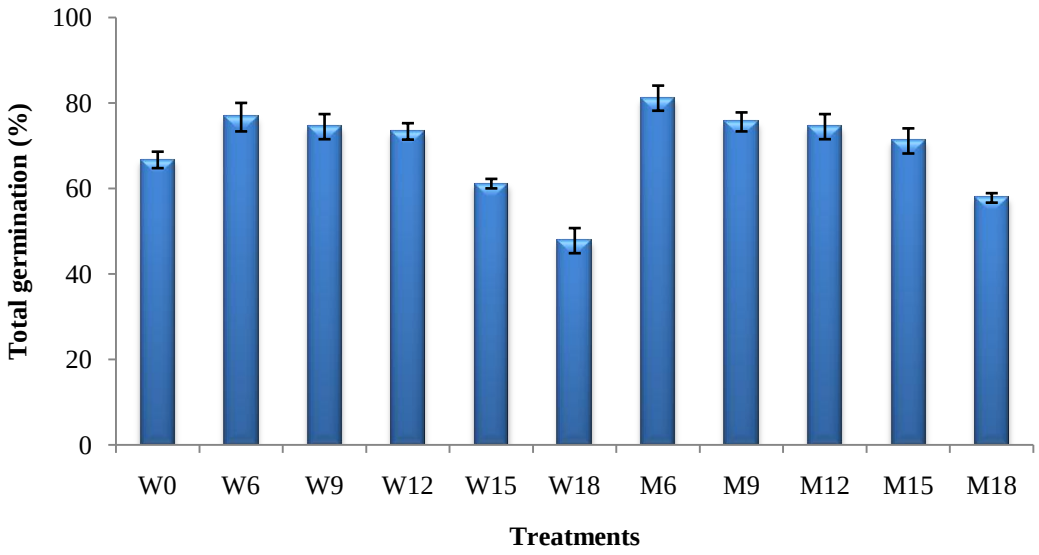

Figure 11. Effect of different priming time on total germination percentage of primed (mannitol and water) and non-primed (control) seeds. (SE= 1.925,3.333,2.939,1.922,1.11,2.939,2.939,2.223,2.939,2.939 and1.113)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours;W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours;M_9 = Seeds primed with 6% mannitol solution for 9 hours;M_{12} = Seeds primed with 6% mannitol solution for 12 hours;M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.2 Effect on mean germination time (days)

Significant variation of mean germination timewas found due to primingwith water andmannitol solution (Appendix XII and Figure 12). With increasing priming time mean germination time was increased in case of both water and mannitol primingwith W_6 and M_6 treatment. However, the minimum mean germination time(4.79 days) was recorded in M_6 treatment which was statistically similar with W_6, W_9, W_{12} and M_9 treatments. The maximum mean germination time (5.20 days) was recorded in W_{18}treatment which was statistically similar withW_6 (5.07 days) treatment. Afzal*et al*. (2004) also found that the osmopriming (jutemat) proved to be the best priming agent in reducing the time to 50%germination and mean germination time among all priming treatments. During emergence test, priming treatments i.e; osmopriming (jute mat) for 24 hours reduced the time to 50% emergence and mean emergence time.

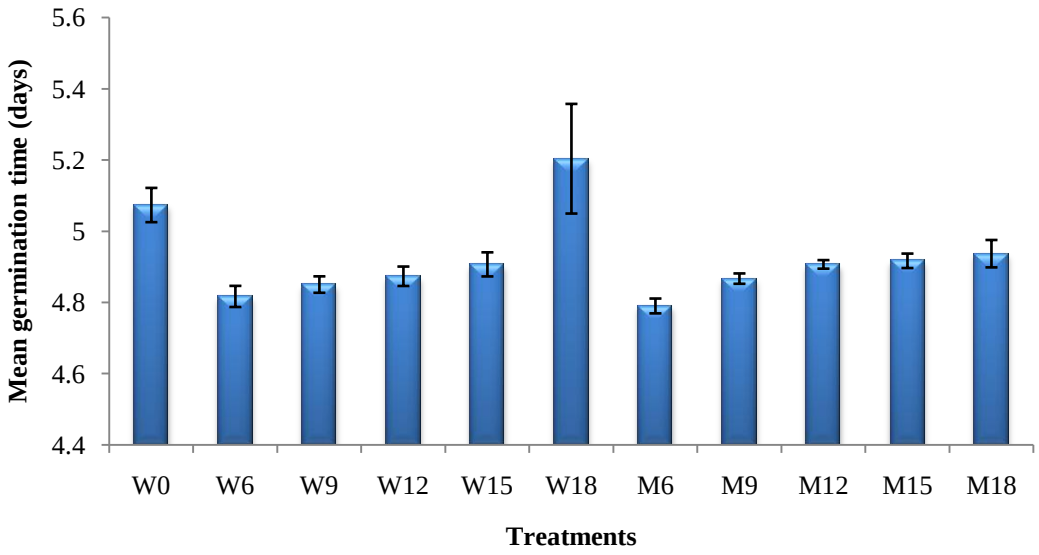

Figure 12. Effect of different priming time on mean germination time (days) of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.048,0.029,0.023,0.027,0.033,0.153,0.02,0.014,0.012,0.02 and0.038)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours;W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours;M_9 = Seeds primed with 6% mannitol solution for 9 hours;M_{12} = Seeds primed with 6% mannitol solution for 12 hours;M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.3 Effect on germination index

Different priming time with water and mannital solution exhibited significant variation in respect of germination index (Appendix XIII and Figure 13). Germination index decreased with increasingthe priming time after 6 hours in both water and mannitol priming.The highest germination index (51.08) was found in M_6 treatmentwhich was statistically similar with W_6, W_9, W_{12}, M_9, M_{12} and M_{15} treatments whereas, the lowestgermination index (28.94) was recorded in M_{18} treatmentwhich was statistically similar with W_0 (35.34) treatment.Increasing germination index in primed seeds as compared with non-primed seeds is due to time duration of water uptake in hydro and osmopriming. In other words, water uptake rate in priming period is slow and seeds had enough time to complete the pre-germination process (Varier*et al.*, 2010).

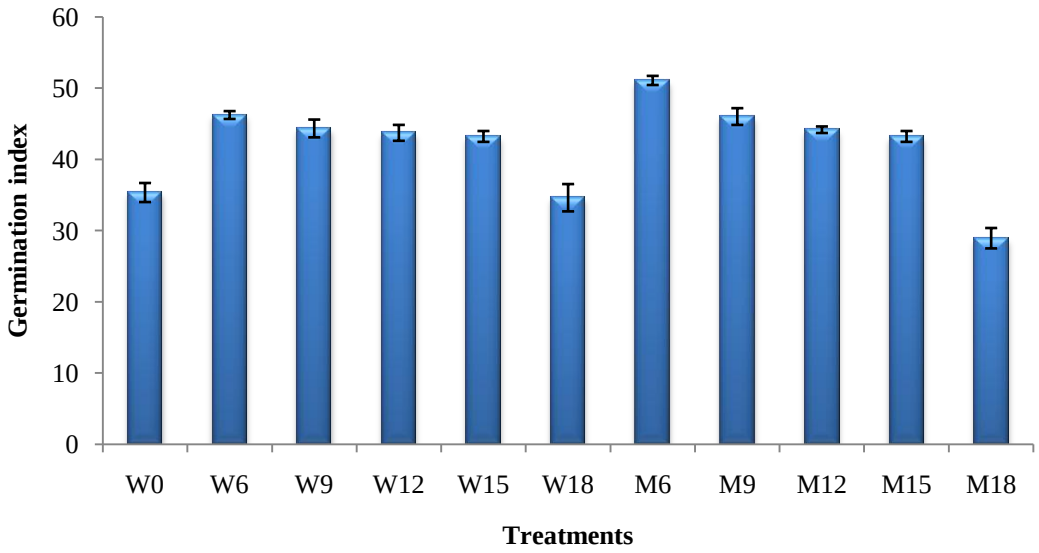

Figure 13. Effect of different priming time on germination index of primed (mannitol and water) and non-primed (control) seeds.(SE= 1.338,0.553,1.25,1.119,0.763,1.918,0.640,1.177,0.459,0.763 and1.4316)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours;W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours;M_9 = Seeds primed with 6% mannitol solution for 9 hours;M_{12} = Seeds primed with 6% mannitol solution for 12 hours;M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.4 Effect on coefficient of velocity

Coefficient of velocity was not significantly influenced by priming timewithwater andmannitol solution (Appendix XIV and Figure 14). Results revealed thatcoefficient of velocity was found highest (21.69) at M_6 treatment whichwas statistically similar with W_6(20.6), W_9(20.49), W_{12}(20.45),W_{15}(20.4),W_{18}(20.30, M_9(20.73), M_{12}(20.51), M_{15}(20.45) and M_{18}(20.38) treatments.On the other hand, the lowest (19.69) coefficient of velocity was found in W_0treatment.Bose and Mishra (1992) found that,improvement in germination and coefficient of velocity in treated fenugreek seeds occur due to increasement of cell division in the seeds.

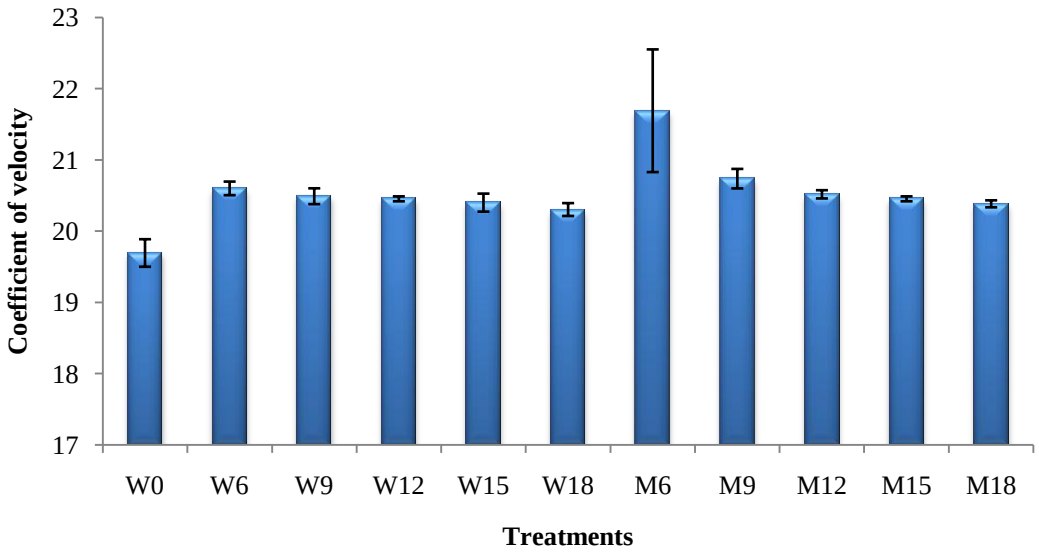

Figure 14. Effect of different priming time on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.193,0.095,0.111,0.034,0.126,0.09,0.86,0.136,0.057,0.034 and0.049)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours; W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours; M_6 = Seeds primed with 6% mannitol solution for 6 hours; M_9 = Seeds primed with 6% mannitol solution for 9 hours; M_{12} = Seeds primed with 6% mannitol solution for 12 hours; M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.5 Effect on energy of emergence (%)

The results regarding energy of emergence of mungbean in different priming time with water and mannitol concentrations (Appendix XV and Figure 15) showed that energy of emergence differed significantly with increasing priming time. After 6 hoursenergy of emergence increased with increasing priming time of both water and mannitolconcentrations. The highestenergy of emergence(93.05 %) was recorded in M_6 treatment whichwas statistically similar with W_6(91.66 %), W_9(88.60 %), W_{12}(87.22 %),M_9(90.77 %), and M_{12}(88.05 %) treatments. The lowestenergy of emergence (58.60 %)was recorded in W_{18} treatment. Priming up to a limit can have positive effect, while extended priming duration will negatively affect germination (Bradford, 1986). Hydrolysis and leaching of certain chemicals from the seed to the aqueous solution, due to priming beyond certain limit, may act as germination inhibitors (Hopkins, 1995). Farooq*et al*. (2006a) reported delayed and poor germination

andenergy of emergence due to over priming in rice. Similarly, Harris *et al*. (2001) also observed damage to seeds of chickpea due to over-soaking.

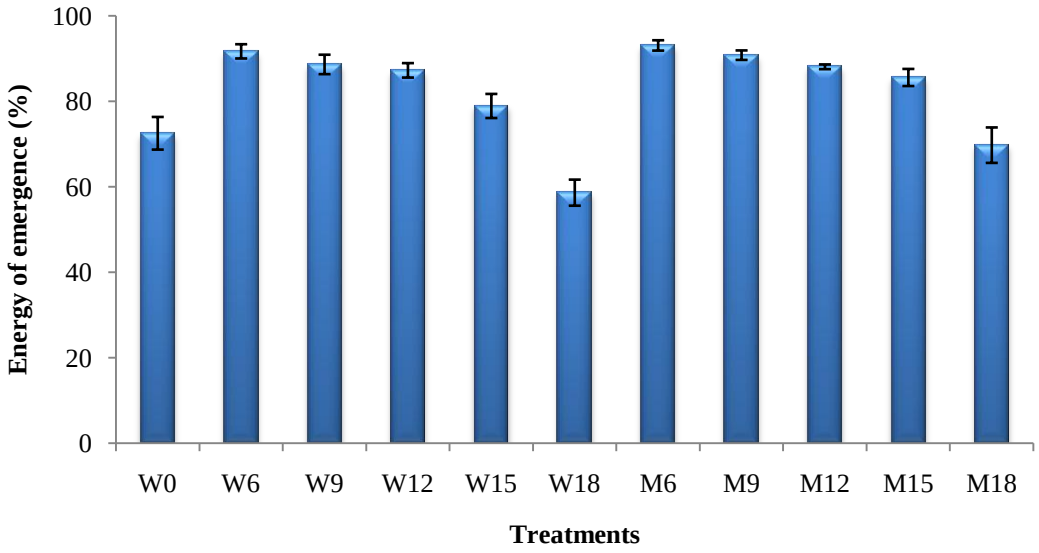

Figure 15. Effect of different priming time on energy of emergence (%) of primed (mannitol and water) and non-primed (control) seeds.(SE= 3.818,1.666,2.274,1.691,2.819,3.053,1.211,1.113,0.553,2.003 and 4.149)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours; W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours; M_9 = Seeds primed with 6% mannitol solution for 9 hours; M_{12} = Seeds primed with 6% mannitol solution for 12 hours; M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.6 Effect on shoot length (mm)

Different priming time of water andmannital solution exhibited significant variation in respect of shoot length (Appendix XVI and Figure 16). Shootlength significantlydecreased with the increasing priming time after 6 hours in both water and mannitol priming. The maximumshootlength (76.13 mm) was found in W_6treatment whichwas statistically similar with M_6(72.60 mm) treatment. The minimum shoot length (40.93 mm) was found in W_{18} treatment whichwas statistically similar with M_{18} (42.13 mm) treatment.Zareh*et al*. (2006) indicated that priming of wheat seed with GA_3germination decreased but has a positive effect on shoot growth. WunGuang*et al*. (2009) reported that when pelleted seeds of tobacco were germinated under 25°C, the priming treatment of 100 mg/L GA_3 under 25°C and 20°C for 36 hours were better than other treatments.

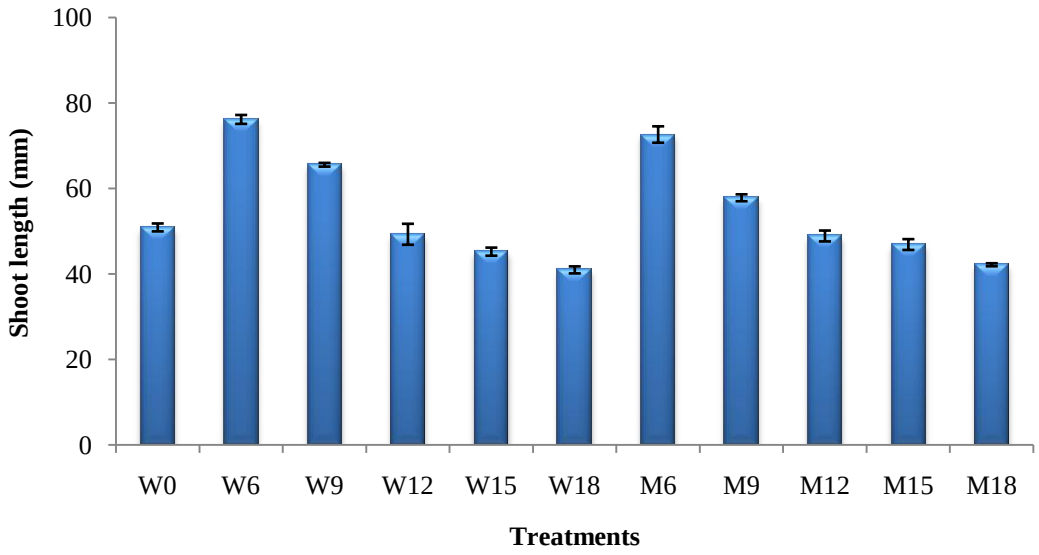

Figure 16. Effect of different priming time on shoot length (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.926,1.041,0.437,2.45,0.945,0.819,1.921,0.808,1.271,1.266 and0.352)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours; W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours; M_6 = Seeds primed with 6% mannitol solution for 6 hours; M_9 = Seeds primed with 6% mannitol solution for 9 hours; M_{12} = Seeds primed with 6% mannitol solution for 12 hours; M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.7 Effect on root length (mm)

Statistical analysis showed significant variation on root length with different priming time of water andmannitol solution (Appendix XVII and Figure 17).Seeds treated with W_6 and M_6 treatment,root length decrease significantly with increasing priming time.Results revealed that,maximum root length (58.67 mm) was obtained atM_6 treatment.On the other hand, the minimum root length (38.06 mm) was obtained at W_{18} treatmentwhich was statistically similar with W_{15}(40.8 mm)and M_{18} (40.06 mm) treatments. Radicle length in both cultivarsdecreased with increasing priming time. The results of the present study are in agreementwith observations of Yari*et al*. (2010b) who reported that maximum radicle length of cultivar Sardari was obtained at 20% PEG6000 solution primed for 24h.

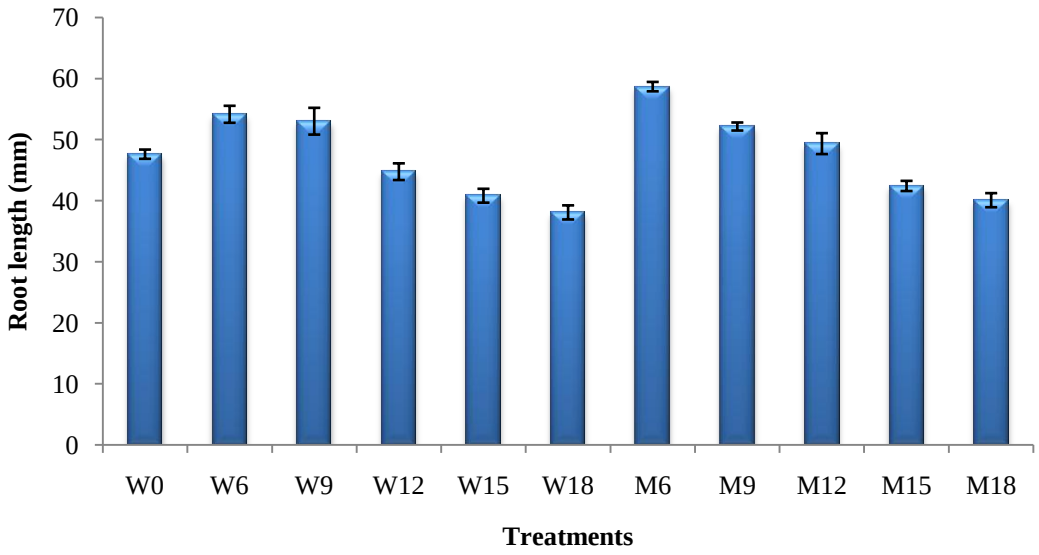

Figure 17. Effect of different priming time on root length (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE= 0.757,1.392,2.193,1.367,1.137,1.156,0.768,0.656,1.713, 0.832 and1.156)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours;W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours;M_9 = Seeds primed with 6% mannitol solution for 9 hours;M_{12} = Seeds primed with 6% mannitol solution for 12 hours;M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.8 Effect on seedling length (mm)

Significant variation was found in respect of seedling length with different priming time of water and mannitol solution (Appendix XVIII and Figure 18). Seedling length was significantly decreased after 6 hours of priming time in both water and mannitol priming. The highest seedling length (116.20 mm) was found in M_6 treatment which was statistically similar with W_6 (114.6 mm)and M_9 (111.26 mm) treatments. On the other hand, the lowest seedling length (81.73 mm) was found in M_{18} treatment.Elkoca*et al*. (2007), recommended that hydropriming for 12 h or osmopriming (PEG -0.5 MPa) for 24 h for a better germination of chickpeas under cold soil conditions. Compared to hydro priming, priming with PEG in a proper priming time was found to have a better effect on seed germination and seedling growth (Yuan-Yuan *et al*., 2010).

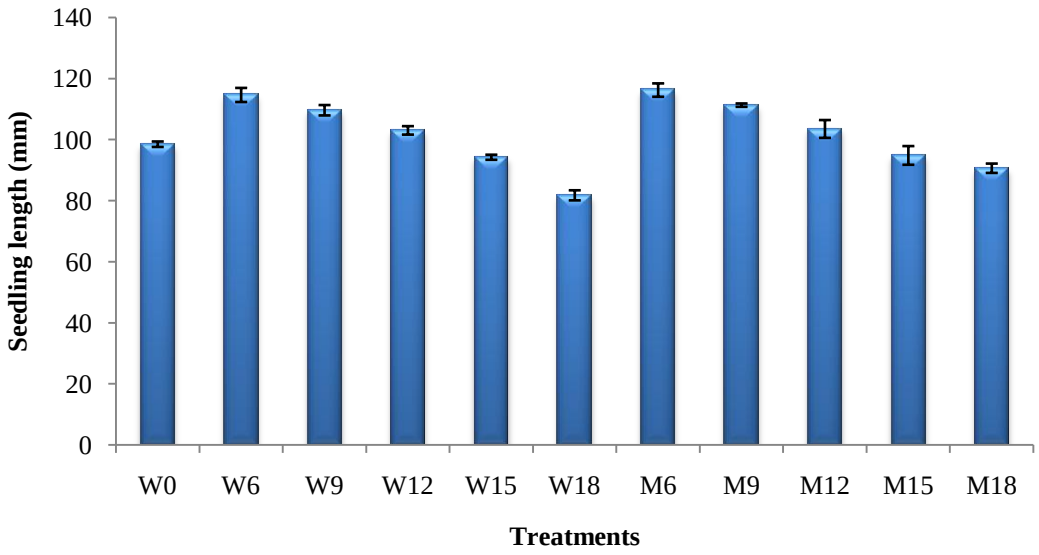

Figure 18. Effect of different priming time on seedling length (mm) of primed (mannitol and water) and non-primed (control) seeds of mungbean (BARI Mung-6) (SE= 0.896,2.306,1.7,1.385,0.832,1.65,2.193,0.545,2.924,3.061 and1.527)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours;W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours;M_6 = Seeds primed with 6% mannitol solution for 6 hours;M_9 = Seeds primed with 6% mannitol solution for 9 hours;M_{12} = Seeds primed with 6% mannitol solution for 12 hours;M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.9 Effect on seedling dry weight (mg)

Seedling dry weight was significantly influenced by different priming time with water andmannitol solution (Appendix XIX and Figure 19). Significant decreased was occurred in respect of seedling dry weight at M_6 and W_6 treatments with increasing priming time.Results revealed that M_6 treatment showed the maximum seedling dry weight(120.93 mg).The lowest seedling dry weight (77.20 mg) was recorded in W_{18} treatment which was statistically similar with M_{18}(77.86 mg) treatment. This result is inverse with MoradiDezfuli*et al*. (2008) who indicated that PEG6000 soakedseeds did not act well from germination point of view, possibly due to low osmotic potential of the solution or long priming duration. Sharifzadeh*et al*. (2006) also found that osmopriming of wheat had no positive significant effect on germination characteristics.

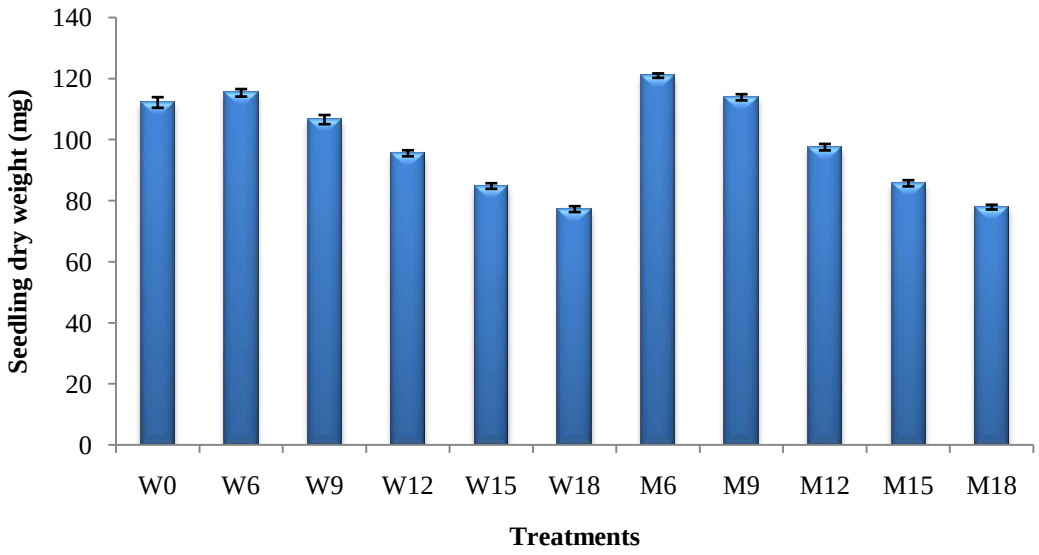

Figure 19. Effect of different priming time on seedling dry weight (mg) of primed (mannitol and water) and non-primed (control) seeds.(SE= 1.738,1.228,1.505,0.986,0.926,0.96,0.731,1.008, 1.058,1.017 and 0.775)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours; W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours; M_6 = Seeds primed with 6% mannitol solution for 6 hours; M_9 = Seeds primed with 6% mannitol solution for 9 hours; M_{12} = Seeds primed with 6% mannitol solution for 12 hours; M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.2.10Effect on vigour index

The results regarding vigour index of mungbean in different priming timewith water and mannitol concentrations showed significant variation (Appendix XX and Figure 20).Vigour indexdecreased with increasing priming time of water and mannitolafter 6 hours of priming. The minimum vigour index (81.68) was obtained in M_6 treatment which was statistically similar with W_6 (79.12), W_9(76.12), W_{12} (75.11), M_9(78.19), M_{12}(75.49) andM_{15} (73.92 mg) treatments. On the other hand, the lowest vigour index (39.09) was recorded in W_{18} treatment. The improvement in germination and vigour index of normal/low-vigour seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes DNA and RNA synthesis start during osmotic priming. Rapid embryo growth resulted when the obstacle to germination was removed (Basra *et at.,* 2003).

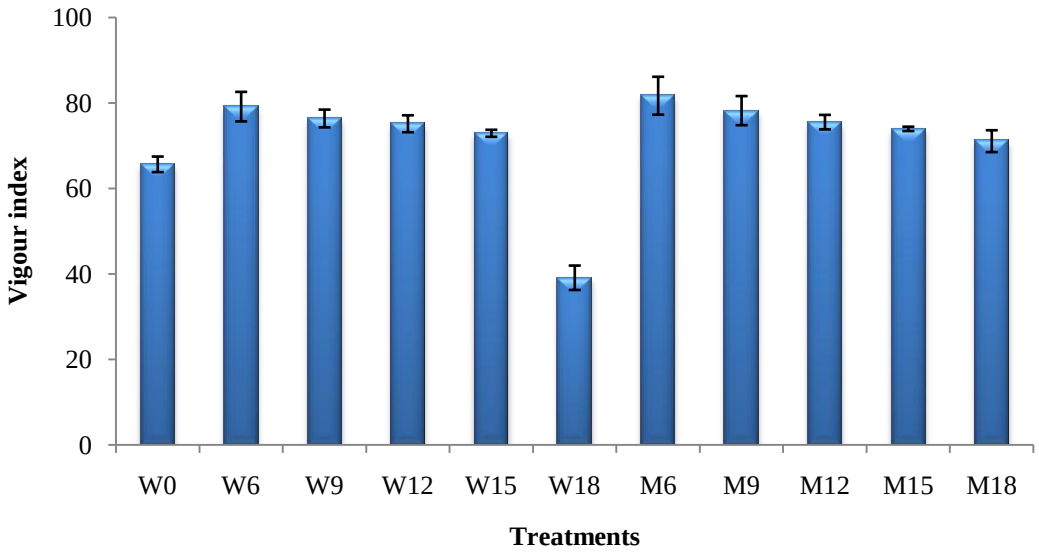

Figure 20. Effect of different priming time on vigour index of primed (mannitol and water) and non-primed (control) seeds.(SE= 1.821,3.454,2.079,1.984,0.819,2.856,4.432,3.402,1.699,0.509 and 2.556)

W_0 = Dry seeds;W_6 = Seeds primed with water for 6 hours; W_9 = Seeds primed with water for 9 hours;W_{12} = Seeds primed with water for 12 hours; W_{15} = Seeds primed with water for 15 hours; W_{18} = Seeds primed with water for 18 hours; M_6 = Seeds primed with 6% mannitol solution for 6 hours; M_9 = Seeds primed with 6% mannitol solution for 9 hours; M_{12} = Seeds primed with 6% mannitol solution for 12 hours; M_{15} = Seeds primed with 6% mannitol solution for 15 hours and M_{18} = Seeds primed with 6% mannitol solution for 18 hours.

4.3 Experiment 3:Germination behavior of primed Seed (mungbean) under salt (NaCl) stress condition of mungbean.

According to the tesults, all 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, mean germination time, germination index, coefficient of velocity, energy of emergence) and growth parameters (shoot length, root length, seedling length, dry weight and vigour Index) of mungbean, as compared with non-primed seeds, under saline condition. The increase in salt stress in culture medium causes a significant decrease in germination percentage (Figure 21), mean germination time (Figure 22), germination index (Figure23), coefficient of velocity (Figure24), energy of emergence (Figure25), shoot length (Figure26), root length (Figure27),seedling length (Figure28), dry weight (Figure29) and vigor index (Figure 30), as well

as for non primed seed. However, the decrease was more significant for non-primed seeds than Mannitol and water seed priming.

4.3.1 Effect on total germination (%)

Different salinity levels exhibited significant variation in respect of total germination percentage (Appendix XXI and Figure 21). Result reveled that total germination from both primed and non-primed seeds decreased significantly with increasing salinity level. Buttotal germination of mannitol and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result. Mannitol priming seed had the maximum total germination(69.99 %) followed closely by water priming (63.33 %) and control seeds had(57.11 %) total germination percentage observed from T_1 treatment.On the other hand minimum total germination percentage (26.66 %)was found in control treatment from T_5 treatment. The results obtained from all other treatments showed intermediate results compared to the highest and the lowest values. Edalat-Pisheh*et al*. (2010) declared that total germination percentage in wheat seeds decreased when salinity of both primed and unprimed (control group) treatments increased; but total germination percentage in primed seeds was higher than unprimed seeds in all salinity conditions because water absorption increased in primed seeds and metabolic activities was formed too soon during germination of primed seeds; and consequently, radicle and plumule appeared sooner (AschermanKock*et al*., 1992). Kaya *et al.* 2006 and Khajeh-Hosseini*et al*. 2003also find that reduction in total germination was significantly lower for non- primed seeds, compared to primed seeds and this may be due to the toxic effects of Na^+ and Cl^- in the process of germination.

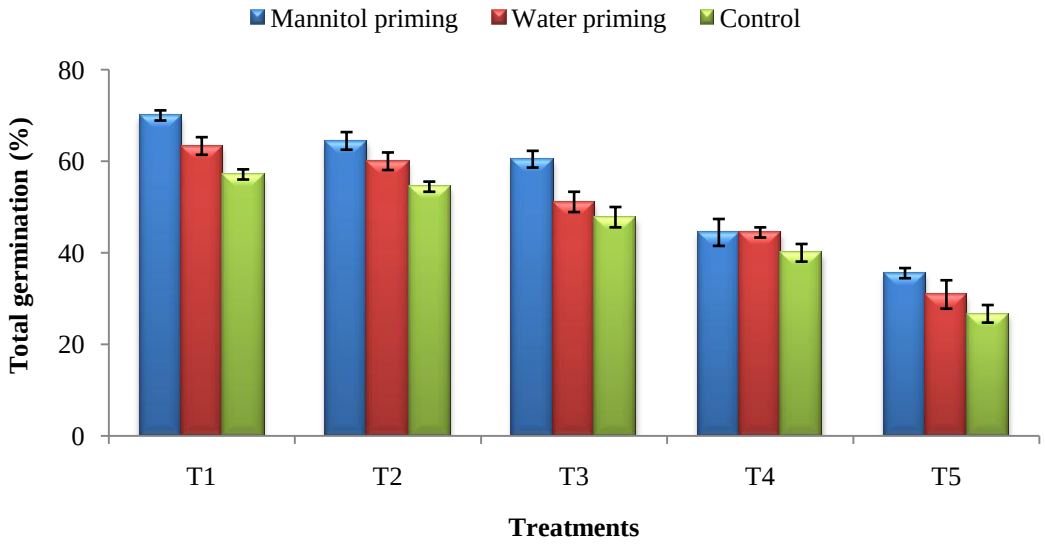

Figure 21. Effect of different salinity levels on total germination percentage of primed (mannitol and water) and non-primed (control) seeds.
(SE=1.11,1.925,1.11,1.925,1.922,1.11,1.828,2.223,2.223,2.939,1.11,1.925,1.11 ,3.109 and 1.925)

T1=Primed (mannitol and water) and non-primed (control) seeds placed withoutsalt: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt:T4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt:T5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.2 Effect on mean germination time (days)

Mean germination time of mungbean significantly delayed with the increasing different salinity levels (Appendix XXII and Figure 22).Results showed that mean germination time increased for both primed (mannitol and water) and non-primed seed due to increasing salinity levels.Butmean germination time of mannitol and water primed seeds was lower compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result. However, mannitolprimed seeds have lower MGT (4.86 days) which is statistically similar with water primed seeds (4.95 days) was recorded from the 0 mM salt treatment. The highest mean germination time(5.58 days) in control treatment was recorded from the 200 mMsalt treatment.Mannitol and water primed mungbean seeds germinated earlier than unprimed ones as it has been reported by Ashraf andRauf (2001)

working with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA.

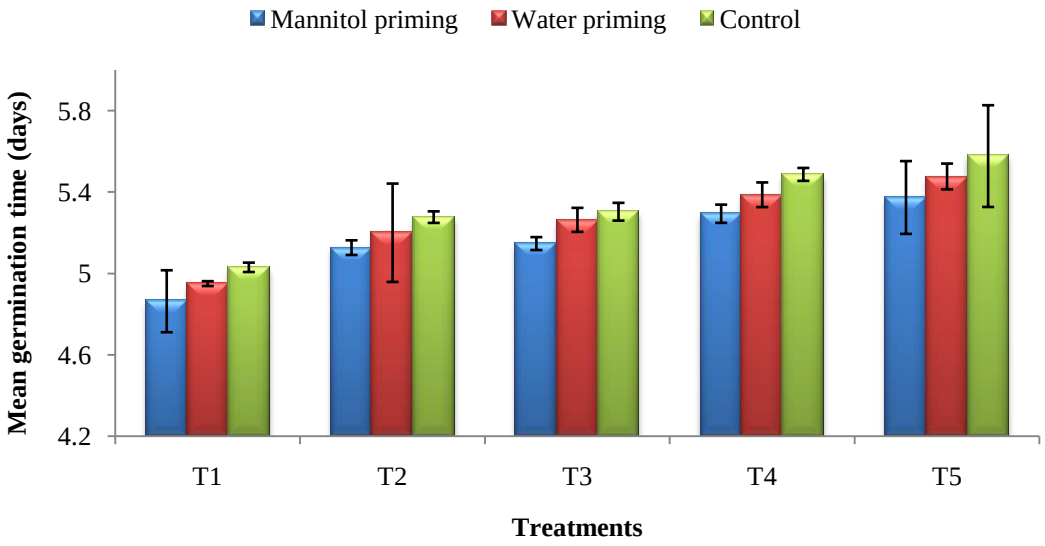

Figure 22. Effect of different salinity levelsmean germination time (days) of primed (mannitol and water) and non-primed (control) seeds.(SE=0.152,0.012,0.023,0.036,0.241,0.028,0.031,0.058,0.043,0.044,0.060 , 0.031,0.178,0.063 and 0.250)

T_1=Primed (mannitol and water) and non-primed (control) seeds placed without salt: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt:T_3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt:T_4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt:T_5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.3 Effect on germination index

The results regarding germination index of mungbean in different salinity level (Appendix XXIII and Figure 23)showed that germination index differed significantly with increasing salinity levels.Result revealed that germination index from both primed and non-primed seeds decreased significantly with increasing salinity level. Butgermination index of mannitol and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result.Highest germination index was recorded (36.36)from mannitol priming seed in T_1treatment compare to (31.41)and (28.09) water priming and control, respectively. Minimum germination index (12.02)was found in control at T_5treatment. Intermediate result was obtained from T_2, T_3 and T_4. The results

under the present study was in agreement with the findings ofRuan*et al*. (2002b) demonstrated that priming the rice seed with KCl and $CaCl_2$ had improved results for germination index.

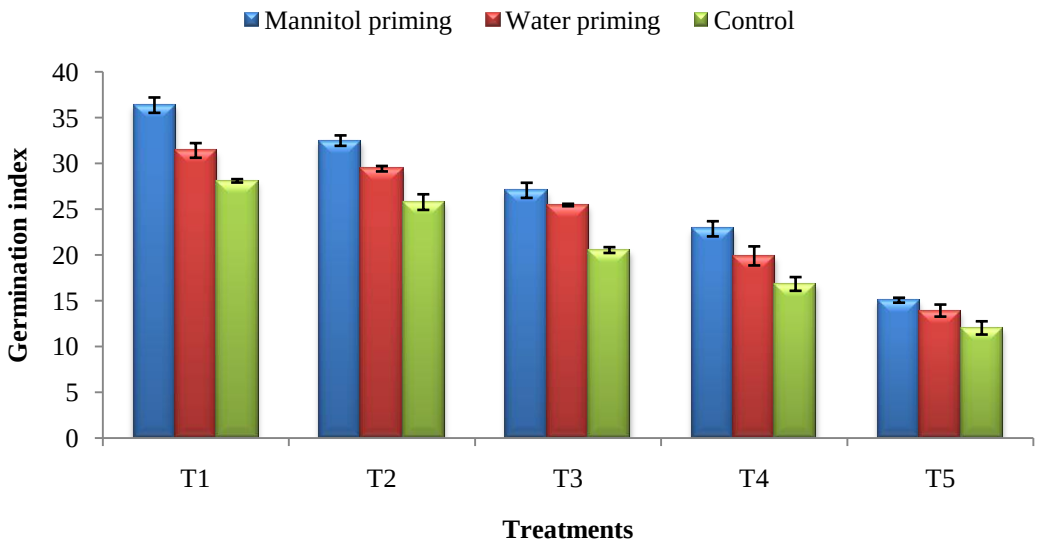

Figure 23. Effect of different salinity levels on germination index of primed (mannitol and water) and non-primed (control) seeds.(SE=0.840,0.800,0.184,0.571,0.298,0.855,0.825,0.133,0.317,0.830,1.041 ,0.750,0.271,0.658 and 0.724)

T1=Primed(mannitol and water) and non-primed (control) seeds placed without salt:T2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt:T3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt;T5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.4 Effect on coefficient of velocity

Coefficient of velocity of mungbean showed significant variation among the treatments (Appendix XXIV and Figure 24). Increasing salinity 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 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result. Coefficient of velocity was found the highest (19.85) with mannitol priming followed by water primed seeds (19.65) in T_1 treatment which was statistically similar with (19.54) and

(19.04) formannitol and water primed seeds, respectively in T_2treatment.On the other hand, lowest coefficient of velocity(17.57) was foundin case of control sees at T_5 treatment. The maximum coefficient of velocity of germination were found in the low salinity treatment and decreased with increasing salinity. Similar results were reported by Okcu*et al*. (2005).

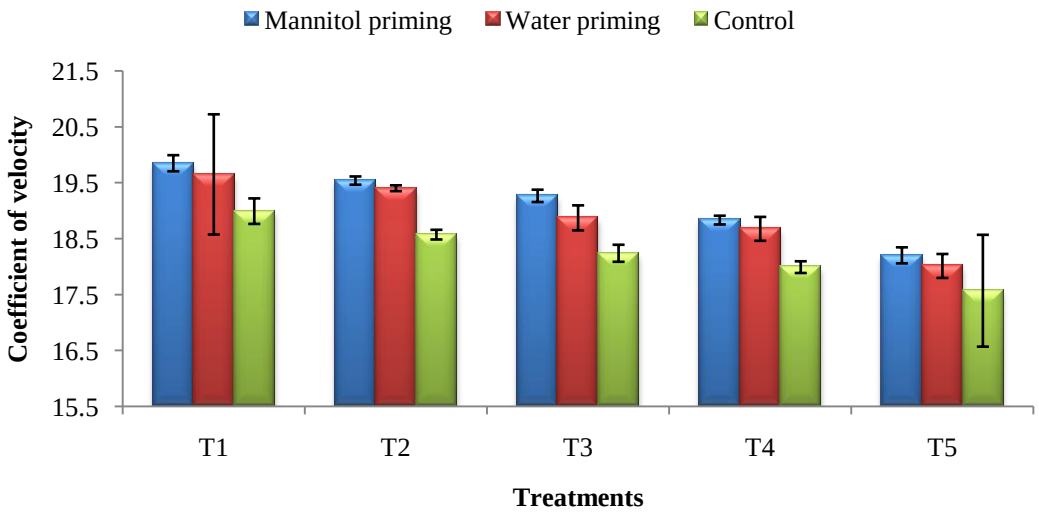

Figure 24. Effect ofdifferent salinity levels on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds.(SE=0.144,1.071,0.228, 0.073, 0.052, 0.086, ,0.110,0.223,0.152,0.078,0.213,0.105,0.143,0.214 and1.000)

T1=Primed (mannitol and water) and non-primed (control) seeds placed without salt: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt; T4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt; T5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.5 Effect on energy of emergence (%)

Energy of emergence wassignificantly influenced by the different salinity levels (Appendix XXV and Figure 25).Increasing salinity level significantly decreases values of energy of emergence. However, this decrease was more pronounced for nonprimed seeds than for primed seeds. Energy of emergence of mannitol and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas

mannitolprimed seed gave the best result.In this study, the maximum energy of emergence (72.50 %)was achievedin mannitolpriming seedfromT_1treatment, compare to (67.22)and (57.22) in water primingand control, respectively.The minimum energy of emergence (26.38 %) incontrolwas achieved from T_5 treatment. That is,energy of emergence decreased in mannitol and water priming solution with increasing salt concentration the decreasing rate is lower in comparison to control seed.The results obtained from T_2, T_3 and T_4 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).

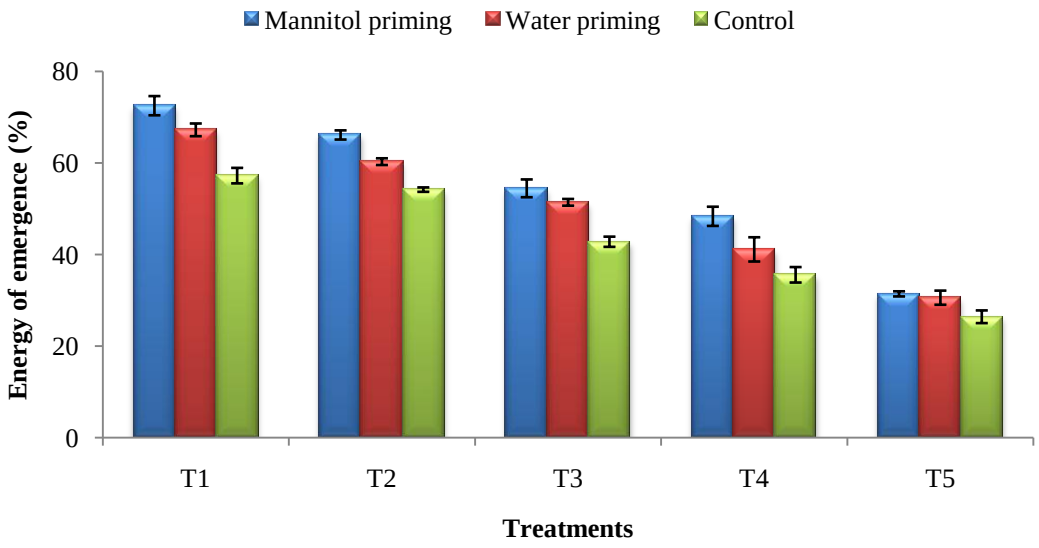

Figure 25. Effect of different salinity levels on energy of emergence (%) of primed (mannitol and water) and non-primed (control) seeds.(SE=2.097,1.39,1.691,1.003,0.734,0.482,1.945,0.734,1.113,2.098,2.649, 1.688,0.556,1.545 and 1.386)

T1=Primed (mannitol and water) and non-primed (control) seeds placed without salt: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.6 Effect on shoot length (mm)

Salinity had a significant inhibitory effect on shoot length (Appendix XXVI and Figure 26) for both primed and non-primed seeds. However, this effect was significantly less pronounced in seedlings priming with mannitol and water in comparison with control seeds. The highest shoot length (122.22 mm) was observed with mannitol primingat T_1 treatment compare to (107.66 mm) and (90.55 mm)with water priming from control seeds, respectively. The lowest shoot length (4.55 mm) from control seeds was found at T_5 treatment. Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita 2000). Similarly, toxic ion accumulation (Na+ and Cl^-) negatively affect plant metabolism (Grieve and Fujiyama 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim etal. 2008), which could adversely affect seedlings growth.

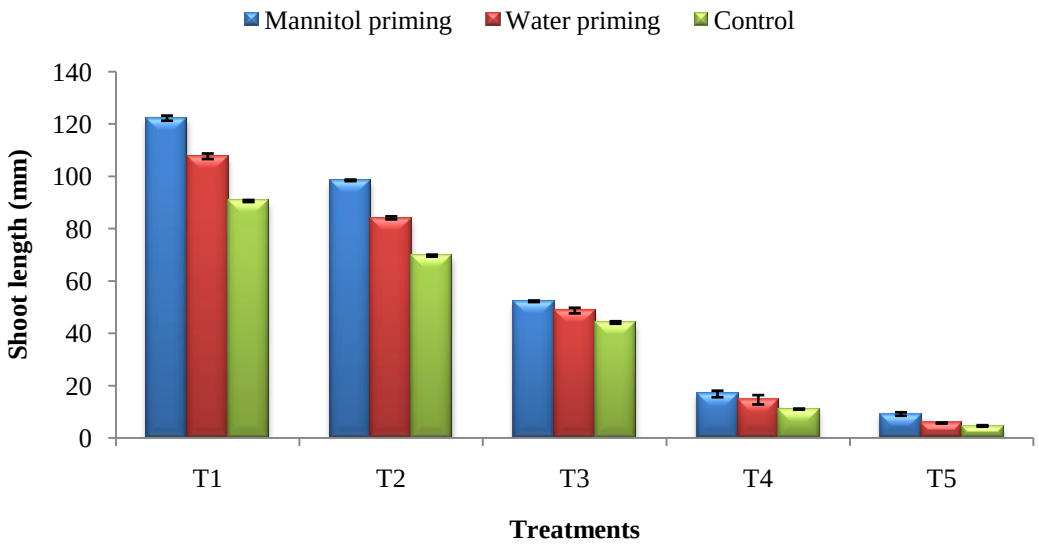

Figure 26. Effect of different salinity levels on shoot length (mm)of primed (mannitol and water) and non-primed (control) seeds.(SE=0.986,1.059,0.399,0.294,0.577,0.383,0.294,1.069,0.485,1.254,1.788 ,0.193,0.674,0.193 and 0.293)

T_1=Primed (mannitol and water) and non-primed (control) seeds placed without salt:T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.7 Effect on root length (mm)

Root length of mungbean significantly influenced by the different salinity levels (Appendix XXVII and Figure 27). Root length increased withmannitol and water priming seedsin comparison to control seeds with increasing salinity levels.The highest root length (72.78 mm) was found withmannitol priming compare to (64.55 mm) and (45.44 mm) with water priming and control seeds, respectivelyat 0 mMsalinity level (T_1) treatment.The lowest root length (4.99 mm) was found in control seedsat 200 mM salinity level (T_5) 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.

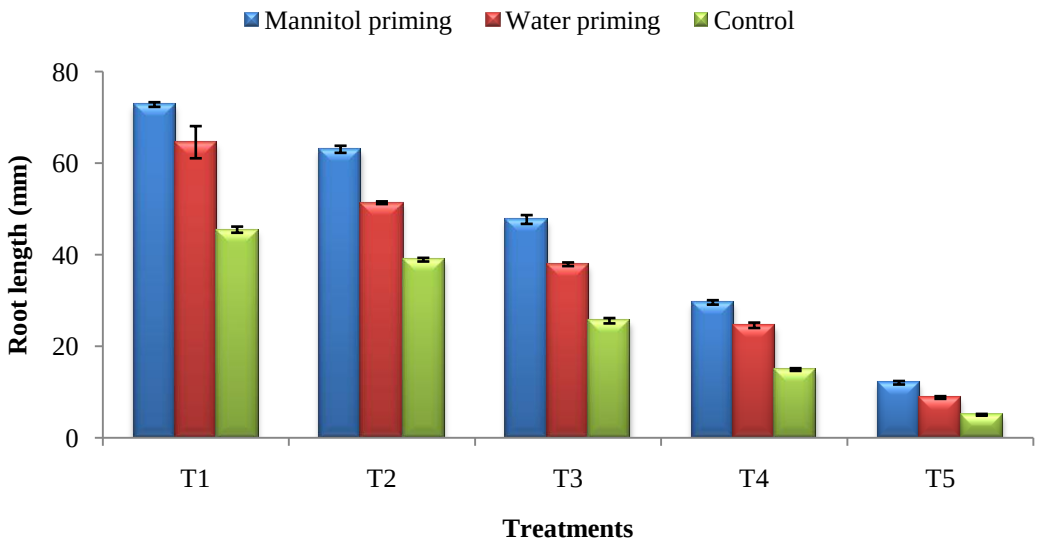

Figure 27. Effect of different salinity levels on root length (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE=0.507,3.511,0.674,0.776,0.293,0.399,0.399,0.587,0.485,0.587,0.294 ,0.383,0.294 and 0.193)

T_1=Primed (mannitol and water) and non-primed (control) seeds placed without salt:T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mMsalt: T_3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.8 Effect on seedling length (mm)

Significant inhibitory effect was found in seedling length of mungbean with increasing salinity levels (Appendix XXVIII and Figure 28)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. Higher the salinity level lesser the seedling growth.Thehigher seedling length was obtained (194.99 mm) in mannitol priming compare to (172.21 mm) in water priming and (127.99 mm) in control seeds at 0 mM salt (T_1) treatment.The minimum seedling length (9.55 mm) was found in case of control seeds at 200 mM salt (T_5) treatment. Result indicated that seed priming significantly improved mungbean seedling growth at different salinity level compare to control seed. Similar result was found by Katembe*et al*. (1998) who investigated the effect of seed priming as a method to improve seedling growth of two *Atriplex*species under salt stress.

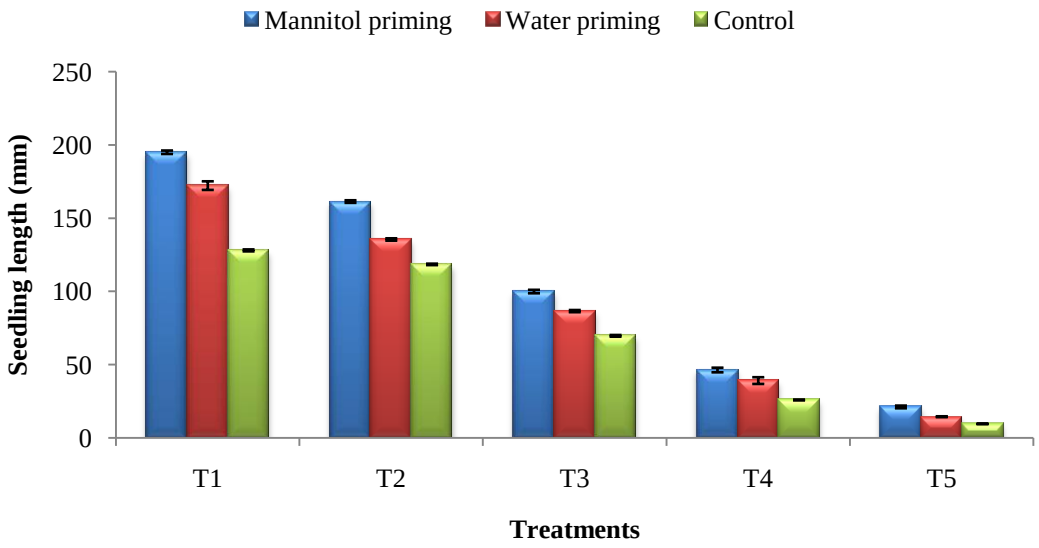

Figure 28. Effect of different salinity levels on seedling length (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE=1.168,2.947,0.666,0.881,0.799,0.482,1.175,0.727,0.577,1.577,2.296 ,0.293,0.909,0.284 and 0.225)

T_1=Primed (mannitol and water) and non-primed (control) seeds placed without salt: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.9 Effect on seedling dry weight (mg)

Increasing salinity significantly decreased mungbean seedlings dry weight for both primed and un-primed seed (Appendix XXIX and Figure 29).Result reveled that seedlings dry weight from both primed and non-primed seeds decreased significantly with increasing salinity level. Butseedlings dry weight of mannitol and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result. Figure 29 shows that the highest dry weight (123.67 mg) of seedlings was recorded from mannitol primingat 0 mM salt(T_1) treatment. On the other hand lowest seedlings dry weight (37.43 mg) was obtained from control seed at 200 mM salt (T_5) treatment. Seedling dry weight decreased linearly with increasing salinity. The similar results were also obtained by other researchers (Mansour *etal*. 2005). Increased dry weight in primed seeds over the non-primed seeds were also observed by Sivritepe*et al*.

(2003) who reported an increase in seedling dry weight in NaCl primed melons seeds under saline conditions as compared to the non-primed seeds.

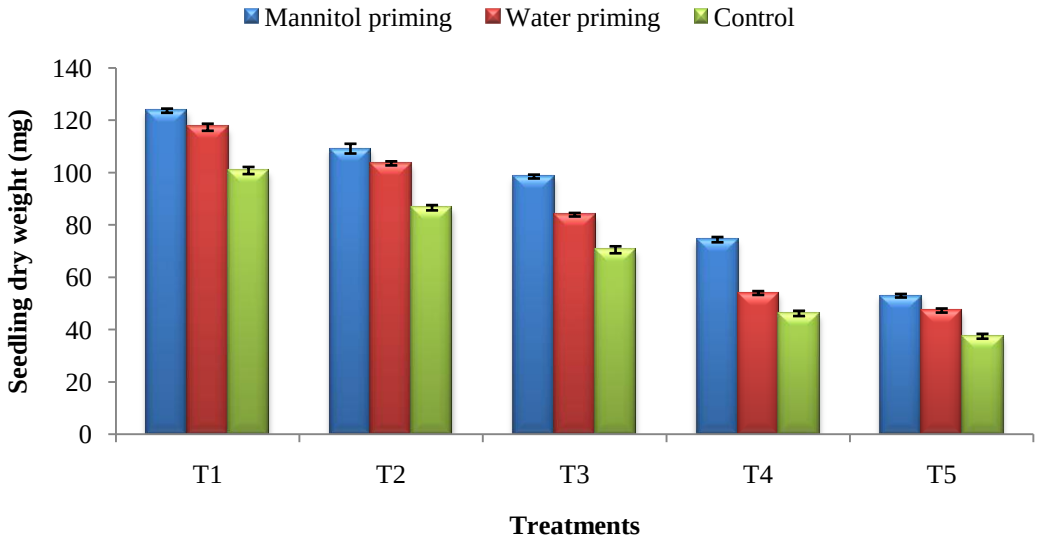

Figure 29. Effect of different salinity levels on seedling dry weight (mm) of primed (mannitol and water) and non-primed (control) seeds.(SE=0.796,1.356,1.357,1.862,0.775,1.013,0.730,0.680,1.331,1.008,0.726 ,1.003,0.674, 0.779 and 0.926)

T_1=Primed (mannitol and water) and non-primed (control) seeds placed withoutsalt: T_2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

4.3.10 Effect on vigour index

The results regarding vigour index of mungbean are given in (Appendix XXX and Figure 30), which showed that vigour index differed significantly with increasing salinity 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 mM salt concentration and various levels of salt stress whereas mannitol primed seed gave the best result. Vigour index was found the highest (121.49) in mannitol priming followed by water priming (112.06) and in control (72.16) at T_1 treatment. The least vigour index (2.55)was found in control seeds at T_5 treatment. Seed priming improve mungbeanvigour index

under saline conditions. Similar results were also found by Ruan*et al*. (2002b) who reported that primed rice seeds showed higher vigour index than non-primed ones.

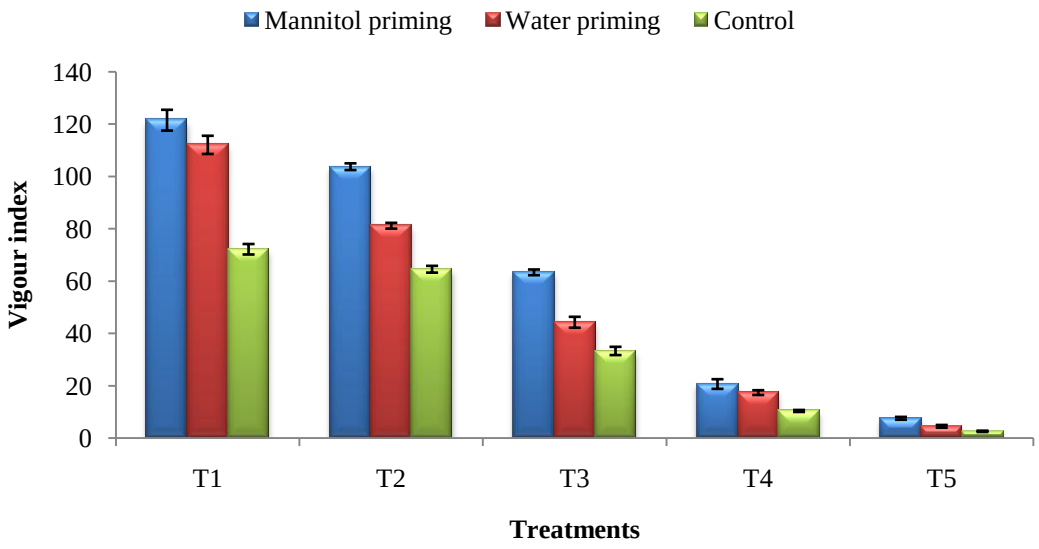

Figure 30. Effect of different salinity levels on vigour indexof primed (mannitol and water) and non-primed (control) seeds.(SE= 3.983,3.482,2.003,1.291,1.106,1.295,1.093,2.090,1.576,1.850,0.915,0.381, 0.544,0.526 and 0.242)

T1=Primed (mannitol and water) and non-primed (control) seeds placed withoutsalt:T2=Primed (mannitol and water) and non-primed (control) seeds placed with 50 mMsalt:T3= Primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt:T4=Primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt: T5=Primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207during the period from August, 2013 to February, 2014 to study the mannitol induced seed priming on salt tolerance capability in mungbean varieties cv.BARI Mung-6 and BU-4 under salt stress condition.

The whole experiment was conducted in three differentexperiments.The experiment was laid out in a Completely Randomized Design (CRD) with five replications.Different priming chemicals such as Mannitol($C_6H_{14}O_6$), salt (NaCl) and distilled water were utilized for osmo and hydro priming. Sodium hypochlorite used as seed treating chemical.Priming was done in room temperature and all the primed seeds were removed from the priming solution at the same time.Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter Petri dishes on whatman No.1filter 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 7 days. The abnormal or dead seedlings with short, thick and spiral formed hypocotyls and stunted primary root were excluded during counting.The dataon germination parameters of mungbean like total germinationpercentage, mean germination time, germination index, coefficient of velocity, energy of emergence and growth parameters like plumule length, root length, seedling length, dry weight and vigour index. Data were analyzed using a computer software SPSS 20.The significance of difference among the treatments means was estimated by the Duncan’s Multiple Range Test (DMRT)at 5% level of probability.

The first experiment was carried out to find the effect of different concentration of mannitol on germination behavior ofmungbean varieties cv. BARI Mung-6 and BU-4 without any stress condition.Four levels of mannitol such as2%, 4%, 6%, and 8% were used forosmopriming and water used ashydropriming agent for12 hours, respectively.The priming treatments were seeds without priming (control)(T_1), seeds primed with distilled water for 12 hours(T_2), seeds primed with 2% mannitol solutionfor 12 hours (T_3), seeds primed with 4% mannitol solutionfor 12 hours (T_4),seeds primed with 6% mannitol solutionfor 12 hours (T_5) andseeds primed with 8% mannitol solutionfor 12 hours(T_6). For BARI Mung-6 and BU-4the maximum total germination percentage(89.99 %) and (85.21 %)was recorded inT_5 andT_3 treatment, mean germination time(6.61 days) and(5.41 days) in T_6andT_1 treatment, germination index (48.64) and (46.61)inT_5 andT_3 treatment, coefficient of velocity(21.04) and (20.21)inT_5 andT_3 treatment, energy of emergence (94.99 %) and (87.88 %) inT_5 andT_3 treatment, shoot length (121.6 mm) and (94.93 mm)inT_5 andT_3 treatment, root length(45.73 mm) and (53.66 mm)inT_5 andT_3 treatment, seedling length(174.73 mm) and (137.00 mm)inT_5 andT_3 treatment, seedling dry weight (124.09 mg) and (108.26 mg)inT_5 andT_3 treatment and vigor index (157.00) and(123.30)inT_5 andT_3 treatment,respectively. Variety BU-4 was not further used as it gives poor result than BARI Mung-6.

The second experiment was conducted to evaluate different pre-sowing priming time on the germination behavior of mungbean. Five different priming times such as 6, 9, 12, 15, and 18 hours for hydropriming andosmopriming were used, respectively in this experiment.Dry seeds (control) (W_0), seeds primed with water for 6 hours (W_6), seeds primed with water for 9 hours(W_9), seeds primed with water for 12 hours(W_{12}), seeds primed with water for 15 hours(W_{15}), seeds primed with water for 18 hours(W_{18}), seeds primed with 6% mannitol solution for 6 hours(M_6), seeds primed with 6% mannitol solution for 9 hours(M_9), seeds primed with 6% mannitol solution for 12 hours(M_{12}), seeds primed with 6% mannitol solution for 15 hours (M_{15}), and seeds primed with 6% mannitol solution for 18 hours (M_{18}) were used as treatment.The total

germination percentage (81.11 %), germination index (51.08), coefficient of velocity (21.69), energy of emergence (93.05 %), root length (58.67 mm), seedling length (116.20 mm), seedling dry weight(120.93 mg) and vigor index (81.68) were observedhighest from M_6treatment, respectively.The maximum mean germination time and shoot length(5.20 days) and (76.13 mm) was recorded in $W_{18}$and W_6 treatment,respectively.

In the third experiment germination behavior of primed seed (mungbean) under different salt (NaCl) stress condition was evaluated.Mannitol solution 6% and distilled water were used as priming solutions, 6 hours as priming time and salt stress levels 50 mM, 100 mM, 150 mM and 200 mM were used in this experiment. Primed (mannitol and water) and non-primed (control) seeds placed without salt (T_1), primed (mannitol and water) and non-primed (control) seeds placed with 50 mM salt (T_2), primed (mannitol and water) and non-primed (control) seeds placed with 100 mM salt (T_3),primed (mannitol and water) and non-primed (control) seeds placed with 150 mM salt (T_4) and primed (mannitol and water) and non-primed (control) seeds placed with 200 mM salt (T_5)were used as treatment.The maximum total germination percentage (69.99 %), germination index (36.36), coefficient of velocity (19.85), energy of emergence (72.50 %), shoot length (122.22 mm), root length (72.78 mm), seedling length (194.99 mm), seedling dry weight (123.67 mg) and vigour index (121.49)were found in mannitolprimid seeds in (T_1) treatment. The maximum mean germination time (5.58 days) was found in control seeds.The minimum total germination percentage (57.11 %), germination index (12.02), coefficient of velocity (17.57), energy of emergence (26.38 %), shoot length(4.55 mm), root length (4.99 mm), seedling length (9.55 mm), seedling dry (37.43 mg) weight and vigour index (2.55)were found in control seeds at (T_5) treatment and mannitolprimed seeds have lower MGT (4.86 days)at (T_1) treatment.

From the results of the study, it may be concluded that the performance of mannitolprimedmungbean cv. BARI Mung-6 was better in respect of germination andgrowth parameters. Priming with 6% mannitolconcentration and 6 hours priming timeincrease the germinationbehabiour of mungbean seeds.Reduction in germination parameters and seedling growth was more profound in control seeds than primed seeds under salt stress condition. Thus, the priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of salt stress. For this reason, further studies are needed to assess the efficacy of seed priming during the later stages of the culture.

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APPENDICES

Appendix I: Mean square values on different concentrations of mannitolfor total germination percentage of mungbean

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	440.121	5	88.024	3.565	.033
Within Groups	296.282	12	24.690		
Total	736.403	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1854.740	5	370.948	8.014	.002
Within Groups	555.422	12	46.285		
Total	2410.162	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.351	5	1.270	.870	.529
Within Groups	17.527	12	1.461		
Total	23.879	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.436	5	.087	3.435	.037
Within Groups	.304	12	.025		
Total	.740	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	108.767	5	21.753	5.703	.006
Within Groups	45.772	12	3.814		
Total	154.539	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1017.795	5	203.559	25.805	.000
Within Groups	94.661	12	7.888		
Total	1112.456	17			

Appendix IV: Mean square values on different concentrations of mannitol for coefficient of valocity of mungbean

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.023	5	.605	1.408	.289
Within Groups	5.152	12	.429		
Total	8.174	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.977	5	.395	9.296	.001
Within Groups	.510	12	.043		
Total	2.487	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	307.809	5	61.562	3.920	.024
Within Groups	188.463	12	15.705		
Total	496.272	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3865.151	5	773.030	21.243	.000
Within Groups	436.680	12	36.390		
Total	4301.830	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5080.507	5	1016.101	84.832	.000
Within Groups	143.733	12	11.978		
Total	5224.240	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1422.971	5	284.594	4.853	.012
Within Groups	703.707	12	58.642		
Total	2126.678	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	612.896	5	122.579	22.036	.000
Within Groups	66.753	12	5.563		
Total	679.649	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	408.224	5	81.645	3.287	.042
Within Groups	298.053	12	24.838		
Total	706.278	17			

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

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8129.092	5	1625.818	86.009	.000
Within Groups	226.833	12	18.903		
Total	8355.925	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2367.060	5	473.412	4.411	.016
Within Groups	1287.840	12	107.320		
Total	3654.900	17			

Appendix IX: Mean square values on different concentrations of mannitol for seedling dry weight of mungbean

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1187.884	5	237.577	78.022	.000
Within Groups	36.540	12	3.045		
Total	1224.424	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2126.978	5	425.396	70.521	.000
Within Groups	72.387	12	6.032		
Total	2199.365	17			

Appendix X: Mean square values on different concentrations of mannitol for vigour index of mungbean

BARI Mung-6	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8213.487	5	1642.697	31.657	.000
Within Groups	622.683	12	51.890		
Total	8836.170	17			
BU-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7563.309	5	1512.662	7.336	.002
Within Groups	2474.271	12	206.189		
Total	10037.580	17			

Appendix XI: Mean square values on different priming time for total germination percentage of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2924.934	10	292.493	15.512	.000
Within Groups	414.830	22	18.856		
Total	3339.764	32			

Appendix XII: Mean square values on different priming time for mean germination time of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.426	10	.043	4.883	.001
Within Groups	.192	22	.009		
Total	.617	32			

Appendix XIII: Mean square values on different priming time for germination index of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1031.411	10	103.141	25.455	.000
Within Groups	89.140	22	4.052		
Total	1120.551	32			

Appendix XIV: Mean square values on different priming time for coefficient of valocity of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.881	10	.688	1.281	.300
Within Groups	11.821	22	.537		
Total	18.701	32			

Appendix XV: Mean square values on different priming time for energy of emergence of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3743.586	10	374.359	20.513	.000
Within Groups	401.489	22	18.250		
Total	4145.075	32			

Appendix XVI: Mean square values on different priming time for shoot length of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4300.383	10	430.038	90.644	.000
Within Groups	104.373	22	4.744		
Total	4404.756	32			

Appendix XVII: Mean square values on different priming time for root length of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1208.456	10	120.846	26.452	.000
Within Groups	100.507	22	4.568		
Total	1308.962	32			

Appendix XVIII: Mean square values on different priming time for seedling length of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3514.623	10	351.462	32.441	.000
Within Groups	238.347	22	10.834		
Total	3752.970	32			

Appendix XIX: Mean square values on different priming time for seedling dry weight of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7537.392	10	753.739	199.386	.000
Within Groups	83.167	22	3.780		
Total	7620.559	32			

Appendix XX: Mean square values on different priming time for vigour index of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4054.939	10	405.494	20.297	.000
Within Groups	439.521	22	19.978		
Total	4494.460	32			

Appendix XXI: Mean square values for totalgermination percentage under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7477.983	14	534.142	47.501	.000
Within Groups	337.346	30	11.245		
Total	7815.329	44			

Appendix XXII: Mean square values for mean germination time under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.443	14	.103	2.628	.013
Within Groups	1.176	30	.039		
Total	2.619	44			

Appendix XXIII: Mean square values for germination index under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2239.651	14	159.975	119.422	.000
Within Groups	40.187	30	1.340		
Total	2279.839	44			

Appendix XXIV: Mean square values for coefficient of valocity under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.336	14	1.238	2.517	.017
Within Groups	14.757	30	.492		
Total	32.093	44			

Appendix XXV: Mean square values for energy of emergence under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8160.984	14	582.927	82.261	.000
Within Groups	212.589	30	7.086		
Total	8373.573	44			

Appendix XXVI: Mean square values for shoot length under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75428.532	14	5387.752	2783.700	.000
Within Groups	58.064	30	1.935		
Total	75486.596	44			

Appendix XXVII: Mean square values for root length under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20078.091	14	1434.149	440.907	.000
Within Groups	97.582	30	3.253		
Total	20175.673	44			

Appendix XXVIII: Mean square values for seedling length under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	169695.776	14	12121.127	2612.889	.000
Within Groups	139.169	30	4.639		
Total	169834.945	44			

Appendix XXIX: Mean square values for seedling dry weight under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35977.497	14	2569.821	771.271	.000
Within Groups	99.958	30	3.332		
Total	36077.455	44			

Appendix XXX: Mean square values for vigour index under salt stress condition of mungbean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81729.511	14	5837.822	587.706	.000
Within Groups	297.997	30	9.933		
Total	82027.507	44			

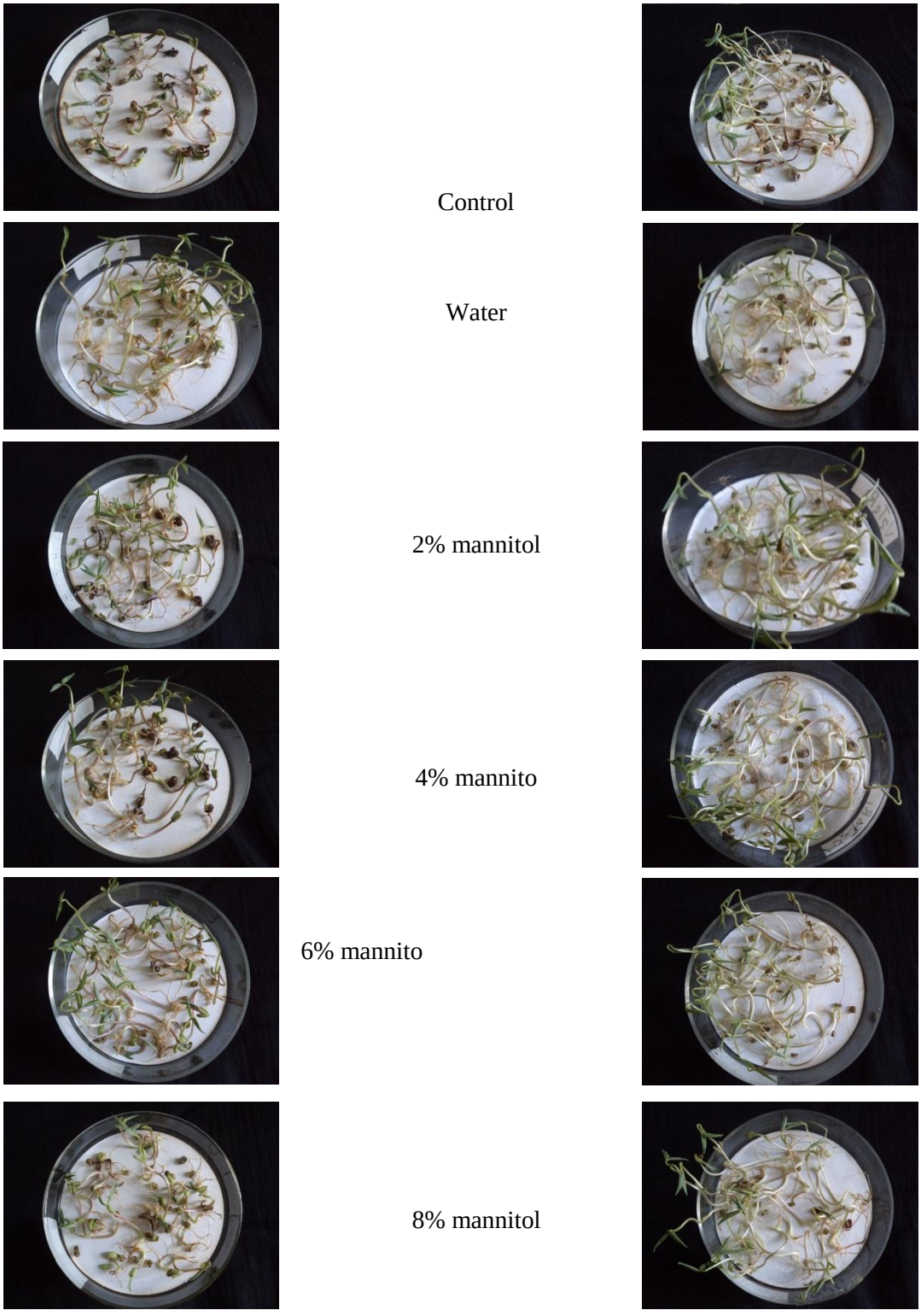


Plate 1: Effect of different concentration of priming solution on germination behabiour of mungbean varieties (BARI Mung-6 and BU-4)

Control

W_6

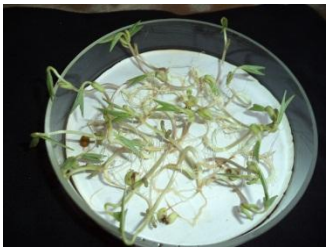
M_6

W_9

M_9

$W_{12}M_{12}$

$W_{15}M_{15}$



$W_{18}M_{18}$

Plate 2:Effect of different priming time (water and mannitol primed) on germination behabiour of mungbean.

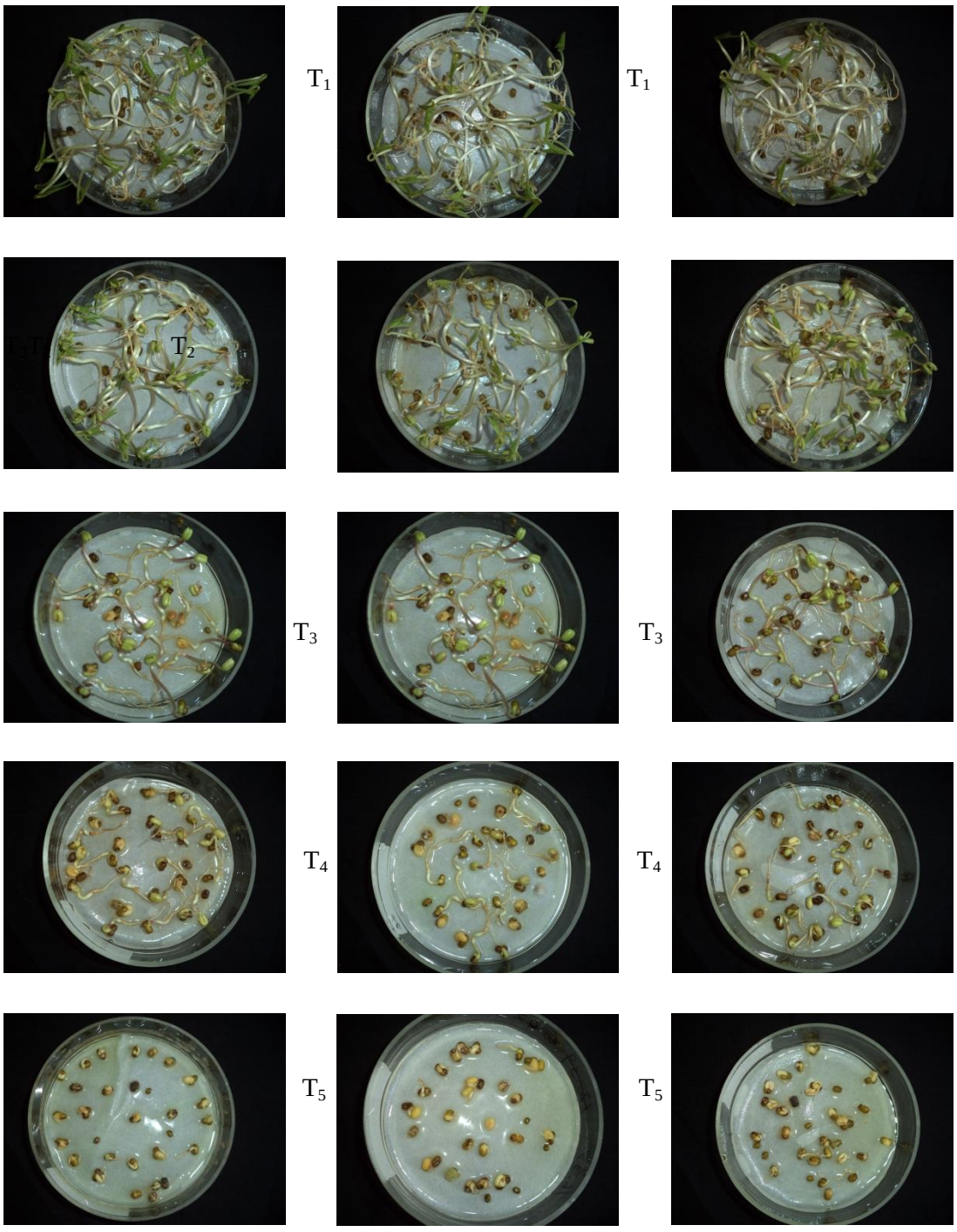


Plate 3: Effect of different salinity levels on (A) mannitol primed, (B) water primed and (C) control seeds of mungbean. Here T_1= Without salt stress, T_2= 50 mM salt, T_3= 100 mM salt, T_4= 150 mM salt and T_5= 200 mM salt stress.