INDUCTION OF DROUGHT TOLERANCE CAPACITY OF MUNGBEAN THROUGH MANNITOL AND HYDROPRIMING

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CERTIFICATE

This is to certify that the thesis entitled **"INDUCTION OF DROUGHT TOLERANCE CAPACITY OF MUNGBEAN THROUGH MANNITOL AND HYDROPRIMING"** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfilment of the requirements for the degree of* ***MASTER OF SCIENCE (M.S.)*** *in* ***AGRONOMY****, embodies the results of a piece of bona fide research work carried out by* **MD. MASUD IBNE ABDULLAH***, Registration no.* **12-04914** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated**:**

Place: Dhaka, Bangladesh

(Prof. Dr. Md. Abdullahil Baque)
Supervisor

Dedicated to

my

Beloved Parents and Wife

LIST OF ACCRONYMS AND ABBREVIATIONS

Agriculture	*Agric.*	Integrative	*Integr.*
Agricultural	*Agril.*	International	*Inter.*
Agronomy	*Agron.*	International Seed Testing Association	ISTA
Association of Official Seed Analysis	AOSA	Journal	*J.*
Bangladesh Agricultural Research Institute	BARI	Least significant difference	LSD
Bangladesh Bureau of Statistics	BBS	Milligram	mg
Botany	*Biot.*	Mean Germination Time	MGT
Biotechnology	*Biotechnol.*	Milliliter	mL
Degree centigrade	^{o}C	Millimeter	mm
Chemistry	*Chem.*	Master of Science	M.S
Chronicle	*Chron.*	Pakistan	*Pak.*
Communications	*Comm.*	Pathology	*Pathol.*
Centimeter	cm	Polyethylene Glycol	PEG
Completely Randomized Design	CRD	Physiology	*Physiol.*
Coefficient of variation	CV	Progressive	*Prog.*
Edition	ed.	Research	*Res.*
Energy of emergence	EG	Reactive Oxygen Species	ROS
Environmental	*Environ.*	Relative water content	RWC
And others	*et al.*	Sher-e-Bangla Agricultural University	SAU
Gazette	*Gaz.*	Science	*Sci.*
Germination Index	GI	Technology	*Technol.*
Germination Percentage	GP	Vigour Index	VI
Horticulture	*Hort.*	Namely	viz.
Hour	hr	Water retention capacity	WRC
idest (L), that is Inst. Institute	i.e.	Water saturation deficit	WSD

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INDUCTION OF DROUGHT TOLERANCE CAPACITY OF MUNGBEAN THROUGH MANNITOL AND HYDROPRIMING

ABSTRACT

A laboratory experiment was set-up at the Agronomy lab of the Central Laboratory, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during 22 April to 15 June, 2018. The experiment was undertaken to evaluate the effect of pre-sowing seed treatment with mannitol in relation to drought tolerance and to optimize the priming time of the best priming solution concentration on mungbean (*Vigna radiata* L.) (BARI Mung 6 and Binamoog 5). The whole experiment was alienated into three experiments. In first experiment, two mungbean varieties were primed in water and mannitol (2%, 4%, 6%, and 8%) for 9 hours and dry seed used as control. The highest germination percentage (96.66%), germination index (80.75) and energy of emergence (98.00%) was were obtained from seeds primed in 4% mannitol for Binamoog 5 and highest coefficient of velocity (22.46), shoot length (153.8 mm), root length (92.98 mm), seedling dry weight (90.49 mg), vigor index (235.3), water content (90.47%) and water retention capacity (20.70) were obtained from seeds primed in 6% mannitol for BARI Mung 6. In second experiment, BARI Mung 6 and Binamoog 5 was primed in under 6% and 4% mannitol solution and distilled water respectively for 0, 3, 6, 9, 12 and 15 hours. 9 hours priming with 6% mannitol showed the best result in BARI Mung 6 and in Binamoog 5, 6 hours priming with 4% mannitol stated the best results for inducing drought tolerance. In the final experiment, seeds were primed with distilled water and 6% mannitol with 9 hours for BARI Mung 6 and 4% mannitol with 6 hours for Binamoog 5; dry seed used as control and were exposed to 0%, 5%, 10%, 15%, and 20% Polyethylene Glycol (PEG) induced drought stress conditions in Petri dishes. Priming with mannitol followed by water were more effective than the control seed in inducing drought tolerance of mungbean cultivars owing to enhance germination and growth parameters under drought stress condition. From the results of the study, it was observed that 9 hours priming with 6% mannitol showed the best result in BARI Mung 6 and in Binamoog 5, 4% mannitol priming for 6 hours stated the best results in comparison to water primed seed and non-primed seed.

CHAPTER I

INTRODUCTION

Mungbean (*Vigna radiata* L.) is a conventional pulse crop in Bangladesh belonging to the family Fabaceae. It has high nutritive value, and due to this, has advantage over the other pulses. Pulses are known as poor man's meat and cheap source of vegetable protein containing 20‑25% protein. The mungbean (*Vigna radiata*) is valued among the entire pulse species because it is an easily digestible pulse (Imran *et al.*, 2016). It holds the 3rd place in protein content and 4th place in both acreage and production among other pulses in Bangladesh (BBS, 2017a).

It is widely cultivated in the tropical and sub-tropical regions of the world and many of countries sprouted seeds are used as vegetable. In Bangladesh, whole or split seeds are used as Dal (soup). The seed contains 348 kcal energy, 24.5 mg protein, 1.2 mg fat, 59.9 mg carbohydrate, 75 mg calcium, 8.5 mg minerals, 0.72 mg thiamin, 0.15 µg riboflavin, 49 µg beta-carotene per 100 g seed, respectively (BARI, 2008). Mungbean is also an excellent source of protein with an ideal essential amino acid profile (Mubarak, 2005). It contains a variety of essential amino acids and is rich in lysine. The intake of mungbean protein may improve the plasma lipid profile by normalizing insulin sensitivity (Tachibana *et al.*, 2013). Mungbean also contains fatty acids such as linoleic acid and linolenic acid that promote the growth and health of organisms. The physical and chemical properties of triglycerides and their applications depend on the fatty acid constituents in molecules (Botinestean *et al.,* 2012). Besides, it has nitrogen-fixing and phosphorous-freeing properties, like other legume crops. Pulses help increase the organic matter and microbial activity (e.g. bacteria, fungi) in the soil. They can also improve soil structure and water retention capacity while helping to reduce wind and water erosion (Biederbeck *et al.,* 2005).

According to FAO (2013) daily consumption rate of pulse should be 80 g/day, but in Bangladesh only 14.2 g/day (BBS, 2017b). At present, total pulse grown area is 0.373 million hectare and production 0.387 million tons, where mungbean

is grown only in the area of 0.041 million hectare with the production of 0.035 million tons (BBS, 2017a) which is insufficient to meet up national demand for low productivity.

Growth and productivity of crops are affected by various types biotic and abiotic stress. Plants are normally faced lot of stresses such as salt, drought, oxidative stress and others. Abiotic stresses such as drought and heat has been shown to be more destructive to crop production at different crop growth stages (Prasad *et al.,* 2011). Stress affected the normal functions of individual life or the conditions in which plants are prevented from fully expressing their genetic potential for growth, development and reproduction (Levitt, 1980). Inadequate soil moisture in the seedbed is a major constrain to the establishment of the crop, because of reducing germination and emergence. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Anjum *et al.*, 2003; Shao *et al.,* 2008). Drought stress has been reported to severely reduce germination and seedling stand (Kaya *et al.*, 2006) also reduces seed yield usually as a result of fewer pods and seeds per unit area (Specht *et al.,* 2001).

A lot of strategies are established to escape the adverse effects of drought. The most effective strategy is the selection of drought tolerant vearities and cultivars (Pavlousek, 2011). However, pre-sowing seed treatments can be an alternative to overcome the drought stress condition of crops (Ghiyasi *et al.*, 2008). Seed priming is a useful treatment, applied prior to planting, which partially hydrates the seeds to a point of germination process initiation, followed by drying which prevents radicle emergence. Seed can be primed by either uncontrolled hydration - hydropriming (Casenave and Tosselli, 2007; Ghassemi-Golezani *et al.*, 2010a) or controlled hydration methods which include osmotic priming, solid matrix priming and hormonal priming (Basra *et al.*, 2006; Foti *et al.*, 2008). Seed priming techniques have been used to accelerate emergence of more vigorous plants and better drought tolerance in many field crops like wheat (Iqbal and

Ashraf, 2007), chickpea, sunflower (Kaya *et al*., 2006), cotton (Casenave and Toselli, 2007) triticale (Yagmur and Kaydan, 2008).

Seed priming induced measurable changes in chemical activities including greater α-amylase activity, increasing free sugars and DNA during seed germination and it may be helped to improve germination (Sung and Chang, 1993). Primed seeds of mungbean exhibited significant difference in germination percentage and seed moisture percentage over non-primed seeds (Saha *et al.*, 2006). It has been found a promising technology to improve rapid and uniform emergence, high vigor, and better yields for vegetable and field crops (Janmohammadi *et al*., 2009; Rouhi *et al*., 2011).

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

1) To analyze the germination behavior of mungbean at different concentrations of priming agents (Mannitol and Distilled water).
2) To estimate the pre-sowing priming time on the germination behavior of mungbean.
3) To evaluate the germination behavior of primed seed (mungbean) under drought (Polyethylene Glycol) stress condition.

CHAPTER II
REVIEW OF LITERATURE

Mungbean is a valued pulse crop in Bangladesh but its production is greatly affected by drought stress. Pre-sowing treatment of seeds improve the germination under various stress conditions. Seed priming is the most important techniques to escape those adverse condition. Available literatures on priming of seeds on different legume and others corps are studied by different authors as followed:

2.1 Seed priming

The history of seed priming dates back to 60 A.D. Attempts to improve seed germination have been reported since Ancient Greeks. Theophrastus (372-287 BC) recommended presoaking of cucumber seeds in milk or water to make them germinate earlier and vigorously (Evanari, 1984). Darwin (1855a, b) tested osmo-priming conditions by soaking the seeds of *Lepidium sativum* and lettuce in seawater and noticed improved germination.

Seed priming is treated of seeds in a solution of priming agent followed by drying that initiates germination processes without the radical emergence (McDonald, 1999). Priming allowed to begin the biochemical processes and metabolism of sugars and hydrolysis inhibitors during the first and the second stages of germination before the emergence of the radical (Parmoon *et al*., 2013). Various advantages are reported in seed priming to eliminate physiological and pathological stresses that results in utilization, activation and enhancement of various cellular defense responses and resistance (Conrath *et al.,* 2002).

Priming effects are related with the fixing and building-up of nucleic acids, improved synthesis of proteins and repairing of the cell membranes (McDonald, 2000). Pre-treatment of seed also boosts the actions of anti-oxidative enzymes (McDonald, 1999; Wang *et al*., 2003 and Hsu and Kao, 2003).

Seed priming increased the germination, seedling emergence and crop yield (Khan, 1992; Ghassemi-Golezani *et al*., 2008a and Ghassemi-Golezani *et al*.,

2010a). Advantages of seed priming have been observed in many crops e.g. maize (Parera and Cantliffe, 1994), sunflower (Singh, 1995), sugar beet (Sadeghian and Yavari, 2004), barley (Abdulrahmani *et al*., 2007), lentil (Ghassemi-Golezani *et al*., 2008a), chickpea (Ghassemi-Golezani *et al*., 2008b), pinto bean (Ghassemi-Golezani *et al*., 2010a) and winter rapeseed (Ghassemi-Golezani *et al*., 2010b). Seeds are primed with H_2O_2 increases germination percentage, shoot emergence, shoot and root lengths and weights (Amjad *et al*., 2004; Cavusoglu and Kabar, 2010).

2.2 Priming effect on germination parameters

2.2.1 Germination percentage

Good germination ensures better establishment of crop and it is a big sign for maximum crop yield. For that sometime seed treatment are necessary before sowing. In the field conditions, primed seeds resulted earlier germination as it restored deteriorated seeder parts and quickened germination because of speedier developing life (Farooq *et al.*, 2008).

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

According to Posmyk and Janas (2007), at low temperature hydropriming and hydro priming along with proline can be practiced as a harmless priming process for betterment of seed germination and growth of *Vigna radiata*. Stress injuries also repaired fast through hydroming. More even germination and emergence were found 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 defined hydropriming for 24 hr betterment hardening, germination rate and percentage in seeds. (Farooq *et al*., 2006).

Kumar *et al.* (2017) reviewed that, osmo-priming treated seed showed significantly higher germination percentage in PEG at 20% followed by mannitol 4% in chick pea. It was informed by Kaya *et al*. (2006) seed priming had an important result on increasing of germination percent; germination speed and seedling dry weight of sunflower. Priming also decreased abnormal seedling in drought stress.

Therefore, the positive effects of priming may be more obvious under unfavorable rather than favorable conditions (Parera and Cantliffe, 1994). In mungbean, 4 hr and 8 hr primed seeds presented substantial variation in germination and seed moisture percentage over un-primed seeds (Saha *et al.,* 2006). Increasing germination rate by 10-15% was reported through hardening (150 gm seeds soaked in 500 mL water for 18 and 24 hr) of older rice seeds, as the process of increase total sugar content and α-amylase activity (Lee and Kim, 2000).

Seed priming hasten germination percentage, lessened emergence time and enhanced yields are reviewed in many crops (Farooq *et al*., 2006b; Afzal *et al*., 2006; Afzal *et al*., 2011a). Judicious doses of PEG (Polyethylene Glycol) showed better tolerance at drought stress condition than hydro-priming, while more doses of PEG had negative effects on germination (Sun *et al.,* 2010).

Seed treated with *Rhizobium* + *Pseudomanas* @ 10% for 12 hr recorded higher germination percentage (87%). It may due to completion of peregrination metabolic activities, increased rate of cell division, apical dominance in shoot and root apex. During seed priming, all those attributes making the seed ready for soon germination after planting and the highest germination percentage (Vishwas *et al.,* 2017).

Natural priming has been shown to increase germination synchrony, rate and final percentage in many species (Gonz_alez-Zertuche *et al.,* 2001; Santini and

Martorell, 2013). Seed priming treatments not only improved germination rate and time, but it also enhanced seedling vigour, as indicated by longer roots and shoots and higher seedling dry weight (Mahajan *et al.*, 2011).

2.2.2 Mean germination time (days)

Mean germination time reflects germination speed that cannot be measured by germination percentage. Speed of germination may because of the accelerated germination of primed seeds might be due to increased rate of cell division (Bose and Mishra, 1992).

According to Basra *et al.* (2005) priming promoted to reduce mean germination time over the un-primed one. The MGT is also dependent on the duration of imbibitions and/or internal metabolic activities after imbibitions (the second stage of germination). Priming activates internal metabolism required for furthering the germination process.

Tavili *et al*. (2011) reported that speed of germination of Bromus increased with seed priming treatments rather than that of control. Similarly, Elkoca *et al*. (2007) determined that hydro priming treatment in chickpea induced faster and more synchronous germination compared with the unprimed seeds. Furthermore, Korkmaz (2005) for sweet pepper and Korkmaz and Pill (2003) for lettuce reported that priming treatments generally improved the germination synchrony. Besides, in terms of priming duration, priming treatment for 24 hr generally reduced the germination synchrony compared with the treatment for 12 hr. This result indicated that longer priming duration may overcome effect of decreased water potential in osmo-priming treatments of lentil seeds.

At the time of different phases of seedling establishment priming plays very significant role to reduce the time between planting and emergence and safe the seeds from environmental stress. Uniform stands and improved yield could be maintained through earlier and synchronized emergence (Farooq *et al*., 2006; Afzal *et al*., 2006; Afzal *et al*., 2011a). Like germination percentage, prime seeds need lower mean emergence time (MET) than non-primed seeds. Priming creates stimulatory effects on the early phases of emergence by occurrence of cell

division in germinating seeds (Hassanpouraghdam *et al.*, 2009; Sivritepe *et al.*, 2003).

Improved seed priming methods were recognized to lessen emergence time, achieve even emergence and ensure good crop stand in several horticultural and field crops (Ashraf and Foolad, 2005). According to Finch-Savage *et al.* (2004) without changing germination percentage priming minimize the optimum and ceiling temperature for germination and also facilitated in progressing the germination time.

Rashid *et al.* (2006) reported that "On-farm" hydropriming was very efficient in speed germination, good crop establishment and increased yields for many crops in various environmental condition.

Yucel (2012) found that Priming treatment influenced the MGT compared with control seeds at all of the germination temperatures. In generally, seeds primed for 24 hr reduced hours required reaching 50 % germination compared with the seeds primed for 12 hr, but there was no significant difference between priming durations.

Ghassemi-Golezani *et al*. (2008a) reported that lentil seeds priming with KNO_3, PEG and water showed the highest germination percentage. Like germination percentage, it has been reported that primed seeds had lower mean germination time. Seeds primed for 24 hr increased the germination percentage and decreased mean germination time compared with seeds primed for 12 hr. This result indicated that longer priming time may overcome adverse effects of decreased water potential in osmo-priming treatments than control seeds (Sadeghi *et al*., 2011, Sağlam *et al*., 2010, and Dezfuli *et al*., 2008).

According to Basra *et al*. (1989) priming with polyethylene glycol or potassium salt (K_2HPO_4 or KNO_3) resulted in quicker emergence in corn seed. When salinity and drought stress cause the reduction of germination speed, hydro priming perform as a decent, economical and simple seed stimulating treatment for inbreed lines of maize (Janmohammadi *et al*., 2008).

Gray *et al*. (1990) said that (-0.5 MPa) reduced the mean germination time of seeds of lettuce, carrot and onion. Goobkin (1989) and Ozbingol *et al.* (1999) also noticed that priming of tomato seeds with PEG, germinate sooner than non-primed seeds and its might be due to quick water uptake. The possible cause for early emergence of the primed seed could be for 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) showed that seed treating may help to overcome dormancy through embryo advancement and leaching of emergence inhibitors that lead to an earlier start of emergence.

According to Harris *et al*. (1999) early emergence and maturity in seed priming treatment could be due to advancement in metabolic state. Musa *et al*. (1999) also concluded that priming improve plant stand and provide benefits in term of maturity. Seed priming resulted in earlier emergence of seedlings by 1-3 days and significantly increased plant stand and initial growth vigour.

Singh *et al.,* found that a steady decrease in number of days taken for seed emergence from control or non-treated seeds to 16 hr of hydropriming. i.e., average number of days taken by control or non-treated seeds for 50% seed emergence was 5.00 which was highest, and average number of days taken by 12 hr of hydropriming was 2.78 days which was lowest. Hydropriming of seeds could have achieved earlier and more uniform germination than the unprimed seeds. These positive effects are probably due to stimulatory effects of priming on early stage of germination process by mediation of cell division in germinating seeds (Ghassemi-Golezani *et al.,* 2010c).

2.2.3 Seed germination index

Hydropriming could have advances the germination rate, speed (germination index) and uniformity even under less optimum field condition (Kant *et al.,* 2006). Hosseein *et al.* (2011) reported that seed priming resulted in anti-oxidant

increment as glutathione and ascorbate in seed. These enzymes led to higher germination speed via reduction of lipid peroxidation activity.

A critical analysis done by Singh *et al.* (2017) on different hydropriming and osmo-priming treatments on pea have significant effect on germination index, 20% Polyethylene glycol (PEG) for 24 hr (9.11) shows significant effect on rest of the treatments except 20% Polyethylene glycol (PEG) for 12 hr (8.92).

Elangbam *et al.* (2017) experimented that, the speed of germination in chickpea as influenced by various seed treatment: Lemon juice, Panchgavya, GA3 and IAA. The maximum speed of germination with GA3 might be due to its influence in early germination and increased percent germination. The results are in conformity with findings of Rajamanickam and Anbu (2001).

Rashid *et al*. (2006) stated that priming boosted speed of germination, better crop stand and amplified yields in varied situations for a lot of crops (Khan *et al*., 2008). Arif (2005) who described that seed priming hasten germination index as it attributed to repair processes, a buildup of germination metabolites or osmotic regulations during priming.

Kaur *et al.* (2003) conducted study to determine the effect of seed priming with mannitol (4%), water and potassium nitrate on chickpea. The response of chickpea seedlings to salt stress was also studied. In general priming with water and mannitol resulted in early germination under salt stress. Priming with 4% mannitol was also as effective as mannitol and water in the enhancement of root and shoot growth. Osmo-priming methods, Mannitol primed seed gave higher germination index than that of NaCl primed.

2.2.4 Coefficient of velocity

Coefficient of velocity (CV) is a measure of vigour (Scott *et al.,* 1984). Generally, CV increases as more seeds germinate and with shorter germination time. The CV gives an indication of the speed and uniformity of seedling growth (i.e., a higher CV means higher vigour).

Khan *et al.* (1980) cited that primed cabbage seeds kept at 15° C had accelerated emergence and gave increased plant fresh weight. Khan *et al.* (1980) stated from

Nyarko *et al.* (2006) also reported that cabbage seeds primed and expose to vernalization temperature (0°- 5° C) for 8 weeks had a higher CV than non-primed seeds. In primed leek seeds, the significant benefit in germination performance was accompanied by marked increases in protein, DNA and nucleotide biosynthesis. During priming of tomato seeds, the breakdown of protein bodies was more extensive in endosperm cells at the micropylar region than was observed prior to germination in non-primed seeds (Haigh, 1988). Therefore, there is ample evidence that priming of Bambara and Sorghum seeds, prior to germination, prevent radicle elongation during germination process by removing the water and drying seeds to original moisture content and improved the rate of germination after sowing. It has been reported that primed seeds showed better germination pattern and higher vigour levels than non- primed (Ruan *et al.,* 2002b).

2.2.5 Energy of emergence (%)

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

Primed seed can quickly uptake and revive the seed metabolism, subsequently advanced germination rate and lessening the internal physiological heterogeneity in germination (Rowse, 1995). The consequential improved crop stand can apparently enhance the drought tolerance, decrease pest damage and hasten crop yield in cereals and legumes (Harris *et al.,* 1999; Musa *et al.,* 1999; Harris *et al.,* 2000; Khan *et al.,* 2005).

Many metabolic processes are related in the early stages of germination and those are stimulated by priming. It is well-known that seedlings from primed seeds germinate faster, grow more rapidly and perform better in negative

conditions (Cramer, 2002). It again stated that seed treatment advances emergence of seedling, stand establishment, tillering, grain and straw yields, and harvest index (Farooq *et al*., 2008).

Roy and Srivastava (1999) found that soaking wheat kernels in water improved their germination rate under saline conditions. It also had pronounced effect on field emergence its rate and early seedling growth of maize crop and it improved the field stand and plant growth both at vegetative and maturity of maize (Nagar *et al*., 1998). Hydro-priming plays an important role in the seed germination, radical and plumule emergence in different crop species. Similar to other priming techniques, hydro-priming generally enhance seed germination and seedling emergence under saline and non-saline conditions and also have beneficial effect on enzyme activity required for rapid germination. Priming with NaCl and KCl was helpful in removing the deleterious effects of salts (Iqbal *et al*., 2006). In sorghum seeds soaked in $CaCl_2$ or KNO_3 solution increased the activity of total amylase and proteases in germinating seeds under salt stress (Kadiri and Hussaini, 1999). In pigeon pea seed treatment with $CaCl_2$ or KNO_3 generally exhibited improvement in proteins, free amino acid and soluble sugars during germinating under salt stress (Jyotsna and Srivastava, 1998).

Zheng *et al*. (1994) reported that rice (*Oryza sativa)* showed earlier and uniform emergence when seeds are osmo-primed with KCl and $CaCl_2$ and mixed salts under flooded conditions. However, Nascimento and West (1999) reported early germination of primed seeds but no improvement in the growth of seedlings in case of muskmelon (*Cucumis melo*) seeds under laboratory conditions. Confounding results, different research workers reported no beneficial results from priming (Mwale *et al*., 2003; Giri and Schillinger, 2003).

2.3 Effect on growth parameters

2.3.1 Shoot length (mm)

Increased shoot and root length may be due to early emergence induced by priming treatment as compared to unprimed seeds (Stofella *et al.*, 1992). Osmo-priming boosted plumule dry weight was reported by Harris *et al*., (2004).

Kumar *et al.*, (2017) experimented on chickpea that shoot length has recorded high in case of osmo-primed seeds than that of unprimed seeds. Among different osmo-priming treatments 20% PEG showed the highest shoot length followed by 4% mannitol and control showed the lowest shoot length.

Sarvjeet *et al*. (2017) found that hydro priming of seeds influenced the seedling length of chickpea and maximum seedling length 17.47 cm and 18.07 cm were associated with 16 hours hydro priming.

It was stated by Kaur *et al*. (2002, 2005) that osmo and hydro priming of chickpea seeds with mannitol and water lightened the negative effects of water deficiency and salt stress on seedling development. The treatment of seeds with water, 2% and 4 % mannitol improved the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions. Similarly, seed priming with P solutions significantly improved fresh and dry weight and plant height of mungbean seedlings 21 days after sowing in the field experiment (Shah *et al.*, 2012).

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

A field experiment was conducted by Gupta and Singh (2012) in inceptisols to find out the effects of seed priming on chickpea. The treatments consisted of seed priming (seed soaking in water for 8 hr). The results revealed that the growth parameters of chickpea were significantly affected by seed priming. Soaking

chickpea seeds in water for about 8 hr significantly influenced plant height and nodule dry weight in comparison to un-soaked seeds.

Shehzad *et al*. (2012) conducted an experiment to study the influence of priming techniques on emergence and seedling growth of forage sorghum. Therefore, this study was designed with different seed priming techniques, un-soaked seed (control), Hydro-priming (soaked with distill water), Halo-priming with KNO_3 and $CaCl_2$ (1% solution). Experiment was conducted in wire house under natural climatic conditions during 2008. All the priming treatments significantly affected the fresh weight, shoot length, number of roots, root length, vigour index, and time to start emergence, time to 50% emergence and energy of emergence of forage sorghum. Seed priming increase cell division and seedling roots which cause an increase in plant height. It is concluded that seed priming may serve as an appropriate treatment for accelerating the emergence of sorghum genotypes studied.

Singh *et al*. (2014) conducted an experiment to study the effect of osmo-priming duration on germination, emergence and early growth of cowpea in Nigeria. Treatment consisted three osmo-priming duration (soaking in 1 % KNO_3 salt for 6, 8 and 10 hr) and one hydro-primed control (10 hr). The results showed that osmo-priming with KNO_3 for different durations were superior to unprimed treatment in term of seed germination, emergence, plant height and dry matter accumulation in cowpea. Primed seeds (both osmo-priming and hydro-priming) increased performance of cowpea. However, osmo-priming with KNO_3 salt (soaked in 1 % KNO_3 salt solution and dried before sowing) for 6 hr could result in greater seed germination and seedling height than hydro-priming.

2.3.2 Root length (mm)

Priming may hasten germination by quickening imbibition, which in turn would simplify the emergence stage and enhancing the division of radicle cells (Kaya *et al.*, 2006).

Kulkarni and Eshanna (1988) said that seed treated prior at sowing with 10 ppm IAA increased root length, germination percentage and seedling vigour. Kathiresan *et al*. (1984) also reported similar results, highest root and shoot growth, seedling height and field emergence was attributed with $CaCl_2$ primed seeds in sunflower.

Kumar *et al.* (2017) conducted an experiment on effect of osmo-priming on seed germination behavior and vigour of chickpea *(Cicer arietinum*) and found that 20% PEG and 4% mannitol produce maximum (22.07 cm) root length. Smallest root length was recorded by (15.38cm) with control. Laghari *et al.* (2016) also stated that maximum mean root length cm (5.324) was recorded at Hydro-priming period 4 hours whereas the lower (3.093) found at no priming or check in case of mungbean.

Singh *et al.* (2017) reported that a significant effect of different hydropriming and osmo-priming on root length of pea. 20% polyethylene glycol for 24 hr (14.11 cm) priming showed better performance over untreated (11.98 cm), 3% mannitol for 12 hr (12.37 cm), 3% mannitol for 24 hr (12.67 cm) and 5% glycerol for 12 hr (12.99 cm) priming. Ashraf and Rauf (2001) found that GA_3 treatment enhanced the vegetative growth of two wheat cultivars. It enhanced the deposition of Na+ and Cl- in both root and shoots of wheat plant. It also caused a significant increase in photosynthetic at the vegetative stage of the crops.

Farahbakhsh (2012) reported that the concentration of 0.25 and 0.5 mM of salicylic acid on germination, germination rate, seed stamina index, hypocotyl length, radical length, seedling fresh and dry weight of fennel (*Foeniculum vulgare*) was more effective as compared to other levels (0 and 0.75 mM). Therefore, seed priming with salicylic acid could be a suitable tool for improving germination characteristics of fennel.

Sarika *et al*. (2013) conducted a lab experiment to study various physiological and biochemical changes by priming in French bean at Bangalore. They reported that chemo priming with GA_3 and Ethrel improved the seed quality and showed improved seedling length, seedling dry weight which in turn improved higher

seedling vigour index, germination speed and mean germination time. Significant increase in initial (6.02 cm) and final (11.5 cm) root length, initial and final shoot length, seedling vigour index and dry seedling weight with GA3 is observed in the crop.

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

2.3.4 Seedling dry weight (mg)

Increase of the synthesis of the hormone gibberellin, which Trigg the activity of α-amylase and other germination specific enzymes like protease and nuclease involved in hydrolysis and assimilation of the starch (Gholami *et al.,* 2009) enhance dry weight of the shoot and dry weight.

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

Kumar *et al.* (2017) experimented on chickpea and found that in case of seedling dry weight it was higher (1.02 mg to 1.59mg) in PEG 20% seeds followed by mannitol 4% when compared with control.

Laghari *et al.* (2016) found that, shoot dry weight (mg) has affected by temperature regimes, hydro-priming periods showed highly significant where as

their interaction was significant for shoot dry weight (mg). The maximum mean shoots dry weight mg (54.74) was recorded at hydro-priming period 4 hours whereas the lower shoot dry weight mg (38.56) found at no priming or check. The maximum mean root dry weight mg (7.898) was observed at hydro-priming period 4 hours whereas the lower root dry weight mg (5.496) found at no priming or check.

At Varanasi, Srivastava and Bose (2012) conducted an experiment on seed priming of rice varieties with or without nitrate salts (Mg $(NO_3)_2$ and KNO_3). Results showed the beneficial effect of priming treatments which was clearly exhibited in plant height, leaf area and number of leaf and yield attribute characteristics i.e. fertile tillers, panicle and grain quality, with nitrate treated varieties. Seed priming treatment resulted in increased crop growth rate in treated sets which encouraged deposition of more photo-assimilates in key plant parts, greatly affecting the dry weight and final yield.

Singh *et al.* (2016) cited on different hydropriming and osmo-priming treatments on dry weight. Polyethylene glycol (PEG) @ 20% for 24 hr (0.54) shows significant effect on Untreated (0.40), Menitol @ 3% for 12 hr (0.44), Menitol @ 3% for 24 hr (0.43), Glycerol @ 5% for 12 hr (0.46) and Glycerol @ 5% for 24 hr (0.48) on dry weight parameters.

Sarika *et al*. (2013) conducted a lab experiment to study various physiological and biochemical changes by priming in French bean at Bangalore. They reported that chemo priming with GA_3 and Ethrel improved the seed quality and showed improved seedling length, seedling dry weight which in turn improved higher seedling vigour index, germination speed and mean germination time. Significant increase in initial (6.02 cm) and final (11.5 cm) root length, initial and final shoot length, seedling vigour index and dry seedling weight with GA_3 is observed in the crop.

2.3.4 Vigour index

During priming, the embryo expands and compresses the endosperm (Liptay and Zariffa, 1993). The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration (Lin *et al.,* 1993), producing free space and facilitating root protrusion after rehydration and enhance vigour of seed.

The probable reason for the highest vigour index might be due to photosynthetic capacity treated with bio fertilizers increases due to increased supply of nutrition (Farnia and Shafie, 2015).

Priming improved seedling vigour experimented by Safiatou (2012). Seedling vigour increased by using seed priming methods in sorghum and Bambara groundnut. Also, highest seedling vigour was achieved by osmo-priming (Mannitol priming) in Bambara groundnut and by hydro-priming in sorghum.

The adverse effects of Reactive Oxygen Species (ROS) lessened and improve the antioxidant enzymes activity through seed priming (Del Ryo *et al*., 2002). It might be meaningfully enhanced the germination rate and vigor of the mungbean seedlings (Umair *et al.,* 2010).

Primed seeds ensured rapid growth, early flowering and high yield (Farooq *et al*., 2008). This practice used for betterment of germination speed, germination vigour, seedling establishment and yield (Talebian *et al*., 2008; Bodsworth and Bewley, 1981). Harris *et al*. (1999) confirmed that on-farm seeds soaking overnight in water noticeably improved establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. Also, vigorous growth is often connected with higher yields (Okonwo and Vanderlip, 1985; Austin, 1989; Carter *et al*., 1992). Seed-treating technology has twofold benefits: greater, speedy and even 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).

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

Relative water content is influenced by seed quality and seed priming technique. Significantly higher relative water content was recorded in leaves obtained from plots sown with higher quality seeds as compared to those obtained from plots sown with lower quality seeds. The leaves obtained from plots having seed primed with $CaCl_2.2H_2O$ (0.5%) showed significantly highest relative water content which was on par with the leaves from plots having seed primed with KH_2PO_4 (50 ppm) followed by leaves obtained from plots having seed primed with GA_3 (20ppm) (84.57%) while the lowest relative water content (79.02 %) was recorded in leaves obtained from plots having seed primed with KCl (100ppm). The interaction effect had also a significant effect with the highest relative water content recorded in leaves obtained from plots sown with the higher quality seeds treated by $CaCl_2.2H_2O$ (0.5) (Assefa, 2008).

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

Sangakkara *et al.* (1996) observed that when *Phasiolous vulgaris* L. plants were subjected to moisture stress, the WRC increase with the increasing potassium concentrations.

CHAPTER III
MATERIALS AND METHODS

A series of experiment were carried out to observe the effect of mannitol induced seed priming for enhancing drought tolerance capability in mungbean (*Vigna radiata*) under drought stress from 22 April to 15 June, 2018. A short illustration of the experimental site, temperature and humidity of the laboratory room, experimental materials, treatments and design, methods of the study, data calculation procedure and data analysis are discussed at this section. The materials and methods used to carry the study are discussed below.

3.1 Description of the experimental site

3.1.1 Location

The experiment was carried at the Agronomy Lab of the Central Laboratory, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207. It was situated in 24.09^0 N latitude and 90.26^0 E longitudes.

3.1.2 Laboratory room Conditions

Laboratory room temperature and relative humidity were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature were 24.4^0 C to 35.2^0 C, respectively and average minimum and maximum relative humidity were 56% and 89%, respectively.

3.2 Test crops

BARI Mung 6 and Binamoog 5 were used for this experiment. Seeds of BARI Mung 6 was collected from Bangladesh Agricultural Research Institute (BARI) and Binamoog 5 was collected from Bangladesh Institute of Nuclear Agriculture (BINA). The collected seeds were free from any visible defects, disease symptoms and insect infestations.

3.3 Experimental materials

Various equipment's such as electric balance, magnetic stirrer, Petri dish, micro pipette, wash bottle, beaker, forceps, filter paper, oven etc. were used for this study.

3.4 Chemicals for seed priming

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

3.5 Experimental design and layout

The study was carried out in a Completely Randomized Design (CRD) with 5 replications.

3.6 Experimental details

The whole study was segmented into three different experiments.

3.6.1 First Experiment

Study on the germination behavior of mungbean at different concentrations of priming agents

3.6.1.1 Weight of seeds

200 g seeds were balanced from the total seed from each of two mungbean variety BARI Mung 6 and Binamoog 5 to avoid the unnecessary loss of seeds. Rest of seeds were kept in refrigerator at airtight condition.

3.6.1.2 Surface treatment

Initially seeds were sterilized with 75% alcohol for 5 minutes then sterilized seeds were rinsed 2 minutes with distilled water for 3 times to reduce the effect alcohol from the seed surface. Finally, seeds were dried in room temperature to regain the normal condition.

3.6.1.3 Treatments

Following six treatments were applied separately for BARI Mung 6 and Binamoog 5:

T0 = Seeds without priming (control)

T1 = Seeds primed with distilled water for 9 hours

T2 = Seeds primed with 2% mannitol for 9 hours

T3 = Seeds primed with 4% mannitol for 9 hours

T4 = Seeds primed with 6% mannitol for 9 hours

T5 = Seeds primed with 8% mannitol for 9 hours

3.6.1.4 Priming solutions

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

3.6.1.5 Preparation of priming solutions

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

   2% mannitol solution was prepared by mixing 5g of mannitol at 250 mL distilled water. Similarly, 10g, 15g, 20g mannitol was mixed with 250 mL of distilled water to prepare 4%, 6% and 8% solution of mannitol respectively.

b) **Distilled water**

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

3.6.1.6 Priming technique

Mannitol priming and hydro priming was applied on both the mungbean varieties. Surface sterilized seeds were sub-divided into three parts. One for control (unprimed), one for hydro priming and another for mannitol priming. For hydro priming seeds were immersed in distilled water and mannitol priming seeds were divided into another four sub-group and treated with 2%, 4%, 6%, and 8% mannitol respectively for 9 hours. Different plastic pots were used with

lids for avoiding evaporation loss. All seeds were taken off form the priming agents at same time after 9 hours. The primed seeds were rinsed with distilled water three times genteelly and removed excess moisture by using tissue paper and finally air dried (Umair *et al.*, 2011) in room temperature for 24 hours to back the original moisture level.

3.6.1.7 Germination of seeds

Thirty seeds were selected randomly from each treatment of both verity and placed on 120 mm diameter Petri dishes. Where saturated (8 mL distillated water) whatman no.1 filter paper was used as growth media. During the test Petri dishes were kept saturated and placed at room temperature 25°C under normal light to facilitate germination for 8 days. Emergence of 2 mm radical considered for germination occurred (Akbari *et al.*, 2007). Every 24 hours interval germination progress was observed and data recorded up to continued 8 days. Shorter, thicker and spiral formed hypocotyls and stunted primary rooted seedlings were considered as abnormal seedlings (ISTA, 2003). Abnormal seedlings and dead seeds were taken off from the Petri dishes when data recorded. At 8^{th} day of germination test, five seedlings were selected randomly from each treatment then root and shoot were separated and packed in brown paper for oven dry. Then seedlings were dried in an oven at 75°C for 72 hours.

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

At 8^{th} day of germination test, five seedlings were selected randomly from each treatment and fresh weight was measured immediate after removing roots. Thereafter, the shoots were submerged at distilled water at room temperature in the dark for 24 hr. Shoots turgid weight was measured after removing the excess water by gently wiping with tissue paper. Then shoots were packed in brown paper and oven dried at 75°C for 72 hours for measuring dry weight. The fresh, turgid and dry weights of shoots were utilized to calculate relative water content (%), water saturation deficit (%) and water retention capacity (Baque *et al.*, 2002).

3.6.2 Second Experiment

Study on the pre-sowing priming time on the germination behavior of mungbean

3.6.2.1 Weight of seeds

200 g seeds were balanced from the total seed from each of two mungbean variety BARI Mung 6 and Binamoog 5 to avoid the unnecessary loss of seeds. Rest of seeds were kept in refrigerator at air airtight condition.

3.6.2.2 Surface treatment

Initially seeds were sterilized with 75% alcohol for 5 minutes then sterilized seeds were rinsed 2 minutes with distilled water for 3 times to reduce the effect alcohol from the seed surface. Finally, seeds were dried in room temperature to regain the normal condition.

3.6.2.3 Treatments

Following eleven types of priming times were used as treatment separately for BARI Mung 6 (Primed with water and 6% mannitol) and Binamoog 5 (Primed with water and 4% mannitol):

T0 = Seeds without priming (control)

T1 = Seeds primed with distilled water for 3 hours

T2 = Seeds primed with distilled water for 6 hours

T3 = Seeds primed with distilled water for 9 hours

T4 = Seeds primed with distilled water for 12 hours

T5 = Seeds primed with distilled water for 15 hours

T6 = Seeds primed with mannitol solution for 3 hours

T7= Seeds primed with mannitol solution for 6 hours

T8 = Seeds primed with mannitol solution for 9 hours

T9 = Seeds primed with mannitol solution for 12 hours

T10 = Seeds primed with mannitol solution for 15 hours

3.6.2.4 Priming solutions

Distilled water, 4% and 6% of mannitol solution were utilized as priming solutions.

3.6.2.5 Preparation of priming solutions

a) Mannitol solutions (4% and 6%)

4% and 6% mannitol were prepared by mixing 10g and 15g of mannitol at 250 mL distilled water respectively.

b) Distilled water

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

3.6.2.6 Priming technique

Surface sterilized seeds were sub-divided into three parts. One for control (unprimed), one for hydro priming and another for mannitol priming. Second parts of seeds were sub-divided into another 5 parts for five different priming times such as 3, 6, 9, 12 and 15 hours hydro priming and third parts seeds also were divided into another five parts for five different priming times such as 3, 6, 9, 12 and 15 hours mannitol priming. Different plastic pots were used with lids for avoiding evaporation loss. Seeds were taken off from the priming solution at the required time. The primed seeds were rinsed with distilled water three times gently and removed excess moisture by using tissue paper and finally air dried (Umair *et al.,* 2011) in room temperature for 24 hours to back the original moisture level.

3.6.2.7 Germination of seeds

Thirty seeds were selected randomly from each treatment of both verity and placed on 120 mm diameter Petri dishes. Where saturated (8 mL distillated water) whatman no.1 filter paper was used as growth media. During the test Petri dishes were kept saturated and placed at room temperature 25°C under normal light to facilitate germination for 8 days. Emergence of 2 mm radical considered

for germination occurred (Akbari *et al.,* 2007). Every 24 hours interval germination progress was observed and data recorded up to continued 8 days. Shorter, thicker and spiral formed hypocotyls and stunted primary rooted seedlings were considered as abnormal seedlings (ISTA, 2003). Abnormal seedlings and dead seeds were taken off from the Petri dishes when data recorded. At 8th day of germination test, five seedlings were selected randomly from each treatment then root and shoot were separated and packed in brown paper for oven dry. Then seedlings were dried in an oven at 75°C for 72 hours.

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

At 8th day of germination test, five seedlings were selected randomly from each treatment and fresh weight was measured immediate after removing roots. Thereafter, the shoots were submerged at distilled water at room temperature in the dark for 24 hr. Shoots turgid weight was measured after removing the excess water by gently wiping with tissue paper. Then shoots were packed in brown paper and oven dried at 75°C for 72 hours for measuring dry weight. The fresh, turgid and dry weights of shoots were utilized to calculate relative water content (%), water saturation deficit (%) and water retention capacity (Baque *et al.,* 2002).

3.6.3 Third Experiment

Study on the germination behavior of primed seed (mungbean) under drought (Polyethylene Glycol) stress condition

3.6.3.1 Weight of seeds

200 g seeds were balanced from the total seed from each of two mungbean variety BARI Mung 6 and Binamoog 5 to avoid the unnecessary loss of seeds. Rest of seeds were kept in refrigerator at air airtight condition.

3.6.3.2 Surface treatment

Initially seeds were sterilized with 75% alcohol for 5 minutes then sterilized seeds were rinsed 2 minutes with distilled water for 3 times to reduce the effect

alcohol from the seed surface. Finally, seeds were dried in room temperature to regain the normal condition.

3.6.3.3 Treatments

Following five treatments were applied separately for BARI Mung 6 and Binamoog 5:

T0 = Non-primed (control) and primed (mannitol and water) seeds placed without PEG (control)

T1 = Non-primed (control) and primed (mannitol and water) seeds placed with 5% PEG concentration

T2 = Non-primed (control) and primed (mannitol and water) seeds placed with 10% PEG concentration

T3 = Non-primed (control) and primed (mannitol and water) seeds placed with 15% PEG concentration

T4 = Non-primed (control) and primed (mannitol and water) seeds placed with 20% PEG concentration

3.6.3.4 Priming solutions and time

6% of mannitol solution and distilled water were used for BARI Mung 6 for 9 hours priming and 4% of mannitol solution and distilled water were used for Binamoog 5 for 6 hours priming.

3.6.3.5 Preparation of priming solutions

a) Mannitol solutions (4% and 6%)

4% and 6% mannitol were prepared by mixing 10g and 15g of mannitol at 250 mL distilled water respectively.

b) Distilled water

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

3.6.3.6 Preparation of drought stress solutions

a) **Polyethylene Glycol (PEG) solutions (5%, 10%, 15% and 20%)**

5% PEG solution was prepared by mixing 12.5g of PEG at 250 mL distilled water. Similarly, 25g, 37.5g, 50g PEG was mixed with 250 mL of distilled water to prepare 10%, 15% and 20% solution of PEG (6000) respectively.

3.6.3.7 Priming technique

Mannitol priming and hydro priming was applied on both the mungbean varieties. Surface sterilized seeds were sub-divided into three parts. One for control (unprimed), one for hydro priming and another for mannitol priming. For hydro priming BARI Mung 6 and Binamoog 5 were immersed in distilled water for 9 and 6 hours respectively. For mannitol priming BARI Mung 6 and Binamoog 5 were emerged at 6% and 4% mannitol solution for 9 and 6 hours respectively. Different plastic pots were used with lids for avoiding evaporation loss. Seeds were taken off from the priming solution at the required time. The primed seeds were rinsed with distilled water three times genteelly and removed excess moisture by using tissue paper and finally air dried (Umair *et al.*, 2011) in room temperature for 24 hours to back the original moisture level.

3.6.3.8 Germination of seeds

Thirty seeds were selected randomly from each treatment of both verity and placed on 120 mm diameter Petri dishes. Where saturated (8 mL distillated water) whatman no.1 filter paper was used as growth media. During the test Petri dishes were kept saturated and placed at room temperature 25°C under normal light to facilitate germination for 8 days. Emergence of 2 mm radical considered for germination occurred (Akbari *et al.*, 2007). Every 24 hours interval germination progress was observed and data recorded up to continued 8 days. Shorter, thicker and spiral formed hypocotyls and stunted primary rooted seedlings were considered as abnormal seedlings (ISTA, 2003). Abnormal seedlings and dead seeds were taken off from the Petri dishes when data

recorded. At 8th day of germination test, five seedlings were selected randomly from each treatment then root and shoot were separated and packed in brown paper for oven dry. Then seedlings were dried in an oven at 75°C for 72 hours.

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

At 8th day of germination test, five seedlings were selected randomly from each treatment and fresh weight was measured immediate after removing roots. Thereafter, the shoots were submerged at distilled water at room temperature in the dark for 24 hr. Shoots turgid weight was measured after removing the excess water by gently wiping with tissue paper. Then shoots were packed in brown paper and oven dried at 75°C for 72 hours for measuring dry weight. The fresh, turgid and dry weights of shoots were utilized to calculate relative water content (%), water saturation deficit (%) and water retention capacity (Baque *et al.*, 2002).

3.7 Data recording

Parameters that's were measured as follows

3.7.1 Germination percentage (GP)

Germination percentage was estimated as the number of seeds which was germinated within total days as a proportion of number of seeds shown (Othman *et al.*, 2006). GP expressed as percentage (%).

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds placed on Petri dish}} \times 100$$

3.7.2 Mean germination time (MGT)

Mean germination time (MGT) was calculated according to the following equation (Ellis and Roberts, 1981):

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

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

3.7.3 Germination index (GI)

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + - - + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

3.7.4 Coefficient of velocity (CV)

Coefficient of velocity (CV) was estimated according to the method described by Kader (2005).

$$CVG = \frac{(N_1 + N_2 + --- + N_x)X\ 100}{T_1N_1 + T_2N_2 + --- + T_xN_x}$$

Where N was number of seeds germinated each day and T was number of days after sowing corresponding to N.

3.7.5 Energy of emergence (EG %)

Energy of emergence (EG) was estimated on the 4th day after placing seeds on Petri dish. It is the percentage of germinating seeds 4 days after sowing relative to the total number of seeds tested (Ruan *et al.,* 2002a). Energy of emergence expressed as percentage.

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

Five seedlings were selected randomly from each treatment and cotyledons were removed from them. Length of shoot and root was measured with ruler at millimeter (mm) scale.

3.7.7 Seedling dry weight(mg)

The mean seedling dry weight were measured with an electric balance at nearest gram (g) and converted to milligram (mg).

3.7.8 Vigour index (VI)

Vigour index in each treatment was estimated from germination percentage and seedlings length according to Abdul- Baki and Anderson (1970).

$$VI = \frac{\text{Germination percentage (GP \%) X Seedling Length}}{100}$$

3.7.9 Relative water content (RWC %)

Relative water content was estimated according to the formula of Baque *et al.* (2002). Relative water content expressed as percentage.

$$\text{Relative water content (RWC)} = \frac{\text{Fresh Weight - Dry Weight}}{\text{Turgid weight - Dry Weight}} \text{ X } 100$$

3.7.10 Water saturation deficit (WSD%)

Water saturation deficit was estimated from RWC according to the formula of Baque *et al.* (2002).

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

3.7.11 Water retention capacity (WRC)

Water retention capacity was estimated from turgid weight and dry weight according to the formula of Baque *et al*., (2002). Relative water content expressed as percentage.

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

3.8 Statistical analysis

Collected data from different treatments were statistically analyzed to notice the significant difference among the treatments. Data compilation and arrangement were done at excel. The mean value of parameters and analysis of variance were calculated by using a computer software MSTAT-C. The significance of difference among the treatment means was observed by the least significant difference (LSD) test at 5% level of significance.

CHAPTER IV
RESULTS AND DISCUSSION

The results are demonstrated and discussed in this chapter which was obtained from the different experiment to induce drought tolerance capacity of mungbean (*Vigna radiata* L.) through mannitol and hydro priming. Germination and growth parameters of mungbean greatly influenced by different concentrations of mannitol and priming time in drought stress condition.

4.1 First experiment

Study on the germination behavior of mungbean at different concentrations of priming agents

Results obtained from the present study regarding the germination behavior of mungbean at different concentrations of priming agents have been demonstrated, discussed and compared in this section. The analytical results have been displayed in Figures 1 to 8 and Tables 1 to 4.

4.1.1 Effect on germination percentage

Osmo-priming technique refers to soaking of seeds for a certain period in solution of sugar, mannitol, PEG etc. followed by air drying before sowing. Osmo-priming not only improves seed germination but also enhance crop performance. In BARI mungben-6, no significant difference was found on total germination percentage at T1, T1, T3 and T5 treatments but T1 and T4 treatments showed significant difference (Figure 1) in priming with different concentrations of mannitol, water and control. In Bainamoog-5, significant difference was found among the treatments. The highest germination percentage (96.66%) of Binamoog 5 was observed in T3 treatment compared to germination percentage (95.33%) of BARI Mung 6 was observed in T4 treatment. The lowest germination percentage (82.00%) and (83.33%) for BARI Mung 6 and Binamoog 5, respectively was found in T0 treatment. This study was in agreement with the findings of Faijunnahar *et al.* (2017); Ahammad *et al.* (2014) and Abnavi and Ghobadi (2012). Kumar *et al.* (2017) found that 4% mannitol

expressed highest germination percentage followed by PEG at 20% in case of chickpea. Kaur *et al.* (2002) also found that priming of pea by water and mannitol (4%) for 12 hours in 25°C can increase the number and biomass of plants knots. Hydro-priming improves the power of germination in plants of sesame species and speeds the germination and solid weight of the plant in lab conditions (Eskandari, 2011). Hydro-priming of bean seeds in water for 7-14 hours can improve the plant performance (Ghassemi-Golezani *et al.*, 2010c). Kaur *et al.* (2003) conducted study to determine the effect of seed priming with mannitol (4%), water and potassium nitrate on chickpea. In general priming with water and mannitol resulted in early germination under salt stress. Priming with 4% mannitol was also as effective as mannitol and water in the enhancement of root and shoot growth.

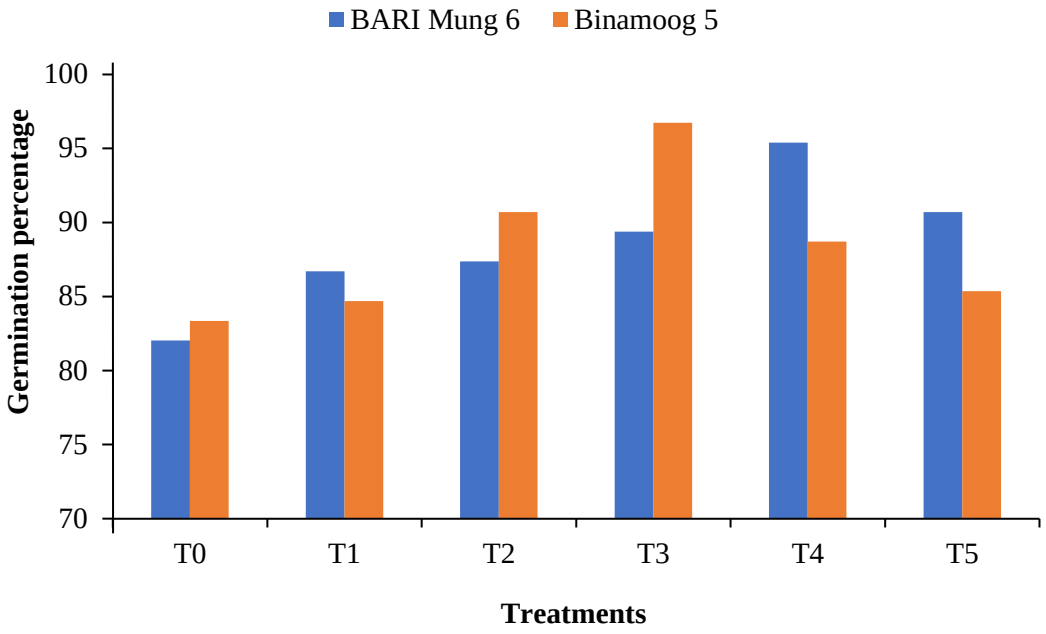

Figure 1. Effect of different concentrations of mannitol and water on germination percentage (LSD $_{(0.05)}$ = 4.424 and 4.897 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.2 Effect on mean germination time (days)

Mean germination time was significantly affected by different concentration of mannitol and water priming (Table 1). Mean germination time was gradually decreased with increasing mannitol concentration up to T4 and T3 for BARI Mung 6 and Binamoog 5 respectively and then increased with increasing mannitol concentration. The maximum mean germination time (4.594 days) was found for BARI Mung 6 compared to mean germination time (4.568 days) was found for Binamoog 5 was found at T0 treatment. The minimum mean germination time (4.454 days) and (4.466 days) for BARI Mung 6 and Binamoog 5 was found at T4 and T3 treatment respectively.

This result was supported by previous findings of Rahman (2014), and Asaduzzaman (2014). Kumar *et al.* (2017) found that 4% mannitol showed maximum speed of germination (71.50) in chickpea. Priming with 2 and 4% mannitol for 24 hr maximally increased final germination percentage, germination capacity, germination index, shoot and root lengths and decreased mean germination time of marigold species as compared to all pre-sowing seed treatments including control. This could be attributed to the effect of mannitol in increasing reducing sugar and total sugars as well as α-amylase activity in primed seeds (Afzal *et al.*, 2011b).

Giri and Schillinger (2003) concluded that soaking of wheat seeds in water for 12 hours causes improvements in the germination process. In another study conducted by Kaya *et al.* (2006) considering hydro-priming effects on sunflower seeds, the results indicated that it accelerates the germination process in dry conditions and shortens the germination period. Tajbakhsh *et al.* (2004) investigated different treating methods on onion and the obtained results indicated that hydro-priming in high humidity leads to shortening the average germination time.

Table 1. Effect of different concentrations of mannitol and water on mean germination time

Treatments	Mean germination time (days)	
	BARI Mung 6	Binamoog 5
T0	4.594 a	4.568 a
T1	4.542 ab	4.534 ab
T2	4.484 bc	4.500 ab
T3	4.476 bc	4.466 b
T4	4.454 c	4.516 ab
T5	4.496 bc	4.526 ab
LSD (0.05)	0.0715	0.0714
CV (%)	1.27%	1.18%

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.2.3 Effect on germination index

Germination index was gradually increased with increasing mannitol concentration up to 6% and 4% for BARI Mung 6 and Binamoog 5 respectively and then decreased with increasing mannitol concentration (Figure 2). Highest germination index (80.70) was observed in T3 treatment for Binamoog 5 compare to germination index (80.40) was observed from T4 treatment for BARI Mung 6. The lowest germination index (61.95) and (63.76) was recorded in T0 treatment for BARI Mung 6 and Binamoog 5 respectively. Germination index of Binamoog 5 was slightly higher than BARI Mung 6. Asaduzzaman, (2014) found significant difference in germination index for control and different priming treatments in BARI Mung 6 and BU-4. 2% and 6% mannitol exposed better result in germination index for BARI mung-3 and BARI Mung 6 respectively (Rahman, 2014). Hosseein *et al.* (2011) reported that seed priming resulted in anti-oxidant increment as glutathione and ascorbate in seed. These enzymes led to higher germination index via reduction of lipid peroxidation activity.

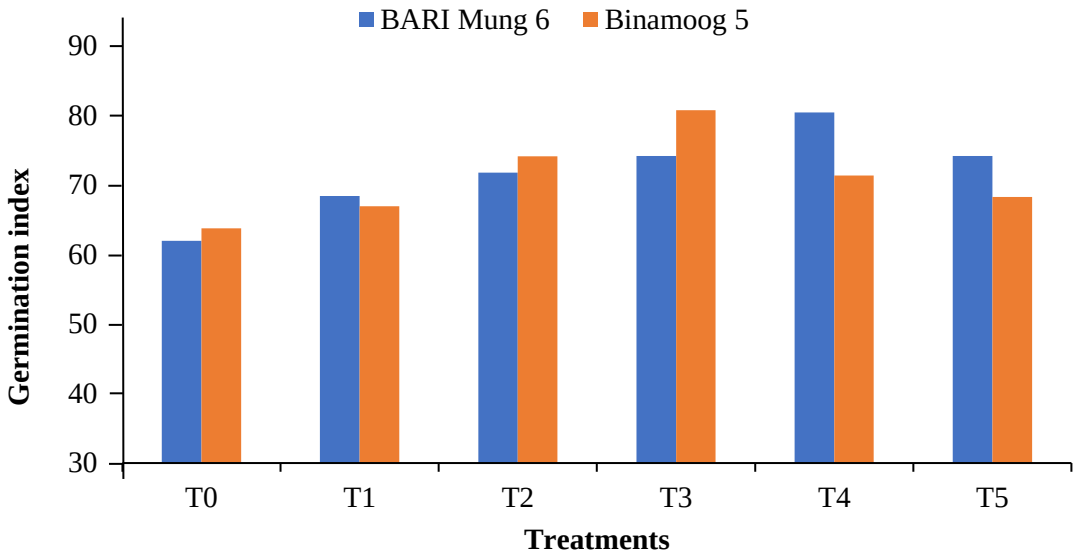

Figure 2. Effect of different concentrations of mannitol and water on germination index (LSD $_{(0.05)}$ = 2.676 and 3.372 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.4 Effect on coefficient of velocity

In both varieties, analysis of variance showed significant difference for coefficient of velocity among the treatments priming with different concentrations of mannitol, water and control (Table 2). Coefficient of velocity increased gradually up to 6% and 4% of mannitol concentration for variety BARI Mung 6 and Binamoog 5 respectively then decreased gradually. The maximum coefficient of velocity (22.46) was founded at T4 (statistically similar with T2, T3 and T4 treatment) treatment for BARI Mung 6 compared to coefficient of velocity (22.15) was founded at T3 (statistically similar with T1, T2, T4 and T5 treatment) treatment for Binamoog 5. The minimum coefficient of velocity (21.77) and (21.90) was found in T0 for BARI Mung 6 and Binamoog 5, respectively. Nyarko *et al.* (2006), reported that cabbage seeds primed and expose to vernalization temperature (0°- 5° C) for 8 weeks had a higher CV than non-primed seeds. In primed leek seeds, the significant benefit in germination performance was accompanied by marked increases in protein, DNA and

nucleotide biosynthesis. Germination and coefficient of velocity was improved in treated fenugreek seeds may be resulted by increase of cell division in the seeds (Gallais *et al.*, 2000).

Table 2. Effect of different concentrations of mannitol and water on coefficient of velocity

Treatments	Coefficient of velocity	
	BARI Mung 6	Binamoog 5
T0	21.77 c	21.90 b
T1	22.03 bc	22.05 ab
T2	22.30 ab	22.24 ab
T3	22.35 ab	22.39 a
T4	22.46 a	22.15 ab
T5	22.24 ab	22.10 ab
LSD (0.05)	0.3551	0.3429
CV (%)	1.22%	1.18%

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.5 Effect on energy of emergence (%)

Energy of emergence was significantly affected by different concentration of mannitol, water and control priming (Figure 3). Energy of emergence was increased with increasing mannitol concentration up to T4 and T3 for BARI Mung 6 and Binamoog 5 respectively and then decreased with increasing mannitol concentration. The highest energy of emergence (98.00%) was recorded in T3 (statistically similar with T3 treatment) treatment of Binamoog 5 compare to energy of emergence (96.66 %) was recorded in T4 (statistically similar with T5 treatment) treatment of BARI Mung 6. The lowest energy of emergence (84.00%) and (84.66%) was recorded in T0 treatment for BARI Mung 6 and Binamoog 5 respectively.

Faster emergence and reduced germination on imbibition periods improved phenology in mungbean due to primed seed (Harris *et al*., 1999). It has been

experimented that priming resulted in more germination speed especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Sivritepe *et al*., 2003; Kaya *et al*., 2006; Foti *et al*., 2002). Soybean seed are made better seedling emergence and yield improvement (Arif *et al*., 2008) through seed priming.

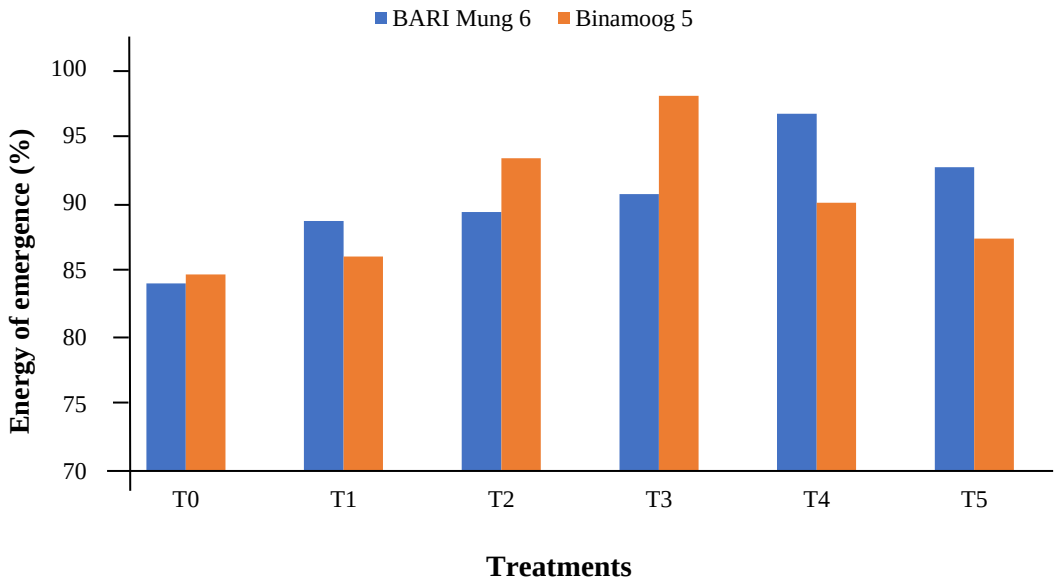

Figure 3. Effect of different concentrations of mannitol and water on energy of emergence (LSD $_{(0.05)}$ = 4.733 and 5.024 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.6 Effect on shoot length (mm)

Significant difference was recorded on shoot length among the treatments priming with different concentrations of mannitol and water (Figure 4). Shoot length was increased up to T4 and T3 treatments for BARI Mung 6 and Binamoog 5, respectively and therefore decreased gradually. The maximum shoot length (153.8 mm) was noticed at T4 treatment for BARI Mung 6 compare to shoot length of Binamoog 5 (148.9 mm) was noticed at T3 treatment. Shoot of BARI Mung 6 was larger than Binamoog 5. The minimum shoot length (113.3 mm) and (110.9 mm) was observed in T0 treatment for BARI Mung 6 and

Binamoog 5, respectively. Kaur *et al.* (2002) conducted study to determine the effect of seed priming on the growth and yield of chickpea. They reported that shoot length and shoot biomass of water and mannitol primed plants were greater compared to these from non-primed plants. Kaur *et al.* (2003) also reported that priming of chickpea seed with 4% mannitol was effective in the enhancement of shoot growth. Kumar *et al.* (2017) experimented on chickpea that shoot length has recorded high in case of osmo-primed seeds than that of unprimed seeds. Among different osmo-priming with PEG 20% found to be highest followed by mannitol 4% and control found to be lowest among the treatments.

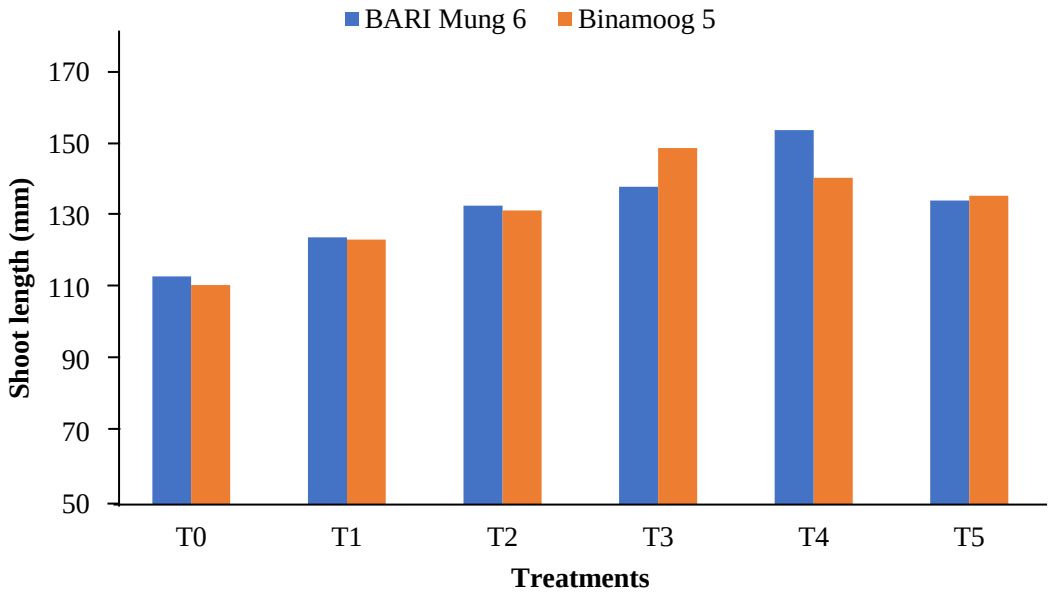

Figure 4. Effect of different concentrations of mannitol and water on shoot length (LSD $_{(0.05)}$ = 7.492 and for 6.363 BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.7 Effect on root length (mm)

Highly significant variation was noticed on root length (Figure 5). Root length was improved up to 6% and 4% of mannitol concentration for BARI Mung 6 and Binamoog 5, respectively and therefore decreased gradually with increasing mannitol concentration. The maximum root length (92.98 mm) was noticed at T4 treatment for BARI Mung 6 compare to root length (75.45 mm) was noticed at T3 treatment for Binamoog 5. Root length of BARI Mung 6 was larger than Binamoog 5. The minimum root length (62.64 mm) for BARI Mung 6 and (47.63 mm) for Binamoog 5 was recorded in T0 treatment.

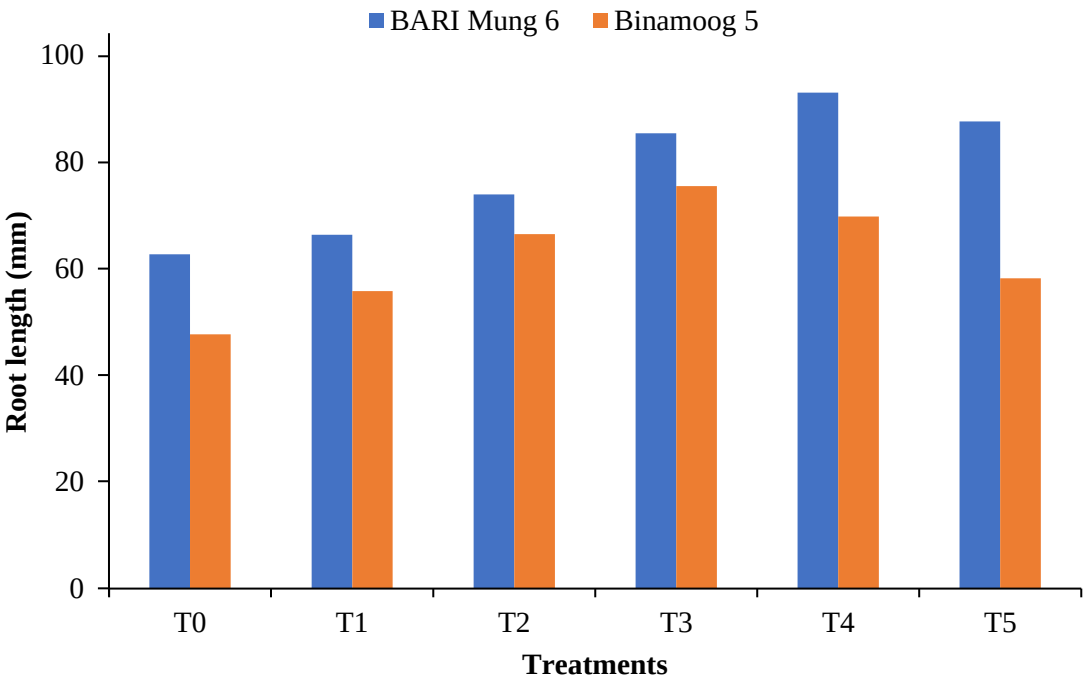

Figure 5. Effect of different concentrations of mannitol and water on root length (LSD $_{(0.05)}$ = 5.305 and 4.102 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

Kaur *et al.* (2003) conducted study to determine the effect of seed priming with mannitol (4%), water and potassium nitrate on chickpea. In general priming with water and mannitol resulted in early germination under salt stress. Priming with 4% mannitol was also as effective as mannitol and water in the enhancement of

root and shoot growth. Singh *et al.* (2017) reported that a significant effect of different hydropriming and osmo-priming on root length of pea. A 20% polyethylene glycol for 24 hr (14.11 cm) priming showed better performance over untreated (11.98 cm), 3% mannitol for 12 hr (12.37 cm), 3% mannitol for 24 hr (12.67 cm) and 5% glycerol for 12 hr (12.99 cm) priming.

4.1.8 Effect on seedling dry weight (mg)

Seedling dry weight was significantly influenced by different mannitol concentration, water and control priming (Figure 6). Seedling dry weight was increased up to T4 and T3 treatment for BARI Mung 6 and Binamoog 5, respectively and then decreased gradually. Results showed that the highest seedling dry weight (90.49 mg) was noticed in T4 treatment for BARI Mung 6 compare to seedling dry weight (82.94 mg) was noticed in T3 treatment for Binamoog 5. Seedling dry weight of BARI Mung 6 was higher than Binamoog 5. The lowest seedling dry weight (70.69 mg) and (65.91 mg) were recorded in T0 treatment for BARI Mung 6 and Binamoog 5, respectively. This result was supported by the findings of previous many scientists practiced in different crops on mannitol priming (Nighat *et al*., 2006; Nishimura *et al*., 2011; Hoekstra *et al*., 2001). Pill and Necker (2001) also suggested that primed seeds compared to non-primed seeds showed higher seedling dry weights. Kumar *et al.* (2017) experimented on chickpea and found that in case of seedling dry weight it was higher (1.02 gm to 1.59mg) in PEG 20% seeds followed by Mannitol 4% when compared with control. Singh *et al.* (2017) also reported that 20% polyethylene glycol for 24 hr (0.54 g) priming showed significant effect over untreated (0.40 g), 3% mannitol for 12 hr (0.44 g), 3% mannitol for 24 hr (0.43 g) and 5% glycerol for 12 hr (0.48 g) priming on seedling dry weight.

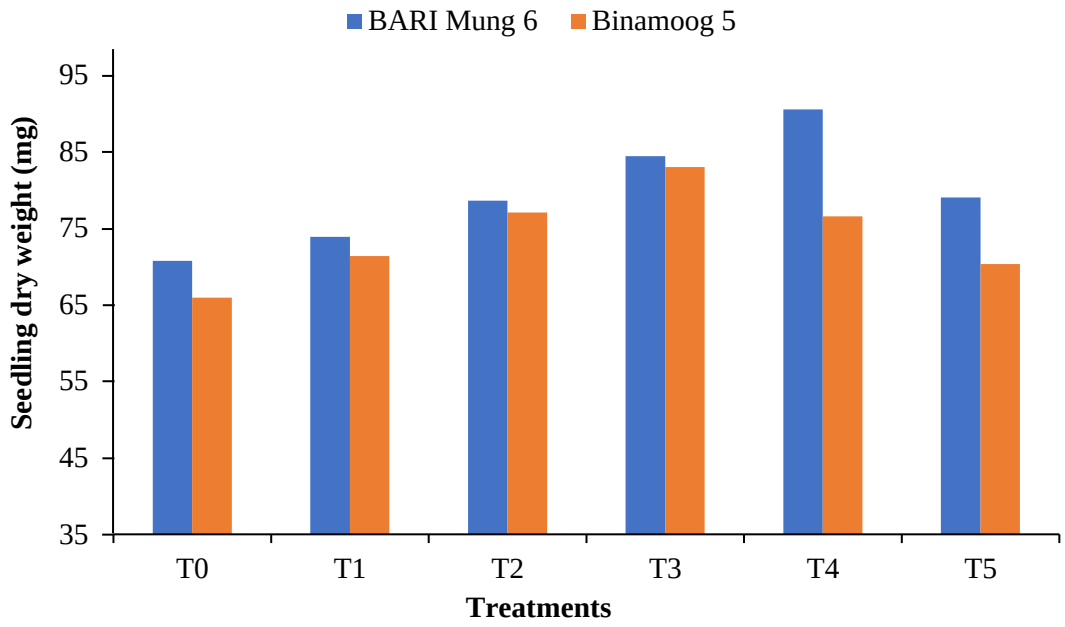

Figure 6. Effect of different concentrations of mannitol and water on seedling dry weight (LSD $_{(0.05)}$ = 1.546 and 2.032 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.9 Effect on vigour index

Vigour index of BARI Mung 6 and Binamoog 5 was significantly varied by different mannitol concentration, water and control priming (Figure 7). An increasing tendency of vigour index was observed up to 6% and 4% of mannitol concentration for BARI Mung 6 and Binamoog 5, respectively and thereafter decreased gradually with the increasing mannitol concentration. The highest vigour index (235.3) was achieved from T4 treatment for BARI Mung 6 compare to vigour index (216.8) was achieved from T3 treatment for Binamoog 5. Vigour index of BARI Mung 6 was higher than Binamoog 5. The lowest vigour index (144.1) and (132.2) was achieved from BARI Mung 6 and Binamoog 5, respectively in T0 treatment. This result was supported by previous findings of Baque *et al*. (2016) and Maiti *et al.* (2013). Maiti *et al.* (2013) also acknowledged that osmo-priming increased seedling vigor of several vegetable crops and concerning sponge gourd. According to Safiatou (2012) seedling vigour

increased by using seed priming methods in sorghum and Bambara groundnut. Also, highest seedling vigour was achieved by osmo-priming (mannitol priming) in Bambara groundnut and by hydro-priming in sorghum. Priming improved seedling vigour. Nascimento and West (1998) reported that, priming enhances the minimization of seed coat adherence during the emergence of muskmelon seeds. During osmotic priming, the reserve mobilization of food material, activation and re-synthesis of some necessary enzymes, DNA and RNA synthesis started and those might be improved of germination and vigor of soybean (Sadeghi *et al.*, 2011).

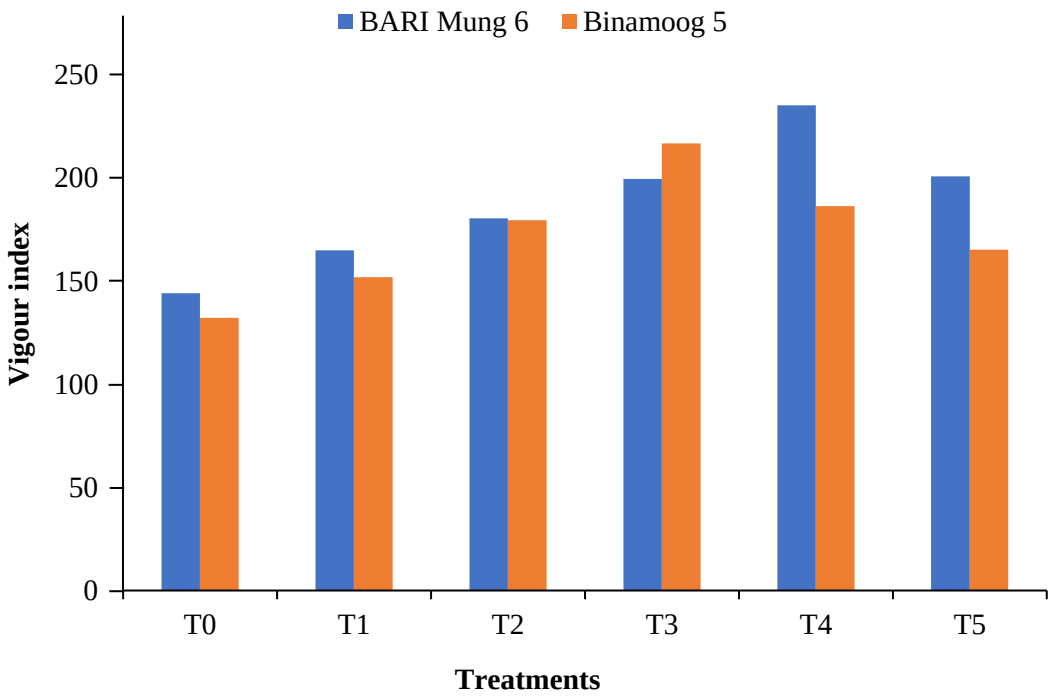

Figure 7. Effect of different concentrations of mannitol and water on vigour index (LSD $_{(0.05)}$ = 10.80 and 11.45 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.10 Effect on relative water content (%)

Relative water content was exposed significant variation by priming with different concentrations of mannitol, water and control treatments in BARI Mung 6 & Binamoog 5 (Table 3). Relative water content was increased up to 6% and 4% of mannitol concentration for BARI Mung 6 and Binamoog 5, respectively and therefore decreased gradually with increasing mannitol concentration. The maximum relative water content (90.47%) was found at T4 treatment for BARI Mung 6 compared to relative water content (88.56%) was found at T3 treatment for Binamoog 5. Relative water content of BARI Mung 6 was higher than Binamoog 5. The minimum relative water content (75.74%) and (74.57%) was recorded in T0 treatment for BARI Mung 6 and Binamoog 5, respectively. This result was supported by previous findings of Rahman (2014). He noticed significant difference on relative water content for mannitol, water and control priming treatments in mungbean. Baghizadeh and Hajmohammadrezaei (2011) showed that soaking seeds in mannitol and salicylic acid solution caused a significant increase in the concentrations of phosphorus and protein in different stages of seed germination and seed protein concentration in the harvest, thus in wheat enriched with higher doses of K, showed the maximum relative water content, higher water retention capacity and exudation rate. Water saturation deficit highly reduced with higher level of K. Fertilizer potassium however, made leaf water potential more negative. The beneficial effect of potassium on water stress tolerance in wheat plants were more noticeable under water stressed conditions than under control conditions (Baque *et al.,* 2002).

Table 3. Effect of different concentrations of mannitol and water on relative water content

Treatments	Relative water content (%)	
	BARI Mung 6	Binamoog 5
T0	75.74 f	74.57 f
T1	78.69 e	78.63 e
T2	82.69 d	83.73 c
T3	86.49 b	88.56 a
T4	90.47 a	85.56 b
T5	84.76 c	82.48 d
LSD (0.05)	1.077	1.161
CV (%)	0.99%	1.08%

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.11 Effect on water saturation deficit (%)

Seedling water saturation deficit was significantly affected by different mannitol concentration, water and control priming (Figure 8). Water saturation deficit was gradually decreased with increasing mannitol concentration up to T4 and T3 for BARI Mung 6 and Binamoog 5 respectively and then increased with the increasing mannitol concentration. The highest water saturation deficit (24.26%) of Binamoog 5 compare to water saturation deficit (25.43%) of BARI Mung 6 was found in T0 treatment. The lowest water saturation deficit (9.53%) and (11.44%) for BARI Mung 6 and Binamoog 5 was found in T4 and T3 treatment respectively. This result also in agreement with the findings of previous researchers in field crops like mungbean (Asaduzzaman, 2014; Rahman, 2014), wheat (Faijunnahar *et al.,* 2017). Baque *et al.* (2002) reported that generally higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. Water saturation deficit highly reduced with higher level of K. Potassium fertilizer also

made the leaf water potential more negative. Under drought stressed conditions the beneficial effect of potassium on drought stress was more noticeable than under control conditions.

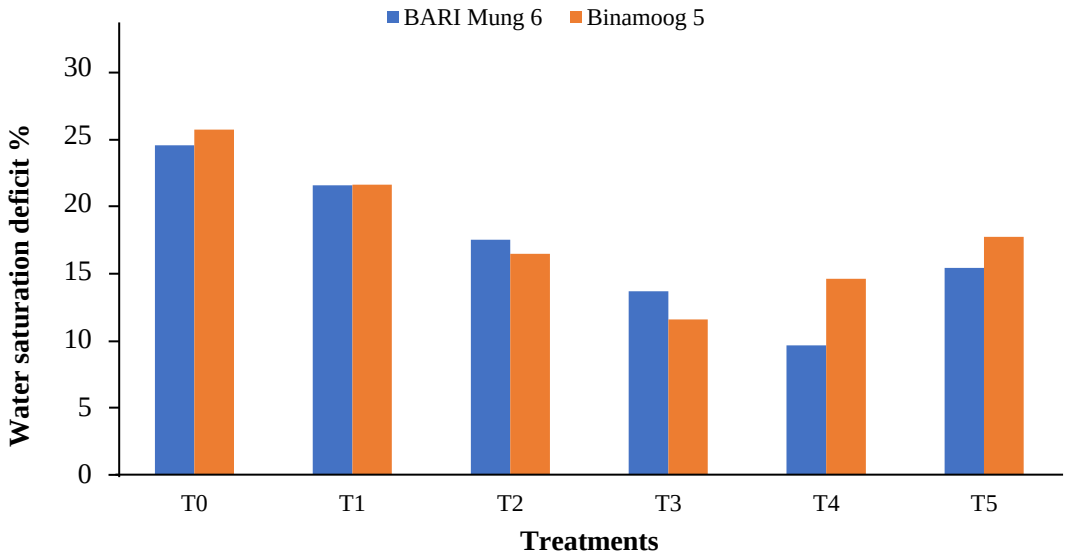

Figure 8. Effect of different concentrations of mannitol and water on water saturation deficit (LSD $_{(0.05)}$ = 1.077 and 1.161 for BARI Mung 6 and Binamoog 5 respectively).

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.1.12 Effect on water retention capacity

Water retention capacity of BARI Mung 6 and Binamoog 5 was significantly varied by different mannitol concentration, water and control priming (Table 4). Water retention capacity was increased up to 6% and 4% of mannitol concentration for BARI Mung 6 and Binamoog 5, respectively and then decreased slightly. The maximum water retention capacity (20.70) was observed in T4 treatment for BARI Mung 6 compare to water retention capacity (19.70) was observed in T3 treatment for Binamoog 5. Water retention capacity of BARI Mung 6 was higher than Binamoog 5. The minimum water retention capacity (16.47) was noticed for BARI Mung 6 and (15.87) was noticed for Binamoog 5 in T0 treatment. This study was in agreement with the findings of Faijunnahar *et al.* (2017); Baque *et al.* (2016) and Rahman (2014). Faijunnahar *et al.* (2017)

also said that priming helps to activate the metabolic enzymes responsible for germination of seed before germination takes place, so the hydro and osmo-primed seedlings can uptake more water than the non-primed ones and obtained the maximum turgid weight, in consequence, they obtained the maximum water retention capacity. Generally higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. Water saturation deficit highly reduced with higher level of K. Potassium fertilizer also made the leaf water potential more negative. Under drought stressed conditions the beneficial effect of potassium on drought stress was more noticeable than under control conditions (Baque *et al.,* 2002).

Table 4. Effect of different concentrations of mannitol and water on water retention capacity

Treatments	Water retention capacity	
	BARI Mung 6	Binamoog 5
T0	16.47 d	15.87 c
T1	17.27 cd	16.61 c
T2	18.41 bc	17.97 b
T3	19.37 ab	19.70 a
T4	20.70 a	18.19 b
T5	18.19 bcd	16.99 bc
LSD (0.05)	1.828	1.327
CV (%)	7.61%	5.79%

T_0 = Seeds without priming (control); T_1 = Seeds primed with distilled water for 9 hours; T_2 = Seeds primed with 2% mannitol for 9 hours, T_3 = Seeds primed with 4% mannitol for 9 hours; T_4 = Seeds primed with 6% mannitol for 9 hours, T_5 = Seeds primed with 8% mannitol for 9 hours.

4.2 Second experiment

Study on the pre-sowing priming time on the germination behavior of mungbean

Results obtained from the present study regarding the pre-sowing priming time on the germination behavior of mungbean have been demonstrated, discussed and compared in this section. The analytical results have been displayed in Figures 9 to 12 and Tables 5 to 12.

4.2.1 Effect on germination percentage

Germination percentage was significantly influenced by different priming (control, water and mannitol) time. Germination percentage gradually increased up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and therefore decreased slowly (Figures 9). In BARI Mung 6, highest germination percentage (96.66%) was observed form T8 treatment which was statistically similar with T3 (92.66%). Lowest germination percentage was recorded from T0 (82.00%) which was statistically similar with T1, T5, T6 and T10. In Binamoog 5, highest germination percentage (96.66%) was observed at T6 treatment which was statistically similar with T2 (92.00%). Lowest germination percentage was recorded from T5 (80.66%) which was statistically similar with T0, T1, T3, T4, T9 and T10. This result was in agreement with the previous findings of Baque *et al.* (2016); Yagmur and Kaydan (2008); Afzal (2005); Afzal *et al.* (2005); Demir and Ermis (2003) and Roy and Srivastava (2000). According to Basra *et al.* (2003) and Salinas (1996) seed priming techniques enhanced the germination percent, emergence and seedling standby. Dastanpoor *et al.* (2013) reported that, *Salvia officinalis* L. expressed final germination percentage (FGP) 25.5% enhanced by hydropriming (12 hr at 30°C) that of non-primed seeds. According to Moradi *et al.* (2012) lower priming duration (i.e., 12 and 24 hr) enhanced germination in normal condition, while in drought stress a higher priming duration (i.e., 36 and 48 hr) provided more protection. Abnavi and Ghobadi (2012); Lemrasky and Hosseini (2012); Giri and Schillinger (2003) also observed that wheat seed priming with water for 12 hr achieved better than non-

primed seed. Whereas Hamidreza *et al.* (2013); Yari *et al.* (2010) concluded that, hydro priming duration from 6 to 12 h expressed better result in germination percentage of wheat over hydro priming duration from 18 to 24 hr.

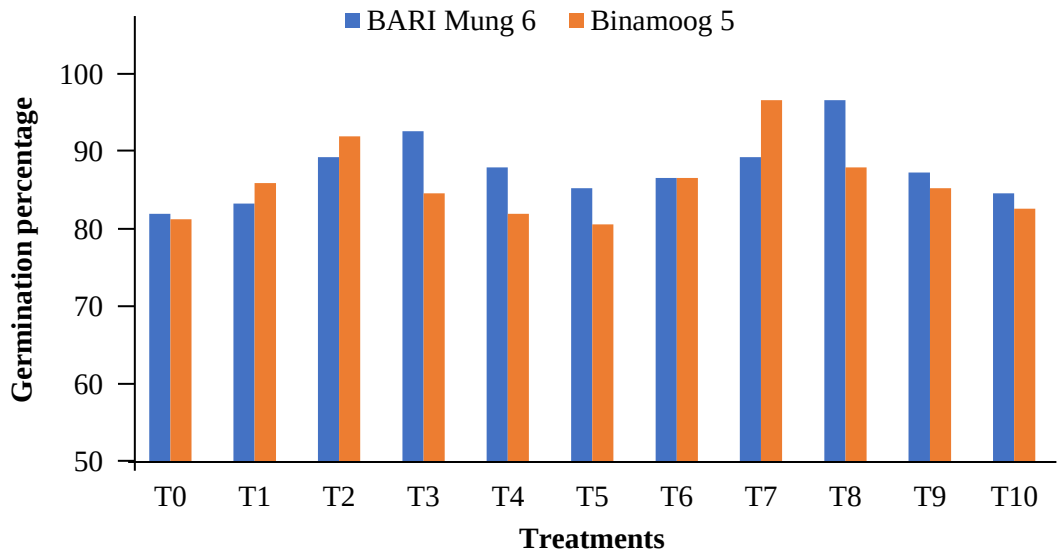

Figure 9. Effect of different priming (mannitol and water) time on germination percentage (LSD $_{(0.05)}$ = 4.706 and 4.861 for BARI Mung 6 and Binamoog 5 respectively).

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.2 Effect on mean germination time (days)

Mean germination time was significantly varied by different priming (control, water and mannitol) time (Figure 10). Mean germination time decreased up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and therefore increased gradually. In BARI Mung 6, maximum mean germination time (4.618 days) was found form T0 treatment and minimum germination percentage was found from T8 (4.438) which was statistically similar with T2, T3, T7 and T9. In Binamoog 5, maximum mean germination time (4.588 days) was recorded from T0 treatment and lowest germination percentage was recorded from T7 (4.462). This result also in agreement with the findings of previous researcher

(Asaduzzaman, 2014). Harris *et al.* (1999) reported that early emergence and maturity in seed priming treatment could be due to advancement in metabolic state. Musa *et al.* (1999) also concluded that priming improve plant stand and provide benefits in term of maturity. Seed priming resulted in earlier emergence of seedlings by 1-3 days and significantly increased plant stand and initial growth vigour. Priming influenced the MGT compared with control seeds at all of the germination temperatures. In generally, lentil seeds primed for 24 hr reduced hours required reaching 50% germination compared with the seeds primed for 12 hr. The interaction between priming and time was significant for MGT and the least values were obtained from water treatment compared with other priming and control treatments in both priming times (Yucel, 2012). Afzal *et al.* (2004) also said that 24 hr osmo-priming with jute mat reduced the time to 50% emergence and mean emergence time.

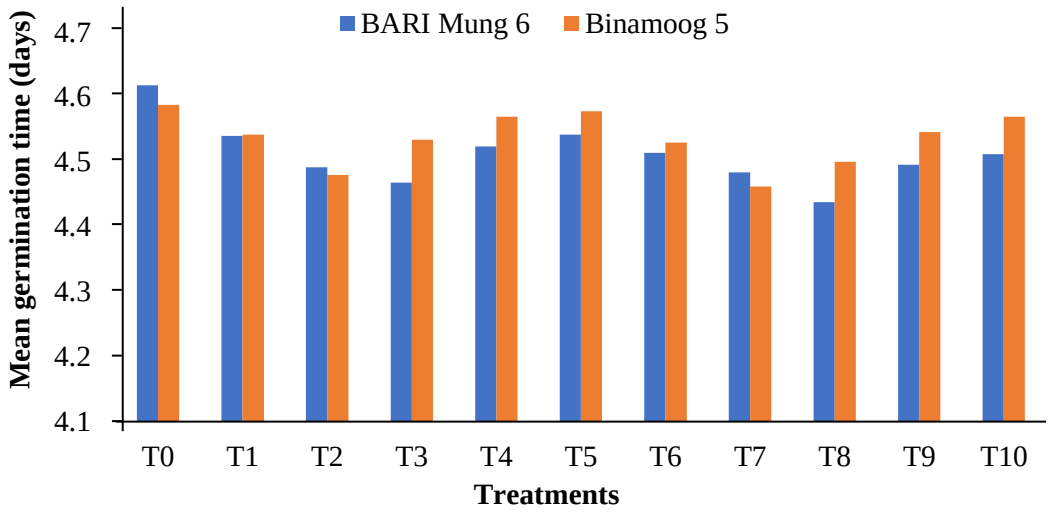

Figure 10. Effect of different priming (mannitol and water) time on mean germination time (LSD $_{(0.05)}$ = 0.06981 and 0.08061 for BARI Mung 6 and Binamoog 5 respectively).

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.3 Effect on germination index

Significant difference was observed in germination index for both BARI Mung 6 and Binamoog 5 (Table 5). Germination index was increased with the increasing of priming time up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and therefore decreased with the increasing of priming time. In BARI Mung 6, highest germination index (79.90) was achieved form T8 treatment which was statistically similar with T3 (77.67) treatment. Lowest germination index was achieved from T0 (59.87). In Binamoog 5, highest germination index (80.07) was calculated from T7 treatment and lowest germination index was calculated from T0 (61.46) which was statistically similar with T4, T5, and T10. Overall vermination index was higher in Binamoog 5 than BARI Mung 6.

Table 5. Effect of different priming (mannitol and water) time on germination index

Treatments	Germination index	
	BARI Mung 6	Binamoog 5
T0	59.87 g	61.46 f
T1	65.59 f	67.91 de
T2	72.32 bc	76.60 b
T3	77.67 a	66.47 e
T4	69.97 de	62.95 f
T5	67.90 ef	62.61 f
T6	70.05 cde	69.45 cd
T7	73.81 b	80.07 a
T8	79.90 a	71.34 c
T9	71.36 cd	66.51 e
T10	66.58 f	63.35 f
LSD (0.05)	2.329	2.701
CV (%)	2.59%	3.11%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

This study was in agreement with the findings of Asaduzzaman (2014). Time duration of water uptake in hydro and osmo-priming plays a vital role in increasing germination index in primed seeds as compared with non-primed seeds. In other words, priming period water uptake rate is slow and seeds get enough time to complete the pre-germination process (Varier *et al.*, 2010). Kumar *et al.* (2017) proposed that osmo-priming increased the germination speed significantly in PEG 20% of 12 hr seeds followed by Mannitol 4% of 12 hr when compared with control.

4.2.4 Effect on coefficient of velocity

In both varieties, analysis of variance showed significant difference in coefficient of velocity (Figure 11). Coefficient of velocity increased up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and therefore decreased with the increasing of priming time. Maximum coefficient of velocity (22.52) was found at T8 treatment which was statistically similar with T2, T3, T7 and T9 treatment and minimum result was found at T0 (21.65) treatment for BARI Mung 6. In Binamoog 5, maximum coefficient of velocity (22.41) was calculated from T7 treatment which was statistically similar with T2, T3, T6 and T8 treatment and lowest germination index (21.79) was calculated from T0 treatment which was statistically similar with T1, T3, T4, T5, T6, T9 and T10. BARI Mung 6 showed higher coefficient of velocity than Binamoog 5.

This study was in agreement with the findings of Asaduzzaman (2014). Tavili *et al*. (2011) reported that speed of germination of Bromus increased with seed priming treatments rather than that of control. Similarly, Elkoca *et al*. (2007) determined that hydro priming treatment in chickpea induced faster and more synchronous germination compared with the unprimed seeds thus increase co efficient of velocity.

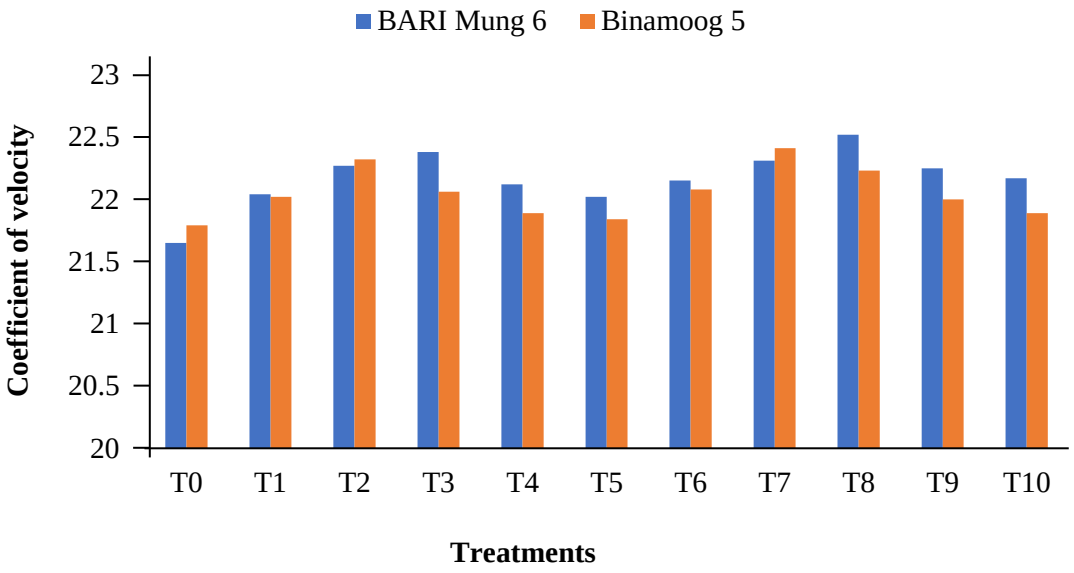

Figure 11. Effect of different priming (mannitol and water) time on coefficient of velocity (LSD $_{(0.05)}$ = 0.3372 and 0.3716 for BARI Mung 6 and Binamoog 5 respectively).

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.5 Effect on energy of emergence (%)

Different priming (mannitol, water and control) time showed significant variation in energy of emergence (Table 6). Energy of emergence increased with the increasing of priming time up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased with the increasing of priming time. In BARI Mung 6, higher coefficient of velocity (97.33) was noticed at T8 treatment which was statistically similar with T3 (93.33) and lower coefficient of velocity was noticed at T0 (84.00) was statistically similar with T1, T5, T6, T9 and T10. In Binamoog 5, higher coefficient of velocity (97.33) was noticed at T7 treatment and lower coefficient of velocity was noticed at T0 (83.33) which was statistically similar with T1, T3, T4, T5, T9 and T10. This study was in agreement with the findings of Asaduzzaman (2014). Assefa and Hunje (2011) reported that the speed of germination and emergence in soybean increased as the priming duration increased from 0 to 14 hours. The germination decreased

with increased priming duration beyond 14 hours. In the early stage of germination seeds of a wide variety of plants can be dried back to 10 per cent moisture without loss of viability, but if they are dried after radical emergence (as the duration increases) the seeds are not able to germinate.

Table 6. Effect of different priming (mannitol and water) time on energy of emergence

Treatments	Energy of emergence (%)	
	BARI Mung 6	Binamoog 5
T0	84.00 e	83.33 de
T1	84.66 e	86.66 cde
T2	89.33 bcd	92.66 b
T3	93.33 ab	85.33 cde
T4	89.33 bcd	82.66 e
T5	87.33 cde	82.66 e
T6	88.00 cde	87.33 cd
T7	90.66 bc	97.33 a
T8	97.33 a	88.66 bc
T9	88.00 cde	86.66 cde
T10	85.33 de	84.00 de
LSD (0.05)	4.419	4.306
CV (%)	3.90%	3.88%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.6 Effect on shoot length (mm)

Different priming time had a significant adverse effect on shoot length for mannitol primed, water primed and non-primed seeds (Table 7). An increasing trend was observed in shoot length up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased gradually. In BARI Mung 6, bigger shoot length (152.8 mm) was achieved from T8 treatment and smaller shoot length was achieved from T0 (111.7 mm). In Binamoog 5, bigger shoot

length (148.8 mm) was achieved at T7 treatment and smaller shoot length was achieved from T0 (109.9 mm).

Table 7. Effect of different priming (mannitol and water) time on shoot length under

Treatments	Shoot length (mm)	
	BARI Mung 6	Binamoog 5
T0	111.7 f	109.9 g
T1	122.3 e	120.5 f
T2	130.3 d	134.4 b
T3	138.8 bc	127.0 cd
T4	130.6 d	125.6 cde
T5	127.8 de	122.1 ef
T6	133.3 cd	128.5 c
T7	142.9 b	148.8 a
T8	152.8 a	135.4 b
T9	142.8 b	127.5 c
T10	138.6 bc	123.6 def
LSD (0.05)	6.940	3.823
CV (%)	4.07%	2.35%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

This result was in agreement with the findings of Faijunnahar *et al.* (2017); Baque *et al.* (2016); Dastanpoor *et al.* (2013); Yari *et al.* (2010) and Moghanibashi *et al.* (2012). According to Faijunnahar *et al.* (2017) priming time might help to augmented enzymatic activities of seed which trigger the vigorous plant growth and in significantly increased the shoot length of wheat; on the other hand, higher priming time could facilitate the ageing of seed that can be resulted lowering the potentiality for better germination, growth and development of seedling. Mannitol and water primed seed expressed higher shoot length because of higher respiration and cell division where in non-primed seeds lower

respiration and cell division shorten shoot length (Daniel *et al.*, 2009). Kumar *et al*. (2002) reported that 8 hours priming of finger millet seeds in water resulted in an increased mean plant height by 9 cm.

4.2.7 Effect on root length (mm)

Root length of BARI Mung 6 and Binamoog 5 significantly affected by the different priming (mannitol, water and control) time. Root length increased up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased with the increasing of priming time (Table 8). In BARI Mung 6, greater root length (92.41 mm) was observed from T8 treatment and shorter root length was observed from T0 (61.79 mm) which was statistically similar with T5 (64.22 mm). In Binamoog 5, greater root length (71.23 mm) was observed at T7 treatment and shorter root length was observed from T0 (109.9 mm) which was statistically similar with T5 and T10. This result was in agreement with the findings of Faijunnahar *et al.* (2017); Baque *et al.* (2016); Dastanpoor *et al.* (2013); Yari *et al.* (2010) and Moghanibashi *et al.* (2012). Murray (1989), who concluded that over priming may cause oxygen deficiency and the build-up of inhibitors. The findings of this study suggested that priming duration of 12 h was generally safer for pea. According to Baque *et al.* (2016) increasing of seed soaking duration(12-24h) improved root length of wheat. Arif *et al*. (2008) conducted a field experiment in Peshawar, Pakistan and they reported that priming improved the seed establishment in soybean which might be due to the completion of pre-germination metabolic activities earlier which makes the seed ready for radical protrusion thus increase root length. Faijunnahar *et al.* (2017) also reported that the maximum root length was recorded when the seed primed with 10% PEG solution for 12 hr.

Table 8. Effect of different priming (mannitol and water) time on root length under

Treatments	Root length (mm)	
	BARI Mung 6	Binamoog 5
T0	61.79 e	47.24 h
T1	54.59 f	51.40 fg
T2	71.77 d	60.38 b
T3	81.34 b	55.85 de
T4	70.97 d	52.04 fg
T5	64.22 e	49.24 gh
T6	73.05 cd	56.44 cd
T7	82.67 b	71.23 a
T8	92.41 a	59.37 bc
T9	76.16 c	52.74 ef
T10	69.99 d	49.24 gh
LSD (0.05)	4.128	3.486
CV (%)	4.46%	4.97%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.8 Effect on seedling dry weight (mg)

Different priming time had a significant positive effect on seedling dry weight for mannitol primed, water primed and non-primed seeds (Table 9). An increasing trend was observed in seedling dry weight up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased gradually. In BARI Mung 6, maximum seedling dry weight (92.41 mg) was recorded from T8 treatment and minimum seedling dry weight was recorded from T0 (61.79 mg) was statistically similar with T5. In Binamoog 5, maximum seedling dry weight (71.23 mg) was recorded from T7 treatment and minimum seedling dry weight was recorded from T0 (47.24 mg) which was statistically similar with T5 and T10.

Table 9. Effect of different priming (mannitol and water) time on seedling dry weight

Treatments	Seedling dry weight (mg)	
	BARI Mung 6	Binamoog 5
T0	65.03 g	60.49 j
T1	70.46 f	68.61 g
T2	76.57 d	77.73 c
T3	85.02 b	71.54 f
T4	80.30 c	66.63 h
T5	76.78 d	63.79 i
T6	73.49 e	73.49 e
T7	84.05 b	88.71 a
T8	92.19 a	82.48 b
T9	85.16 b	74.85 d
T10	77.70 cd	68.65 g
LSD (0.05)	2.818	1.046
CV (%)	2.81%	1.13%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

From current findings; dry weight 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). Osmo-priming at 6 hr seed treatment significantly affected shoot dry weight had highest shoot dry weight of wheat (Hamidreza *et al.*, 2013). But Moghanibashi *et al.* (2012) revealed that, 24 hr hydropriming increased shoot dry weight of sunflower as compared to non-primed seed. According to Faijunnahar *et al.* (2017) enzymatic activities of seed increased with increasing optimum time, which generate the proliferous root growth and in significantly increased the root dry weight of wheat. Over priming time facilitate the ageing of seed which caused loose the potentiality for better germination, growth and development of seedling.

4.2.9 Effect on vigour index

Vigour index of BARI Mung 6 and Binamoog 5 significantly varied by the different priming (mannitol, water and control) time. Vigour index increased up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased with the increasing of priming time (Table 10). In BARI Mung 6, greater seedling virour index (237.2) was found at T8 treatment and lower vigour index was found at T0 (142.3) was statistically similar with T1. In Binamoog 5, greater seedling virour index (212.8) was found at T7 treatment and minimum seedling dry weight was found at T0 (127.9) which was statistically similar with T5.

Table 10. Effect of different priming (mannitol and water) time on vigour index

Treatments	Vigour index	
	BARI Mung 6	Binamoog 5
T0	142.3 f	127.9 f
T1	147.3 f	147.8 de
T2	180.5 d	179.3 b
T3	204.0 b	154.8 cd
T4	177.1 d	145.7 de
T5	163.8 e	138.1 ef
T6	178.8 d	160.3 c
T7	201.5 bc	212.8 a
T8	237.2 a	171.4 b
T9	191.1 c	153.8 cd
T10	176.3 d	142.8 e
LSD (0.05)	10.41	10.77
CV (%)	4.49%	5.36%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

Seedling vigour increased by using seed priming methods in sorghum and Bambara groundnut. Also, highest seedling vigour was achieved by osmo-priming (Mannitol priming) in Bambara groundnut and by hydro-priming in sorghum (Safiatou, 2012). The probable reason for the highest vigour index might be due to photosynthetic capacity treated with bio fertilizers increases due to increased supply of nutrition found from priming treatment (Farnia and Shafie, 2015).

4.2.10 Effect on relative water content (%)

Different priming time had a significant influential effect on relative water content up to 9 hr and 6 hr priming for BARI Mung 6 and Binamoog 5 respectively and then decreased gradually (Figure 12). Relative water content of seedling valued significand difference among the treatments.

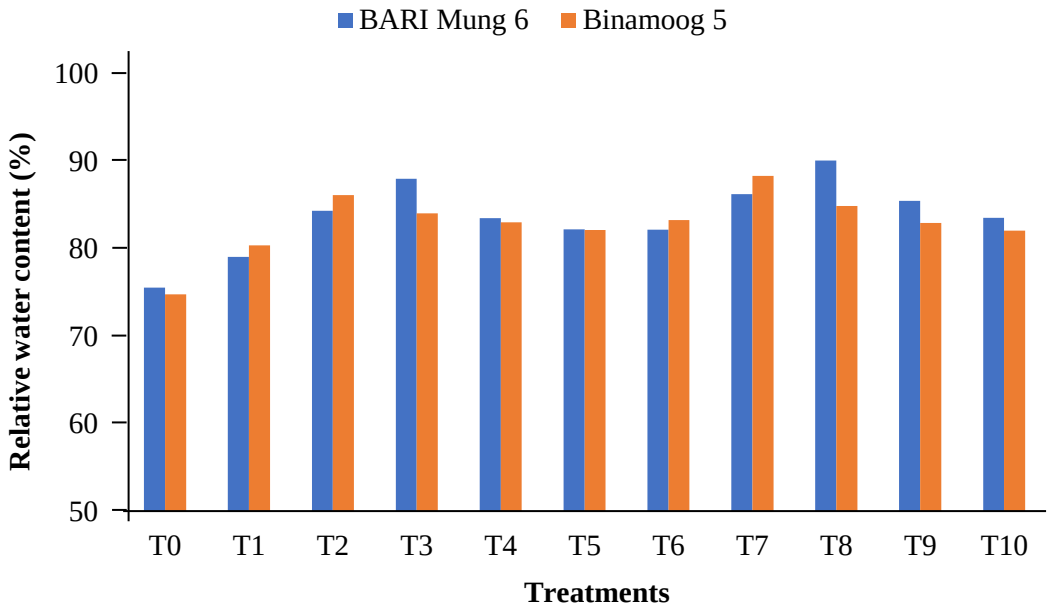

Figure 12. Effect of different priming (mannitol and water) time on relative water content (LSD $_{(0.05)}$ = 1.238 and 1.079 for BARI Mung 6 and Binamoog 5 respectively).

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

In BARI Mung 6, highest seedling relative water content (90.07%) was noticed from T8 treatment and lowest seedling relative water content from T0 (75.54%). In Binamoog 5, highest seedling relative water content (88.34%) was noticed at T7 treatment and lowest seedling relative water content from T0 (74.77%).

This result also in agreement with the findings of previous researcher (Asaduzzaman, 2014). Faijunnahar *et al.* (2017) reported that growth of healthy and vigorous seedling through enhanced enzymatic activities of seed with optimum priming time, which might have the capacity to provide higher relative water content. Over priming time prompt the ageing process of primed seed, produced weak and lean seedling ultimately exhibited lower relative water content.

4.2.11 Effect on water saturation deficit (%)

Seedling water saturation deficit of BARI Mung 6 and Binamoog 5 was decreased up to 9 hr and 6 hr respectively and then increased gradually (Table 11). Seedling water saturation deficit noticed significant difference among the treatments. In BARI Mung 6, greater seedling water saturation deficit (24.46%) was found at T0 treatment and lower seedling water saturation deficit was found at T8 (9.93%). In Binamoog 5, greater seedling water saturation deficit (25.23%) was found at T0 treatment which was statistically similar with T5 and T10. Lowest lower seedling water saturation deficit was found at T7 (11.66%).

This result also in agreement with the findings of previous worker (Asaduzzaman, 2014). Lower priming time induced lower enzymatic activities which result weak and lean seedling. On the other hand, over priming time accelerate ageing process and produced weak and lean seedling which were failed to uptake enough water and provided more water saturation deficit value (Faijunnahar *et al.*, 2017).

Table 11. Effect of different priming (mannitol and water) time on water saturation deficit

Treatments	Water saturation deficit (%)	
	BARI Mung 6	Binamoog 5
T0	24.46 a	25.23 a
T1	20.95 b	19.61 b
T2	15.68 de	13.88 f
T3	11.99 g	15.97 de
T4	16.52 d	17.00 cd
T5	17.78 c	17.88 c
T6	17.84 c	16.71 d
T7	13.77 f	11.66 g
T8	9.93 h	15.13 e
T9	14.53 ef	17.04 cd
T10	16.46 d	17.95 c
LSD (0.05)	1.238	1.079
CV (%)	5.94%	4.94%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.2.12 Effect on water retention capacity

Increasing priming (mannitol, water and control) time increased the seedling water retention capacity of BARI Mung 6 and Binamoog 5 up to 9 hr and 6 hr respectively and then decreased gradually (Table 12). Seedling water retention capacity valued significand difference among the treatments. In BARI Mung 6, maximum seedling water retention capacity (20.79) was noticed from T8 treatment and minimum seedling water retention capacity from T0 (16.28). In Binamoog 5, maximum seedling water retention capacity (19.67) was noticed at

T7 treatment and minimum seedling water retention capacity from T0 (15.47) which was statistically similar with T10.

Lower priming time induced lower enzymatic activities which result weak and lean seedling. On the other hand, over priming time accelerate ageing process and produced weak and lean seedling which were failed to uptake enough water and provided more water saturation deficit value. Vigorous seedling can uptake enough water than the weaker seedling which ensured maximize the turgid weight of seedling so the water retention capacity might be higher than the lower and over priming time (Faijunnahar *et al.,* 2017).

Table 12. Effect of different priming (mannitol and water) time on water retention capacity

Treatments	Water retention capacity	
	BARI Mung 6	Binamoog 5
T0	16.28 h	15.47 g
T1	17.13 g	17.71 cd
T2	18.28 cd	18.94 ab
T3	19.58 b	17.82 cd
T4	17.57 efg	17.07 de
T5	17.30 fg	16.05 fg
T6	17.01 g	18.25 bc
T7	18.56 c	19.67 a
T8	20.79 a	18.65 b
T9	18.06 cde	17.59 cd
T10	17.75 def	16.67 ef
LSD (0.05)	0.5686	0.8191
CV (%)	2.48%	3.65%

T0 = Seeds without priming (control), T1 = Seeds primed with distilled water for 3 hours, T2 = Seeds primed with distilled water for 6 hours, T3 = Seeds primed with distilled water for 9 hours, T4 = Seeds primed with distilled water for 12 hours, T5 = Seeds primed with distilled water for 15 hours, T6 = Seeds primed with mannitol solution for 3 hours, T7 = Seeds primed with mannitol solution for 6 hours for, T8 = Seeds primed with mannitol solution for 9 hours, T9 = Seeds primed with mannitol solution for 12 hours, T10 = Seeds primed with mannitol solution for 15 hours (BARI Mung 6 primed with 6% mannitol solution and Binamoog 5 primed with 4% mannitol solution).

4.3 Third experiment

Study on the germination behavior of primed seed (mungbean) under drought (Polyethylene Glycol) stress condition

Results obtained from the present study regarding the germination behavior of primed seed (mungbean) under drought (Polyethylene Glycol) stress condition. The analytical results have been displayed in Figures 13 to 25.

4.3.1 Effect on germination percentage

Different drought stress condition exhibited significant variation in germination percentage (Figure 13). Germination percentages from mannitol primed, water primed and non-primed seeds gradually decreased with increasing drought stress level. But germination percentage of mannitol and water primed seeds were higher than the non-primed seeds. The highest germination percentage (96.66%) and (95.66%) was achieved in mannitol primed control stress (0% PEG) condition for Binamoog 5 and BARI Mung 6 respectively. Lowest germination percentage (45.33%) was found in BARI Mung 6 and (42.66%) was found in Binamoog 5 under 20% PEG stress condition in non-primed seed. Seed priming enhance a wide range of biochemical changes which was crucial for germination, *i.e.*, breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibitions and enzymes activation (Ajouri *et la.*, 2004). Primed seed can rapidly consume and revitalize the seed metabolism; thus, germination percentage was increased and the physiological heterogeneity was triggered down (Rowse, 1995). Moreover, seed priming most likely contributes to fix-up of damage membrane caused by deterioration and lead to better germination pattern and higher vigor level compared with non-primed seeds (Jisha *et al.*, 2013 and Ruan *et al.*, 2002b). Priming showed stimulating effects in the early stages of germination by the initiating cell division in germinating seeds (Hassanpouraghdam *et al.*, 2009). Similar mechanisms seem to perform in the course of our experiments so that mannitol-primed seeds resulted in higher germination attributes and rapid seedling growth under osmotic stress. Hydropriming meaningfully boost germination rate (Ghassemi-Golezani *et al*., 2010a) and is a useful technique for

optimized overall germination percentage (Maiti *et al*., 2013) these beneficial effects of hydropriming have been attributed when seeds imbibe, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986).

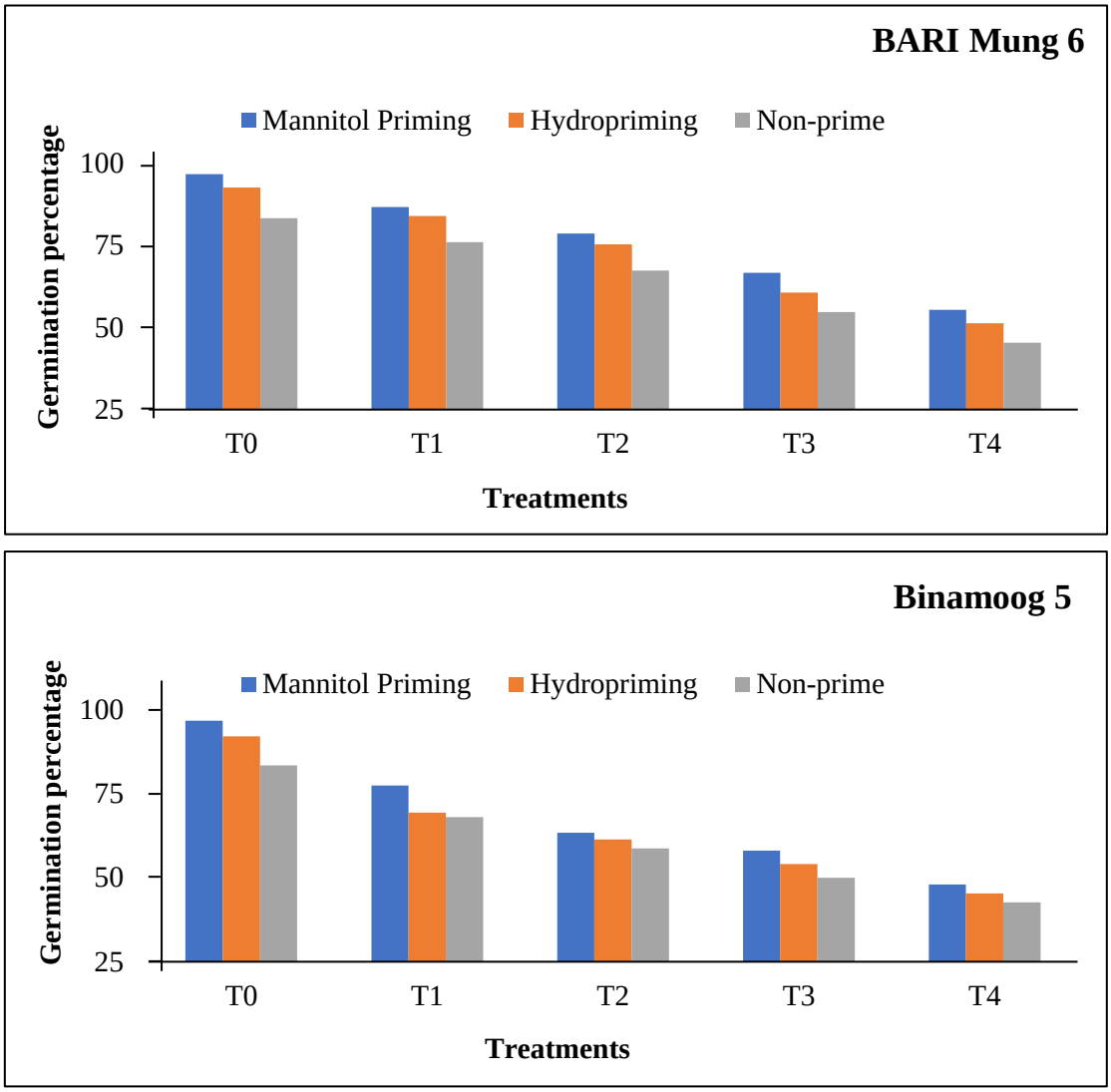

Figure 13. Effect of different drought levels on germination percentage of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 3.740 and 3.156 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.2 Effect on mean germination time (days)

Mean germination time of BARI Mung 6 and Binamoog 5 were significantly increased with the increasing of drought stress (PEG solution) levels (Figure 14). But mean germination time of mannitol and water primed seeds was lower than non-primed seeds. Highest mean germination time (5.114 days) was recorded in BARI Mung 6 and (4.936 days) was recorded in Binamoog 5 under 20% PEG stress condition in non-primed seed. And the lowest mean germination time (4.441 days) and (4.459 days) was recorded in mannitol primed control stress (0% PEG) condition for BARI Mung 6 and Binamoog 5 respectively. Seedling emergence percentage for seeds primed with water was higher than that for other primed and unprimed seeds. Seed priming with water enhanced seedling emergence rate in the field. Afzal *et al.* (2011b) carried out a laboratory study to investigate the influence of priming with mannitol (2, 4 and 6%) on germination and seedling growth of African and French marigold seeds. He found that effect of mannitol in increasing reducing sugar and total sugars as well as α-amylase activity in primed seeds and reduce mean germination time.

This result was supported by previous findings of Rahman (2014) and Asaduzzaman, (2014). Kumar *et al.* (2017) found that 4% mannitol showed maximum speed of germination (71.50) in chickpea. Priming with 2 and 4% mannitol for 24 hr maximally increased final germination percentage, germination capacity, germination index, shoot and root lengths and decreased mean germination time of marigold species as compared to all pre-sowing seed treatments including control. This could be attributed to the effect of mannitol in increasing reducing sugar and total sugars as well as α-amylase activity in primed seeds (Afzal *et al.*, 2011b). Rahman *et al,* (2016) found that Priming with pure water had reduced MGT from 28.3 hr as in dry seeds (unprimed) to 10.5 hr.

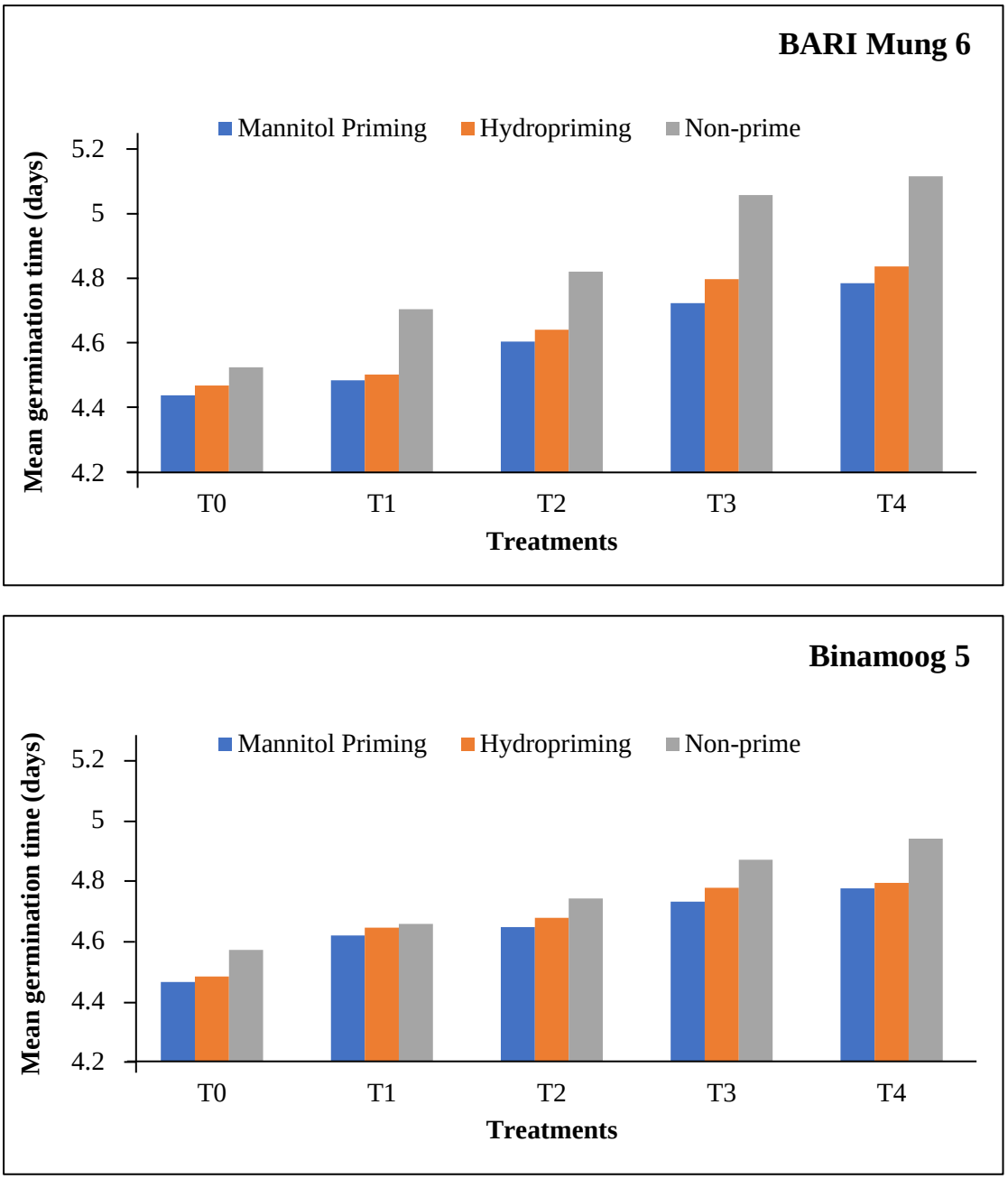

Figure 14. Effect of different drought levels on mean germination time of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 0.08001 and 0.05658 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.3 Effect on germination index

The results regarding germination index of BARI Mung 6 and Binamoog 5 exposed that germination index decreased significantly with increasing of drought stress (PEG solution) levels (Figure 15). But germination index of mannitol and water primed seeds were higher than the non-primed seeds. The maximum germination index (80.07) was observed in Binamoog 5 and (79.70) observed in BARI Mung 6 at in mannitol primed control stress (0% PEG) condition. Minimum germination index (24.70) and (22.65) was observed in Binamoog 5 and BARI Mung 6 respectively.

This result was in agreement with the previous work of Rahman (2014), and Asaduzzaman (2014). The benefits of seed priming were reported by Harris *et al*. (2001) in crops like wheat, rice and maize which included faster emergence (rate and speed), better and uniform stands, less need to re-sow, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield.

Moghanibashi *et al*. (2012) reported that growth rate of all parameters reduced under salinity and/or drought condition in case of primed and un-primed seeds. Higher GI found on primed seeds under all salinity and/or drought levels as compared with non-primed seeds. Germination index inhibited due to drought stress, but it can be overcome by using osmo-priming treatments in soybean (Ghiyasi and Tajbakhsh, 2013).

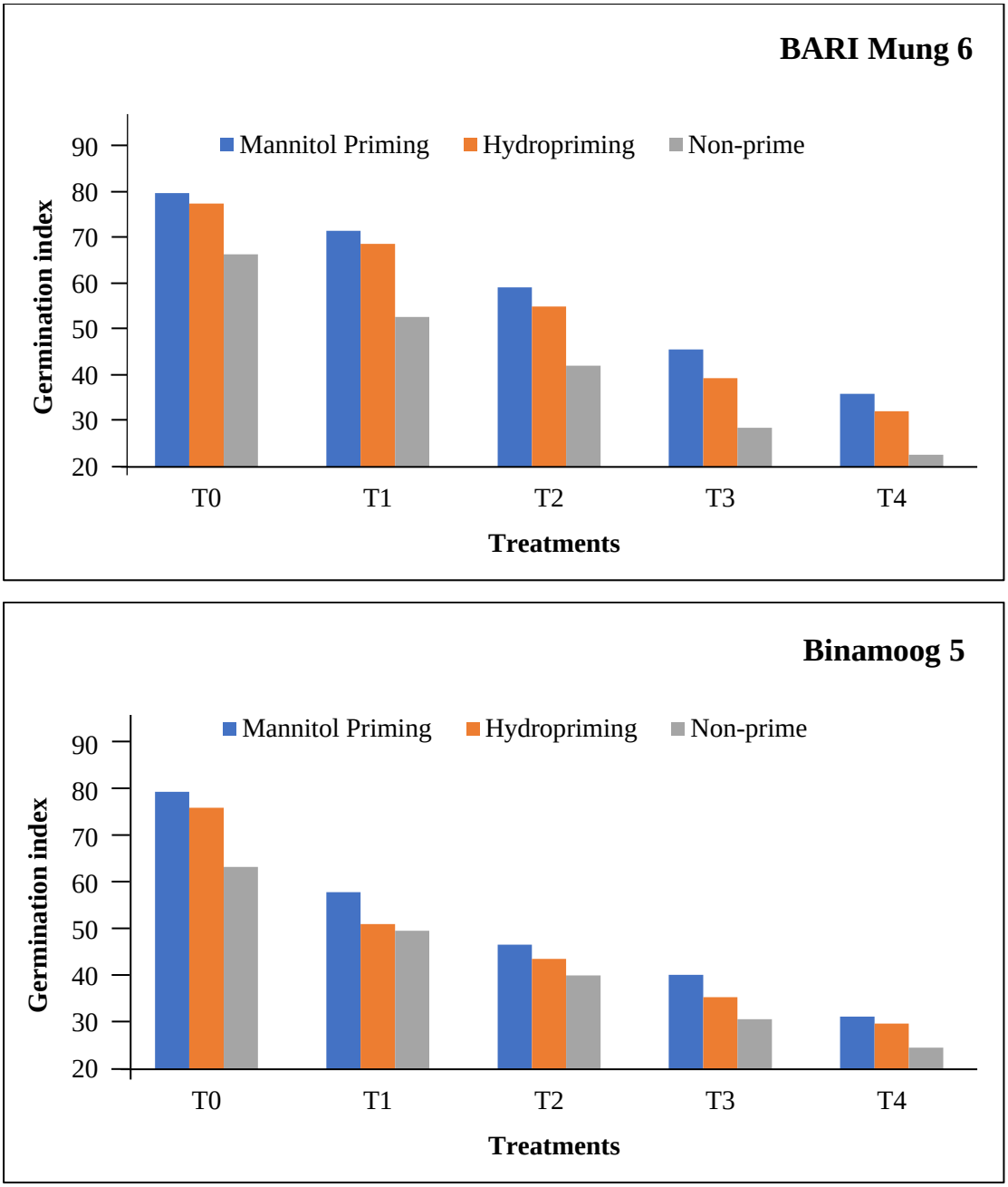

Figure 15. Effect of different drought levels on germination index of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 2.181 and 1.812 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.4 Effect on coefficient of velocity

Coefficient of velocity of BARI Mung 6 and Binamoog 5 showed significant variation among the treatments (Figure 16). Increasing the drought stress (PEG solution) level significantly decreased the values of coefficient of velocity. However, this decreasing trend was more noticeable for non-primed seeds than for primed seeds. Highest coefficient of velocity (22.54) was noticed in BARI Mung 6 and (22.37) was noticed in Binamoog 5 under mannitol primed control stress (0% PEG) condition. The lowest coefficient of velocity (19.57) and (20.27) was noticed under 20% PEG stress condition in non-primed seed for BARI Mung 6 and Binamoog 5 respectively.

Generally, CV increases as more seeds germinate and with shorter germination time. The CV gives an indication of the speed and uniformity of seedling growth (i.e., a higher CV means higher vigour). During priming of tomato seeds, the breakdown of protein bodies was more extensive in endosperm cells at the micropylar region than was observed prior to germination in non-primed seeds (Haigh, 1988). So, speed of germination and uniformity of seed through priming was observed in tomato and enhanced coefficient of variance.

Tavili *et al*. (2011) reported that speed of germination of Bromus increased with seed priming treatments rather than that of control. Similarly, Elkoca *et al*. (2007) determined that hydro priming treatment in chickpea induced faster and more synchronous germination compared with the unprimed seeds thus increase co efficient of velocity.

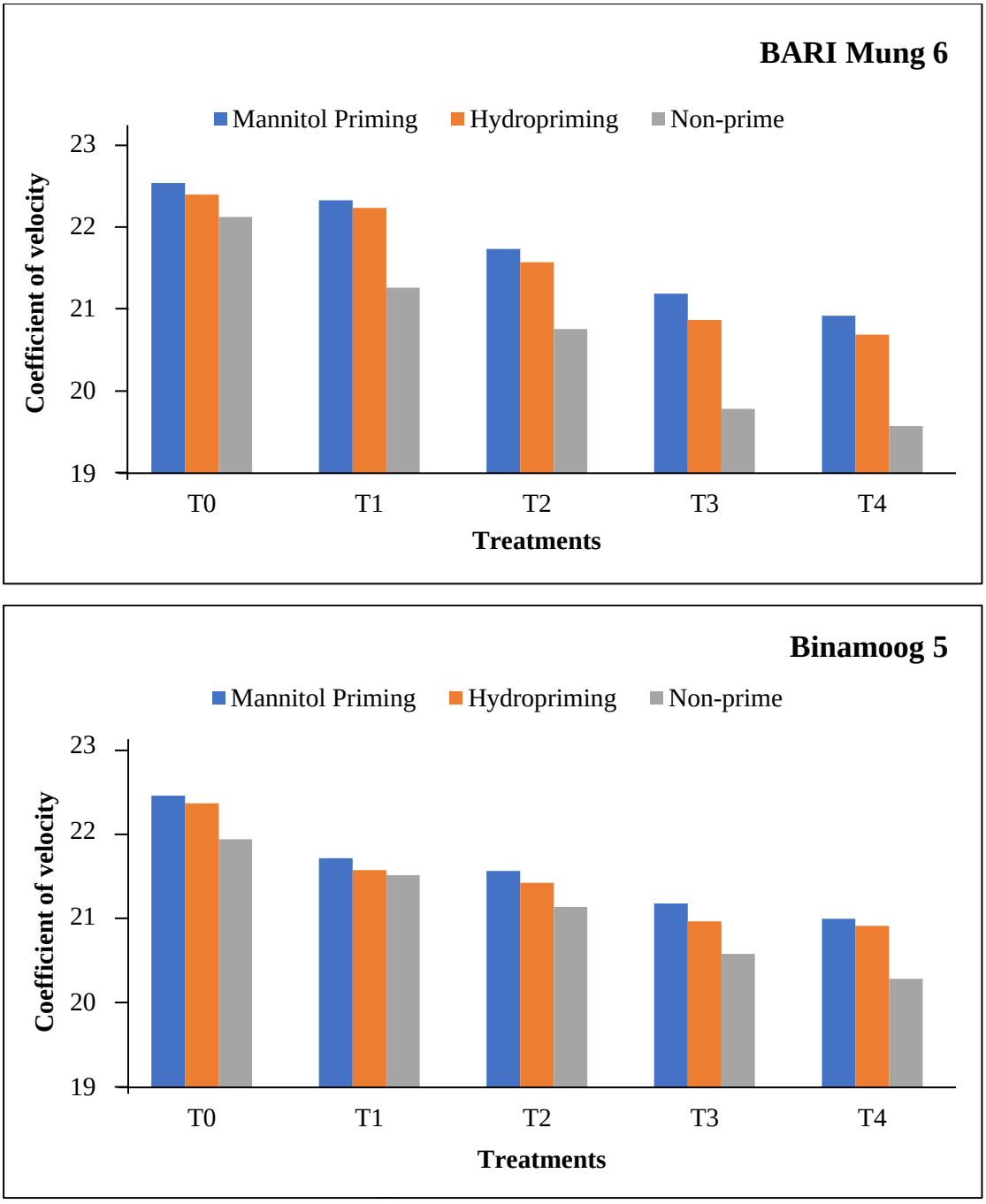

Figure 16. Effect of different drought levels on coefficient of velocity of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 0.3511 and 0.2530 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.5 Effect on energy of emergence (%)

Different drought stress condition showed significant variation in energy of emergence (Figure 17). Energy of emergence from mannitol primed, water primed and non-primed seeds gradually decreased with increasing drought stress level. But energy of emergence of mannitol and water primed seeds were higher than the non-primed seeds. Maximum energy of emergence (98.66%) and (97.33%) was recorded in mannitol primed control stress (0% PEG) condition for Binamoog 5 and BARI Mung 6 respectively. Lowest germination percentage (51.33%) was found in BARI Mung 6 and (46.66%) was found in Binamoog 5 under 20% PEG stress condition in non-primed seed.

This result was in agreement with the previous work of Rahman (2014), and Asaduzzaman (2014). Rajpar *et al*. (2006) revealed that seedlings were significantly faster in emergence, took fewer days to mature and gave significantly higher grain yield. Hydro-priming generally enhance seed germination and seedling emergence under saline and non-saline conditions and also have beneficial effect on enzyme activity required for rapid germination (Singh *et al.*, 2015).

An experiment was conducted by Rahimi (2013) to study the effect of seed priming improves the germination performance and emergence of cumin. Osmo-priming improves germination performance it produces high seed vigour and radical and plumule length at low temperature. Seed priming has been found a double technology to enhance rapid and uniform emergence and to achieve high vigour and better yields in cumin (Nematollahi *et al*., 2009).

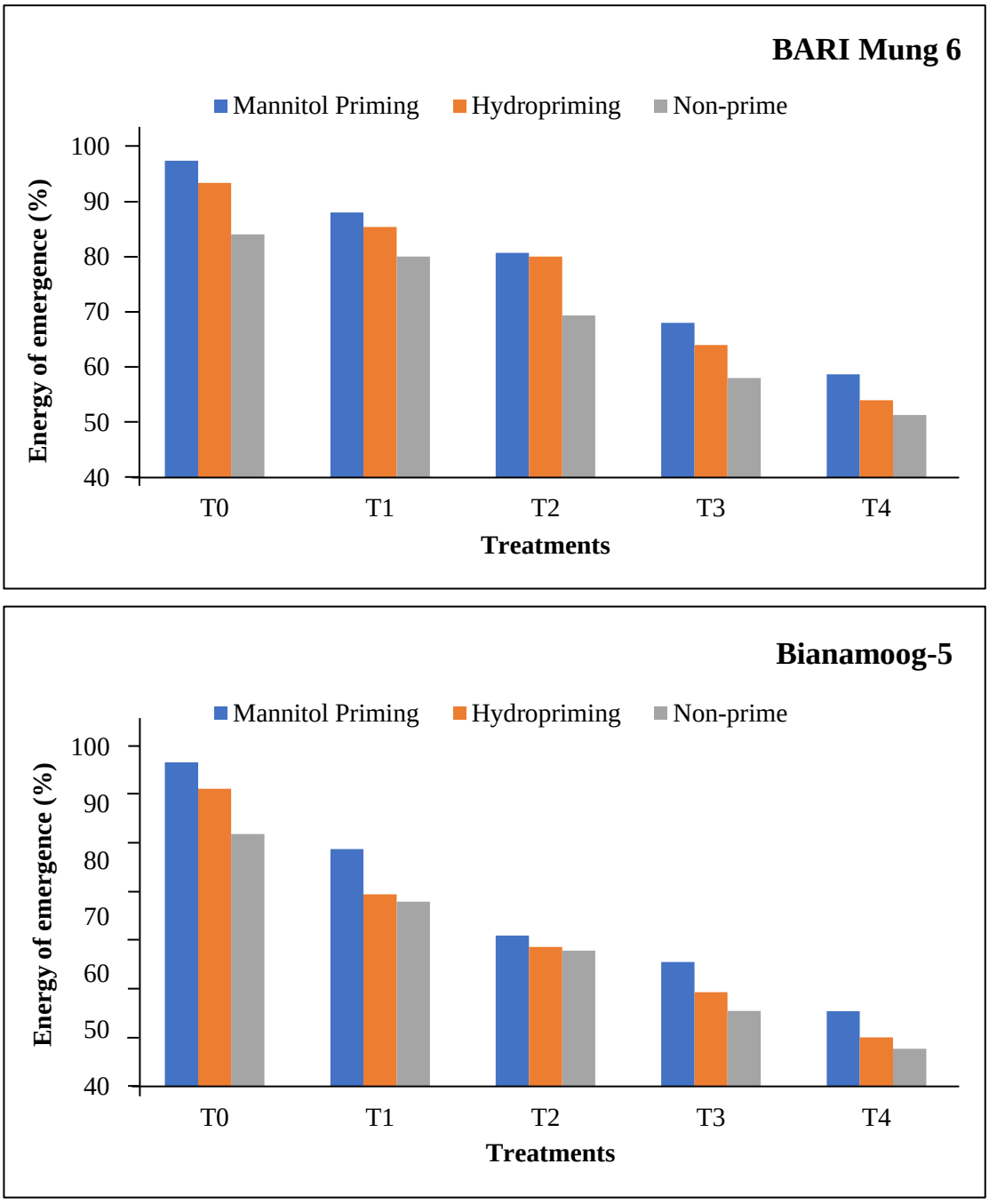

Figure 17. Effect of different drought levels on energy of emergence of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 3.692 and 3.769 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.6 Effect on shoot length (mm)

Drought stress had a significant adverse effect on shoot length for mannitol primed, water primed and non-primed seeds (Figure 18). However, this effect was less noticed in seeds priming with mannitol and water than non-primed seed. The largest shoot length (153.7 mm) was observed in BARI Mung 6 and (147.9 mm) was observed in Binamoog 5 at in mannitol primed control stress (0% PEG) condition. Smallest shoot length (35.75 mm) and (47.34 mm) was observed in BARI Mung 6 and Binamoog 5 respectively under 20% PEG stress condition in non-prime seed.

This result was in agreement with the previous work of Rahman (2014), and Asaduzzaman (2014). Rennick and Tiernan (1978) reported that treated seeds are showed faster and rapid elongation of coleoptile than non-treated and over primed seeds. Lee and Kim (2000) revealed that, priming increased the metabolic activities of seed and finally obtained larger shoot length than non-primed seed. Kaur *et al.* (2003) conducted study to determine the effect of seed priming with mannitol (4%), water and potassium nitrate on chickpea. In general priming with water and mannitol resulted in early germination under salt stress. Priming with 4% mannitol was also as effective as mannitol and water in the enhancement of root and shoot growth.

A field experiment was conducted by Gupta and Singh (2012) in inceptisols to find out the effects of seed priming on chickpea. The treatments consisted of seed priming (seed soaking in water for 8 hr). The results revealed that the growth parameters of chickpea were significantly affected by seed priming. Soaking chickpea seeds in water for about 8 hr significantly influenced plant height and nodule dry weight in comparison to un-soaked seeds.

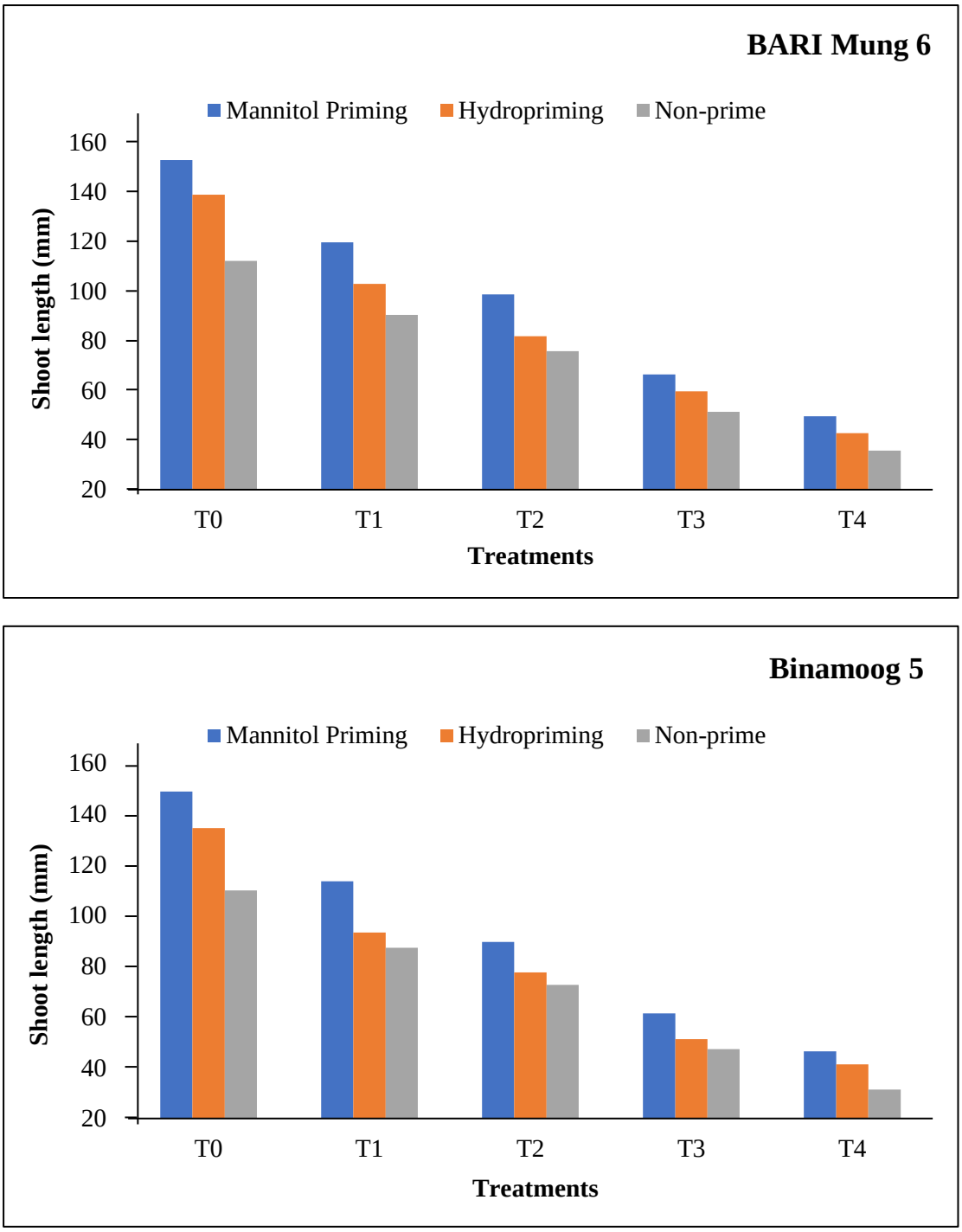

Figure 18. Effect of different drought levels on shoot length of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 3.953 and 3.687 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.7 Effect on root length (mm)

Root length of BARI Mung 6 and Binamoog 5 significantly influenced by the different drought stress (PEG solution) levels (Figure 19). Root length increased with mannitol and water priming seeds in comparison to control seeds with increasing drought stress level. The largest root length (91.88 mm) and (72.53 mm) was accounted in mannitol primed control stress (0% PEG) condition for BARI Mung 6 and Binamoog 5 respectively. Smallest root length (17.18 mm) was accounted in BARI Mung 6 and (14.68 mm) was accounted in Binamoog 5 under 20% PEG stress condition in non-primed seed.

This result was in agreement with the previous work of Rahman (2014) and Asaduzzaman (2014). Arif *et al*. (2008) conducted a field experiment in Peshawar, Pakistan and they reported that priming improved the seed establishment in soybean which might be due to the completion of pre-germination metabolic activities earlier which makes the seed ready for radical protrusion thus increase root length.

Sir…Experiments conducted by Ashraf and Abu-shakra (1978) exposed that priming of wheat seed in osmoticum or water might advance germination and emergence and magnify vigorous root growth. Carceller and Soriano (1972) found that osmo and hydro primed seed exerted the highest root length than non-primed seed. Increased metabolic activities in the primed seeds enhance considerable root length than non-primed Baque *et al.* (2016) and Lee and Kim (2000). Jisha *et al.* (2013) reported that overall growth of plants was enhanced owing to the seed-priming treatments.

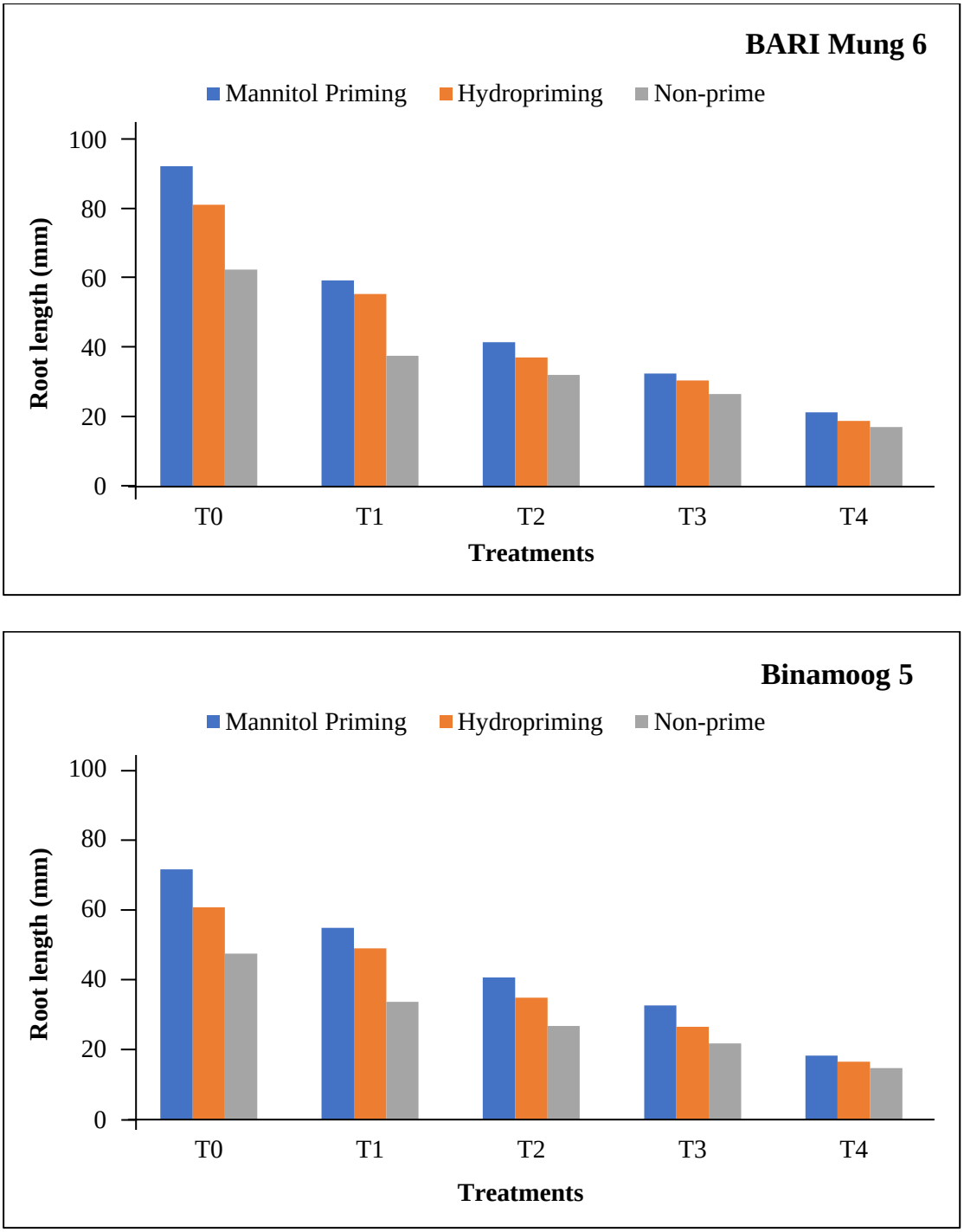

Figure 19. Effect of different drought levels on root length of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 2.631 and 2.324 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.8 Effect on seedling dry weight (mg)

Significant inhibitory effect was found in seedling dry weight of BARI Mung 6 and Binamoog 5 with increasing drought stress (PEG solution) level for mannitol primed, water primed and non-primed seeds (Figure 20). But seedling dry weight of mannitol and water primed seeds were higher than the non-primed seeds. The higher seedling dry weight (92.19 mg) was found in BARI Mung 6 and (88.70 mg) was found in Binamoog 5 under mannitol primed control stress (0% PEG) condition. And lower seedling dry weight (30.77 mg) and (25.78 mg) was found under 20% PEG stress condition in non-primed seed for BARI Mung 6 and Binamoog 5 respectively.

This result was supported by the findings of previous works of Rahman (2014) and Asaduzzaman (2014). Dry weight 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). Pill and Necker (2001) also suggested that primed seeds compared to non-primed seeds showed higher seedling dry weights. Kumar *et al.* (2017) experimented on chickpea and found that in case of seedling dry weight it was higher (1.02 gm to 1.59mg) in PEG 20% seeds followed by Mannitol 4% when compared with control.

Increase of the synthesis of the hormone gibberellin, which trigger the activity of α-amylase and other germination specific enzymes like protease and nuclease involved in hydrolysis and assimilation of the starch enhance dry weight of the shoot and root (Gholami *et al.,* 2009). Ghassemi-Golezani *et al*., (2008c) exhibited that hydro-priming meaningfully improved shoot and root dry weights and Sarwar *et al.* (2006) also stated that shoot length and biomass of shoots and root were better when treated with water and mannitol.

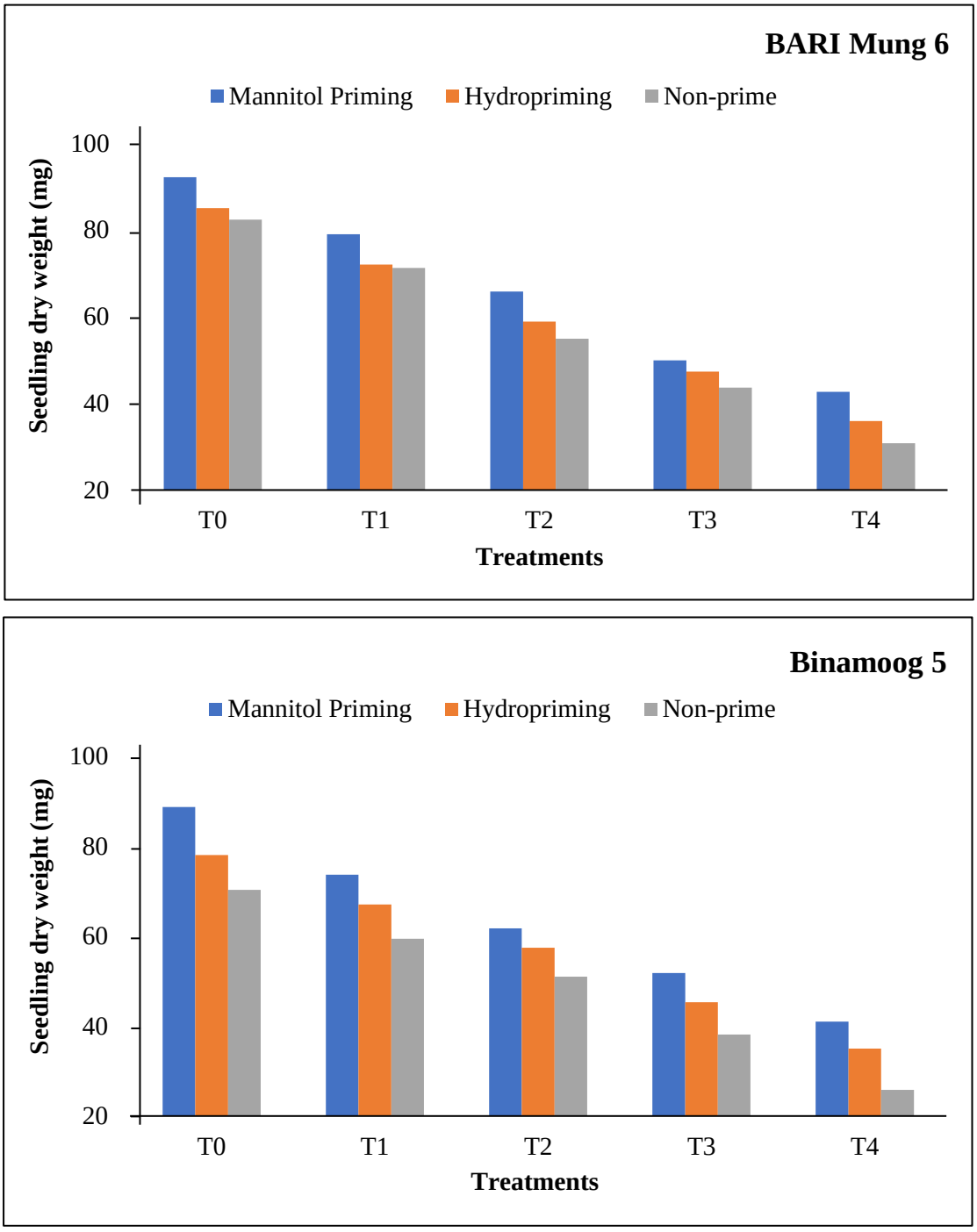

Figure 20. Effect of different drought levels on seedling dry weight of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 2.632 and 1.996 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.9 Effect on seedling vigour index

Increasing drought stress (PEG solution) condition significantly decreased the seedling vigour index of BARI Mung 6 and Binamoog 5 for mannitol primed, water primed and non-primed seed (Figure 21). But seedlings vigour index of mannitol and water primed seeds was higher than the non-primed seeds. Maximum seedling vigour index (236.5) and (215.5) was obtained from mannitol primed control stress (0% PEG) condition for BARI Mung 6 and Binamoog 5 respectively. Lowest seedling vigour index (24.00) was obtained from BARI Mung 6 and (19.68) was obtained from Binamoog 5 under 20% PEG stress condition in non-primed seed. This result was supported by previous findings of Baque *et al*. (2016) and Maiti *et al.* (2013). Maiti *et al.* (2013) also acknowledged that osmo-priming increased seedling vigor of several vegetable crops and concerning sponge gourd. The probable reason for the highest vigour index might be due to photosynthetic capacity treated with bio fertilizers increases due to increased supply of nutrition. (Farnia and Shafie, 2015). Safiatou (2012) stated that seedling vigour increased by using seed priming methods in sorghum and Bambara groundnut. Also, highest seedling vigour was achieved by osmo-priming (Mannitol priming) in Bambara groundnut and by hydro-priming in sorghum. Priming improved seedling vigour.

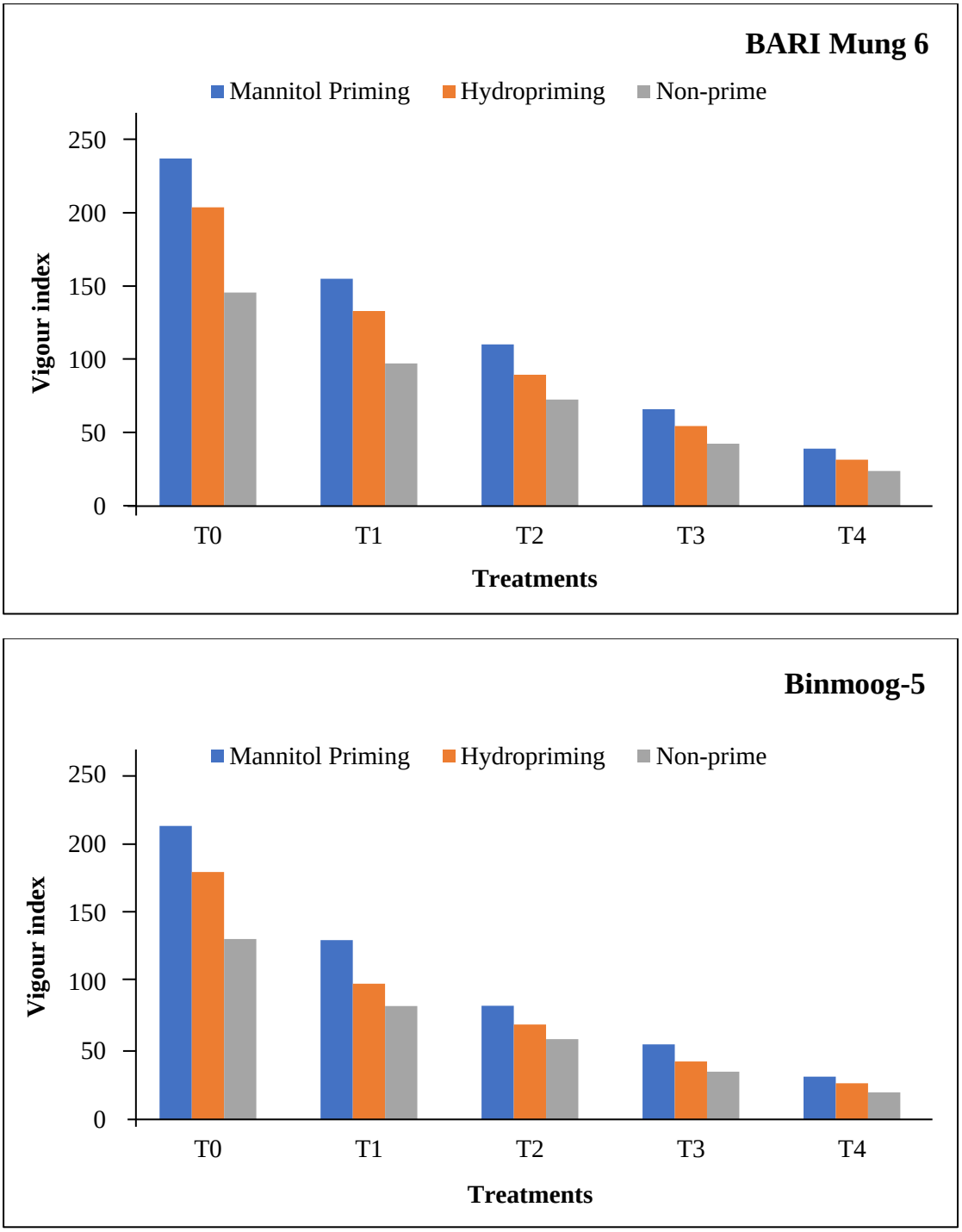

Figure 21. Effect of different drought levels on vigour index of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 7.111 and 6.139 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.10 Effect on relative water content (%)

Relative water content was significantly more pronounced in control seeds in compare to mannitol and water primed seed (Figure 22). Relative water content from mannitol primed, water primed and non-primed seedlings gradually decreased with increasing drought stress level. The highest relative water content (90.12%) was observed in BARI Mung 6 and (88.62%) was observed in Binamoog 5 at in mannitol primed control stress (0% PEG) condition. Lowest relative water content (63.47%) and (61.80%) was observed in BARI Mung 6 and Binamoog 5 respectively under 20% PEG stress condition in non-prime seed.

Under stress condition, osmo and hydro primed seedling ensures better water use efficiency thus vigorous plant growth observed than non-primed seeds Flower *et al.* (1998). A similar finding was reported by Sairam *et al.* (2002). Faijunnahar *et al.* (2017) reported that growth of healthy and vigorous seedling through enhanced enzymatic activities of seed with optimum priming time, which might have the capacity to provide higher relative water content. Over priming time prompt the ageing process of primed seed, produced weak and lean seedling ultimately exhibited lower relative water content.

Baque *et al.* (2002) reported that generally higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. Water saturation deficit highly reduced with higher level of K. Potassium fertilizer also made the leaf water potential more negative. Under drought stressed conditions the beneficial effect of potassium on drought stress was more noticeable than under control conditions.

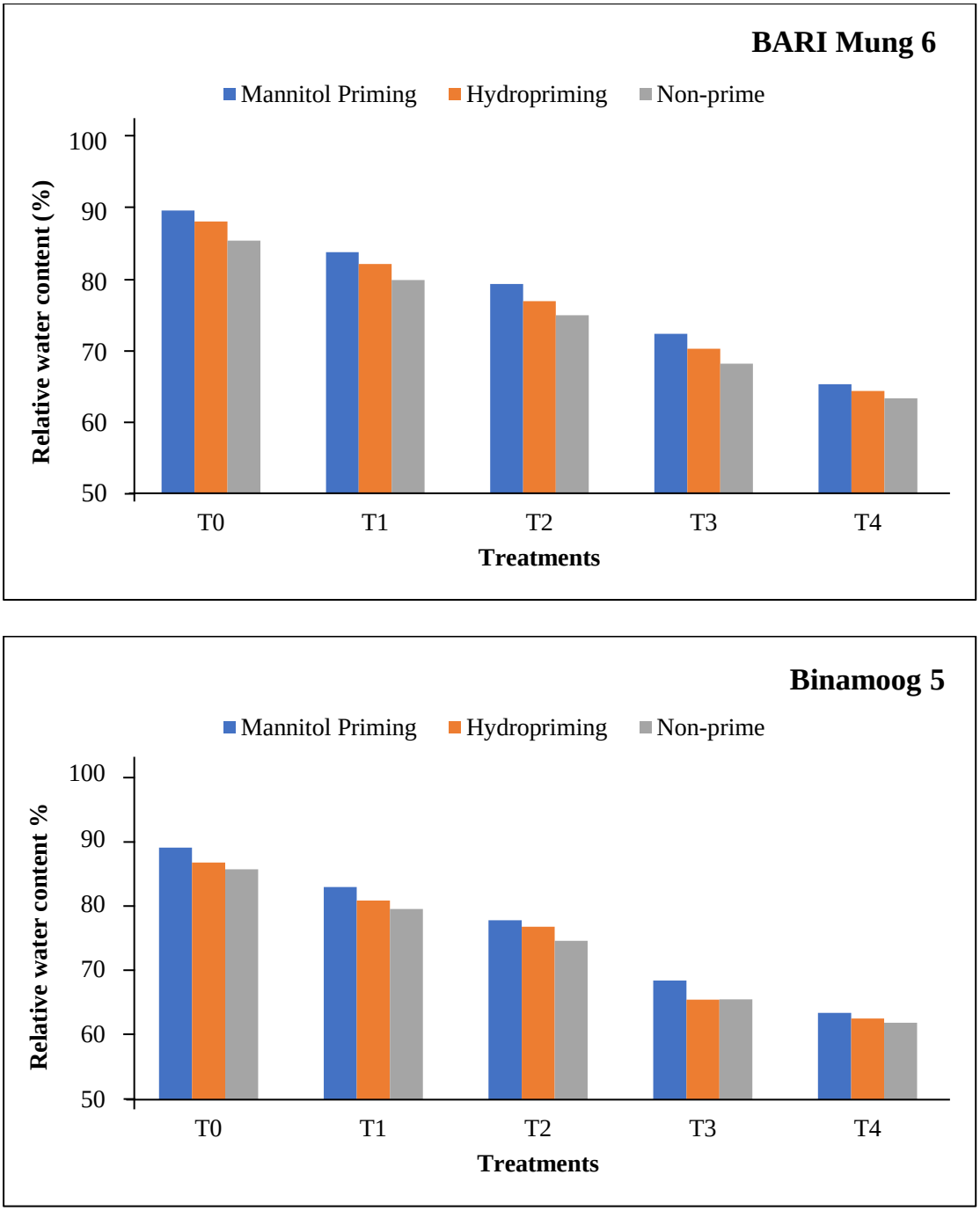

Figure 22. Effect of different drought levels on relative water content of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 1.177 and 0.3667 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.11 Effect on water saturation deficit (%)

Water saturation deficit of BARI Mung 6 and Binamoog 5 were significantly increased with the increasing of drought stress (PEG solution) levels (Figure 23). But water saturation deficit of mannitol and water primed seeds was lower than non-primed seeds. Highest water saturation deficit (38.20%) was achieved in Binamoog 5 and (36.53%) was achieved in BARI Mung 6 under 20% PEG stress condition in non-primed seed. And the lowest water saturation deficit (11.38%) and (9.87%) was achieved in mannitol primed control stress (0% PEG) condition for Binamoog 5 and BARI Mung 6 respectively.

This result was in agreement with the previous work of Rahman (2014) and Asaduzzaman (2014). Faijunnahar *et al.* (2017) reported that relative water content and water saturation deficit had an inverse relation between them. The enzymatic activities were lower in non-prime seed which result produced the weak and lean seedling on the other hand due to over priming time, ageing process was accelerated and produced weak and lean seedling which were failed to uptake enough water and provided more water saturation deficit value.

According to Baque *et al.* (2002) higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. Water saturation deficit highly reduced with higher level of K. Potassium fertilizer also made the leaf water potential more negative. Under drought stressed conditions the beneficial effect of potassium on drought stress was more noticeable than under control conditions.

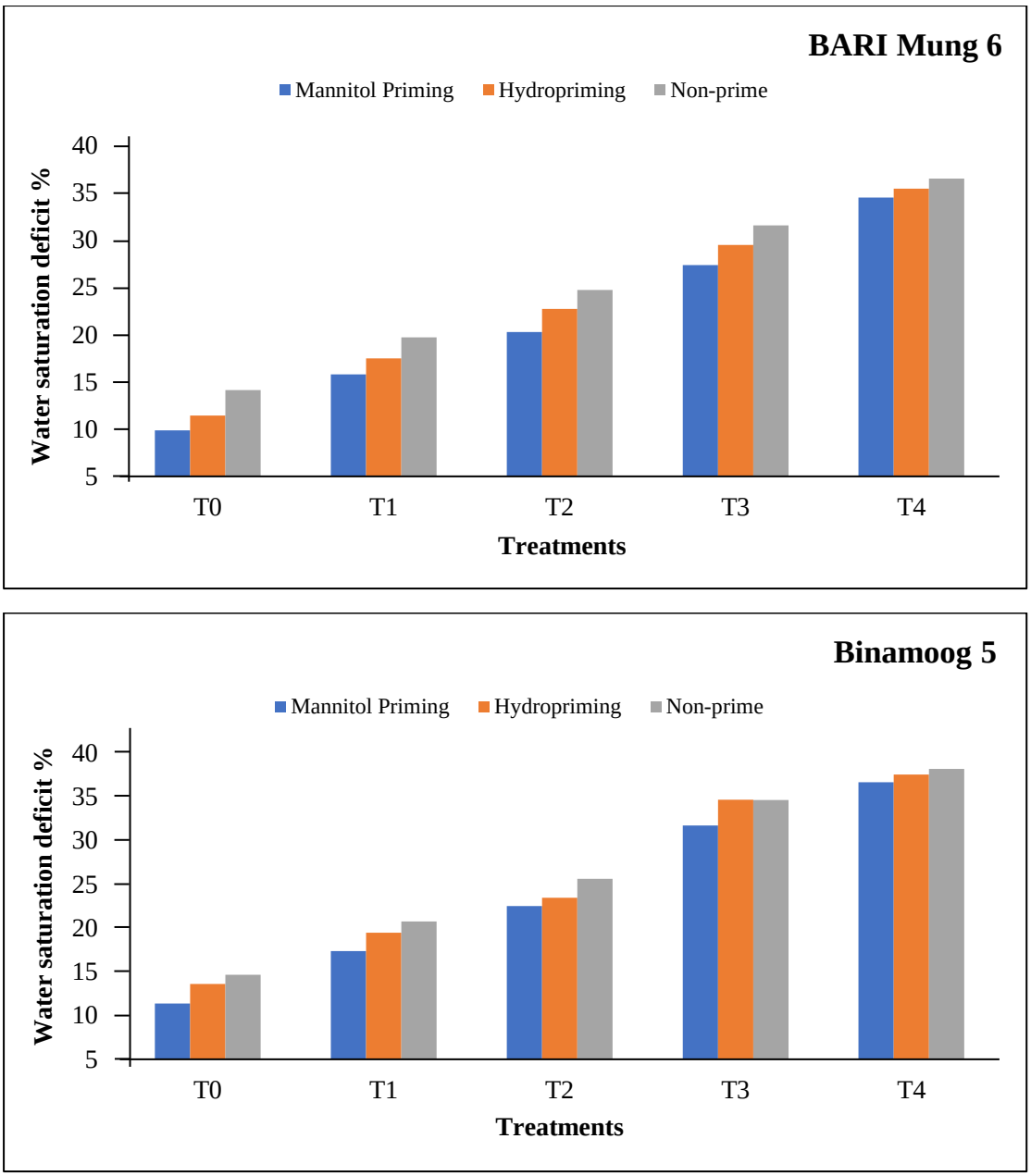

Figure 23. Effect of different drought levels on water saturation deficit of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 1.177 and 0.3667 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

4.3.12 Effect on water retention capacity

Water retention capacity of BARI Mung 6 and Binamoog 5 showed significant variation among the treatments (Figure 24). Increasing the drought stress (PEG solution) level significantly decreased water retention capacity. However, this decreasing trend was more noticeable for non-primed seeds than for primed seeds. Maximum water retention capacity (20.80) was noticed in BARI Mung 6 and (19.78) was noticed in Binamoog 5 under mannitol primed control stress (0% PEG) condition. Minimum water retention capacity (15.16) and (15.06) was noticed under 20% PEG stress condition in non-primed seed for BARI Mung 6 and Binamoog 5 respectively.

This result was in agreement with the previous work of Rahman (2014) and Asaduzzaman (2014). Faijunnahar *et al.* (2017) also said that priming helps to activate the metabolic enzymes responsible for germination of seed before germination takes place, so the hydro and osmo-primed seedlings can uptake more water than the non-primed ones and obtained the maximum turgid weight, in consequence, they obtained the maximum water retention capacity.

Baque *et al.* (2002) reported that generally higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. Water saturation deficit highly reduced with higher level of K. Potassium fertilizer also made the leaf water potential more negative. Under drought stressed conditions the beneficial effect of potassium on drought stress was more noticeable than under control conditions.

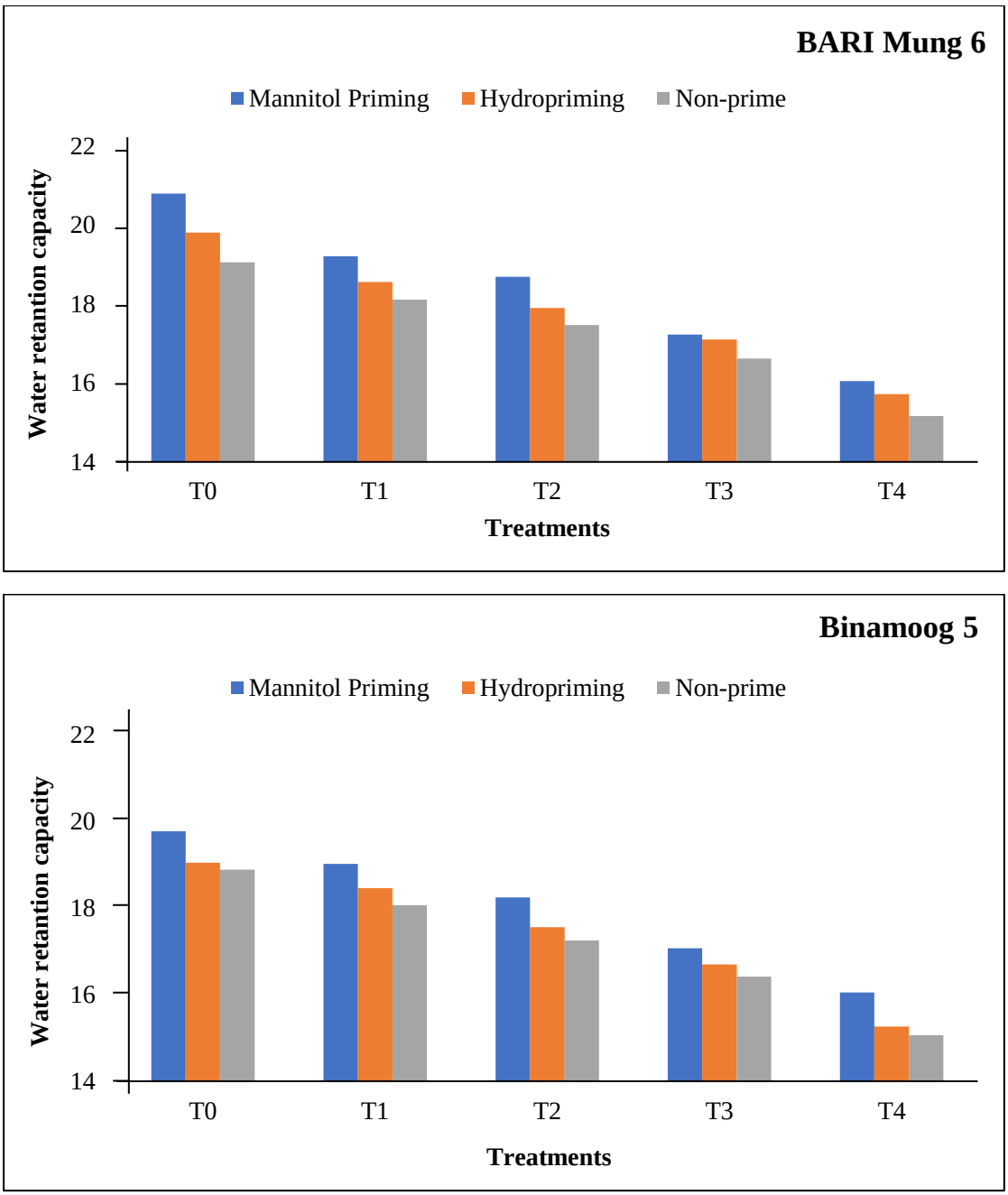

Figure 24. Effect of different drought levels on water retention capacity of primed (mannitol and water) and non-primed (control) seeds (LSD $_{(0.05)}$ = 0.1960 and 0.2994 for BARI Mung 6 and Binamoog 5 respectively).

T0= Primed (mannitol and water) and non-primed (control) seeds placed in control (without PEG); T1=Primed (mannitol and water) and non-primed (control) seeds placed with 5% PEG: T2=Primed (mannitol and water) and non-primed (control) seeds placed with 10% PEG, T3= Primed (mannitol and water) and non-primed (control) seeds placed with 15% PEG: T4 = Primed (mannitol and water) and non-primed (control) seeds placed with 20% PEG. BARI Mung 6 primed with 6% mannitol and water for 9 hours and Binamoog 5 primed with 4% mannitol and water for 6 hours.

CHAPTER V
SUMMARY AND CONCLUSION

The experiment was carried out at the Agronomy Laboratory of the Central Laboratory, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from 22 April, 2018 to 15 June, 2018 to study seed priming with mannitol induced drought tolerance capacity under stress condition in BARI Mung 6 and Binamoog 5.

The entire study was carried out under three individual experiments with Completely Randomized Design (CRD). Different chemicals such as mannitol and distilled water were used as priming agent, Polyethylene Glycol (PEG) was used for induction of drought stress condition and 75% alcohol was used for surface sterilization. Priming was done in room temperature and all the primed seeds were removed from the priming solution at required time. Thirty seeds were selected randomly and placed on 120 mm diameter Petri dishes on saturated whatman no.1 filter paper for germination. Emergence of 2 mm radical considered for germination occurred and every 24 hours interval germination progress was observed and data recorded up to continued 8 days. Shorter, thicker and spiral formed hypocotyls and stunted primary rooted seedlings and dead seeds were taken off from the Petri dishes. The data on germination parameters of mungbean like germination percentage, mean germination time, germination index, coefficient of velocity, energy of emergence and growth parameters like shoot length, root length, seedling dry weight and vigour index, Relative water content (RWC), water saturation deficit (WSD) and water retention capacity (WRC). Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the least significant difference (LSD) at 5% level of probability.

The first experiment was conducted to find the germination behavior of mungbean (BARI Mung 6 and Binamoog 5) at different concentrations of priming agents (Mannitol and Distilled water). For BARI Mung 6 and Binamoog 5, the highest germination percentage 95.33% and 96.66%, germination index

80.40 and 80.75, energy of emergence 96.66% and 98.00%, coefficient of velocity 22.46 and 22.34, shoot length 153.8 mm and 148.9 mm, root length 92.98 mm and 75.45 mm, seedling dry weight 90.49 mg and 82.94 mg, vigor index 235.3 and 216.8, relative water content 90.47% and 88.66%, water retention capacity 20.70 and 19.70 were recorded at T4 and T3 treatment, respectively. The lowest mean germination time (4.454 days) and (4.466 days), water saturation deficit 9.53% and 11.44% in T4 and T3 treatment were observed for BARI Mung 6 and Binamoog 5, respectively. From the first experiment, 6% and 4% mannitol show better performance over control, water primed, 2% and 8% mannitol for germination percentage, mean germination time, germination index, coefficient velocity, energy of emergence, shoot length, root length, seedling dry weight, vigour index, relative water content, water saturation deficit and water retention capacity for BARI Mung 6 and Binamoog 5 respectively.

The second experiment was conducted to calculate the pre-sowing priming time on the germination behavior of mungbean (BARI Mung 6 and Binamoog 5). For BARI Mung 6 and Binamoog 5, the highest germination percentage 96.66% and 96.66%, germination index 79.9 and 80.05, coefficient of velocity 22.52 and 22.41, energy of emergence 97.33% and 97.33%, shoot length 152.8 mm and 148.8 mm, root length 92.41 mm and 71.23 mm, seedling dry weight 92.19 mg and 88.71 mg, vigor index 237.2 and 212.8, relative water content 90.07% and 88.34%, water retention capacity 20.79 and 19.67 were recorded at T8 and T7 treatment, respectively. The lowest mean germination time (4.462 days) and (4.438 days), water saturation deficit 9.93% and 11.66% in T8 and T7 treatment were observed for BARI Mung 6 and Binamoog 5, respectively. From the second experiment, 9 hours show better performance in BARI Mung 6, 6 hours for Binamoog 5 over control, 3, 12 and 15 hours priming for germination percentage, mean germination time, germination index, coefficient velocity, energy of emergence, shoot length, root length, seedling dry weight, vigour index, relative water content, water saturation deficit and water retention capacity.

The third experiment was conducted to calculate the germination behavior of primed seed (BARI Mung 6 and Binamoog 5) under drought (Polyethylene Glycol) stress condition. For BARI Mung 6 and Binamoog 5, the highest germination percentage 95.66% and 96.66%, germination index 79.7 and 80.07, coefficient of velocity 22.54 and 22.37, energy of emergence 97.33% and 98.66%, shoot length 153.7 mm and 147.9 mm, root length 91.88 mm and 72.53 mm, seedling dry weight 92.19 mg and 88.71 mg, vigor index 236.5 and 215.5, relative water content 90.12% and 88.62%, water retention capacity 20.80 and 19.78 were recorded at T8 and T7 treatment, respectively. The lowest mean germination time (4.459 days) and (4.441 days), water saturation deficit 9.87% and 11.38% in T8 and T7 treatment were observed for BARI Mung 6 and Binamoog 5, respectively. From the third experiment, priming with 6% mannitol solution with 9 hours priming for BARI Mung 6 and 4% mannitol with 6 hours priming for Binamoog 5 expressed better results over non primed and water primed for germination percentage, mean germination time, germination index, coefficient velocity, energy of emergence, shoot length, root length, seedling dry weight, vigour index, relative water content, water saturation deficit and water retention capacity at drought stress condition.

From the above discussion, it may be concluded that primed seeds expressed the expected results to alleviate drought stress in case all of the parameters studied. In the conditions of drought stress, priming of seeds may be the effective way in installation of field trial as well as suggested method for farmers to cope with the changing environment. However, further studies with different field crops, various types of priming agents, different concentrations and optimum time of priming could be explored before drawing valid conclusions.

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APPENDICES

Appendix I: Analysis of variance of different concentrations of mannitol and water on germination percentage of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	495.226	99.045	8.624	0.0001
Error	24	275.645	11.485		
Total	29	770.645			
Binamoog 5					
Treatment	5	611.806	122.361	8.694	0.0001
Error	24	337.796	14.075		
Total	29	949.602			

Appendix II: Analysis of variance of different concentrations of mannitol and water on mean germination time of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	0.066	0.013	4.061	0.0082
Error	24	0.078	0.003		
Total	29	0.144			
Binamoog 5					
Treatment	5	0.029	0.006	2.060	0.0106
Error	24	0.068	0.003		
Total	29				

Appendix III: Analysis of variance of different concentrations of mannitol and water on germination index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	968.098	193.620	46.078	0.0000
Error	24	100.847	4.202		
Total	29	1068.945			
Binamoog 5					
Treatment	5	907.561	181.512	27.201	0.0000
Error	24	160.155	6.673		
Total	29	1067.716			

Appendix IV: Analysis of variance of different concentrations of mannitol and water on coefficient of velocity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	1.591	0.318	4.321	0.0060
Error	24	1.768	0.074		
Total	29	3.359			
Binamoog 5					
Treatment	5	0.697	0.139	2.032	0.1101
Error	24	1.646	0.069		
Total	29	2.342			

Appendix V: Analysis of variance of different concentrations of mannitol and water on energy of emergence of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	447.949	89.590	6.813	0.0004
Error	24	315.578	13.149		
Total	29	763.527			
Binamoog 5					
Treatment	5	632.935	126.587	8.545	0.0001
Error	24	355.525	14.814		
Total	29	988.459			

Appendix VI: Analysis of variance of different concentrations of mannitol and water on shoot length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	4630.430	926.086	28.109	0.0000
Error	24	790.717	32.947		
Total	29	5421.717			
Binamoog 5					
Treatment	5	4448.670	889.734	37.443	0.0000
Error	24	570.299	23.762		
Total	29	5018.969			

Appendix VII: Analysis of variance of different concentrations of mannitol and water on root length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	3793.623	758.725	45.937	0.0000
Error	24	396.403	16.517		
Total	29	4190.026			
Binamoog 5					
Treatment	5	2605.366	521.073	52.759	0.0000
Error	24	237.035	9.876		
Total	29	2842.402			

Appendix VIII: Analysis of variance of different concentrations of mannitol and water on seedling dry weight of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	1276.922	255.384	182.119	0.0000
Error	24	33.655	1.402		
Total	29	1310.577			
Binamoog 5					
Treatment	5	909.315	181.863	75.060	0.0000
Error	24	58.150	2.423		
Total	29	967.465			

Appendix IX: Analysis of variance of different concentrations of mannitol and water on vigour index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	25249.184	5049.837	73.756	0.0000
Error	24	1643.212	68.467		
Total	29	26892.396			
Binamoog 5					
Treatment	5	21533.458	4306.692	55.956	0.0000
Error	24	1847.175	76.966		
Total	29	23380.633			

Appendix X: Analysis of variance of different concentrations of mannitol and water on relative water content of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	711.918	142.384	209.102	0.0000
Error	24	16.342	0.681		
Total	29	728.260			
Binamoog 5					
Treatment	5	625.037	125.007	158.122	0.0005
Error	24	18.974	0.791		
Total	29	644.011			

Appendix XI: Analysis of variance of different concentrations of mannitol and water on water saturation deficit of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	711.918	142.384	209.102	0.0000
Error	24	16.342	0.681		
Total	29	728.260			
Binamoog 5					
Treatment	5	625.037	125.007	158.122	0.0005
Error	24	18.974	0.791		
Total	29	644.011			

Appendix XII: Analysis of variance of different concentrations of mannitol and water on water retention capacity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	5	56.433	11.287	5.755	0.0000
Error	24	47.067	1.961		
Total	29	103.500			
Binamoog 5					
Treatment	5	46.283	9.257	8.958	0.0000
Error	24	24.799	1.033		
Total	29	71.083			

Appendix XIII: Analysis of variance of different priming (mannitol and water) time on germination percentage of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	890.600	89.060	6.534	0.0000
Error	44	599.710	13.630		
Total	54	1490.311			
Binamoog 5					
Treatment	10	1168.951	116.895	8.037	0.0000
Error	44	639.974	14.545		
Total	54	1808.925			

Appendix XIV: Analysis of variance of different priming (mannitol and water) time on mean germination time of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	0.110	0.011	3.822	0.0009
Error	44	0.126	0.003		
Total	54	0.236			
Binamoog 5					
Treatment	10	0.084	0.008	2.288	0.0292
Error	44	0.162	0.004		
Total	54	0.246			

Appendix XV: Analysis of variance of different priming (mannitol and water) time on germination index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	1572.271	157.227	47.078	0.0000
Error	44	146.948	3.340		
Total	54	1719.219			
Binamoog 5					
Treatment	10	1781.323	178.132	39.661	0.0000
Error	44	197.618	4.491		
Total	54	1978.941			

Appendix XVI: Analysis of variance of different priming (mannitol and water) time on coefficient of velocity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	2.568	0.257	3.685	0.0012
Error	44	3.066	0.070		
Total	54	5.633			
Binamoog 5					
Treatment	10	2.005	0.200	2.352	0.0252
Error	44	3.751	0.085		
Total	54	5.756			

Appendix XVII: Analysis of variance of different priming (mannitol and water) time on energy of emergence of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	764.873	76.487	6.363	0.0000
Error	44	528.925	12.021		
Total	54	1293.798			
Binamoog 5					
Treatment	10	1024.084	102.408	8.972	0.0000
Error	44	502.231	11.414		
Total	54	1526.315			

Appendix XVIII: Analysis of variance of different priming (mannitol and water) time on shoot length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	6267.802	626.780	21.145	0.0000
Error	44	1304.251	29.642		
Total	54	7572.052			
Binamoog 5					
Treatment	10	4872.870	487.287	54.167	0.0000
Error	44	395.826	8.996		
Total	54	5268.697			

Appendix XIX: Analysis of variance of different priming (mannitol and water) time root length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	5523.149	552.315	52.657	0.0000
Error	44	461.515	10.489		
Total	54	5984.664			
Binamoog 5					
Treatment	10	2337.825	233.783	31.254	0.0000
Error	44	329.127	7.480		
Total	54	2666.953			

Appendix XX: Analysis of variance of different priming (mannitol and water) time seedling dry weight of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	2928.329	292.833	59.917	0.0000
Error	44	215.043	4.887		
Total	54	3143.371			
Binamoog 5					
Treatment	10	3408.470	340.847	506.217	0.0000
Error	44	29.626	0.673		
Total	54	3438.096			

Appendix XXI: Analysis of variance of different priming (mannitol and water) time on vigour index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	35885.737	3588.574	53.750	0.0000
Error	44	2937.623	66.764		
Total	54	38823.356			
Binamoog 5					
Treatment	10	27298.533	2729.853	38.228	0.0000
Error	44	3142.031	71.410		
Total	54	30440.564			

Appendix XXII: Analysis of variance of different priming (mannitol and water) time on relative water content of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	809.616	80.962	85.819	0.0000
Error	44	41.509	0.943		
Total	54	851.125			
Binamoog 5					
Treatment	10	594.339	59.434	82.838	0.0000
Error	44	31.569	0.717		
Total	54	625.908			

Appendix XXIII: Analysis of variance of different priming (mannitol and water) time on water saturation deficit of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	809.616	80.962	85.819	0.0000
Error	44	41.509	0.943		
Total	54	851.125			
Binamoog 5					
Treatment	10	594.339	59.434	82.838	0.0000
Error	44	31.569	0.717		
Total	54	625.908			

Appendix XXIV: Analysis of variance of different priming (mannitol and water) time on water retention capacity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	10	80.423	8.042	40.334	0.0000
Error	44	8.773	0.199		
Total	54	89.197			
Binamoog 5					
Treatment	10	78.627	7.863	19.016	0.0000
Error	44	18.193	0.413		
Total	54	96.820			

Appendix XXV: Analysis of variance of different drought levels on germination percentage of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	17440.003	1245.715	142.553	0.0000
Error	60	524.316	8.739		
Total	74	17964.319			
Binamoog 5					
Treatment	14	19353.390	1382.385	222.058	0.0000
Error	60	373.520	6.225		
Total	74	19726.911			

Appendix XXVI: Analysis of variance of different drought levels on mean germination time of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	3.000	0.214	57.237	0.0000
Error	60	0.225	0.004		
Total	74	3.225			
Binamoog 5					
Treatment	14	1.207	0.086	39.578	0.000
Error	60	0.131	0.002		
Total	74	1.388			

Appendix XXVII: Analysis of variance of different drought levels on germination index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	23676.060	1691.147	569.283	0.0000
Error	60	178.240	2.971		
Total	74	23854.300			
Binamoog 5					
Treatment	14	19642.151	1403.011	683.628	0.0000
Error	60	123.138	2.052		
Total	74	19765.289			

Appendix XXVIII: Analysis of variance of different drought levels on coefficient of velocity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	58.956	4.211	54.902	0.0000
Error	60	4.602	0.077		
Total	74	63.558			
Binamoog 5					
Treatment	14	24.865	1.776	43.882	0.0000
Error	60	2.428	0.040		
Total	74	27.294			

Appendix XXIX: Analysis of variance of different drought levels on energy of emergence of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	15107.544	1079.110	126.670	0.0000
Error	60	511.143	8.519		
Total	74	15618.687			
Binamoog 5					
Treatment	14	17267.700	1233.407	138.938	0.0000
Error	60	532.644	8.877		
Total	74	17800.343			

Appendix XXX: Analysis of variance of different drought levels on shoot length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	88289.749	6306.411	645.899	0.0000
Error	60	585.827	9.764		
Total	74	88875.575			
Binamoog 5					
Treatment	14	86569.565	6183.540	728.098	0.0000
Error	60	509.564	8.493		
Total	74	87079.129			

Appendix XXXI: Analysis of variance of different drought levels on root length of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	35720.559	2551.469	589.842	0.0000
Error	60	259.541	4.326		
Total	74	35980.100			
Binamoog 5					
Treatment	14	20217.810	1444.129	426.560	0.0000
Error	60	203.132	3.386		
Total	74	20420.941			

Appendix XXXII: Analysis of variance of different drought levels on seedling dry weight of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	25576.502	1826.893	422.027	0.0000
Error	60	259.732	4.329		
Total	74	25836.233			
Binamoog 5					
Treatment	14	21462.042	1533.003	615.707	0.0000
Error	60	149.389	2.490		
Total	74	21611.432			

Appendix XXXIII: Analysis of variance of different drought levels on vigour index of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	287286.657	20520.476	649.464	0.0000
Error	60	1895.761	31.596		
Total	74	289182.418			
Binamoog 5					
Treatment	14	232324.714	16594.622	704.758	0.0000
Error	60	1412.793	23.547		
Total	74	133737.508			

Appendix XXXIV: Analysis of variance of different drought levels on relative water content of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	5461.392	390.099	451.058	0.0000
Error	60	51.891	0.865		
Total	74	5513.283			
Binamoog 5					
Treatment	14	6167.559	440.540	5244.358	0.0000
Error	60	5.040	0.084		
Total	74	6172.599			

Appendix XXXV: Analysis of variance of different drought levels on water saturation deficit of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	5461.392	390.099	451.057	0.0000
Error	60	51.891	0.865		
Total	74	5513.283			
Binamoog 5					
Treatment	14	6167.559	440.540	5244.359	0.0000
Error	60	5.040	0.084		
Total	74	6172.599			

Appendix XXXVI: Analysis of variance of different drought levels on water retention capacity of mungbean

SV	df	SS	MS	F value	Prob.
BARI Mung 6					
Treatment	14	173.694	12.407	516.100	0.000
Error	60	1.442	0.024		
Total	74	175.136			
Binamoog 5					
Treatment	14	143.957	10.283	184.740	0.0000
Error	60	3.340	0.056		
Total	74	147.297			

PLATES

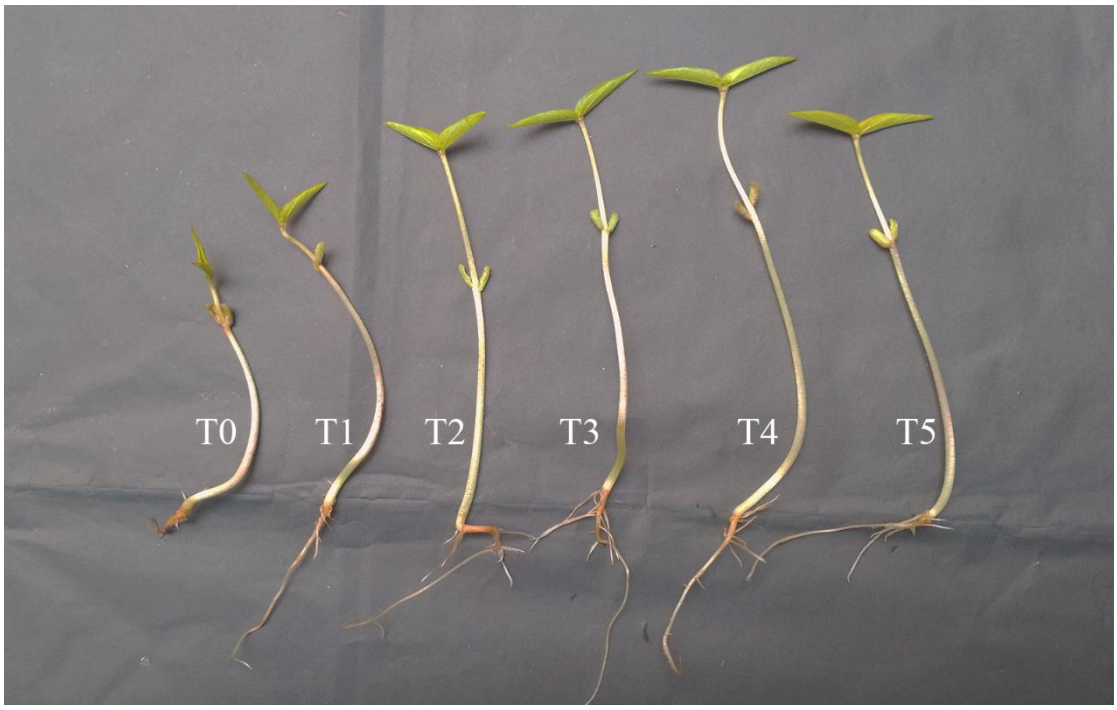

Plate 1: Effect of different concentration of priming solution on germination behavior of BARI Mung 6

Plate 2: Effect of different concentration of priming solution on germination behavior of Binamoo-5

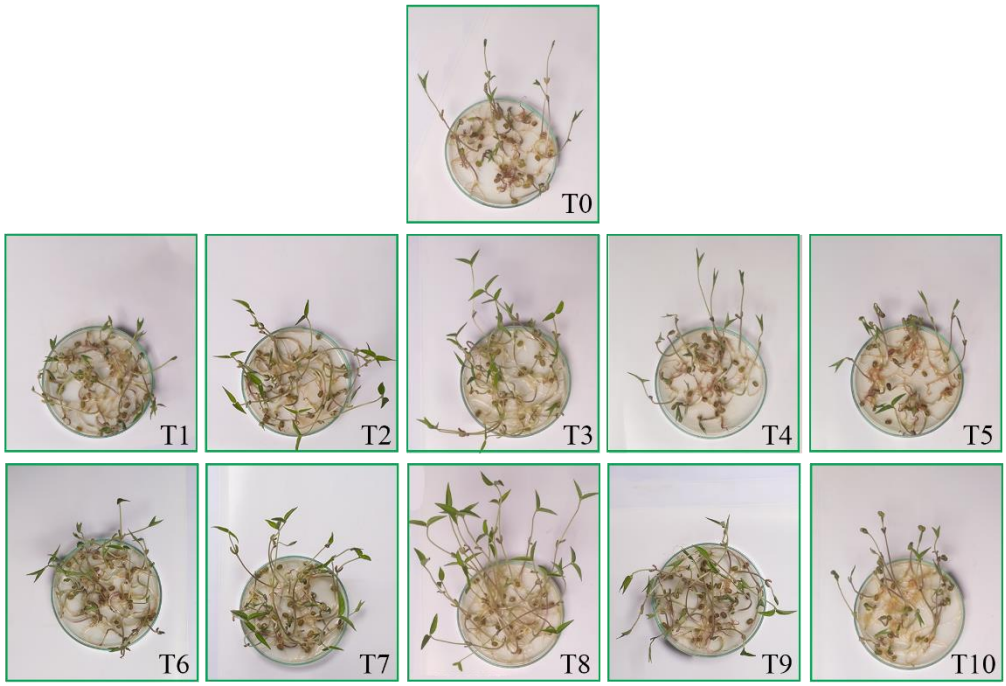

Plate 3: Effect of different priming time (water and mannitol primed) on germination behavior of BARI Mung 6.

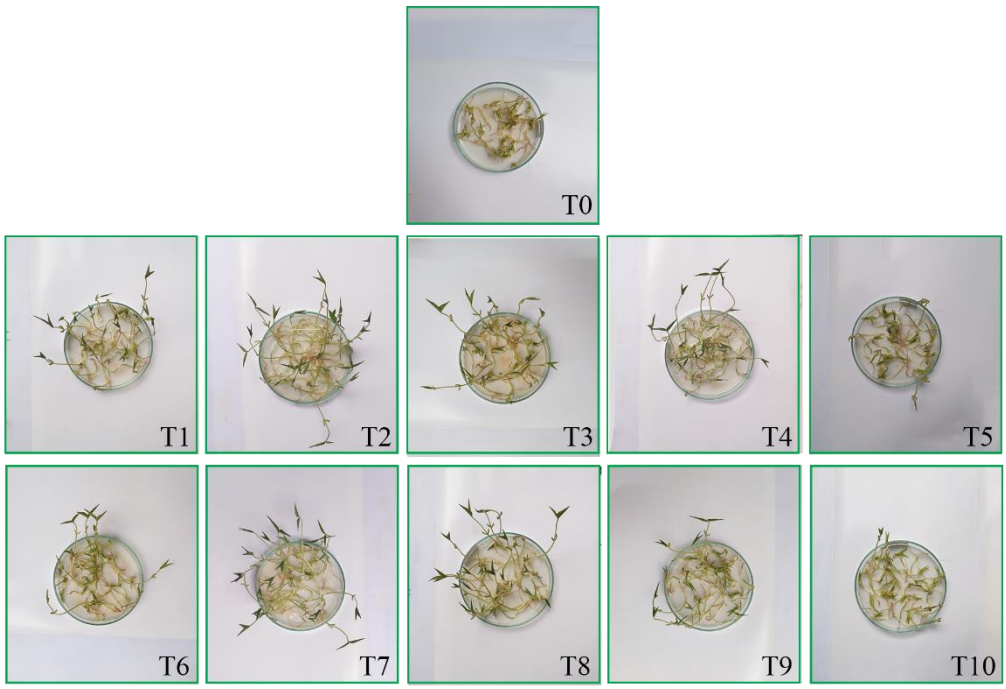

Plate 3: Effect of different priming time (water and mannitol primed) on germination behavior Binamoog 5.

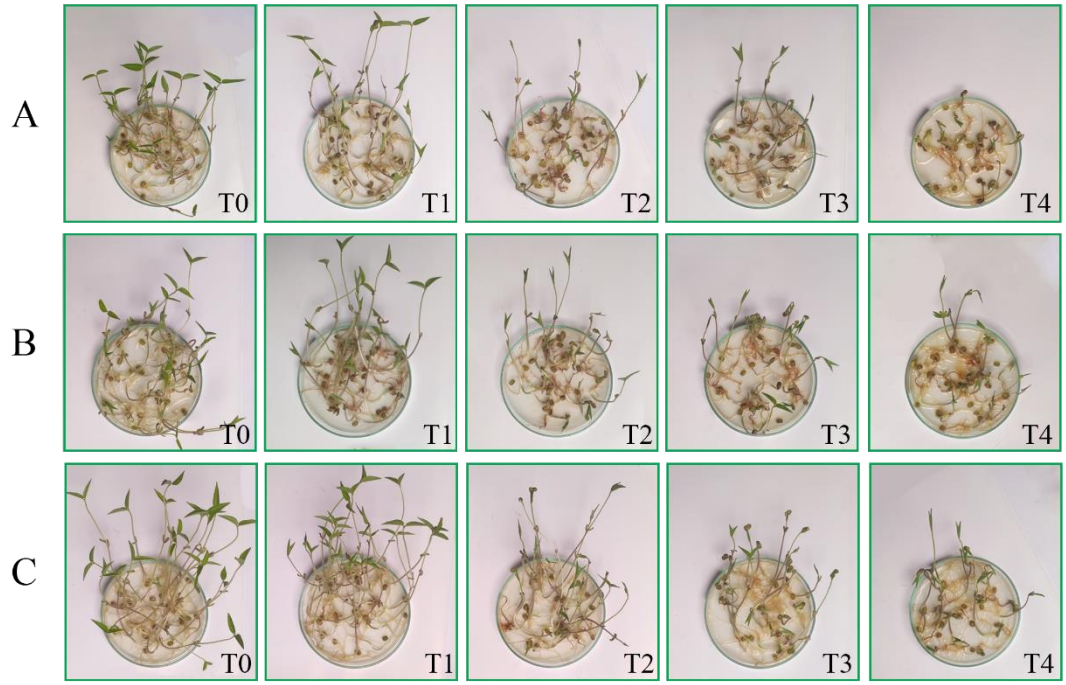

Plate 5: Effect of different drought levels on (A) control, (B) water primed and (C) 6% mannitol primed seeds of BARI Mung 6.

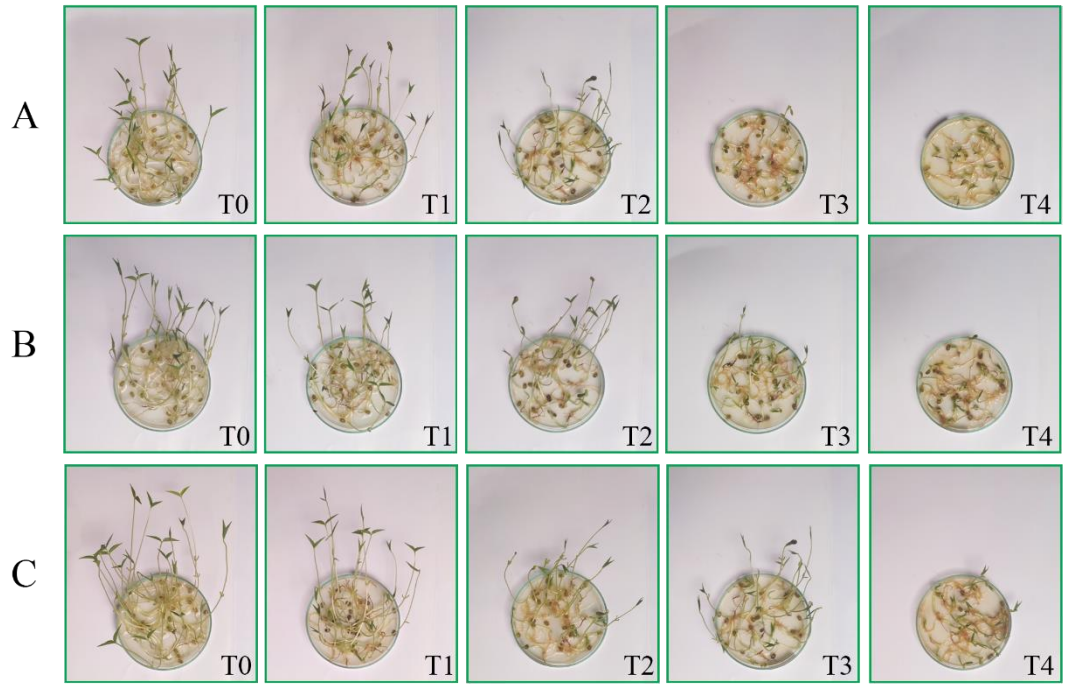

Plate 5: Effect of different drought levels on (A) control, (B) water primed and (C) 4% mannitol primed seeds of Binamoog 5.