INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH MANNITOL AND HYDROPRIMING

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CERTIFICATE

This is to certify that the research work entitled, **"INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH MANNITOL AND HYDROPRIMING"** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) in AGRONOMY, embodies the results of a piece of bona fide research work carried out by MUNMUN AKTER, Registration No. 12-05221, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution**.**

I further certify that any help or sources of information received during the course of this investigation has duly been acknowledged.

Dated: June, 2018

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INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH MANNITOL AND HYDROPRIMING

ABSTRACT

The experiments were conducted at the Agronomy Laboratory, Sher-e-Bangla Agricultural University, during the period from April to May 2018 to investigate the potentiality of seed priming for induction of drought tolerance capability and the pre-sowing seed treatment with mannitol on germination behavior and growth of soybean (BARI soybean 5 and BARI soybean 6) under drought stress conditions. The whole study was divided into three experiments. In the first experiment, two soybean varieties were surface sterilized with 75% of alcohol for 5 min and soaked in water and 2%, 4%, 6%, and 8% mannitol for 6 hours and dry seed used as control. Then the seeds were dried in room temperature to regain normal condition. Seeds primed with 6% mannitol of BARI soybean 6 showed higher germination percentage (96.70%), shoot length (86.92mm), root length (55.44mm), relative water content (95.41%), coefficient of germination (22.82%) and vigor index (137.7) than seeds without priming(control) of BARI soybean 5 in germination percentage (69.00%), shoot length (28.30mm), root length (17.20mm) , relative water content (67.58%), coefficient of germination (15.50%) and vigor index (31.38). In the second experiment, BARI soybean 6 was primed in 3, 6, 9, 12, 15, and 18 hours under both 6% mannitol solution and distilled water, respectively. Priming time 6 hours with 6% mannitol showed the best result for increasing the effectiveness in inducing drought tolerance in all parameters except WSD (water saturation deficit). In the third experiment, seeds were primed with distilled water and 6% mannitol for 6 hours and primed seeds were placed under control and drought stress condition induce by 5% PEG,10% PEG, 15% PEG and 20% PEG solution in Petri dish. Drought tolerance capability increased in osmopriming over hydropriming. These result suggest that soybean seeds primed with 6% mannitol for 6 hours showed the best result than hydropriming. This priming concentration and time helps to induce drought tolerance capability of soybean and also helps to enhance germination, growth of seedlings and water relation behavior under such stress conditions.

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LIST OF ACCRONYMS AND ABBREVIATIONS

Agric.	Agriculture
Agril.	Agricultural
Agron.	Agronomy
App.	Applied
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Biol.	Biological
Biotechnol.	Biotechnology
Bot.	Botany
Cm	Centi-meter
CRD	Completely Randomized Design
Environ.	Environmental
et al.	And others
Exp.	Experimental
g	Gram
h	Hour
Hort.	Horticulturae
Int.	International
ISTA	International Seed Testing Association
J.	Journal
Kcal	Kilocalory
mg	Miligram
ml	Mililiter
mm	Milimeter
MPa	Megapascal
Mmol/l	Milimole per liter
%	Percent
ppm	Parts per million
^{o}C	Degree centigrade
Physiol.	Physiological
Publ.	Publication
PEG	Polyethylene glycol
Regul.	Regulation
Res.	Research
Sci.	Science
Tech.	Technique
Technol.	Technology
Trop.	Tropical

CHAPTER I
INTRODUCTION

Soybean (*Glycine max* L.) belonging to family Papilionaceae, possesses a very high nutritional value. It is one of the most important protein and oil seed crops throughout the world. The crop is of a high commercial value as it contains about 40- 45% protein, 20-22% oil, 20-26% carbohydrate, a high amount of Ca, P and vitamins (Rahman *et al*., 2011). Its oil is the largest component of the world's edible oils. The oil contains about 0.5-1.0 per cent lecithin which is essential for building up of human nerve tissues. Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals is deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin A and Vitamin C.

Soybean is a multipurpose crop grown for edible oil, industrial use, human food, livestock feed and as a source of bio-energy. Processed soybean products such as flour, oil, soya milk, soya beverage, snacks and chunks have a long shelf life and soybean milk is important for feeding babies with lactose intolerance (Karuga *et al.*, 2004). The crop therefore has the potential to improve food and nutrition security, alleviate poverty in rural areas, protect the environment and increase incomes through increased productivity and value addition (Mathu *et al.*, 2010). Edible oils play vital roles in human nutrition by providing calories and aiding in digestion of several fat soluble vitamins, for example Vitamin A (National Research Council, 1989). The per capita recommended dietary allowance of oil is 6 g/day for a diet with 2700 Kcal (BNNC, 1984). At least 15% (405 Kcal) of the total calories must come from visible and invisible oils or fats for maintaining good health. Oil cake is also an important manure for crop production and livestock feed.

At present, the area under oil crop is 0.484 million hectare out of 14.418 million hectare total cropped areas and total production of the country is 0.975 million tons (BBS, 2017). Plant growth and productivity adversely affected by various biotic and abiotic stress factors. Water stress, drought and salinity are known to inhibit the germination and growth of most crop species. All these stress factors prevent them from reaching their full genetic potential and limit the crop productivity worldwide. Soybean is grown in different agro-ecological conditions, thus seed germination and vigor are affected by various unfavourable environmental factors such as drought, extreme temperatures, untimely sowing, etc. (Casenave and Toselli, 2007). One of the methods which can overcome this problem is priming, i.e. soaking the seed prior to sowing (Ashraf and Foolad, 2005).

Seed priming is a process which leads to fast and even germination and sprouting with the aim of achieving high vigor and better yield. It is reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions (Heydecker *et al*., 1973, 1975; Harris *et al*., 1999). Hydropriming is a very simple, cost-effective, and eco-friendly technique which basically involves soaking seeds in water for a pre-determined time followed by re-drying to their initial moisture content (Farooq *et al.*, 2006a; Lutts *et al.*, 2016).

Priming ensures optimum running of molecular-biological processes during germination, stimulates activation of different enzymes, mobilizes proteins reserves and prepares cells for division (Soleimanzadeh, 2013). This technology is mainly applied with vegetable crops (Basra 2004, Farooq 2006b, Soltani 2001), and in some cases with field crops: wheat, sugar beet, maize, soybean, and sunflower (Khajeh-Hosseini *et al*., 2003). Basra *et al*., (2003) reported that priming treatment significantly affected growth parameters and recorded an increase in LAI and dry matter accumulation due to priming in canola. During priming, seeds are partially hydrated so that pre-germinative metabolic activities proceed, while radical protrusion is prevented, then are dried back to the original moisture level (McDonald, 2000).

Earlier reports have shown that seed priming was influenced by many complex interactions of factors and conditions such as plant species, water potentiality of priming agent, soaking duration in priming agent, variation of temperature, seed vigor and dehydration, and storage conditions of the primed seed (Hussain, *et al.*, 2006). Seong *et al*., (1988) reported that the moisture content and the seedling length decreased when the mannitol concentration increased, concluding that germination in mannitol was useful for the selection of soybean cultivar for emergency capacity under conditions of water deficit.

Quite a good number of works have been done on seed priming of soybean but under Bangladesh condition such works are scanty. The seed priming technology can enhance seedling emergence and ensure good plant stand which in turn can maximize yield and improve quality of the crop.

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

- To evaluate the effect of pre-sowing seed treatment with mannitol on germination and water relation behavior of soybean.
- To optimize the priming time of the best priming chemical on germination behavior of soybean.
- To evaluate the effect of seed priming on germination and water relation behavior of soybean under drought stress.

CHAPTER II

REVIEW OF LITTERATURE

The effect of priming on soybean and other crops in term of germination behavior and other performance has been studied throughout the world. Different priming techniques such as osmopriming , hydropriming, nutrientpriming etc used with different priming time on different crops were found. Some importent findings about hydropriming and osmopriming are cited here . The review of literature given below was based on the performance of different priming on different crops cultivation.

Senaratna and McKersie (1983) studied the effects on germination of imbibing soybean seeds followed by a dry down. They found that both the length of the imbibition period and the moisture content following drying affected germination percentage. Imbibing soybean seeds for 6 hours followed by drying to 10% moisture did not exhibit a significant statistical difference in germination percentage than the nondehydrated control, which had a germination percentage of 93%. Water imbibitions for 12, 18, and 24 hours resulted in germination percentages of 60, 65, and 65%, respectively, after the seeds were dried to 10% moisture. When the soybean seeds was imbibed for a period of 36 hours then dehydrated to moisture levels of 60, 40, and 20%, germination percentages were 90% or 5 greater. However when the 36 hour imbibed seeds were dehydrated to 10% moisture, seed germination percentage dropped to 0%. Senaratna and McKersie (1983) concluded that soybean seed viability after an imbibition-dehydration treatment was influenced by the severity of dehydration and the length of the germination period prior to dehydration.

Kaya *et al., (*2006) examined that the treated seeds (control, KNO_3 and hydropriming) of sunflower (*Helianthus annuus* L.) cultivars were evaluated at germination and seedling growth for tolerance to salt (NaCl) and drought conditions induced by PEG-6000 at the same water potentials of 0.0, −0.3, −0.6, −0.9 and −1.2 MPa. Results revealed that germination delayed in both solutions, having variable germination with different priming treatments. Germination, root and shoot length were higher but mean germination time and abnormal germination percentage were lower in NaCl than PEG at the same water potential. Seeds were able to germinate at all concentrations of NaCl but no seed germination was observed at −1.2 MPa of PEG treatments. NaCl had less inhibitor effect on seedling growth than the germination. It was concluded that inhibition of germination at the same water potential of NaCl and PEG resulted from osmotic effect rather than salt toxicity. Hydropriming increased germination and seedling growth under salt and drought stresses.

Moghanibashi *et al*., (2012) carried out a laboratory experiment to evaluate the effect of aerated hydropriming (24h) on two cultivar of sunflower (Urfloar and Blazar) seed germination under a range of drought stress and salt stress. Cultivar Urfloar had the more germination rate (R50), days to 50% germination (D50), germination index (GI), root and shoot length and dry weight as compared with cultivar Blazar. Hydropriming for 24 h increased germination percentage, germination rate, germination index, root and shoot length, root and shoot weight of seed sunflower as compared with the control. Primed seeds produced higher germination rate and percentage, D50 and GI under all salinity and drought levels as compared with non-primed seeds. The results suggested that hydropriming for 24 h was enhanced germination and seedling growth of sunflower under stress conditions. Therefore, this treatment may be used to improved seed performance of sunflower under normal and stress conditions.

Bijanzadeh *et al*., (2010) studied to enhance the germination and emergence of rapeseed (*Brassica napus* L.) cv. Hyola 401 seeds using different priming treatments. Priming treatments examined were hydropriming, osmopriming with polyethylene glycol (PEG 6000), and solid matrix priming (SMP) with animal compost. Seeds from each of these 3 techniques were primed for 12 and 24 hours. Germination times and percentages, seedling growth, and electrical conductivity of seed leachates were measured. Solid matrix priming with compost was effective in improving germination percentage as compared to the non-primed control. Solid matrix priming with compost for 12 and 24 hours, and hydropriming for 24 hours were the most efficient techniques for reducing the mean germination time. Highest electrical conductivity was recorded for the control group and seeds osmo-primed with PEG 6000 and the lowest electrical conductivity in hydro-primed and SMP treatments after 12 and 24 hours. During emergence tests, the SMP treatment for 24 hours reduced the mean emergence time and also recorded the highest final emergence.

Arif *et al*., (2008) conducted an study to determine the effects of osmo and hydro-priming on phenology, yield components and biomass yield of soybean (*Glycine max*) cv. William-82. After a laboratory experiment to determine the optimum combination of priming duration and polyethylene glycol 8000 (PEG 8000) concentration, field experiment was conducted in 2003 and 2004 with three priming durations (6, 12 and 18 h) and five different concentrations of PEG 8000 solution (0, -0.2, -0.5, -1.1, -1.8 , -3.0 and -4.2 MPa), together with a dry seed (non primed) control. Primed and non-primed seeds were sown in the field. During both years, plants from primed seed flowered and matured faster than plants from non-primed seed. Primed seed gave taller plants. Averaged over all treatments, priming for 6 h or with -1.1 MPa, were the most beneficial treatments. It is concluded that priming with PEG was much effective but priming with water alone was also better than control.

Pegah *et al*., (2008) conducted study to evaluate the influence of seed priming techniques on germination and early growth of two maize inbred lines including B73 and MO17. Seeds were hydroprimed for 12, 24, 36 and 48h, osmoprimed in urea solution and in solution of polyethylene glycol-6000 (PEG-6000) for 96h (4 days) (water potential -1.2 MPa). Priming techniques affected seed germination and early growth of both inbred lines. Hydropriming resulted in lower time taken to germination and higher germination index, vigor index and final germination percentage in both genotypes. Maximum invigoration was observed in seeds hydroprimed for 36h as indicated by higher germination rate, radical length. Conversely, for most germination parameters osmoprimed seeds behaved similar to or even poor than that of control.

Dkhil *et al.,* (2014) conducted an experiment to evaluate the effects of priming on the germination, emergence and seedling growth of *Abelmoschus esculentus* (cultivar Marsaouia) under low temperature and salinity conditions. Seeds were primed for 24h at 20°C in three priming media (KCl 4%, mannitol 0.75M, CaCl2 10Mm) and control (non-primed seeds) and were examined at different salinity levels (0, 40 and 100 mM NaCl). Result indicated that KCl priming increased final germination percentage, radicle length and seedling dry weight 100%, 40.94 mm and 0.3 g, respectively, as compared with non-primed seeds. Mannitol and $CaCl_2$ have been found to be better treatments for improving final emergence percentage. Overall increased

NaCl level, led to the reductions in final germination and emergence percentage but these reductions were higher for non-primed compared to primed seeds.

Harris *et al*., (2001) reported the germination of maize without priming from less than 40hr to more than 70hr in laboratory experiment. Priming seeds of maize for 12hr reduced the time for germination. The treatment reduced the range of germination times to between 20hr and 40hr in the field experiments, primed maize seed in water for 16hr or 18hr resulted in statistically significant positive benefits. Where priming was effective, the extra produce varied from 0.3 t/ha to about 14 t/ha and represented increases ranging from 17% to 76%.

Ghassemi- Golezani *et al.*, (2008) concluded that hydropriming is a simple, low cost and environmentally friendly technique for improving seed and seedling vigor of lentil. Seedling emergence rate was also enhanced by priming seeds with water. Hydropriming significantly improved imbibition rate, germination rate, seed vigor index, shoot, root and seedling dry weights, compared to other seed treatments. The different soaking-drying treatments are highly effective in maintaining viability and vigor in most seeds, except leguminous seeds because soaking injury are observed due to rapid uptake of water (Som and Chattopadhyay, 1996).

A field experiment on a sandy soil in Rajasthan, India showed that emergence of pearl millet was only around 50 %, even in moist soils. However, primed seeds emerged better across a range of soil moisture levels and the relative increase due to priming increased from 15 % in moist soil to 45 % in dry soil. Priming was not able to compensate completely for the effects of low soil moisture at sowing but made a significant contribution across a range of soil moisture contents and was relatively more effective in drier soils (Harris, 2006).

Toklu (2015) conducted an experiment under laboratory and field conditions in order to evaluate the effects of different priming treatments, specifically KNO_3(1%), KCl (2%), KH_2PO_4 (1%), $ZnSO_4$ (0.05%), PEG-6000 (20%), IBA (100 ppm), Mannitol (4%), GA_3 (100 ppm) and distilled water on seed germination properties and several agro-morphological plant characteristics of red lentil. Seeds not primed were used as a control. GA_3 treatment increased shoot length. The control (non-primed seeds) treatment resulted in increased seedling root number and length. Distilled water, $ZnSO_4$ and control treatments increased germination rate and percentage. In the pot experiments, GA_3 treatment increased plant height and seedling emergence rate, whereas KCl treatment improved the number of nodules, as well as root and shoot dry weight when compared to the control. $ZnSO_4$ treatment increased yield components and grain yield in field conditions. The results of this study showed that $ZnSO_4$, GA_3 and PEG-6000 seed priming treatments may be useful tools due to their positive effects on germination rate, germination percentage, yield component and grain yield of lentil.

Dastanpoor *et al*., (2013) in Iran showed that the germination of *Salvia officinalis* L. (sage) seeds is a problem of great concern that may be overcome by employing seed priming techniques. Seed priming is an efficient technique for improvement of seed vigor, increasing germination and seedling growth. Little information has been reported on seedling development of sage subsequent to seed priming. The influence of hydropriming treatments on seed parameters of *S. officinalis* L. was investigated. Seeds of sage were treated by hydropriming at three temperatures 10, 20, 30°C for 0, 12, 24 and 48 h. The hydropriming clearly improved the final germination percentage (FGP), mean germination time (MGT) and synchronized the germination of seeds at each three temperature. All the seed treatments resulted in germination enhancement except hydroprimed seeds for 48 h at temperature 30°C. However, hydropriming (12 h at 30°C) was most effective in improving seed germination that FGP was increased by 25.5% as compared to that of non-primed seeds.

To overcome the problem of slow and erratic emergence in okra, two experiments, i.e., laboratory and field were carried out during 2013. In the laboratory experiment, okra seeds were primed in PEG-8000 (polyethylene glycol) and Mannitol solutions (0, -0.4, -0.8, -1.2, -1.6, -2 and -2.4 Mpa osmotic potential) for 6, 12, 18 and 24 hours while dry seeds were used as control. Same primed seeds were used in the field experiment. Priming proved effective in improving percent germination and reducing the mean germination time (MGT) as compared to unprimed seeds. Priming agents, its osmotic potential and duration significantly affected germination percentage, which increased as osmotic potential lowered from 0 to -1.2 Mpa while further lowering osmotic potential to -1.6 Mpa and below adversely affected germination. Similarly, germination percentage significantly enhanced as priming duration increased from 6h to 12h but further increase in duration from 12h to 18h caused insignificant increase in germination percentage. Increase in priming duration beyond 18h, however, resulted in non-significant decrease in percent germination. Amongst priming agents, PEG gave better germination than

Mannitol, however, priming agents were ineffective in lowering MGT. Increase in osmotic potential from -2.4 to 0 Mpa and duration from 6h to 24h caused significant reduction in MGT. Field experiment results showed significant improvement in yield from primed seeds as compared to unprimed seeds. Seeds primed with PEG gave significantly a higher yield than Mannitol. Maximum yield was observed at -1.2 Mpa osmotic potential. Like MGT, days to 50% emergence in the field were unaffected by priming agent but significantly affected by osmotic potential, reduction in days to 50% emergence occurred as osmotic potential increased from -2.4 to 0 Mpa (Inayat-Ur-Rahman *et al.,* 2013).

A study was conducted by Elkoca (2007) under controlled environmental conditions in Turkey. The effects of various seed priming treatments and seed soaking durations on germination performance of pea (*Pisum sativum* L. cv. Winner) seeds were examined. Seeds were osmoprimed in polyethylene glycol (PEG 6000) (-0.5, -1.0 and -1.5 bar) or in mannitol (1%, 2% and 3%) and hydroprimed with water for 12 or 24 h at 25 ± 0.5 °C in darkness. Primed seeds were subjected to germination tests at ten different constant temperatures ranging from 5 to 32 ± 0.5 °C. Priming treatments had no significant effect on germination percentage. But, osmo- and hydroprimig treatments improved germination rate and decreased thermal time requirements significantly and induced more synchronous germination at some of the temperatures tested. Reductions in thermal time requirements ranged between 3.4 °C d and 11.3 °C d, 6.6 °C d and 17.4 °C d, and 11.6 °C d and 27.5 °C d for 10%, 50% and 90% germination, respectively. As compared with the priming duration of 12 h, priming duration of 24 h had generally negative effect on the 50% germination time and thermal time requirement. Among the osmopriming treatments, seeds treated with -0.5 bar solution of PEG and 1% solution of mannitol, and also hyropriming gave the best results. Consequently, above osmo- and hydropriming treatments for 12 h might be recommended for better germination of pea.

Afzal *et al*., (2011) 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. Priming with 2 and 4% mannitol for 24h maximally increased final germination percentage, germination capacity, germination index, shoot and root lengths of both marigold species as compared to all pre-sowing seed treatments including control. Similarly, at those concentrations mannitol significantly reduced mean emergence time and days to 50%

emergence, increased seedling emergence uniformity, final seedling emergence percentage and seedling growth. In conclusion, priming with 2% mannitol proved to be the most effective priming agent in both marigold cultivars. This could be attributed to the effect of mannitol in increasing reducing and total sugars as well as alpha-amylase activity in primed seeds.

Mir-Mahmoodi *et al.*, (2013) conducted a field experiments and laboratory evaluations to investigate the effects of hydropriming durations on sunflower seed. Field tests were conducted in a RCB design in 2011 and 2012 to evaluate the effects of various durations of hydropriming treatment on evaluations for seed germination, seedling vigor and field establishment of sunflower seeds. Seeds were divided into five sample sets, one of which was used as the control (non-primed) and the other four sample sets were soaked in distilled water for durations of 6, 12, 18 and 24h and then dried off to reach a moisture content of about 10-12%. Linear regression showed that a longer duration of hydropriming improved evaluations for germination percentage and mean time for germination. Records were taken for seed response in terms of dry weight of shoot, root and seedling and electrical conductivity. Evaluations for dry weight max, root dry weight max and seedling dry weight max were obtained from durations of 13.7, 15.1 and 14.3h hydropriming respectively. The lowest evaluation for electrical conductivity was recorded in the hydropriming duration of 7.15h. This resulted in better stand establishment of seedlings from primed seeds and the optimum duration of hydropriming sunflower seeds was recorded in the range of 13.7-15.1h. Hydropriming in field conditions enhanced evaluations for percentage of seedling emergence (0.63%/1h priming) and reduced time to emergence (-0.29h/1priming). Thus, hydropriming presents a simple method for improving seed germination and seedling emergence of sunflower in field conditions.

Geraldo *et al*., (2016) in Brazil showed that abiotic stress directly influences seed performance, so poor-vigor seeds under adverse conditions tend to show lower germination speed and rate. By controlling the hydration level of seeds (i.e., priming) with the addition of chemical agents, it is possible to elicit the maximum physiological potential of seeds, even under stress conditions. This study aimed to evaluate the priming effect of different chemical agents on the physiological potential of maize seeds under abiotic stress (polyethylene glycol induced water stress, hypoxia, low temperature and salt stress after controlled deterioration). Priming with calcium nitrate lead to a greater germination and higher emergence rate of the seedlings under suboptimal temperature conditions, and seeds that underwent controlled deterioration showed greater germination levels with the use of calcium nitrate + phenylalanine in the priming process, regardless of the lot used. In general, seed priming allowed a greater expression of seed vigor, even though an interaction with lots was observed in some variables. this study aimed to evaluate the priming effect of different chemical agents on the physiological potential of maize seeds submitted to abiotic stressful conditions (polyethylene glycol - PEG induced water stress, hypoxia, low temperature and salt stress after controlled deterioration).

Seed priming has proven beneficial in many important agricultural crops. The present study was conducted to explore the role (if any) of hormonal and vitamin seed priming to improve the germination, seedling emergence, early seedling establishment, electrolyte leakage and nutrients uptake in wheat seedlings. The wheat seeds were soaked for 48 hours in aerated solution of salicylic acid, ascorbic acid, kinetin and GA_3 with 20 ppm concentration of each solution, whereas untreated seeds were taken as control. Seed priming with ascorbic acid resulted in maximum final germination and emergence percentage (FGP and FEP), radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root dry weight, shoot dry weight and seedling dry weight compared to control (untreated seeds). Minimum mean germination and emergence time (MGT and MET) was recorded in seeds primed with kinetin and GA_3. While in case of biochemical attributes, seedling potassium contents were decreased by hormonal seed priming while total soluble sugars were increased by salicylic acid and ascorbic acid seed priming. Hormonal seed priming had non-significant effect on phosphorus seedling contents. Untreated (Control) seeds showed maximum electrical conductivity at 0.5, 1, 2, 6, 12 and 24 h after imbibition than primed seeds against the minimum electrical conductivity that was recorded in seeds primed with salicylic acid and ascorbic acid. In conclusion, the wheat seeds primed with 20 ppm solution of ascorbic acid may be used for wheat seed invigoration (Khan *et al*., 2010)

Seed osmopriming could be a sustainable method to increase crop establishment, uniform emergence, and growth of plant on the field. Laboratory and field studies were carried out in 2010 cropping season at Usmanu Danfodiyo University, Sokoto, to study the effect of seed osmopriming duration on the germination, emergence, and growth of cowpea seeds. Treatments consisted of three osmopriming duration (soaking in 1% KNO_3 salt for 6, 8, and 10 hrs), one hydroprimed control (10 hr), and an unprimed control.These five treatments were laid out in a completely randomized design (CRD) replicated four times. The results showed that osmopriming with KNO_3 for different durations was at par but was superior to unprimed treatments in terms of seed germination, emergence, plant height, and dry matter accumulation at 3 weeks after sowing. From this study, it can therefore be concluded that seeds of cowpea could be primed (both hydro and osmopriming) for increased performance. However, osmopriming with KNO_3 salt (soaked in 1% KNO_3 salt solution and dried before sowing) for 6 hours could result in greater seed germination, emergence, seedling growth and seedling height than hydropriming in semiarid environment. (Singh *et al*., 2013).

Kiani *et al*., (2017) examined the effect of drought stress induced by mannitol at three levels (0, 88 and 176 mM) on biochemical and polyphenolic traits of six F1 broccoli hybrids, a factorial experiment based on completely randomized design in three replications was implemented at research station of Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran in 2016. The results showed that mannitol stress reduced dry weight and shoot length significantly for all varieties but with a different rate. Total phenolic, flavonoid, and anthocyanin contents, the activity of enzymatic antioxidants, and DPPH activity were significantly higher under 176 Mm mannitol application condition than control. In addition, results showed that mannitol stress increased the content of sulforaphane. Marathon genotype showed the highest content of sulforaphane among all varieties under both normal (6.139) and under stress (14.122) conditions. Marathon and Heraklion genotypes could be suggestively used for breeding program to increase content of sulforaphane coupled with other traits. Since the content of sulforaphane along with phenolic compounds and antioxidant activities were higher under both severe and moderate stress conditions, using moderate mannitol stress treatment can be implemented for increasing the content of these suitable compounds in broccoli .

Plants are exposed to various stress factors which might lead to structural damage and physiological function abnormalities. Drought is one of the environmental stress factors that reduce the productivity of plants. The aim of our study was to determine the influence of drought stress induced by mannitol (-0.5 and -1.5MPa) on selected physiological processes in Z. mays L. In the first stage we studied the effect of mannitol on the germination. In the second stage the effect of mannitol on the growth of plants germinated on distilled water and watered with mannitol in growth phase were measured. Mannitol, which decreased the water content in a concentration-dependent manner, had an inhibitory effect on germination and growth of seedlings and adult plants. Electrolyte leakage of cell membranes of the Z. mays seedlings showed high disturbances in the functioning of the membrane structures in the osmotic drought conditions. Similar results were obtained for maize roots, shoots and leaves in both treatment studies. Chlorophyll content showed only significant differences in plants from treated during the growth phase. Drought stress caused a decrease in chlorophyll content by almost a half compared to the control plants. Measurements of chlorophyll fluorescence of plant leaves from the second stage of experiments showed changes in fluorescence activity parameters Fv/Fm, NPQ, Rfd, qP, ect.; gas exchange measurements also showed changes in activity in each of the two phases.(Mozdzen *et al*., 2015).

Jalilian *et al*., (2014) conduct an experiment in green house resulted that seed priming was used to reinforcement of barley seedling growth under water deficit stress in a greenhouse condition. Barley seeds were primed with humic acid, *Pseudomonas* Spp., Marmarin, distilled water (hydropriming) and none (as control) under four levels of water deficit stress (irrigation at 20 (I1), 40 (I2), 60 (I3) and 80% (I4) field capacity). Results indicated that all measured parameters were decreased with increasing the stress levels, except root length and root-shoot ratio. The highest value of root length (18.42 cm) and root-shoot ratio (2.84) was obtained in the I1 irrigation regime. However, I2, I3 and I4 irrigation regimes did not significantly affect on barley seedling traits, but I1 irrigation regime exhibited better growth. Seed priming with Pseudomonas affected root length, root and shoot dry weight, plant height and SPAD, significantly. Seed priming with Marmarin showed best results on SPAD. The maximum (0.37 g/plant) shoot dry weight was obtained from I2 irrigation regime with hydropriming treatment. Whereas, the lowest shoot dry weight (0.14 g/plant) was observed in I1 irrigation regimes in control condition. Hydropriming shows better response in root length, root and shoot and plant height and was equal with Pseudomonas, so it could be considered as a suitable substitute in organic agricultural systems.

An experiment was conducted in Post Graduate Laboratory, Department of Genetics and Plant Breeding, SHUATS, Allahabad, U.P. during Rabi (2016), in order to standardize the best method of Osmopriming specific to chickpea. One method of priming viz., osmopriming, on two duriations that is 6hrs and 12 hrs were evaluated by screening a range of durations and concentrations viz., T0 - Unprimed Control, T1–Polyethylene glycol (PEG) (for 6hrs & 12hrs), T2 –Mannitol 4% (6hrs & 12hrs) T3–Glycerol 5% (6hrs & 12hrs). It was found that all the priming methods showed significance difference with the control and the highest germination %, seedling length (cm), seedling fresh weight (g), seedling dry weight (g) and vigor index were observed in T2 for PEG 6000 priming for 12 hrs. Among all the osmopriming treatments, osmopriming PEG 6000 was found to be the best osmopriming treatment followed by Mannitol. The study helps to improve the quality of seeds with the help of seed osmopriming treatments which are cost effective and economic, non toxic, eco friendly sources.(Kumar *et al*., 2017).

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

Musa *et al*., (2014) conducted two field trials to evaluate the effect of hydropriming duration on the growth and yield of amaranth. Treatments consisted of four hydropriming durations (2, 4, 6, and 8 hours) and control (where no priming was applied). The treatments were laid out in a completely randomized design (CRD) replicated three times for the germination test and randomized complete block design (RCBD) for the field trial. Data were collected on days to 50% germination, percentage germination, days to 50% emergence, and percentage emergence. Results revealed significant effect of hydropriming duration on days to 50% germination, percentage germination, and days to 50% emergence. Soaking seeds for 2 hours reduced the number of days to 50% germination and emergence and also recorded higher germination. Thus, from the findings of this study, it could be concluded that hydropriming amaranth seeds for 2 hours could be applied to enhance amaranth production.

Yucel (2012) in order to develop suitable techniques to improve lentil seed germination capacity, a research was conducted with cultivar Local Red lentil cultivar. Seeds were fully soaked in KH_2PO_4 (1%), KNO_3 (1%) solutions for osmopriming, and distilled water for hydropriming treatments, for 12 and 24 hours at a 24°C and untreated seeds as control. After the priming treatments, seeds were germinated at six different (5, 10, 15, 20, 25 and 30 ± 0.5°C) constant temperatures. In terms of both germination percentage and MGT, the highest results were obtained from priming treatment of water at germination temperature of 20°C in priming times of 12h and 24h. The best germination synchrony value was obtained from water treatment for 12h at 15°C. Consequently, seeds treated with water for 12h produced the highest germination percentage and the least mean germination time and synchrony, in this way this treatment may be recommended for germination of lentil under different germination temperatures.

An experiment was carried out at the laboratory of Botany Department, Kohat University of Science and Technology, Kohat in December 2012. Two wheat cultivars (Janbaz and Atta Habib) were subjected to different mannitol levels (0, 125, 250 and 500 mM) under room temperature in petridishes. The objectives of the study were to investigate the effect of mannitol induced drought stress on seedling traits and protein profile of two wheat cultivars. Analyses of variance revealed significant differences for shoot and root length for mannitol levels as well as for interaction. Genotypes, however, showed significant variability for root length only. It was evident from the data that increased levels of mannitol decreased the shoot and root lengths linearly. The longest shoot (8.57 and 9.57 cm) and root (9.73 and 10.13 cm) were recorded from control treatments while the shortest shoot (3.03 and 2.67 cm) and roots (2.37 and 2.67 cm) were observed in 500 mM treatment for Janbaz and Atta Habib, respectively. 96.1 and 88.6 % of variation in shoot length and 72.3 and 71.5 % variation in root length was due to the mannitol induced drought stress for Janbaz and Atta Habib, respectively (Ullah *et al.*, 2014).

Karakas (2008) examined that tobacco plants (*Nicotiana tabacum* L.) were transformed with a mannitol-1-phosphate dehydrogenase gene resulting in mannitol accumulation. Experiments were conducted to determine whether mannitol provides salt and/or drought stress protection through osmotic adjustment. Non-stressed transgenic plants were 20–25% smaller than non-stressed, non-transformed (wild-type) plants in both salinity and drought experiments. However, salt stress reduced dry weight in wild-type plants by 44%, but did not reduce the dry weight of transgenic plants. Transgenic plants adjusted osmotically by 0.57 MPa, whereas wild-type plants did not adjust osmotically in response to salt stress. Calculations of solute contribution to osmotic adjustment showed that mannitol contributed only 0-003-0-004 MPa to the 0.2 MPa difference in full turgor osmotic potential (%) between salt-stressed transgenic and wild-type plants. Assuming a cytoplasmic location for mannitol and that the cytoplasm constituted 5% of the total water volume, mannitol accounted for only 30–40% of the change in of the cytoplasm. Inositol, a naturally occurring polyol in tobacco, accumulated in response to salt stress in both transgenic and wild-type plants, and was 3-fold more abundant than mannitol in transgenic plants.

Drought stress reduced the leaf relative water content, leaf expansion, and dry weight of transgenic and wild-type plants. However, π_o was not significantly reduced by drought stress in transgenic or wild-type plants, despite an increase in non-structural carbohydrates and mannitol in droughted plants. We conclude that (1) mannitol was a relatively minor osmolyte in transgenic tobacco, but may have indirectly enhanced osmotic adjustment and salt tolerance; (2) inositol cannot substitute for mannitol in this role; (3) slower growth of the transgenic plants, and not the presence of mannitol, may have been the cause of greater salt tolerance, and (4) mannitol accumulation was enhanced by drought stress but did not affect π_o or drought tolerance.

Carlos *et al*., (1992) conduct an experiment on 'Verina' leek (*AIlium porrum* L.). Seed germination is normally reduced at temperatures > 25^0C. Leek seeds were primed in aerated solutions (- 1.5 MPa, 10 days at 15^0C) of d-mannitol (mannitol), polyethylene glycol-8000 (PEGa), KNO_3 , and a non-aerated solution of PEG-8000 (PEGna). At high temperatures mannitol, PEGa , and PEGna significantly enhanced germination percentage relative to KNO_3 , or the control. At constant 30^0C, the mannitol, PEGa , and PEGna treatments increased final germination almost 10 times and the coefficient of velocity (COV) was improved compared to KNO_3 , and the control. 10 growth chambers with alternating day/night temperatures (38 to 28^0C or 32 to 22C, 10 to 14 hours, respectively), primed seeds had significantly higher emergence and a larger COV than the control. In a greenhouse study under good conditions for germination, total emergence of primed and nonprimed seeds was similar; however, mannitol, PEGa , and PEGna led to a significantly higher COV than the control or KNO_3 , treatments. These controlled-environment results demonstrate that priming leek seeds via mannitol, PEGa , and PEGna may promote early emergence at high temperature and improve stand uniformity for container transplant production.

Yan (2015) studied that drought stress influence seed germination and seedling growth of many plants. Seed priming could be used to alleviate the depressive effects of drought stress. The improving effects are influenced by many factors including priming methods, plant species and drought intensity. The mechanisms of drought tolerance induced by seed priming have not been clearly elucidated. The study was carried out to assess whether drought tolerance could be enhanced by seed priming at the germination stage and characterize the potential physiological and biochemical alternation of drought tolerance in Chinese cabbage. The seeds were soaked at 20°C for 8h in distilled water, 200 m mol/l potassium nitrate (KNO_3), 200 m mol/l urea, respectively. Both primed and unprimed seeds germinated under six levels of drought stress (0, −1.0, −2.0, −3.0, −4.0 and −5.0 MPa osmotic potential) induced by PEG 6000. Results indicated that germination traits (germination percentage, potential and seedling vigor index) of Chinese cabbage all decreased gradually with increasing drought intensity. Three seed priming types including water, KNO_3 and urea all increased germination traits at all levels of drought stress as compared to the unprimed seed. The enhanced drought tolerance conferred by seed priming treatments is associated with the modulating peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities and levels of soluble sugar and proline. The results suggested that priming could serve as an appropriate treatment to increase the germination and early seedling growth of Chinese cabbage under drought stress conditions.

It has been estimated that about nine million square kilometers of the world's arid rangelands have been turned into man- made deserts over the past half century. *B. inermis* was introduced as a livestock improvement crop, it has since invaded natural prairies and grasslands, outcompeting native grasses and decreasing biodiversity. The increasing frequency of dry periods in many regions of the world and the problems associated with salinity in irrigated areas frequently result in the consecutive occurrence of drought and salinity on cultivated land. The objective of this study was to determine the effect of seed priming on germination characteristics of Bromus species under stressful conditions. For osmopriming treatment, Bromus seeds were immersed in -0.6 MPa of PEG solutions at 25°C for 12 hours under dark conditions and seed were soaked for 12h in distilled water for hydropriming treatment.Drought condition was simulated by using PEG6000 according to Kuffman formula. Our results showed that Bromus could be categorized as a salt tolerant plant and its more tolerate to salinity than drought stress. Seed priming is a good seed enhancement technique for improving seed germination and faster seed germination of Bromus seeds (Tavili *et al*., 2011).

Papastylianou *et al*., (2012) study the effect of osmopriming on germination performance of cottonseeds was investigated under suboptimal thermal conditions (12 to 24°C). Seeds were primed in mannitol solutions at three water potentials (-0.5, -1.0 and -1.5 MPa) for 6, 12 or 18 hours at four incubation temperatures (16, 19, 22 and 25°C) in darkness. The treated seeds and non-primed controls were then subjected to germination tests at 12, 15, 18, 21 and 24°C. Germination performance was evaluated by final germination percentage, time to reach 10, 50 and 90% germination, germination uniformity, and thermal time requirement. Osmotic potentials of -0.5 MPa and, to a lesser extent -1.0 MPa, tended to increase germination rate and uniformity in comparison with non-primed seeds. In addition, osmopriming resulted in a substantial lowering of the thermal time requirements for seed germination. In most cases, seed performance based on all the parameters considered was better for priming durations of 6 or 12 hours than for 18 hours. A trend for higher germination uniformity was apparent when seeds were primed at 19 and 22°C.

CHAPTER III

MATERIALS AN D METHODS

The experiment was conducted during the period from April to May, 2018 to study the effect of mannitol induced seed priming for enhancing drought tolerance capability in soybean (*Glycine max*) under drought stress. A short description of the experimental site, climatic condition of the culture room, experimental materials, treatments and design, methods of the study, data collection procedure and data analysis in materials and methods. The detailed materials and methods that were used to conduct the study are presented below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

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

3.1.2 Conditions of laboratory room

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study period of the culture room was 24.2^0 C to 32.4^0 C respectively. The average minimum and maximum relative humidity was 56% and 81% respectively.

3.2 Test crops

Two soybean varieties namely BARI soybean 5 and BARI soybean 6 were used for this experiment. BARI soybean 5 and BARI soybean 6 were collected from Bangladesh Agricultural Research Institute (BARI). The collected soybean varieties were free from any visible defects, disease symptoms and insect infestations .

3.3 Experimental materials

Different equipments such as electric balance, Petri dish, filter paper, micro pipette, forcep, oven etc were used to conduct the study.

3.4 Experimental chemicals

Different priming chemicals such as Mannitol ($C_6H_{14}O_6$) and distilled water were utilized for osmo and hydro priming. Mannitol ($C_6H_{14}O_6$) used as seed treating chemical and PEG (Polyethylene glycol) used for drought induction.

3.5 Experimental treatments and design

The experiment comprises of

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

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

(c) Six levels of priming agent concentration viz. water, 2%, 4%, 6% and 8% mannitol ($C_6H_{14}O_6$). The experiment was laid out in a Completely Randomized Design (CRD) with 5 replications.

3.6 Experimental details

Three experiments were conducted under the whole experiment.

3.6.1. 1st Experiment: Study on the effect of different concentrations of Mannitol on the germination behavior of Soybean.

3.6.1.1 Weight of seeds

Two hundred gram seeds were weighted from the total seed from each of two soybean variety BARI soybean 5 and BARI soybean 6 to reduce the unnecessary loss of seeds.

3.6.1.2 Surface treatment

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

3.6.1.3 Treatments

The experiment was comprised with two soybean variety and six types of priming solutions.

Factor A: Soybean variety (02)

V_1: BARI soybean 5

V_2: BARI soybean 6

Factor B: Six types of priming solution

1. P_0 = Control (no priming)
2. P_1 = Distilled water priming
3. P_2 = 2% mannitol priming
4. P_3 = 4% mannitol priming
5. P_4 = 6% mannitol priming
6. P_5 = 8% mannitol priming

3.6.1.4 Priming solutions

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

3.6.1.5 Preparation of priming solution

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

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

b) Distilled water

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

3.6.1.6 Priming technique

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

3.6.1.7 Germination of seeds

Twenty seeds from each of the treatments were selected randomly and placed in 120 mm diameter Petri dishes on whatman No.1 filter paper moist with 8 ml of distilled water. Here, whatman No.1 filter paper were used as growth media for germination. Experimental units (60 Petri dishes) were arranged in a completely randomized design with five replications. During the test filter 20 papers in the Petri dishes were kept saturated condition with water. Seeds were kept at room temperature 25°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles emerge by rupturing seed coat. Germination progress was inspected and data were collected everyday and continued up to 8 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (8 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven approximately 75°C for 72 hours.

3.6.2 2nd Experiment: Optimization of pre-sowing priming time on the germination behavior of soybean

3.6.2.1 Weight of seeds

Seeds were weighted 200g from the total seed of BARI soybean 6 for this experiment to reduce the unnecessary loss of seeds. Remaining seeds are taken in poly bag and preserved in refrigerator for further use.

3.6.2.2 Surface treatment

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

3.6.2.3 Treatments and design

Soybean variety: BARI soybean 6

Factor A: Priming of seeds

P_1: Osmopriming (6% mannitol solution)

P_2: Hydropriming

Factor B: Six types of priming times

T_1 = Seeds primed for 3 hours,

T_2 = Seeds primed for 6 hours,

T_3 = Seeds primed for 9 hours,

T_4= Seeds primed for 12 hours

T_5= Seeds primed for 15 hours and

T_6 = Seeds primed for 18 hours.

3.6.2.4 Priming solutions

Mannitol 6% was used as osmopriming and distilled water was used as hydro priming solutions.

3.6.2.5 Preparation of priming solutions

a) Mannitol solutions (6%)

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

b) Distilled water

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

3.6.2.6 Priming technique

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

3.6.2.7 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter petri dishes on whatman No.1 and filter paper was moistened with 8 ml of distilled water. Here, whatman No.1 filter paper were used as growth media for germination. Experimental units (60 Petri dishes) were arranged factorialy in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept saturated condition with water. Seeds were kept at room temperature 25°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were emerged by rupturing seed coat. Germination progress was inspected and data were collected at every day and continued up to 8 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (8 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven approximately 75°C for 72 hours**.**

3.6.3 3rd experiment: Germination behavior of primed Seed (soybean) under drought (PEG) stress condition

3.6.3.1 Weight of seeds

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

3.6.3.2 Surface treatment

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

3.6.3.3 Treatments

This experiment was comprises of osmopriming and hydropriming with four drought stress levels (5%, 10%, 15%, 20%). Drought stress was simulated by highly osmotic substance PEG.

The treatments are as follows:

Factor A: Priming

P_1: Osmopriming for 6 hours with 6% manniol

P_2: Hydropriming for 6 hours

Factor B : Drought stress level

1. T_0 = Primed seeds placed without drought (control),
2. T_1 = Primed seeds placed with 5% level of PEG,
3. T_2 = Primed seeds placed with 10% level of PEG,
4. T_3 = Primed seeds placed with 15% level of PEG and
5. T_4 = Primed seeds placed with 20% level of PEG

3.6.3.4 Priming solutions and time

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

3.6.3.5 Preparation of priming solutions

a) Mannitol solutions (6%)

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

b) Distilled water Distilled

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

3.6.3.6 Preparation of stress solutions

Drought stress (0%, 5%, 10%, 15%, 20%)

To prepare 5% PEG solution, 12.5g of PEG was dissolved in 250 ml of distilled water. Similarly, 25 g, 37.5 g, 50 g PEG was dissolved in 250 ml of water to prepare 10%, 15%, 20% solution of PEG respectively.

3.6.3.7 Priming technique

Two priming techniques viz., osmopriming and hydropriming were applied on BARI soybean 6. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were primed with priming chemicals. Seeds of a sub-sample were soaked in distilled water for hydropriming and seeds of another sub-sample were pretreated with mannitol for osmopriming at a concentration of 6% for 6 hours, respectively. Priming is done in different Petri dishes. Containers were covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were cleaned with tissue paper properly and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al.,* 2011) in room temperature for 24 hours back to the original moisture level**.**

3.6.3.8 Germination of seeds

The standard germination test was performed by placing randomly selected 20 seeds in 90-mm-diameter Petri dishes on whatman No.1. Petri dishes containing primed and control seeds were irrigated with solutions of8 ml salt stress levels. Here whatman No.1 filter paper were used as growth media for germination. Experimental units (50 Petri dishes) were arranged factorialy in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept water saturated state. Seeds were kept at room temperature 25±1°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were emerged by rupturing seed coat. Germination progress was inspected and data were collected every day and continued up to 8 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting.

At the end of germination test (8 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75°C for 72 hours

3.7 Data recording

Parameters that are measured as follows:

3.7.1 Total germination (TG%)

Total germination (TG) was calculated as the number of seeds which was germinated within 7 days as a proportion of number of seeds shown in each treatment, expressed as a percentage (Othman *et al.*, 2006).

$$TG\% = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds set for germination}} \times 100$$

3.7.2 Shoot length (mm), root length (mm)

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

3.7.3 Shoot dry weight (mg), root dry weight (mg)

By using an electrical balance, the dried shoots and roots were weighted to the nearest milligram (mg) .

3.7.4 Vigor Index (VI)

Vigor Index (VI) was calculated from total germination and seedlings length by using the formula of Abdul- Baki *et al.*, (1970).

$$VI = \frac{TG (\%) \times Seedlings\ length(mm)}{100}$$

Here,

TG = total germination.

3.7.5 Germination coefficient (GC)

Germination coefficient (GC) was calculated using the following formula Copeland, 1976.

$$\text{Germination coefficient (\%)} = \frac{A1 + A2 + \ldots\ldots + Ax}{A1T1 + A2T2 + \ldots\ldots + AxTx} \times 100$$

Where,

A= Number of seeds germinated

T= Time corresponding to A

x= Number of days to final count.

3.7.6 Relative water content (RWC)

Relative water content (RWC) was measured using following formula of Baque *et al.*, (2002).

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

3.7.7 Water saturation deficit (WSD)

Water saturation deficit (WSD) was recorded using following formula of Baque *et al.*, (2002).

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

3.7.8 Water retention capacity (WRC)

Water retention capacity (WRC) was measured following formula of Baque *et al*., (2002).

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

3.7.9 Statistical analysis

The data obtained for different parameters were statistically analyzed to observed the significant difference among the treatment. The mean value of all the parameters was calculated. Analysis of variance was performed. The significance of difference among the treatments means was estimated by using a computer software MSTAT-C. The least significant difference (LSD) test at 1% level of significance. Drawings were made using Excel software.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter results obtained from the study to investigate the effect of seed priming with mannitol which enhance the drought tolerant capability of soybean varieties cv. BARI soybean 5 and BARI soybean 6 are discuss and present below. The results of the germination and growth parameters of soybean as influenced by different concentration of priming agents (mannitol) and priming times in drought stress condition have been presented in this chapter.

4.1 First Experiment: Study on the effect of different concentrations of Mannitol on the germination behavior of soybean

The effects of different concentrations of mannitol on the germination behavior of soybean varieties such as BARI soybean 5 and BARI soybean 6 have been presented, discussed and compared in this chapter. Different concentrations have significant effects on germination of soybean. The results have been presented in Figures 1 to 20 and Tables 1 to 10.

4.1.1 Effect of variety on germination

Soybean variety exhibited non-significant difference in respect of the germination percentage (Figure 1). Between the varieties, BARI soybean 6 (V_2) showed the maximum germination percentage (83.11%) and BARI soybean 5 (V_1) showed the minimum germination percentage (79.60%). The variety/genotype Shohag, BARI soybean 6 and BD 2331 is considered as drought tolerant because of their higher proline, soluble sugar accumulation, chlorophyll content and cell membrane stability (Chowdhury *et al*., 2017).

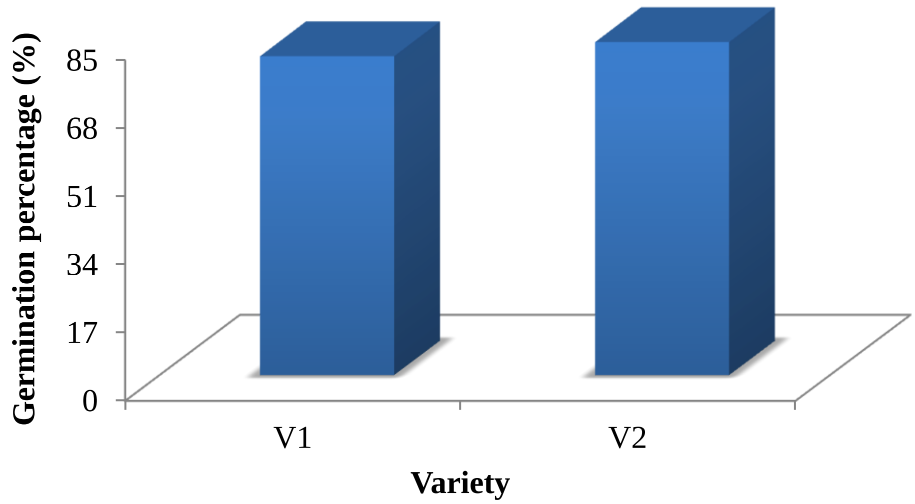

Figure 1. Effect of variety on the germination percentage of soybean ($LSD_{0.01}$=2.53)

V_1= BARI soybean 5, V_2= BARI soybean 6

4.1.2 Effect of priming solutions on germination

Priming with different concentrations of mannitol and water showed a significant variation in respect of germination percentage (Figure 2). P_4 (primed with 6% mannitol concentration) showed the maximum germination percentage (94.60%) and thereafter decrease due to increasing concentration of mannitol. The lowest germination percentage was found in P0 (71.35%). Total germination of BARI soybean 6 was higher than BARI soybean 5. Khalil *et al*., (2010) reported that seed priming with phosphorus solutions enhanced uniformity and speed of germination in wheat. Mohammadi (2009) reported that soaking seed in potassium nitrate had the best effect on germination and soybean seed vigor with late sowing.

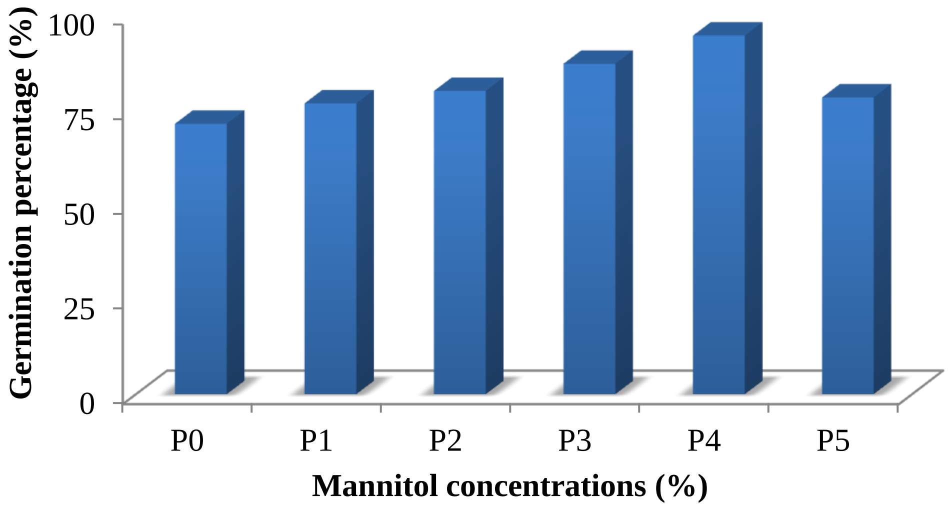

Figure 2. Effect of priming solutions on the germination percentage of soybean ($LSD_{0.01}$=4.37)

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4=Seeds primed with 6% mannitol solution, P_5= Seeds primed with 8% mannitol solution.

4.1.3 Effect of variety on shoot length

Soybean varieties exhibit non-significant differences in respect of shoot length (Figure 3). Between the varieties, BARI soybean 6 (V_2) showed the maximum shoot length (65.58mm) than BARI soybean 5 (V_1) (56.58mm). Study conducted by different researcher showed that shoot length depend on varietal characteristic of crops. Faijunnahar *et al.*, (2017) showed that among four wheat varieties such as BARI Gom 28, ESWYT-5, ESWYT-6 and ESWYT-7 growth performance of ESWYT-7 was best. Kaya (2009) showed that Kernel type seeds exhibited higher longer root and shoot length compared to the achene type seeds at all NaCl levels.

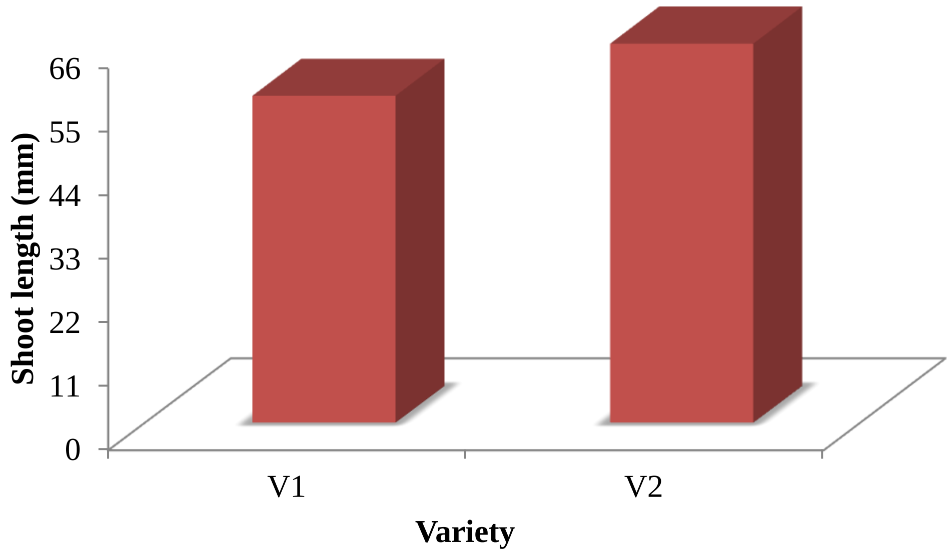

Figure 3. Effect of variety on the shoot length of soybean ($LSD_{0.01}$=2.30)

V_1=BARI soybean 5, V_2=BARI soybean 6

4.1.4 Effect of priming on shoot length

Treatment P_4 (primed with 6% mannitol concentration) showed the maximum shoot length (80.08mm) and P_0 (control) showed the minimum shoot length (35.27mm). In rice, Priming showed a significant variation in respect of shoot length (Figure priming with 5-aminolevulinic acid improved shoot elongation (Kanto *et al.*, 2015) while priming with picomolarrutin augmented both root and shoot length in relation to an increase in photosyn-thetic pigments, phenolic and flavonoid contents (Singh *et al.*, 2016).

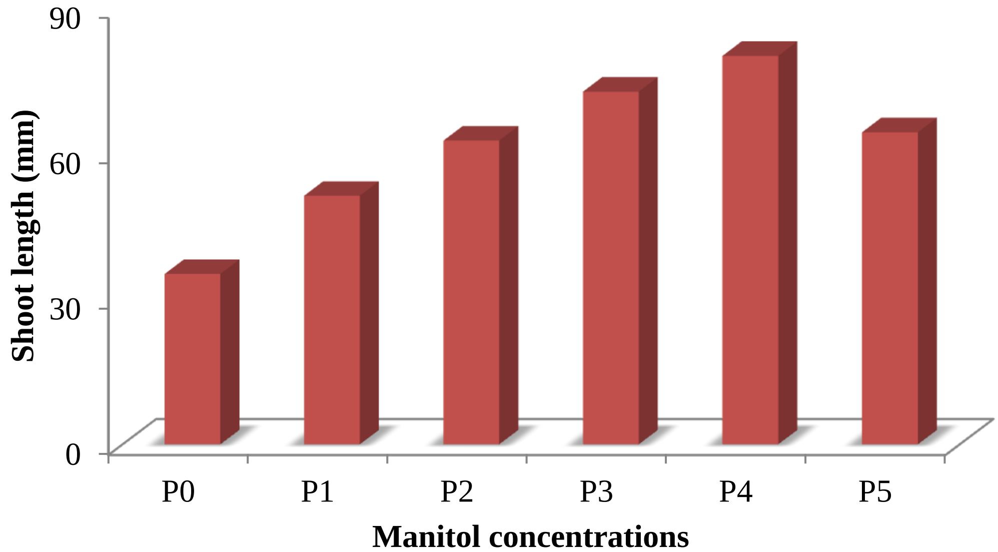

Figure 4. Effect of priming solutions on the shoot length of soybean ($LSD_{0.01}$=3.97)

P0= Seeds without priming (control), P1= Seeds primed with distilled water, P2= Seeds primed with 2% mannitol soluion,P3=Seeds primed with 4% mannitol solution, P4=Seeds primed with 6% mannitol solution, P5=Seeds primed with 8% mannitol solution.

4.1.5 Effect of variety on root length

Soybean variety exhibited non-significant difference in respect of root length (Figure 5). Between the varieties, BARI soybean 6 (V_2) showed the maximum root length (39.16mm) and BARI soybean 5 (V_1) showed minimum root length (30.64mm). Our result is in agreement with Ramesh *et al.,* (2006) who did seed priming in rice with K_2SO_4 and ascorbic acid with four rice genotypes (Pusa Basmati-1, Basmati-385, Saket-4 and IR-36) and found seed priming with ascorbic acid was effective in enhancing root and shoot length. Present results agrees to the study of previous researcher Sarwar *et al*., (2006) who reported that seeds treated with water and mannitol over control helps to increase root length.

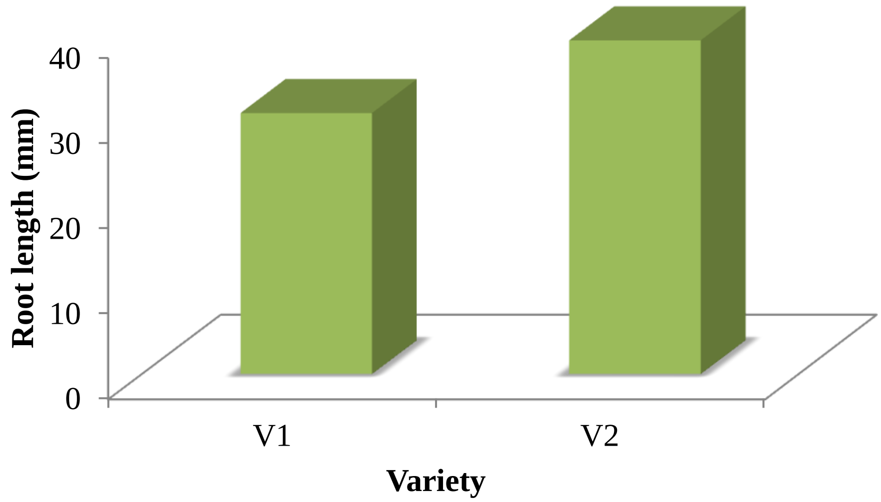

Figure 5. Effect of variety on the root length of soybean ($LSD_{0.01}$=1.27)

V_1= BARI soybean 5, V_2= BARI soybean 6

4.1.6 Effect of priming on root length

Priming showed a significant variation in respect of root length (Figure 6). Treatment P_4 (primed with 6% mannitol concentration) showed the maximum root length (50.56mm) and P_0 showed the minimum root length (20.01mm). The faster shoot growth of maize plants from primed seeds were the result of higher capacity of osmotic adjustment because plants from primed seeds have more Na^+ and Cl^- in roots and leaves than plants from non primed seeds. This might have helped to increase water absorption and turgor pressure that speed up growth parameters (Nasim *et al.*, 2008).

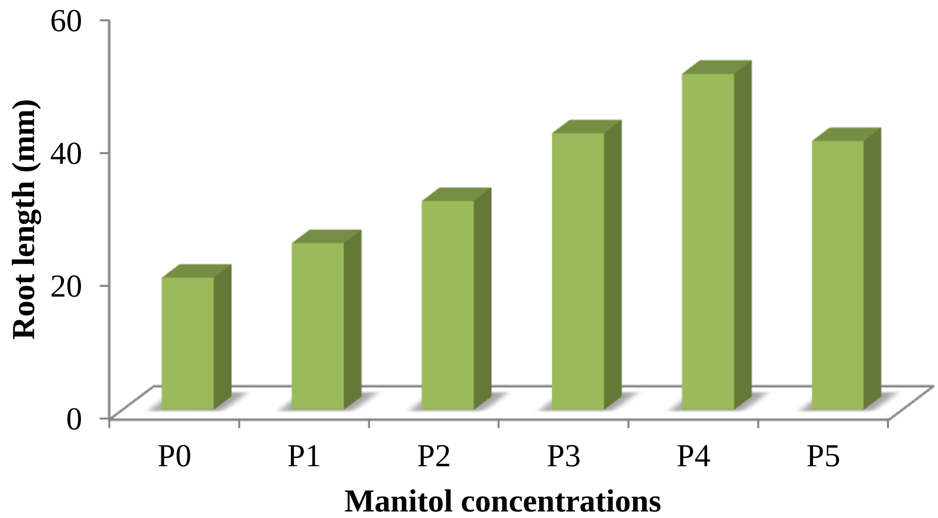

Figure 6. Effect of priming solutions on the root length of soybean ($LSD_{0.01}$=2.20)

P0= Seeds without priming (control), P1= Seeds primed with distilled water,P2= Seeds primed with 2% mannitol soluion,P3=Seeds primed with 4% mannitol solution, P4=Seeds primed with 6% mannitol solution, P5=Seeds primed with 8% mannitol solution.

4.1.7 Effect of variety on shoot dry weight

Soybean variety exhibited non-significant difference in respect of shoot dry weight (Figure 7). Between the varieties, BARI soybean 6 (V_2) showed the maximum shoot dry weight per plant (84.91mg) and BARI soybean 5 (V_1) showed minimum shoot dry weight (72.20mg). Faijunnahar *et al.*, (2017) showed that among four wheat varieties, shoot dry weight was best in ESWYT-7 variety.

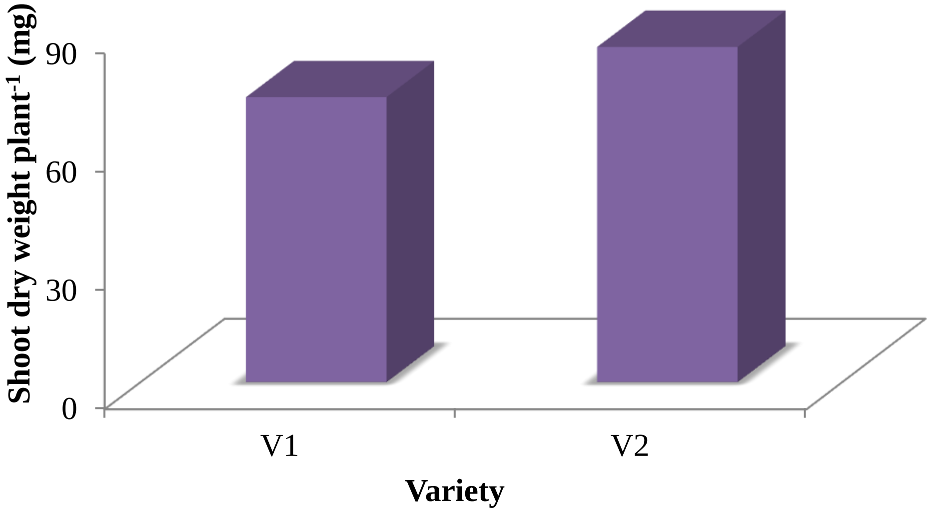

Figure 7. Effect of variety on the shoot dry weight plant^{-1} of soybean ($LSD_{0.01}$=2.83).

V1= BARI soybean 5, V2= BARI soybean 6

4.1.8 Effect of priming concentration on shoot dry weight

Priming showed a significant variation in respect of shoot dry weight per plant (Figure 8). Treatment P_4 (primed with 6% mannitol concentration) showed the maximum shoot dry weight per plant (96.50mg) and P_0 showed the minimum shoot dry weight per plant (52.58mg). According to Farooq *et al*., (2006) Hydro-priming for 48 h improved the growth and dry weight of rice seedlings.

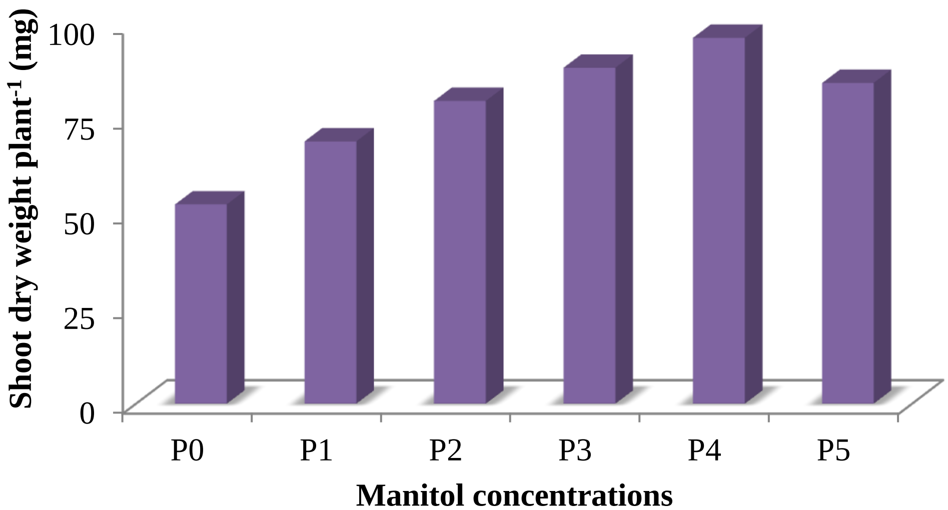

Figure 8. Effect of priming solutions on the shoot dry weight plant^{-1} of soybean ($LSD_{0.01}$=4.89).

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4=Seeds primed with 6% mannitol solution, P_5=Seeds primed with 8% mannitol solution.

4.1.9 Effect of variety on root dry weight

Soybean variety exhibited non-significant difference in respect of root dry weight (Figure 9). Between the varieties, BARI soybean 6 (V_2) showed the maximum root dry weight per plant (6.97mg) and BARI soybean 5 (V_1) showed the minimum root dry weight (5.31mg). Anuradha (2014) showed that fresh weight and dry weight of seedlings (root, shoot and total seedling) of all the twelve genotypes decreased significantly with increased salt (NaCl) stress.

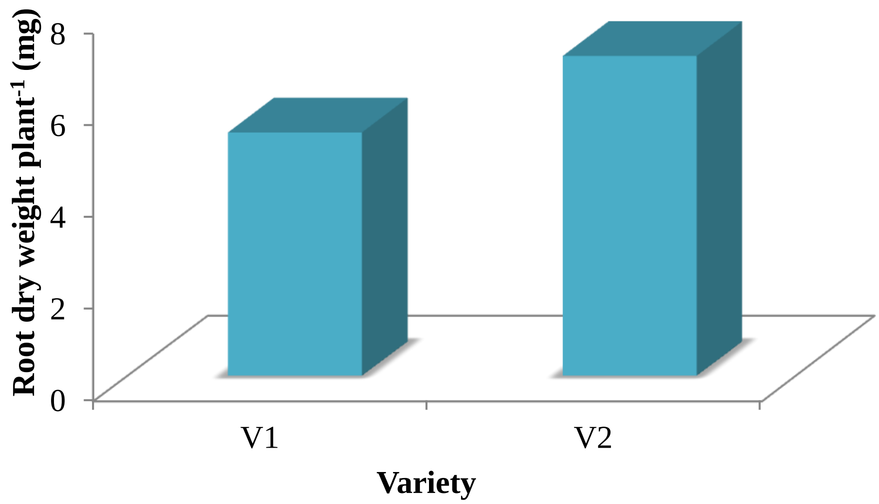

Figure 9. Effect of variety on the root dry weight plant^{-1} of soybean ($LSD_{0.01}$=0.21) .

V_1= BARI soybean 5, V_2= BARI soybean 6

4.1.10 Effect of priming on root dry weight per plant

Priming showed a significant variation in respect of shoot dry weight per plant (Figure 10). Treatment P_4 (primed with 6% mannitol concentration) showed the maximum root dry weight per plant (8.465mg) and P0 showed the minimum root dry weight per plant (4.108mg). Mostafavi *et al.*, (2012) point out that Hisun cultivar had the highest value of plumule dry weight, plumule fresh weight, seedling fresh weight, and plumule length. In addition, Azargol cultivar had the maximum value of radicle dry weight, seedling dry weight and radicle length.

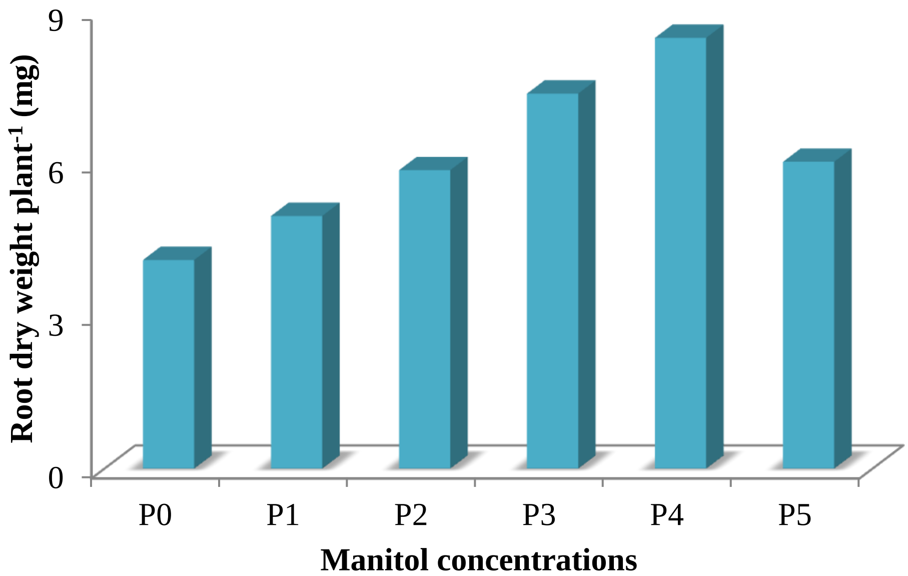

Figure 10. Effect of priming solutions on the root dry weight plant^{-1} of soybean ($LSD_{0.01}$=0.37)

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4=Seeds primed with 6% mannitol solution, P_5=Seeds primed with 8% mannitol solution.

4.1.11 Interaction effect of variety and different priming concentrations

Interaction of variety and different priming concentrations showed significant variation and advances of germination and growth behaviors of soybean (Table 1). At germination percentage, highest germination showed in V_2P_4 (96.70%) which was statistically similar with V_1P_4. Lowest germination was in V_1P_1 (75.77%). V_1P_2, V_1P_5 and V_2P_1 are statistically identical. In case of shoot length, highest shoot length was found in V_2P_4 (86.92mm) and lowest shoot length was found in V_1P_0 (28.30mm). In case of root length, highest root length was found in V_2P_4 (55.44mm). Second highest result was found in V_2P_3 (48.84mm) which was statistically similar with V_2P_5 (46.70mm) and V_1P_4 (45.68mm). Lowest root length was found in V_1P_0 (17.20mm). In shoot dry weight, highest shoot dry weight was found in V_2P_4 (101.0mg) which is statistically similar with V_2P_3 (94.20mg), V_1P_4 (92.00mg), V_2P_6 (88.20mg), V_2P_2 (87.20mg), V_1P_3 (83.02mg). Lowest shoot dry weight was found in V_1P_0 (41.04mg). In root dry weight, highest root dry weight was found in V_2P_4 (9.380mg) and second highest was in V_2P_3 (8.580mg). Lowest root dry weight was found in V_1P_0 (3.456mg). Basra *et al.,* (2003) and Arif *et al.*, (2008) have reported improved germination, emergence and establishment in field trials of PEG primed seed. Improvements in later growth have been noted in chickpea (*Cicer arienatum*) using mannitol (Kaur *et al.,* 2002). Elkheir *et al.,* (2016) mentioned that soaking rice seed in the water and PEG solutions 100 g L-1 and 200 g L-1 until priming indicator will lead to accelerated and improved germination behaviors shown by parameters observed such as: germination percentage, Germination Index (GI), days of 50% germination, seedling fresh and dry weight (mg), seedling shoot fresh and dry weight (mg), root fresh dry and weight (mg), shoot/root ratio, seedling length (cm), seedling root length (cm), shoot length (cm) and seed vigor index under laboratory and greenhouse condition.

Table 1. Interaction effect of variety and priming concentrations on the germination behaviors and seedling growth of soybean

Treatment combinations	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
V_1P_0	69.00 g	28.30 i	17.20 g	41.04 i	3.46 h
V_1P_1	75.77 f	47.65 h	23.14 f	63.65 h	4.60 g
V_1P_2	78.02 ef	59.20 fg	29.08 e	72.51 g	5.00 fg
V_1P_3	85.32 cd	68.72 cd	34.48 d	83.02 de	6.16 e
V_1P_4	92.51 ab	73.24 bc	45.68 c	92.00 bc	7.55 c
V_1P_5	77.00 ef	62.36 ef	34.28 d	81.00 ef	5.06 fg
V_2P_0	73.71 fg	42.24 h	22.82 f	64.13 h	4.76 g
V_2P_1	77.66 ef	55.01 g	27.24 e	74.76 fg	5.34 f
V_2P_2	82.00 de	66.16 de	33.94 d	87.20 cde	6.74 d
V_2P_3	89.00 bc	76.74 b	48.84 b	94.20 ab	8.58 b
V_2P_4	96.70 a	86.92 a	55.44 a	101.0 a	9.38 a
V_2P_5	79.60 def	66.40 de	46.70 bc	88.20 bcd	7.01 d
$LSD_{(0.01)}$	6.18	5.62	3.11	6.91	0.53
CV (%)	4.48	5.42	5.25	5.19	5.05

Note : V_1= BARI soybean-5, V_2= BARI soybean-6

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4=Seeds primed with 6% mannitol solution, P_5=Seeds primed with 8% mannitol solution.

4.1.12 Effect of variety and different priming concentration

Effect of variety and different priming concentrations on growth and water relation behaviors of soybean (Table 2). Relative water content (%), water relation capacity (WRC), co-efficient of germination and vigor index was higher in BARI soybean 6 (V_2) than BARI soybean 5 (V_1).Water saturation deficit (WSD) was higher in BARI soybean 5 (V_1) than BARI soybean 6 (V_2). In case of relative water content, higher RWC was found in P_4 (93.61%) and lower RWC was found in P_0 (70.12%) which was statistical similar with P_1 (74.04%). In case water saturation deficit, higher WSD was found in P_0 (29.88) and lowest WSD was found in P_3 (12.93). In water retention capacity, higher WRC was found in P_4 (6.740). Lowest WRC was found in P_0 (4.886) which is statistically identical with P_1 (5.206). Highest CG (coefficient of germination) was found in P_4 (21.48) which was statistically identical with P3 (20.38). Lowest CG was found in P_0 (16.23) which was statistically similar with P_1 (17.38). Vigor index was highest in P_4 (123.80) and lowest in P_0 (39.64). Marur *et al*., (1994), water restriction acted slowing physiological and biochemical processes and soybean seedlings at low water deficit showed a weak growing leading to a lower accumulation of dry matter.

Table 2. Effect of variety and different priming concentrations on the growth and water relation behaviors of soybean

Treatments	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
Effect of variety					
V_1	78.43 b	21.57 a	5.44 b	17.76 b	71.06 b
V_2	82.56 a	17.44 b	5.95 a	19.73 a	88.87 a
LSD $_{(0.01)}$	2.72	0.79	0.23	0.69	3.18
CV (%)	4.88	5.87	5.9	5.31	5.73
Effect of priming concentrations					
P_0	70.12 e	29.88 a	4.89 c	16.23 c	39.64 f
P_1	74.04 de	25.96 b	5.21 c	17.38 bc	58.73 e
P_2	80.43 c	19.58 d	5.72 b	18.48 b	75.49 d
P_3	87.07 b	12.93 e	5.97 b	20.38 a	99.94 b
P_4	93.61 a	6.387 f	6.74 a	21.48 a	123.8 a
P_5	77.69 cd	22.31 c	5.64 b	18.53 b	82.13 c
LSD $_{(0.01)}$	4.71	1.37	0.40	1.20	5.50
CV (%)	4.88	5.87	5.90	5.31	5.73

Note: V_1= BARI Soybean-5, V_2= BARI Soybean-6

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4= Seeds primed with 6% mannitol solution, P_5= Seeds primed with 8% mannitol solution.

4.1.13 Interaction on growth and water relation behavior

Interaction effect of variety and different priming concentration on growth and water relation behaviors in BARI soybean 6 (V_2). In case of relative water content (RWC), highest RWC was found in V_2P_4 (95.41). V_1P_4 (91.82) and V_2P_3 (88.86) are statistically identical. Lowest RWC was found in V_1P_0 (67.58) which was statistically similar with V_1P_1 (73.09), V_1P_5 (74.11), V_2P_0 (72.67). In water saturation deficit was higher in V_1P_0 (32.42) and lowest WSD was found inV_2P_4 (4.592). In water retention capacity, higher WRC was found in V_2P_4 (6.862) which was statistically similar with V_1P_4 (6.618), V_2P_3 (6.290), V_2P_2 (6.066). Lowest WRC was found in

V_1P_0 (4.554) which was statistically similar with V_1P_1 (4.942). Coefficient of germination (CG) was highest in V_2P_4 (22.82) which was statistically similar with V_2P_3 (21.74), V_1P_3 (20.13), V_2P_2 (19.38). Vigor index (VI) was highest in V_2P_4 (137.7), second highest VI was V_2P_3 (111.8) which is identical with V_1P_4 (110.0). Lowest VI was found in V_1P_0 (31.38).In cucumber, Torres *et al.*, (2000) reported that water deficits lower than -0.4MPa were harmful to germination, where a reduction in germination and growth of seedlings could be observed. Mannitol, as a high molecular weight substance, owing to inducing a condition with osmotic solution identical to natural environment, is often used to control water potential in drought stress studies and controlled environments (Guo *et al.*, 2011).

Table 3. Interaction effect of variety and different priming concentrations on the growth and water relation behaviors of soybean

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
V_1P_0	67.58 f	32.42 a	4.55 g	15.50 g	31.38 i
V_1P_1	73.09 ef	26.91 bc	4.94 fg	16.87 fg	53.65 h
V_1P_2	78.69 c-e	21.31 d	5.38 ef	17.59 ef	68.92 fg
V_1P_3	85.28 bc	14.72 f	5.64 de	19.03 c-e	88.06 cd
V_1P_4	91.82 ab	8.182 h	6.62 ab	20.13 bc	110.0 b
V_1P_5	74.11 ef	25.89 bc	5.49 ef	17.44 ef	74.38 ef
V_2P_0	72.67 ef	27.33 b	5.22 ef	16.96 fg	47.91 h
V_2P_1	75.00 de	25.00 c	5.47 ef	17.88 def	63.81 g
V_2P_2	82.16 c	17.84 e	6.07 b-d	19.38 cd	82.07 de
V_2P_3	88.86 ab	11.14 g	6.29 bc	21.74 ab	111.8 b
V_2P_4	95.41 a	4.592 i	6.86 a	22.82 a	137.7 a
V_2P_5	81.28 cd	18.72 e	5.79 c-e	19.61 c	89.89 c
$LSD_{(0.01)}$	6.66	1.94	0.57	1.69	7.77
CV (%)	4.88	5.87	5.90	5.31	5.73

Note: V_1= BARI soybean 5, V_2= BARI soybean 6

P_0= Seeds without priming (control), P_1= Seeds primed with distilled water, P_2= Seeds primed with 2% mannitol soluion, P_3=Seeds primed with 4% mannitol solution, P_4= Seeds primed with 6% mannitol solution, P_5= Seeds primed with 8% mannitol solution.

4.2 Second Experiment: Optimization of pre-sowing priming time on the germination behavior and seedling growth of soybean

Result obtained from the study of the effects of different priming time of mannitol on the germination behavior and seedling growth of BARI soybean 6 have been presented and discussed below.

4.2.1 Effect of priming

Osmopriming and hydropriming have effect on the germination and growth of soybean variety (BARI soybean 6). In case of germination percentage (%) osmopriming (P_1) showed higher germination (82.23%) than hydropriming (P_2) (77.60%). In case of shoot length, P_1 showed higher shoot length (77.59mm) than P_2 (64.91mm). P_1 showed higher root length (70.17mm) than P_2 (59.45mm). Shoot dry weight and root dry weight in mg was higher in osmopriming (P_1) than hydropriming (P_2). Priming has also been reported to enhance seedling emergence and improve yields of soybean (Arif *et al.,* 2008).

4.2.2 Effect of priming time

Germination percentage was highest in T_2 (94.17%) and lower in T_0 (71.16%) which is statistically similar with T_6 (73.43%), T_4 (77.76%) and T_5 (76.81%). Shoot length was highest in T_2 (86.70mm) which is statistically identical with T_3 (81.78mm). Lowest shoot length was found in T_1 (55.20mm). Highest root length was found in T_2 (80.25mm) and lowest root length was in T_1 (53.93mm) which was statistically identical withT_6 (57.40mm). Shoot dry weight was found highest in T_2 (89.17mm) and lowest in T_1 (54.50) which was statistically identical with T_6 (59.74mm). Root dry weight was found highest in T_2 (8.333mm) and lowest in T_1 (4.930mm) which was statistically identical with T_6 (5.185mm).Similar reductions in germination parameters with increasing priming duration were observed for soybean (Khalil *et al.,* 2001), chickpea (Elkoca *et al.,* 2007) and bean (Ghassemi-Golezani *et al.,* 2010). This is supported by 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 compare with 24h.

Table 4. Effect of priming agents and priming times on the germination and growth behavior of soybean

Treatments	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
Effect of osmo and hydro priming					
P_1	82.23 a	77.59 a	70.17 a	75.46 a	6.94 a
P_2	77.60 b	64.91 b	59.45 b	66.97 b	5.68 b
LSD $_{(0.01)}$	3.23	3.17	3.24	3.36	0.25
CV (%)	4.33	4.78	5.37	5.06	4.2
Effect of priming times					
T_1	71.16 d	55.20 e	53.93 d	54.50 e	4.93 d
T_2	94.17 a	86.70 a	80.25 a	89.17 a	8.33 a
T_3	86.14 b	81.78 a	69.77 b	81.93 b	6.98 b
T_4	77.76 c	73.95 b	64.00 c	74.29 c	6.25 c
T_5	76.81 c	68.27 c	63.52 c	67.67 d	6.20 c
T_6	73.43 cd	61.59 d	57.40 d	59.74 e	5.19 d
LSD $_{(0.01)}$	5.59	5.50	5.62	5.82	0.43
CV (%)	4.33	4.78	5.37	5.06	4.20

Note: P_1=Osmopriming(6% mannitol solution), P_2= Hydropriming

T_1= Seeds primed for 3 hours, T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours

Lemrasky *et al.,* (2012) reported that shoot length of wheat seeds which primed for 45 hours was higher than non primed. Priming time help to increase enzymatic activities of seeds which trigger plant growth. Over priming time might facilitate the ageing of seed which degrade the germination, growth and development.

4.2.3 Interaction of priming and priming time on germination and growth

Interaction effect of osmo and hydropriming and different priming times on the germination and growth of soybean variety (BARI soybean 6). In case of germination percentage (GP), highest germination found in P_1T_2 (95.85%) which was statistically similar with P_2T_2 (92.49%), P_1T_3 (87.69%) and P_2T_3 (84.59%). Lowest germination was found in P_2T_1 (67.53%) which was statistically similar with P_2T_6 (71.46%). P_2T_5 (74.02%), P_1T_1 (74.78%) and P_1T_6 (75.39%) were statistically identical. Shoot length found in P_1T_2 (91.07mm) was highest and statistically similar with P_1T_3 (86.73mm), P_2T_2 (82.33mm). Lowest shoot length was found in P_2T_1 (44.33mm). Highest root length was found in P_1T_2 (81.16mm) which is statistically identical with P_2T_2 (79.33mm). Lowest root length was found in P_2T_1 (46.00mm) which is statistically similar with P_2T_6 (51.41mm). Highest shoot dry weight was found in P_1T_2 (92mg) and lowest shoot dry weight was found in P_2T_1 (52.00mg). P_1T_3 (87.00mg) and P_2T_2 (86.33mg) was statistically identical. In case of root dry weight, highest root dry weight was found in P_1T_2 (9.267mg) and lowest root dry weight in P_2T_1 (4.33mg) which was statistically identical with P_2T_6 (4.74mg).The results agree with the findings of Basra *et al.*, (2003) who reported higher dry matter accumulation following seed priming. They further reported that CGR (crop growth rate) was greater for primed seed sown fresh or after storage as compared to unprimed seed. Earlier and faster germination and emergence has been associated with a lower value of thermal time requirement (Mohamed *et al.*, 1988). Ajirloo *et al*., (2013) reported that optimum seed soaking duration improved root and shoot length. Moghanibashi *et al.*, (2012) also concluted that sunflower seed hydroprimed for 24 hours help to increase shoot dry weight than non-primed seed.

Table 5. Interaction effect of priming agents and priming times on the germination and growth behavior of soybean

Treatment combinations	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
P_1T_1	74.78 e-g	66.07 fg	61.87 de	57.00 f-h	5.53 f
P_1T_2	95.85 a	91.07 a	81.16 a	92.00 a	9.27 a
P_1T_3	87.69 bc	86.73 ab	74.00 ab	87.00 ab	7.62 b
P_1T_4	80.04 c-e	78.80 cd	70.34 bc	78.96 bc	6.93 cd
P_1T_5	79.60 de	73.20 d-f	70.26 bc	73.33 cd	6.67 d
P_1T_6	75.39 e-g	69.67 e-g	63.40 c-e	64.48 ef	5.63 f
P_2T_1	67.53 g	44.33 i	46.00 g	52.00 h	4.33 g
P_2T_2	92.49 ab	82.33 bc	79.33 a	86.33 ab	7.40 bc
P_2T_3	84.59 b-d	76.83 c-e	65.53 cd	76.86 cd	6.33 de
P_2T_4	75.48 ef	69.10 e-g	57.67 d-f	69.63 de	5.73 ef
P_2T_5	74.02 e-g	63.33 g	56.78 ef	62.00 e-g	5.57 f
P_2T_6	71.46 fg	53.52 h	51.41 fg	55.00 gh	4.74 g
$LSD_{(0.01)}$	7.91	7.77	7.94	8.23	0.60
CV (%)	4.33	4.78	5.37	5.06	4.20

Note: P_1=Osmopriming(6% mannitol solution), P_2= Hydropriming

T_1= Seeds primed for 3 hours, T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours

4.2.4 Effect of relative water content

Soybean variety exhibited non-significant difference in respect of relative water content (Figure 11). Between the varieties, osmopriming (P_1) showed the maximum relative water content (80.70%) and hydropriming (P_2) showed the minimum relative water content (74.97%). Relative water content of soybean influenced by priming time. Priming time helps to enhance enzymatic activities of seed which facilitated growth and higher relative water content.

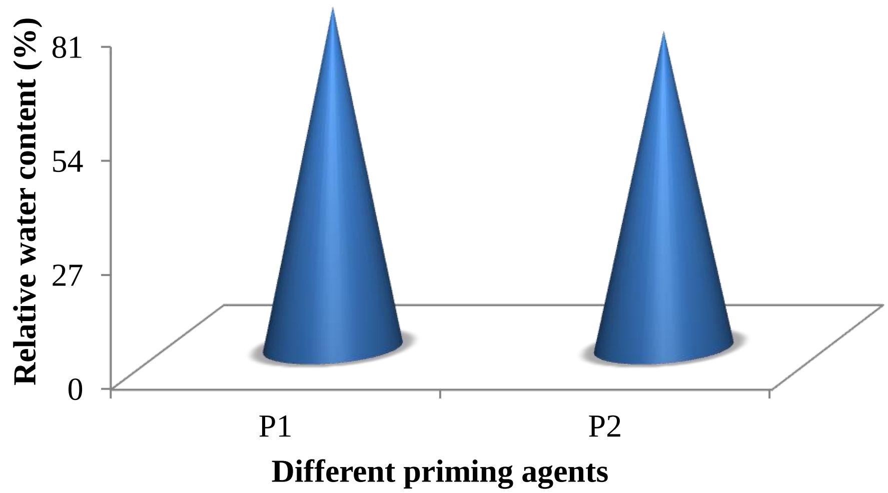

Figure 11. Effect of priming agent on the relative water content(%) of soybean ($LSD_{0.01}$=3.69).

P_1=Osmopriming (6% mannitol solution), P_2= Hydropriming

4.2.5 Effect of relative water content on priming time

Priming times showed different effect on relative water content of BARI soybean 6 at 6% mannitol concentration (Figure 12). Treatment T_2 (6hours priming) showed higher relative water content (92.91%) and lower relative water content was in T_1 (69.62%) which was statistically similar with T_5 (73.95%) and T_6 (71.44%). Priming time helps to enhanced enzymatic activities of seed which facilitated the growth of healthy and vigorous seedling, which might have the capacity to provide higher relative water content. Over priming time trigger the ageing process of primed seed, produced weak and lean seedling ultimately exhibited lower relative water content (Faijunnahar *et al.*, 2017).

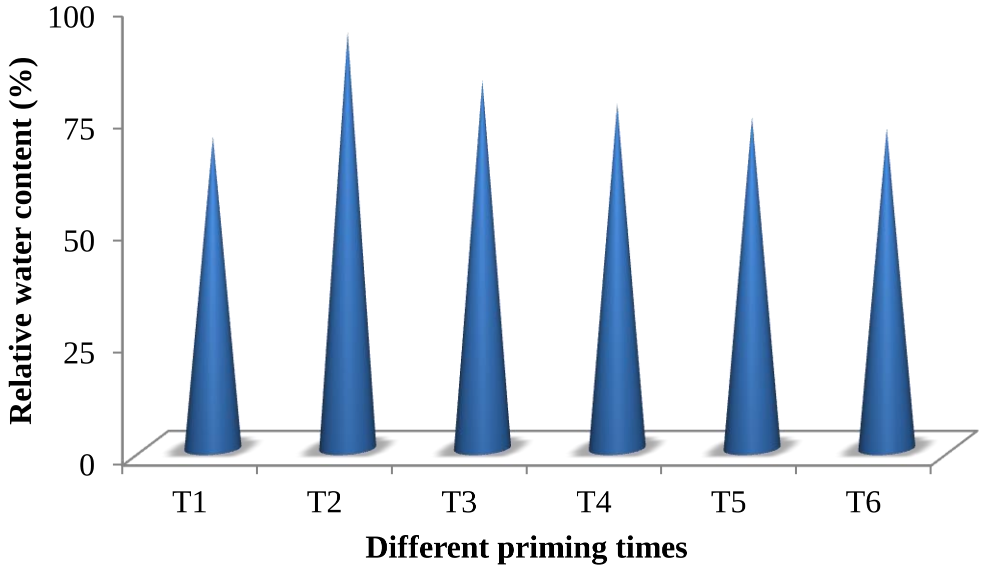

Figure 12. Effect of priming times on the relative water content (%) of soybean ($LSD_{0.01}$=6.39).

T_1= Seeds primed for 3 hours, T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours.

4.2.6 Effect of priming on water saturation deficit

Effect of hydropriming (P_2) in relation to water saturation deficit was higher than osmopriming (P_1) (Figure 13). P_2 showed higher water saturation deficit (25.03) and P_1 showed lower (19.30). Faijunnahar *et al.*, (2017) showed that the wheat genotype ESWYT-7 performed poor for all parameter but maximum water saturation deficit was observed among four varieties.

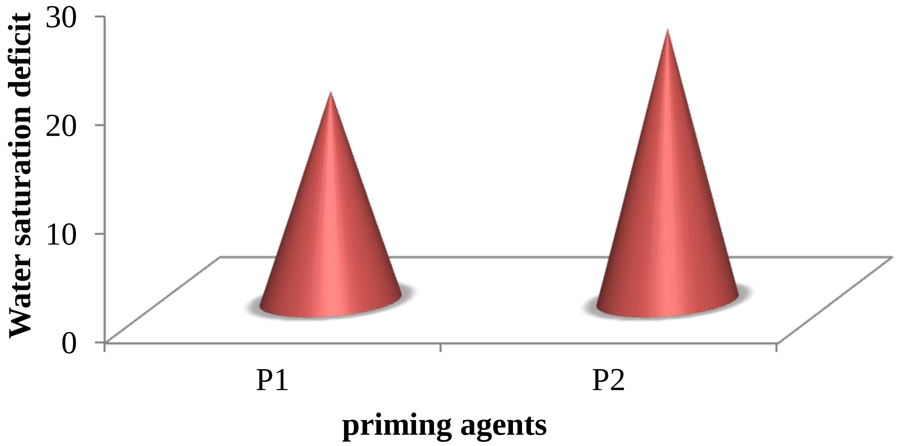

Figure 13. Effect of priming agents on the water saturation deficit (%) of soybean ($LSD_{0.01}$=1.10)

P_1= Osmopriming (6% mannitol solution), P_2= Hydropriming

4.2.7 Effect of priming times on water saturation deficit

Different priming times showed different effect on water saturation capacity (WSD) at 6% priming concentration (Figure 14). Treatment T_1 showed highest WSD (30.38) which was statistically identical with T_6 (28.56). Lowest WSD was found in T2 (7.085). Kramer (1974) reported that the first effect measurable due to water deficit was the growth reduction, caused by the declining in the cellular expansion. The cellular elongation process and the carbohydrates wall synthesis were very susceptible to water deficit (Wenkert *et al.*, 1978). Water saturation deficit exhibited an inverse relation with water relation behavior. The enzymatic activities was lower under lower priming time which result produce the weak and lean seedling and over priming also produce weak and lean seedling which were failed to uptake enough water and provided more water saturation deficit value.(Faijunnahar *et al*., 2017).

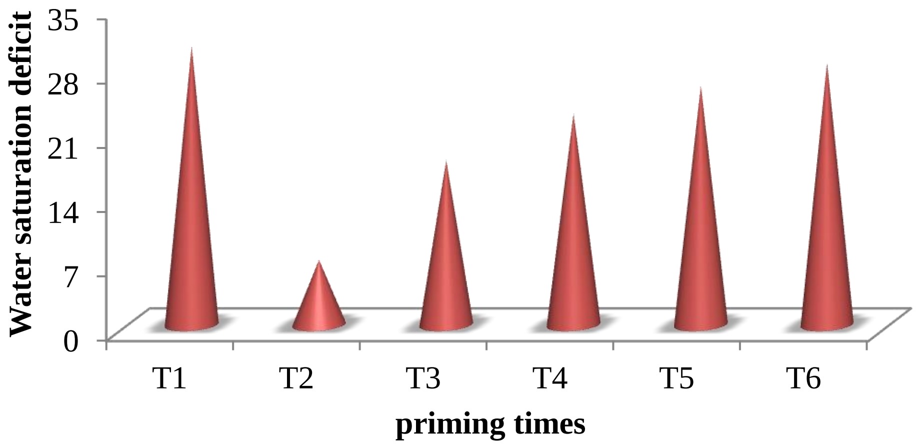

Figure 14. Effect of priming times on the water saturation deficit of soybean ($LSD_{0.01}$=1.91)

T_1= Seeds primed for 3 hours, T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours.

4.2.8 Effect of priming time on water retention capacity

Water retention capacity was higher in osmopriming (P_1) than hydropriming (P_2). P_1 showed higher water retention capacity (6.23) than P_2 (5.48). water retention capacity of soybean influenced by different priming agents. Osmopriming helps to increase water retention capacity of plants. The water retention capacity of Freanch bean increase with the increase of potassium concentration.(Sangakkara *et al*., 1996).

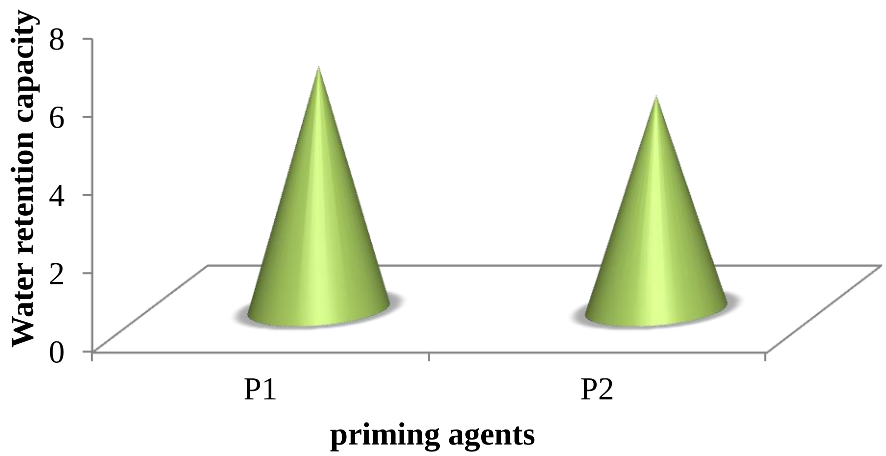

Figure 15. Effect of priming agent on the water retention capacity of soybean ($LSD_{0.01}$=0.27)

P_1= Osmopriming (6% mannitol solution), P_2= Hydropriming

4.2.9 Effect of priming times on water retention capacity:

Priming times effect on water retention capacity (WRC) of soybean variety (Figure 16). T2 (6 hours priming) showed higher WRC (6.942) which was statistically identical with T3 (6.625). Lowest WRC was found in T1 (4.932) which was statistically identical with T6 (5.112) .Faijunnahar *et a*l., (2017) also showed that lower priming time might reduce the activation of enzymes responsible for germination of seed and proper growth and development of seedling. So, the seedling became weak and lean. On the other hand optimum priming time facilitated the enzymatic activities of seed which ensure vigorous growth and development of seedling and over priming might accelerate ageing of seed which reduced the seed potentiality for proper seedling growth and development. Vigorous seedling might have the capacity to uptake enough water than the weaker seedling which ensured maximize the turgid weight of seedling in consequence the water retention capacity might be higher than the lower and over priming time.

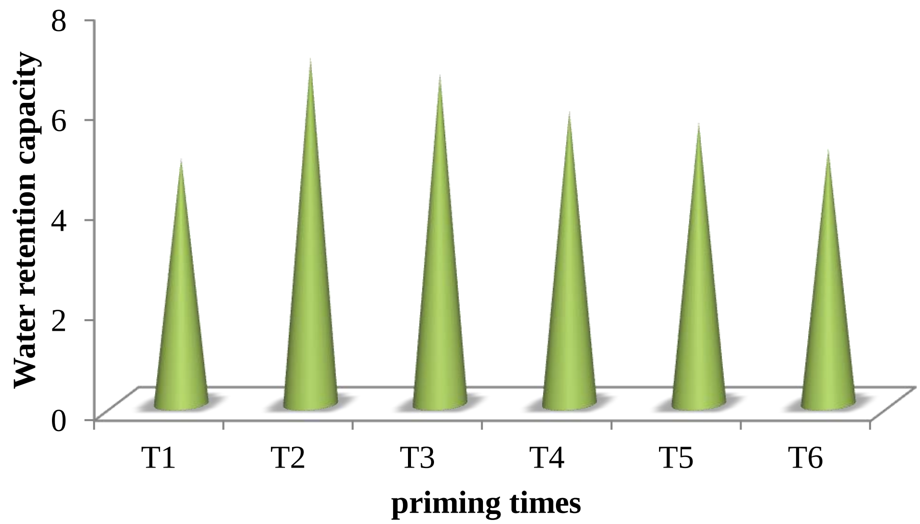

Figure 16. Effect of priming times on the water retention capacity of soybean ($LSD_{0.01}$=0.47).

T_1= Seeds primed for 3 hours , T_2= Seeds primed for 6 hours, T3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours.

4.2.10 Effect of priming agents on coefficient of germination

In soybean variety showed higher coefficient of germination (CG) in P1 (19.77). Lower CG was found in P_2 (17.57). Osmopriming helps to increase coefficient of germination of soybean. Sadeghi *et al*., (2011) reported that, the highest germination coefficient was attained from -1.2 osmotic potential and 12 h seed priming duration treatments (21.15 and 20.15 respectively). Coefficient of germination reduced by the reduction of osmotic potential.

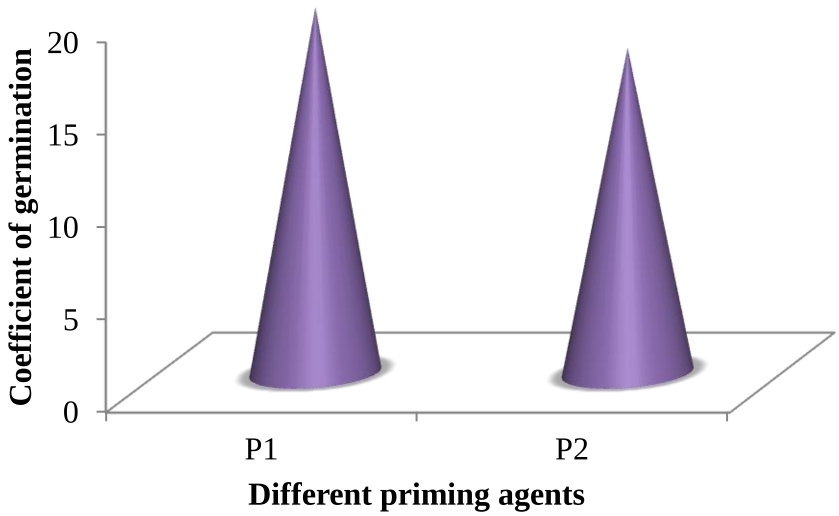

Figure 17. Effect of priming on the coefficient of germination of soybean ($LSD_{0.01}$=0.95).

P1=Osmopriming (6% mannitol solution), P2= Hydropriming

4.2.11 Effect of priming times on coefficient of germination:

Different priming times had different effect on coefficient of germination (Figure 8). Highest coefficient of germination (CG) was found in T_2 (21.56) which was statistically identical with T_3. Lowest CG was found in T_1 (16.41) which was statistically similar with T_6. Moghanibashi *et al.*, (2012) reported that the impact of hydropriming for 24 h enhanced germination coefficient of sunflower seed as compared with the control. Sadeghi *et al*., (2011) also reported that, the highest germination coefficient was attained at 12 h seed priming duration. This result of the experiment similar with the report of (Nahar *et al*., 2016, Moghanibashi *et al*., 2012, Lemrasky *et al*., 2012, Sadeghi *et al*., 2011 and Yari *et al*., 2010), who reported that priming time had significant effect on germination coefficient of wheat.

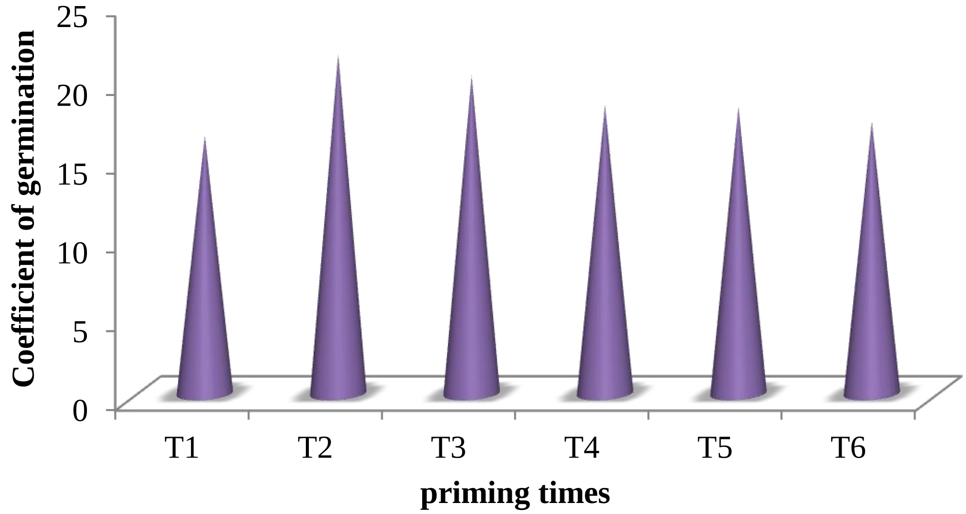

Figure 18. Effect of priming times on the coefficient of germination of soybean ($LSD_{0.01}$=1.65)

T_1= Seeds primed for 3 hours , T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours.

4.2.12 Effect of priming on vigor index

At 6 hours priming on soybean variety showed different vigor index (Figure 19). Higher vigor index (VI) was found in P1 (122.60) which was lower than P2 (98.46). Ghassemi-Golezani *et al.*, (2008) concluded that hydropriming is a simple, low cost and environmentally friendly technique for improving seed and seedling vigor of lentil.

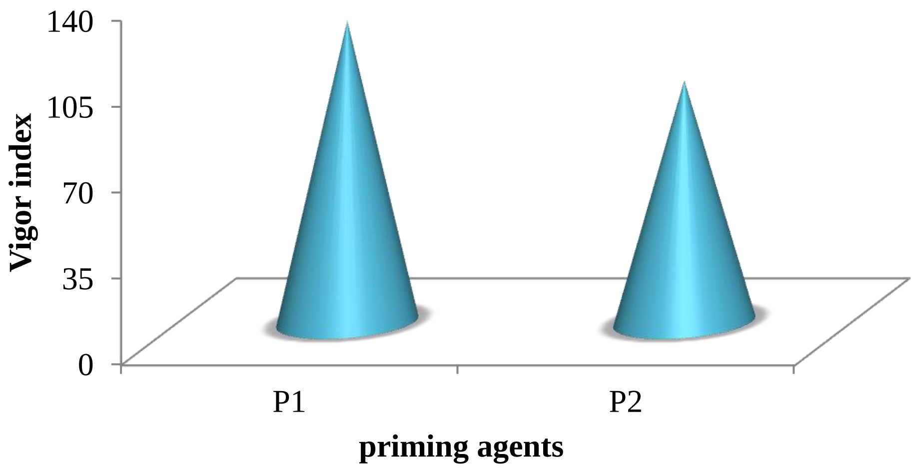

Figure 19. Effect of priming on the vigor index of soybean ($LSD_{0.01}$=5.67)

P1=Osmopriming (6% mannitol solution), P2= Hydropriming

4.2.13 Effect of priming time on vigor index

Different priming times had different effect on vigor index (Figure 20). Highest vigor index (VI) was found in T2 (157.3) and lowest VI was found in T1 (78.30) which was statistically identical with T6. Pegah *et al.*, (2008) showed that hydropriming resulted in lower time taken to germination and higher germination index, vigor index and final germination percentage in maize. Nahar *et al.*, 2016 and Sadeghi *et al.*, 2011 mentioned that priming time had significant affected vigor index and also it was observed that 12 h priming time had most effect on the studied traits of wheat. Priming time helps to vigorous plant growth and in consequence increased the vigor index of plant. on the other hand over priming time caused lower growth and vigor.

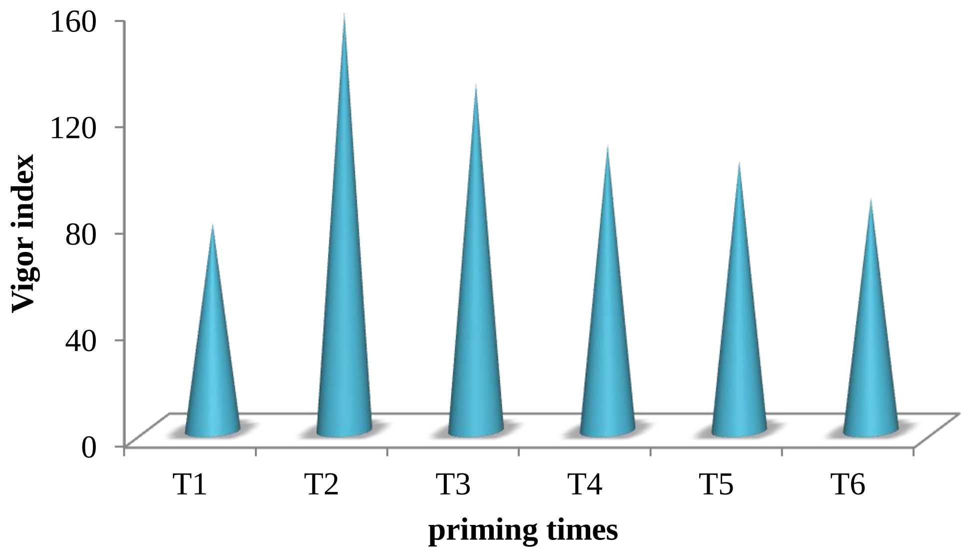

Figure 20. Effect of priming times on the vigor index of soybean ($LSD_{0.01}$=9.83)

T1= Seeds primed for 3 hours , T2= Seeds primed for 6 hours, T3= Seeds primed for 9 hours, T4= Seeds primed for 12 hours , T5= Seeds primed for 15 hours, T6= Seeds primed for 18 hours.

4.2.14 Interaction of priming times on the water relation behavior

Interaction effect of osmo and hydro priming and different priming times on the water relation behavior are different (Table 6). In relative water content (RWC), highest RWC was found in P_1T_2 (94.03%) which was statistically identical with P_2T_2. Lowest RWC was found in P_2T_1 (67. 06) which was statistically similar with P_2T_5, P_2T_6, P_1T_1. For water saturation deficit (WSD), highest WSD was found in P_2T_1 (32.94) which was statistically similar with P_2T_6. Lowest WSD was found in P_1T_2 (5.97) which was statistically identical with P_2T_2. In case of water retention capacity (WRC), highest WRC was found in P_1T_2 (7.270) which was statistically similar with P_1T_3, P_2T_2. P_1T_4 and P_2T_3 were statistically identical. Lowest WRC was found in P_2T_1 (4.523).In coefficient of germination (CG), highest CG was found in P_1T_2 (22.55) which was statistically similar with P_1T_3 and P_2T_2. Lowest CG was found in P_2T_1 (15.30) which was statistically similar with P_2T_4, P_2T_5, P_2T_6. Vigor index (VI) was highest in P_1T_2 (165.2) and lowest VI was found in P_2T_1 (61.02). P_1T_3 and P_2T_2, P_1T_4 and P_2T_3, P_1T_1 and P_2T_4 were statistically identical. Priming time helps to increased enzymatic activities of seed which trigger the vigorous plant growth and in consequence increased the vigor index of wheat on the other hand over priming time facilitate the ageing of seed which resulted loose the potentiality for better germination, growth and development of seedling. This finding was similar with the findings of (Hamidreza *et al*., 2013, Yari *et al*., 2010, Sadeghi *et al.*, 2011 and Nahar *et al*., 2016) who reported that priming time significantly influenced vigor index.

Table 6. Interaction effect of osmo and hydro priming and different priming times on the water relation behavior of soybean

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
P_1T_1	72.18 c-e	27.82 c	5.34 de	17.53 c-f	95.58 e
P_1T_2	94.03 a	5.97 g	7.27 a	22.55 a	165.2 a
P_1T_3	87.15 ab	12.85 f	6.88 ab	21.16 ab	140.9 b
P_1T_4	80.74 bc	19.26 e	6.45bc	19.40 b-d	119.2 c
P_1T_5	76.51 cd	23.49 d	5.99 cd	19.80 bc	114.3 cd
P_1T_6	73.58 c-e	26.42 c	5.43 de	18.17 c-e	100.4 de
P_2T_1	67.06 e	32.94 a	4.52 f	15.30 f	61.02 g
P_2T_2	91.80 a	8.20 g	6.61 a-c	20.56 ab	149.4 b
P_2T_3	77.03 cd	22.97 d	6.37 bc	19.23 b-d	120.5 c
P_2T_4	73.24 c-e	26.76 c	5.32 e	17.26 d-f	95.75 e
P_2T_5	71.39 de	28.61 bc	5.29 e	16.63 ef	88.91 ef
P_2T_6	69.30 de	30.70 ab	4.79 ef	16.42 ef	75.13 f
$LSD_{(0.01)}$	9.04	2.70	0.67	2.34	13.90
CV (%)	5.08	5.33	5.02	5.49	5.51

Note: P_1= Osmopriming(6% mannitol solution), P_2= Hydropriming

T_1= Seeds primed for 3 hours, T_2= Seeds primed for 6 hours, T_3= Seeds primed for 9 hours, T_4= Seeds primed for 12 hours , T_5= Seeds primed for 15 hours, T_6= Seeds primed for 18 hours

4.3 Third experiment: Germination behavior of primed Seed under drought stress condition

According to the results, there are significant difference between control (non primed) and primed seeds. Mannitol and water priming increase the germination percentage (%), relative water content, vigor index and coefficient of germination. Growth factors such as shoot length, root length, shoot dry weight, root dry weight of soybean are compared in primed and drought stress condition. The increase of drought stress causes reduction of germination percentage, shoot length, root length. However, drought stress was more significant in non primed seeds than primed seeds.

4.3.1 Effect of drought on growth

Effect of osmopriming and hydro priming and different drought concentration (PEG) on the germination and growth behavior of soybean (Table 7). Osmopriming (P_1) showed higher germination percentage (%), shoot length (mm), root length (mm), shoot dry weight (mg) and root dry weight (mg). In case of germination percentage (%), highest GP was found in T0 (93.10%) then GP% was gradually decrease with increase of concentration. Same result was found for shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW). Kaya *et al.,* (2005) examined that hydropriming increased germination and seedling growth under salt and drought stresses. Dkhil *et al.,* (2014) examined that overall increased NaCl level, led to the reductions in final germination and emergence percentage but these reductions were higher for non-primed compared to primed seeds. It was reported that seed priming had meaningful effect on acceleration of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in water scarcity and salinity condition (Kaya *et al.,* 2006). Priming of seeds in osmoticums such as mannitol, polyethylene glycol (PEG) and sodium chloride (osmopriming) and in water (hydropriming) has been reported to be an economical, simple and a safe technique for increasing the capacity of seeds to osmotic adjustment and enhancing seed germination, seedling establishment and crop production under stress conditions (Kaur *et al.,* 2002; Elkoca *et al*., 2007).

Table 7. Effect of priming agents and drought stress on the germination and growth behavior of soybean

Treatments	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
Effect of priming agents					
P_1	85.45 a	81.61 a	76.10 a	80.62 a	7.73 a
P_2	77.67 b	71.96 b	59.58 b	71.84 b	6.69 b
LSD $_{(0.01)}$	3.98	3.80	3.76	3.96	0.36
CV (%)	4.70	4.76	5.34	5.01	4.87
Effect of drought stress					
T_0	93.10 a	88.09 a	80.50 a	86.60 a	9.27 a
T_1	89.28 ab	83.90 ab	76.93 ab	83.75 ab	8.59 b
T_2	84.14 b	78.57 bc	71.01 b	78.36 b	7.53 c
T_3	76.72 c	72.75 c	62.91 c	71.10 c	5.87 d
T_4	64.57 d	60.61 d	47.85 d	61.32 d	4.81 e
LSD $_{(0.01)}$	6.29	6.01	5.95	6.27	0.58
CV (%)	4.70	4.76	5.34	5.01	4.87

Note: P_1=Osmopriming for 6 hours with 6% mannitol, P_2=Hydropriming for 6 hours

T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed with 5% level of PEG, T_2= Primed seeds placed with 10% level of PEG, T_3= Primed seeds placed with 15% level of PEG, T_4= Primed seeds placed with 20% level of PEG.

4.3.2 Interaction with drought stress and growth

Interaction of osmo and hydro priming and different drought concentration on the germination and growth behavior of soybean (Table 8).In case of germination percentage (%), highest germination was found in P_1T_0 (94.62%) which was statistically identical with P_1T_1 and P_2T_0 , statistically similar with P_1T_2 and P_2T_1. Lowest germination was found in P_2T_4 (56.96%). For shoot length (SL), highest SL was found in P_1T_0 (91.24mm) which was statistically identical with P_1T_1 and statistically similar with P_1T_2, P_2T_0, P_1T_3 and P_2T_1. Lowest SL was found in P_2T_4 (54.11mm). For root length (RL) , highest root length was found in P_1T_0 (86.00mm) which was statistically identical with P_1T_1 and statistically similar with P_1T_2,P_1T_3 and P_2T_0. Lowest RL was found in P_2T_4 (35.67mm). For shoot dry weight (SDW), highest SDW was found in P_1T_0 (88.67mg) which was statistically identical with P_1T_1. For root dry weight (RDW), highest RDW was found in P_1T_0 (9.600mg) which was statistically identical with P_1T_1. Lowest RDW was found in P_2T_4 (4.347mg). Several authors have found that soaking with high PEG concentrations is detrimental to germination, e.g. Murungu *et al.*, (2005) in cotton (*Gossypium hirsutum*) and maize. The lower germination with high osmotic potentials could be due to solute leakage during priming. Water may have come out of the primed seed into the PEG solution, possibly by osmosis, thereby arresting the germination process (Murungu *et al*., 2005). Kaya *et al*., 2006; Sivritepe *et al*., 2003 and Foti *et al.,* 2002 affirmed that priming resulted in more germination speed especially in saline stress, drought stress and low temperatures in sorghum, sunflower and melon.

Table 8. Interaction effect of osmo and hydro priming and different drought concentration on the germination and growth behavior of soybean

Treatment combinations	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
P_1T_0	94.62 a	91.24 a	86.00 a	88.67 a	9.60 a
P_1T_1	91.86 a	88.62 a	83.67 a	86.73 a	9.20 a
P_1T_2	87.69 ab	83.14 ab	78.41 ab	82.01 a-c	8.21 bc
P_1T_3	80.91 bc	77.93 bc	72.40 b	76.00 b-d	6.39 d
P_1T_4	72.18 c	67.12 d	60.03 de	69.67 de	5.26 e
P_2T_0	91.58 a	84.94 ab	75.00 b	84.52 ab	8.93 ab
P_2T_1	86.70 ab	79.18 bc	70.20 bc	80.77 a-c	7.97 c
P_2T_2	80.59 bc	74.00 cd	63.62 cd	74.72 c-e	6.85 d
P_2T_3	72.54 c	67.57 d	53.43 e	66.20 e	5.34 e
P_2T_4	56.96 d	54.11 e	35.67 f	52.97 f	4.35 f
$LSD_{(0.01)}$	8.90	8.50	8.42	8.87	0.81
CV (%)	4.70	4.76	5.34	5.01	4.87

Note: P_1=Osmopriming for 6 hours with 6% mannitol, P_2=Hydropriming for 6 hours

T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed with 5% level of PEG, T_2= Primed seeds placed with 10% level of PEG, T_3= Primed seeds placed with 15% level of PEG, T_4= Primed seeds placed with 20% level of PEG

4.3.3 Effect of drought stress on water relation

Effect of osmo and hydro priming and different drought concentration (control, 5% PEG, 10% PEG, 15% PEG and 20% level of PEG) on the water relation behavior of soybean (Table 9). Treatment P_1 (osmopriming) showed higher relative water content (%), water retention capacity (WRC), coefficient of germination (CG) and vigor index (VI). Water saturation deficit (WSD) was higher in P_2 (hydropriming). Moghanibashi *et al.,* (2012) suggested that hydropriming for 24 h was enhanced germination and seedling growth of sunflower under stress conditions. Therefore, hydropriming may be used to improved seed performance of sunflower under normal and stress conditions. RWC was highest in T_0 (92.24%) which was statistically identical with T_1. Lowest RWC was found in T_4 (59.38%). In case of WSD, highest deficit was observed in T_4 (40.62) and lowest was found in T_0 (7.762). For WRC, highest result was found in T_0 (7.120) which was statistically identical with T_1. Lowest WRC was found in T_4 (3.740). In CG, T_0 (21.69) showed highest result which was statistically identical with T_1 and lowest CG was in T_4 (15.26). For VI, T_0 (157.0) showed highest result where T_4 (71.49) showed lowest.

Table 9. Effect of priming agents and drought stress on the water relation behavior of soybean

Treatments	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
Effect of priming agents					
P_1	81.81 a	18.19 b	6.56 a	20.34 a	136.24 a
P_2	74.23 b	25.77 a	4.75 b	17.54 b	105.15 b
LSD $_{(0.01)}$	3.98	1.40	0.33	1.08	6.25
CV (%)	4.91	6.11	5.63	5.51	4.99
Effect of drought stress					
T_0	92.24 a	7.762 e	7.12 a	21.69 a	157.0 a
T_1	87.23 a	12.77 d	6.64 a	21.05 a	144.0 b
T_2	80.51 b	19.49 c	5.85 b	19.10 b	126.3 c
T_3	70.74 c	29.26 b	4.94 c	17.58 b	104.7 d
T_4	59.38 d	40.62 a	3.74 d	15.26 c	71.49 e
LSD $_{(0.01)}$	6.30	2.21	0.52	1.71	9.89
CV (%)	4.91	6.11	5.63	5.51	4.99

Note: P_1= Osmopriming for 6 hours with 6% mannitol, P_2= Hydropriming for 6 hours

T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed with 5% level of PEG, T_2= Primed seeds placed with 10% level of PEG, T_3= Primed seeds placed with 15% level of PEG, T_4= Primed seeds placed with 20% level of PEG

4.3.4 Interaction effect of drought stress on water relation

Interaction effect of osmo and hydro priming and different drought concentration (control, 5%, 10%, 15%, 20% level of PEG) on the water relation behavior of soybean .In case of relative water content (RWC), highest RWC was found in P_1T_0 (92.68%) which was statistically identical with P_1T_1, P_2T_0 and P_2T_1. Lowest RWC was found in P_2T_4 (52.08%). For water saturation deficit (WSD), P_2T_4 (47.92) showed highest result and lowest result was found in P_1T_0 (7.323) which was statistically similar with P_2T_0. In case of water retention capacity (WRC), highest result was found in P_1T_0 (7.843) which was statistically similar with P_1T_1, P_1T_2, P_1T_3 and P_2T_0. Lowest WRC was found in P_2T_4 (2.727). For coefficient of germination (CG), highest result was found in P_1T_0 (22.98) which was statistically similar with P_1T_1. P_1T_2, P_2T_0 and P_2T_1 were statistically identical. Lowest CG was found in P_2T_4 (13.22). In case of vigor index (VI), P_1T_0 (167.7) showed highest result which was statistically similar with P_1T_1, P_2T_0 and P_1T_2. Lowest VI was found in P_2T_4 (51.15). Priming has the capability to repair some damages that have been produced from seed erosion and improve seed quality (Arif *et al*., 2008). Priming helps to increase germination, vigor and water content of plants under stress condition. Moghanibashi *et al.*, (2012) reported that, when salinity or drought level increased, all the growth parameter reduced except water saturation deficit. Primed seeds produced higher growth and water relation behavior under drought condition than non-primed seeds. Ghiyasi *et al.*, (2013) showed that osmopriming treatments helped to overcome the inhibition of germination index caused by drought stress.

Table 10. Interaction effect of priming agent and PEG concentration on the water relation behavior of soybean

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
P_1T_0	92.68 a	7.32 f	7.84 a	22.98 a	167.7 a
P_1T_1	88.81 a	11.19 e	7.45 ab	22.23 ab	158.4 ab
P_1T_2	84.51 ab	15.49 d	6.74 bc	20.15 bc	141.7 cd
P_1T_3	76.38 b	23.62 c	6.03 cd	19.03 cd	121.6 ef
P_1T_4	66.68 c	33.32 b	4.75 e	17.30 de	91.82 g
P_2T_0	91.80 a	8.20 ef	6.40 cd	20.41 bc	146.4 bc
P_2T_1	85.65 a	14.35 d	5.83 d	19.87 bc	129.5 de
P_2T_2	76.51 b	23.49 c	4.95 e	18.05 c-e	110.9 f
P_2T_3	65.10 c	34.90 b	3.85 f	16.12 e	87.77 g
P_2T_4	52.08 d	47.92 a	2.73 g	13.22 f	51.15 h
$LSD_{(0.01)}$	8.91	3.12	0.74	2.42	13.98
CV (%)	4.91	6.11	5.63	5.51	4.99

Note: P_1=Osmopriming for 6 hours with 6% mannitol, P_2=Hydropriming for 6 hours

T_0= Primed seeds placed without drought (control), T_1= Primed seeds placed with 5% level of PEG, T_2= Primed seeds placed with 10% level of PEG, T_3= Primed seeds placed with 15% level of PEG, T_4= Primed seeds placed with 20% level of PEG

CHAPTER V

SUMMARY AND DISCUSSION

The experiment was conducted at Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka 1207 during the period from April to May, 2018 to study the mannitol induced seed priming on drought tolerance capability in soybean varieties cv. BARI soybean 5 and BARI soybean 6 under drought stress condition.

The whole experiment was conducted in three different experiments. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Priming chemicals such as Mannitol ($C_6H_{14}O_6$) and distilled water were utilized for osmo and hydro priming. Mannitol used as seed treating chemical. Priming was done in room temperature and all the primed seeds were removed from the priming solution at the same time. Twenty seeds from each of the treatments were selected randomly and placed in 90 mm diameter Petri dishes on whatman No.1 filter paper and filter paper was moistened with 8 ml of distilled water .Germination was considered to have occurred when radicles emerged by rupturing seed coat. Germination progress was inspected and data were collected at every 24 h intervals and continued up to 8 days. The abnormal or dead seedlings with short, thick and spiral formed hypocotyls and stunted primary root were excluded during counting. The data on germination parameters of soybean like total germination percentage (%), growth parameters like shoot length, root length, shoot dry weight, root dry weight, water retention capacity (WRC), relative water content (RWC), water saturation deficit (WSD), coefficient of germination (CG) and vigor index (VI). Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated at 1% level of probability.

The first experiment was carried out to find the effect of different concentration of mannitol on germination behavior of soybean varieties cv. BARI soybean 5 (V_1) and BARI soybean 6 (V_2) without any stress condition .Four levels of mannitol such as 2%, 4%, 6%, and 8% were used for osmopriming and water used as hydropriming agent for 6 hours, respectively. The priming treatments were seeds without priming (control) (P_0), seeds primed with distilled water for 6 hours (P_1), seeds primed with 2% mannitol solution for 6 hours (P_2), seeds primed with 4% mannitol solution for 6 hours (P_3), seeds primed with 6% mannitol solution for 6 hours (P_4) and seeds primed with 8% mannitol solution for 6 hours (P_5). V_2 (BARI soybean 6) showed maximum germination percentage (%), shoot length (mm), root length (mm), shoot dry weight (mg) and root dry weight (mg) than V_1 (BARI soybean 5). Maximum germination percentage, shoot length, root length , shoot dry weight and root dry weight was recorded in P_4 (seeds primed with 6% mannitol solution for 6 hours). Maximum germination and other growth parameter was recorded in V_2P_4 (BARI soybean 6 primed with 6% mannitol solution for 6 hours). The growth and water relation behaviors was maximum in V_2 (BARI soybean 6). P_4 (6% mannitol priming) showed maximum growth and water relation behaviors. Variety BARI soybean 5 was not further used as it gives poor result than BARI soybean 6.

The second experiment was conducted to evaluate different pre-sowing priming time on the germination behavior of soybean. Six different priming times such as 3, 6, 9, 12, 15, and 18 hours for osmopriming (P_1) and hydropriming (P_2) were used, respectively in this experiment. Seeds primed with 6% mannitol solution for 3 hours (T_1), seeds primed with 6% mannitol solution for 6 hours (T_2), seeds primed with 6% mannitol solution for 9 hours (T_3), seeds primed with 6% mannitol solution for 12 hours (T_4), seeds primed with 6% mannitol solution for 15 hours (T_5), seeds primed with 6% mannitol solution for 18 hours (T_6). Germination percentage was maximum in P_1 (82.23%) and minimum in P_2 (77.60%). Shoot length, root length , shoot dry weight and root dry weight was recorded maximum in P_1 than P_2 . T2 (primed with 6% mannitol for 6 hours) was recorded maximum shoot length (86.70mm), root length (80.25mm), shoot dry weight (89.17mg) and root dry weight (8.333mg). Interaction effect of priming and priming times on germination and growth was recorded in P_1T_2 (seeds osmoprimed for 6 hours). For relative water content (80.70%), water retention capacity (6.23), coefficient of germination (19.77%) and vigor index (122.60) was maximum in P_1 (osmopriming) . T_2 (6 hours priming) was showed maximum relative water content (92.91%), water retention capacity (6.942), coefficient of germination (21.56%) and vigor index (157.3). Interaction effect of priming and priming times on the water relation behaviors was maximum in P_1T_2 (osmopriming for 6 hours). Water saturation deficit (32.94) was maximum in P_2T_1 (hydropriming for 3 hours).

In the third experiment germination behavior of primed seed (soybean) under different drought (PEG) stress condition was evaluated. Five different concentration of stress such as 0% PEG, 5%

PEG, 10% PEG, 15% PEG and 20% PEG for osmo and hydro priming was used respectively. Osmopriming for 6 hours with 6% mannitol (P_1) and hydropriming for 6 hours (P_2) was used. Different level of drought stress , primed seeds placed without drought (control) (T_0), primed seeds placed with 5% level of PEG (T_1), primed seeds placed with 10% level of PEG (T_2), primed seeds placed with 15% level of PEG (T_3), primed seeds placed with 20% level of PEG (T_4). P_1 was recorded maximum germination percentage (85.45%), shoot length (81.61mm), root length (76.10mm), shoot dry weight (80.62mg) and root dry weight (7.73mg). T_0 (control) was showed maximum germination percentage (93.10%), shoot length (88.09mm), root length (80.50mm), shoot dry weight (86.60mg) and root dry weight (9.267mg). Interaction of priming with drought stress concentration on growth behavior was recorded in P_1T_0 (osmoprimed with 6% mannitol in control). P_1 was recorded maximum relative water content (81.81), water retention capacity (6.56), coefficient of germination (20.34%) and vigor index (136.24). T_0 (control) was showed maximum relative water content (92.24%), water retention capacity (7.120), coefficient of germination (21.69%) and vigor index (157.0). Water saturation deficit was recorded lowest in P_2 and T_0. Interaction effect of priming and drought stress on the water relation behaviors was maximum in P_1T_0 (osmopriming with 6% mannitol for 6 hours at control).Germination and growth was more profound in control condition. Thus, the priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of drought stress. For this reason, further studies are needed to assess the efficacy of seed priming during the later stages of the culture.

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APPENDICES

Appendix I. Analysis of variance of the data on germination and growth behavior of soybean as influenced by combined effect of different varieties and priming solution

Source of variation	df	Mean square of				
		Germination percentage	Shoot length	Root length	Shoot dry weight	Root dry weight
Variety (A)	1	184.486**	1215.360**	1088.686**	2423.034**	41.450**
Priming solution (B)	5	683.877**	2542.782**	1299.344**	2446.661**	25.008**
Variety (A) X Priming solution (B)	5	2.797**	39.347**	46.086**	80.302**	0.832**
Error	48	13.265	10.971	3.352	16.608	0.096

**Significant at 1% level of significance

^{NS} Non significant

Appendix II. Analysis of variance of the data on growth and water relation behavior of soybean as influenced by combined effect of different varieties and priming solution

Source of variation	df	Mean square of				
		Relative water content	Water saturation deficit	Water retention capacity	Germination Coefficient	Vigor index
Variety (A)	1	256.515**	256.515**	3.912**	58.115**	4755.449**
Priming solution (B)	5	744.814**	744.814**	4.127**	36.899**	8851.814**
Variety (A) X Priming solution (B)	5	8.050**	8.050**	0.095**	1.165**	110.451**
Error	48	15.414	1.313	0.113	0.992	20.996

**Significant at 1% level of significance

^{NS} Non significant

Appendix III. Analysis of variance of the data on germination and growth behavior of soybean as influenced by combined effect of different priming agent and priming time

Source of variation	df	Mean square of				
		Germination percentage	Shoot length	Root length	Shoot dry weight	Root dry weight
Variety (A)	1	192.839**	1447.295**	1033.623**	648.891**	14.263**
Priming solution (B)	5	450.088**	860.065**	525.965**	1044.249**	9.271**
Variety (A) X Priming solution (B)	5	3.667**	40.225**	37.107**	9.792**	0.186**
Error	24	11.999	11.587	12.100	12.971	0.070

**Significant at 1% level of significance

^{NS} Non significant

Appendix IV. Analysis of variance of the data on growth and water relation behavior of soybean as influenced by combined effect of different priming agent and priming time

Source of variation	df	Mean square of				
		Relative water content	Water saturation deficit	Water retention capacity	Germination Coefficient	Vigor index
Variety (A)	1	295.668**	295.725**	4.951**	43.714**	5243.691**
Priming solution (B)	5	443.664**	443.724**	3.872**	21.552**	5089.257**
Variety (A) X Priming solution (B)	5	11.265**	11.258**	0.070**	0.382**	58.805**
Error	24	15.663	1.395	0.086	1.048	37.022

**Significant at 1% level of significance

^{NS} Non significant

Appendix V. Analysis of variance of the data on germination and growth behavior of soybean as influenced by combined effect of different priming agent and drought concentration

Source of variation	df	Mean square of				
		Germination percentage	Shoot length	Root length	Shoot dry weight	Root dry weight
Variety (A)	1	453.574**	698.612**	2046.498**	578.075**	8.164**
Priming solution (B)	4	767.325**	689.001**	1015.423**	625.852**	20.729**
Variety (A) X Priming solution (B)	4	32.088**	8.744**	41.357**	35.773**	0.109**
Error	20	14.670	13.373	13.120	14.562	0.123

**Significant at 1% level of significance

^{NS} Non significant

Appendix VI. Analysis of variance of the data on growth and water relation behavior of soybean as influenced by combined effect of different priming agent and drought concentration

Source of variation	Df	Mean square of				
		Relative water content	Water saturation deficit	Water retention capacity	Germination Coefficient	Vigor index
Variety (A)	1	431.302**	431.302**	24.589**	58.912**	7247.856**
Priming solution (B)	4	1040.638**	1040.638**	10.994**	41.170**	6855.674**
Variety (A) X Priming solution (B)	4	47.894**	47.894**	0.133**	0.895**	74.691**
Error	20	14.697	1.805	0.101	1.088	36.208

**Significant at 1% level of significance

^{NS} Non significant

PLATES

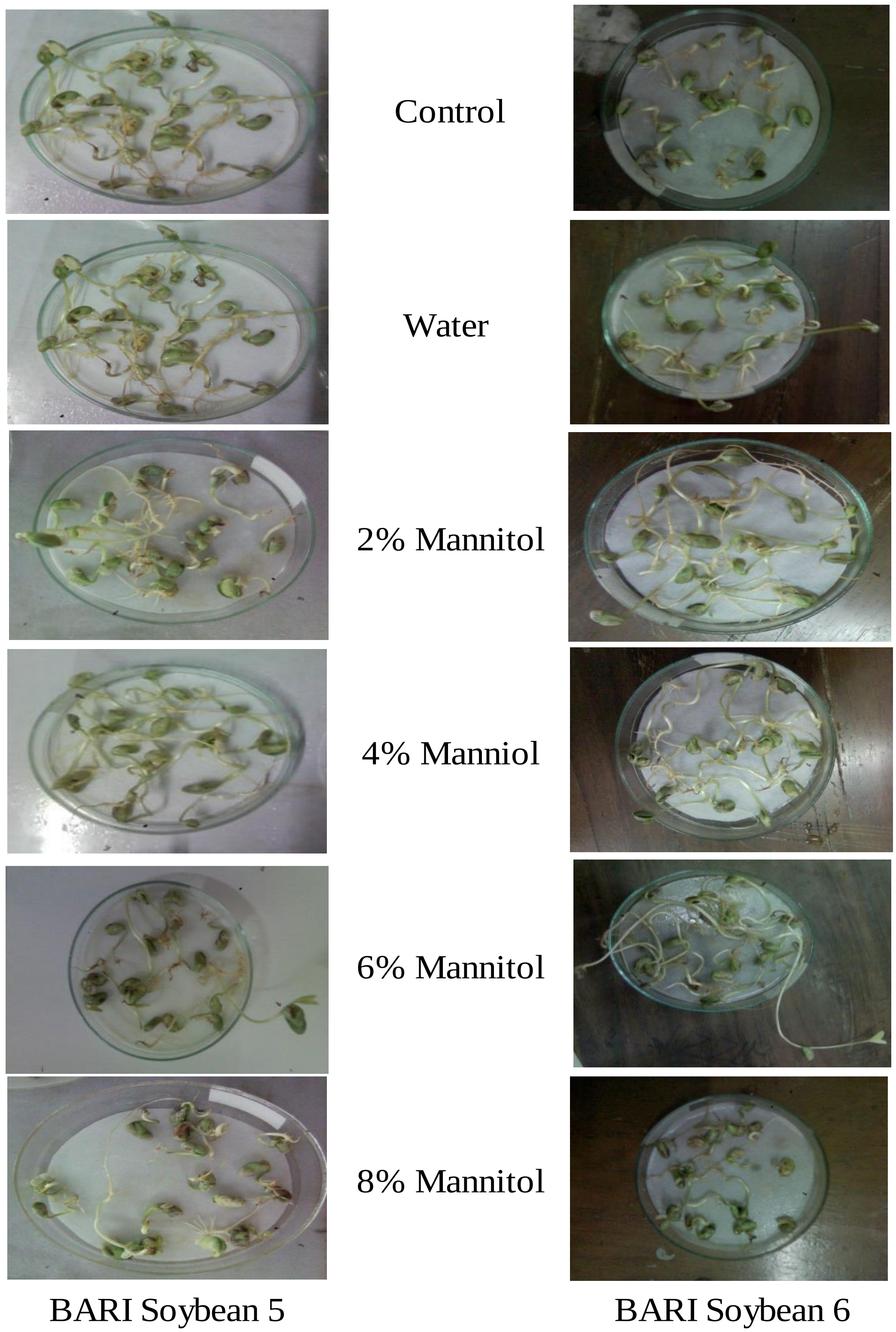

Plate 1: Effect of different concentration of priming solution on germination behavior of soybean varieties (BARI soybean 5 and BARI soybean 6)

Osmo Priming

Hydropriming

3 hr

6 hr

9 hr

12 hr

15 hr

18 hr

Plate 2: Effect of osmo and hydro priming and priming time on germination behavior of BARI soybean 6

Osmo Priming

Hydro Priming

0% PEG stress

5% PEG stress

10% PEG stress

15% PEG stress

20% PEG stress

Plate 3: Effect of osmo and hydro priming and stress concentration on germination behavior of BARI soybean 6