INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH POLYETHYLENE GLYCOL AND HYDROPRIMING

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CERTIFICATE

This is to certify that the thesis entitled **"INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH POLYETHYLENE GLYCOL AND HYDROPRIMING"** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfilment of the requirements for the degree of* ***MASTER OF SCIENCE*** *in* ***AGRONOMY****, embodies the results of a piece of bona fide research work carried out by* **NUSRAT JAHAN KEYA**, *Registration no.* **12-04762** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated**:**

Place: Dhaka, Bangladesh

(Prof. Dr. Md. Abdullahil Baque)
Supervisor

Dedicated to

My

Beloved Family

LIST OF ACCRONYMS AND ABBREVIATIONS

Agron.	Agronomy
Agric.	Agriculture
Agril.	Agricultural
Ann.	Annals
Appl.	Applied
AOSA	Association of Official Seed Analysis
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Biot.	Botany
Biotechno.	Biotechnology
^{0}C	Degree centigrade
Chem.	Chemistry
Chron.	*Chronicle*
Comm.	Communications
cm	Centimeter
CRD	Completely Randomized Design
CG	Coefficient of germination
Curr.	Current
Compt.	Comptes
Eng.	Engineering
ed.	Edition
Environ.	Environmental
et al.	And others
Expt.	Experimental
Gaz.	*Gazette*
GI	Germination Index
GP	Germination Percentage
Hort.	Horticulture
hr	Hour
i.e.	idest (L), that is
Inst.	Institute
Integr.	Integrative
Inter.	International
ISTA	International Seed Testing Association
J.	Journal
LSD	Least significant difference
mg	Milligram
mL	Milliliter
mm	Millimeter
M.S	Master of Science
Nut.	Nutrition
Nat.	Nature
Opin.	Opinion
Pak.	Pakistan
Pathol.	Pathology
PEG	Polyethylene Glycol
Physiol.	Physiology
Prog.	Progressive
Rev..	Revolution
Res.	Research
ROS	Reactive Oxygen Species
RWC	Relative water content
SAU	Sher-e-Bangla Agricultural University
Sci.	Science
Technol.	*Technology*
VI	Vigour Index
viz.	Namely
WRC	Water retention capacity
WSD	Water saturation deficit

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The Author

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INDUCTION OF DROUGHT TOLERANCE CAPABILITY OF SOYBEAN THROUGH POLYETHYLENE GLYCOL AND HYDROPRIMING

ABSTRACT

The study was conducted under the laboratory condition of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka from March to April, 2018 to evaluate the effect of pre-sowing seed treatment with Polyethylene Glycol (PEG) on germination behavior and water relation behavior of soybean in relation to drought tolerance and to optimize priming time. In the 1st experiment, seeds of BARI soybean 5 and BARI soybean 6 were primed with distilled water, 5%, 10%, 15% and 20% PEG solution. Results revealed that BARI soybean 6 showed better performance than BARI soybean 5 in case of germination, seedling growth and water relation behavior of soybean except water saturation deficit (WSD) at 10% PEG. BARI soybean 6 showed GP (85.56%), root and shoot length (67.55 mm and 40.12 mm respectively), root and shoot dry weight (83.28 mg and 7.78 mg respectively), Relative Water Content (82%), Water Retention Capacity (7.61), Coefficient of Germination (19.79%) and Vigor index (84.81). In the 2nd experiment, BARI soybean seeds were soaked with 10% PEG and distill water for different priming time *viz.* 3,6.9.12,15 and 18hr. Osmopriming (10% PEG) showed better performance than hydropriming when seeds were soaked for 6 hrs in case of all parameters except WSD. In the 3rd experiment, osmoprimed and hydroprimed seeds were placed under drought stress condition induced by 0%, 5%, 10%, 15% and 20% PEG. Osmopriming showed better result over hydropriming under low to moderate stress condition and this value decreased with increasing stress condition in relation to all parameters except WSD. Drought tolerance capability increased in osmopriming over hydropriming in case of GP (7.41%), RWC (8.96%), CG (26.05%) and VI (26.32%) under 10% PEG stress. From this study it can be concluded that when BARI soybean 6 primed with 10% PEG for 6 hr showed better performance and stress tolerance than hydroprimed with distill water. These results suggest that seed priming had significant effect to boost up the germination, seedling growth and water relation behavior of soybean.

CHAPTER I

INTRODUCTION

Soybean (*Glycine max* L.) belongs to Fabaceae family native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse by the UN Food and Agricultural Organization (FAO). Soybean contains 40-45% protein and 19-22% oil. As a good source of protein, unsaturated fatty acids, minerals like Ca and P including vitamin A, B, C and D, soybean can up different nutritional needs (Rahman, 1982). It is a rich source of amino acids, vitamins and minerals. Recently, soybean has become an important crop in Bangladesh for its increasing demand as an ingredient of poultry and fish meal as well as for the consciousness of its healthy nutrition as human food. It is one of the most economic and nutritious crops in the world (Yaklich *et al*., 2002).

It is a high value and profitable crop. The economic viability of soybean production is determined by the commercial utilization of both its sub-products, meal and oil, which, account for about two thirds and one third of the crops economic value, respectively. Soybean is the most important oil crops in Bangladesh. Out of the total cropped areas of 14.418 million ha, oil crops occupy about 0.484 million ha and the total production of the country stands at 0.975 million tones. Out of total oil copped area, Soybean occupies 0.062 million ha and production of soybean is 0.097 million tones (BBS, 2017). Given normal weather conditions, soybean production is expected to increase 1.96 percent to 156 thousand MT in MY 2017/18 (Jul-Jun). Greater farmer interest in planting soybeans will drive an increase in planted area of 1.23 percent, to a total of 82 thousand hectares in MY 2017/18. Among oilseeds in Bangladesh, in FY 2015-16 soybeans are the fourth crop in terms of total planted area at 9% of total oilseed planted area (Hossain, 2017). About 71,000 hectares of land in Bangladesh is cultivation of soybean with an annual approximate production of 135,000 tons (Anon., 2015). Soybean is a major oil seed crop of world grown in an area of 91million hectare with production of 204 metric ton and productivity of 2,233 kg/ha. Soybean accounts for approximately 50 % of the total production of oilseed crops in the world (FAO, 2007). The crop is mainly cultivated in USA, China, Brazil, Argentina and India. India contributes more than 90 per cent of world's acreage. In India it is grown over an area of 8.17 million hectare with production of 9.46 metric ton and productivity of

1,069 kg per ha (Anon., 2007). Major soybean growing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh and Gujarat. In Karnataka, soybean occupies an area of 1.62 lakh hectare with the production of 1.53 lakh tonnes and productivity of 950 kg per ha (Anon., 2007). As a grain legume, it is gaining important position in the agriculture of tropical countries including Bangladesh. Now, soybean producing areas are Barisal, Bhola, Faridpur, Patuakhali, Meherpur, Jessore, Rangpur, Kurigram, Thakurgaon, Tangail, Mymensingh, Jamalpur, Chandpur, Feni, Noakhali and Laxmipur (Chowdhury *et al*., 2013). Soybean production area is increasing day by day and in the year 2013 it reaches above 61000 ha (Chowdhury *et al*., 2014). In Bangladesh, about five thousand hectares of land is under soybean cultivation and annual production is approximately 4 thousand metric tons with an average yield of 1.5-2.3 t/ha (BARI, 2006). The low productivity of soybean both at national and state level is attributed to abiotic and biotic stresses like drought, salinity, weeds, insect pests and diseases. Among these, drought a great threat to soybean production by increasing cost of cultivation and impairing quality of produce in many ways.

Soybean can play a vital role in balancing the protein-calorie malnutrition in Bangladeshi diet. The present nutritional situation of third world and some developing countries like Bangladesh is matter of great concern since the most of the people are suffering from malnutrition. Soybean can play an important role in this case and can help to meet up the nutritional deficiency problem (Mahbub *et al*., 2015). Soybean could be regarded as an ideal food for the people of poor and some developing countries as it contains high quality of protein and reasonable quantity of oil as a source of energy. Furthermore, being a leguminous crop it improves soil fertility and productivity by fixing the atmospheric nitrogen through Rhizobium bacteria that lives in root nodules *(*Jaiswal *et al*., 2012 and Youseif *et al*., 2014). As a result it is very suitable crop to fit into the cropping systems of Bangladesh. It is the world's leading economic oil seed crop (Manavalan *et al*., 2009). It is also an important source of plant protein of the people in semi-arid and tropical region.

The production of the crop is often limited by the erratic nature of rainfall. Among the crops soybean has highest sensitivity to drought. (Maleki *et al*., 2013). Drought is a worldwide problem and a major proportion of agriculture land is affected with varying degrees of drought. Water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the most limiting factors for better plant performance and higher crop yield (Szilgyi., 2003; Hirt & Shinozaki., 2003). Water deficit is reported to inhibit soybean growth and yield (Ahmadvand *et al*., 2012). Plants are exposed to various abiotic factors throughout the course of their growth and development (Zhao *et al*., 2007). The major abiotic stress to which plants are exposed include extreme temperature, drought and high salinity. These stresses are the most significant factors leading to substantial and unpredictable loss in crop production in agriculture (Jakab *et al*., 2005). Though stress has positive impact on seed but it is not good for seed germination especially for drought stress. And for this reason seed priming is considered as a promising approach to increase stress tolerance capacity of crop plants including drought. Priming the seeds has been successfully demonstrated to improve germination and emergence in seed of many crops. Priming also allows for controlling the rate of seed water imbibition so that seed could become resistant to stress and apart from stimulating growth (Ghassemi *et al*., 2011). Seed priming is the induction of particular physiological state in plant by the treatments of natural and synthetic compounds to the seed before germination. It enhances seed and seedling vigor leading to better stand establishment and yield, so that plants can grow in unfavorable environmental conditions (Khalil *et al*., 2010). Improvement Seed priming can accomplished through different methods such as hydro-priming (soaking in DW), osmo priming (soaking in osmotic solutions such as PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth inducers (CCC, Ethephon, IAA) (Capron *et al*., 2000;Chiu *et al*., 2002; Harris *et al*., 1999;Chivasa *et al*., 1998). Primed seeds usually exhibit an increased germination rate, greater germination uniformity, and at times, greater total germination percentage (Basra *et al*., 2005). Therefore there is a strong interest in the seed industry to find suitable priming agent(s) that might be used to increase the tolerance of plants under adverse field conditions. (Job *et al*., 2000). For drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG) (Turkan *et al*., 2005; Landjeva *et al*., 2008). The study has been presented here that deals with responses of soybean cultivars in case of drought when seeds are primed with PEG and distill water. As soybean cultivating in drought prone areas like

Laxipur, Noakhali, Bhola, Kushtia and other district in Bangladesh. Priming of seed may help to germination, growth and establishment of this crop.

The present study was undertaken with the following objectives -

Objectives:

- To evaluate the effect of pre-sowing seed treatment on germination and water relation behavior of soybean.
- To optimize the pre-sowing priming time on the germination and water relation behavior of soybean
- To understand the effect of priming under different levels of drought stress on germination and water relation behavior of soybean.

CHAPTER II

REVIEW OF LITERATURES

Literatures which are related to the performances of various priming like hydropriming, osmopriming, halopriming etc. used alone or together with different priming time on various crops cultivation were obtainable. But work of different priming on soybean, a minor oilseed crop in our country were not so much accessible. The review of literature presented below was based on work of different priming on different crops cultivation. The review includes reports of several investigators which appear right on understanding the problem and which may lead to the interpretation of results of the present experiment.

2.1 Seed priming

Stand establishment is of primary importance for optimizing field production of any crop plant. At suboptimal conditions of environment conditions, poor seed germination and subsequently poor field establishment is a common phenomenon.

Mwale *et al*. (2003) reported that one of the major obstacles to high yield and production of crop plants is the lack of synchronized crop establishment due to poor weather and soil conditions. On the other hand seeds are occasionally sown in seedbeds having unfavorable moisture because of the lack of rainfall at sowing time which results in poor and unsynchronized seedling emergence (Angadi and Entz, 2002).

According to Singh *et al*. (2015) strategies for improving the growth and development of crop species have been investigated for many years. Rapid germination and emergence are essential for successful crop establishment, for which seed priming could play an important role. Seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigour, leading to better stand establishment and yield. It is a simple and low cost hydration technique in which seeds are partially hydrated to a point where pre-germination metabolic activities start without actual germination, and then re-dried until close to the original dry weight. Seed priming is employed for better crop stand and higher yields in a range of crops.

Harris *et al*. (2007) reported that seed priming led to better establishment and growth, earlier flowering, increase seed tolerance to adverse environment and greater yield in maize. The beneficial effects of seed priming have been demonstrated for many field crops such as wheat, sweet corn, mung bean, barley, lentil, cucumber etc. (Sadeghian and Yavari, 2004).

Rehman *et al*. (2011) reported that seed priming is a cost effective technology that can enhance early crop growth leading to earlier and more uniform stand with yield associated benefits in many field crops including oilseeds.

Various seed priming techniques have been developed which include hydro-priming, halo-priming, osmo-priming and hormonal priming. Hydro-priming soaking the seeds in water before sowing and may or may not be followed by air drying of the seeds. Hydro-priming may enhance seed germination and seedling emergence under both saline and non- saline conditions.

Roy and Srivastava (1999) found that soaking wheat kernels in water improved their germination rate under saline conditions.

Nagar *et al*. (1998) also found that hydropriming had pronounced effect on field emergence its rate andearly seedling growth of maize crop and it improved the field stand and plant growth both at vegetative and maturity of maize. Hydro-priming plays an important role in the seed germination, radical and plumule emergence in different crop species. Similar to other priming techniques, hydro-priming generally enhance seed germination and seedling emergence under saline and non-saline conditions and also have beneficial effect on enzyme activity required for rapid germination.

Halo-priming refers to soaking of seeds in solution of inorganic salts i.e NaCl, KNO_3, $CaCl_2$ and $CaSO_4$ etc. Khan *et al*. (2009) evaluated the response of seeds primed with NaCl solution at different salinity levels 0, 3, 6 and 9 dSm-1 in relation to early growth stage and concluded that seed priming with NaCl has found to be better treatment as compared to nonprimed seeds.

Iqbal *et al*. (2006) found that Priming with NaCl and KCl was helpful in removing the deleterious effects of salts.

Afzal *et al*. (2006) reported that wheat cultivar Auqab- 2000 was treated with different priming agents i.e. Abscisic acid (ABA), Salicylic acid (SA) and ascorbic acid and were sown under normal and saline condition (15 dSm^{-1}), and showed that under saline conditions these treatment reduced the time for 50% germination, increased final germination count, and significantly increased the fresh and dry weight but ascorbic acid did not show such results. Hormonal priming has reduced the severity of the effect of salinity but the amelioration was better due to 50 ppm SA and 50 ppm ascorbic acid treatments as these showed the best results on seedling growth, fresh and dry weights under non-saline and saline conditions whereas hormonal priming with ABA as not effective under present experimental material and conditions.

Osmo-priming technique refers to soaking of seeds for a certain period in solution of sugar, PEG etc followed by air drying before sowing. Osmo-priming not only improves seed germination but also enhance crop performance under nonsaline or saline conditions. Salehzade *et al*. (2009) conducted a study to enhance germination and seedling growth of wheat seeds using osmo-priming treatments. Seeds were osmo-primed with PEG-8000 solution for 12 hours. Osmo-priming treatments improved the seedling stand establishment parameters.

Hormonal priming is the pre - seed treatment with different hormones like GA_3, kinetin, ascorbate etc. which promotes the growth and development of the seedlings.

Ashraf *et al*. (2001) found that GA_3 treatment enhanced the vegetative growth of two wheat cultivars. It enhanced the deposition of Na^+ and Cl^- in both root and shoots of wheat plant. It also caused a significant increase in photosynthetic at the vegetative stage of the crops.

2.2 Effect of priming on different crops

Miladinov *et al*. (2018) conducted an experiment on the effect of primers on seed quality parameters using different starting values of germination. For this purpose, ten soybean lines selected at the Institute of Field and Vegetable Crops, Novi Sad were evaluated. The starting values of germination ranged between 48% and 89%. Seeds were surface sterilized with 3% sodium hypochlorite and immersed in different primers: 1% potassium nitrate, 1% potassium chloride and 1% hydrogen peroxide. Untreated seeds were used as the control. The obtained results revealed that the effects of priming depended on soybean line and treatment, whereas the efficiency of this pre-sowing treatment was not affected by the starting value of seed germination. Some lines responded favorably to immersion, while in others priming had an inhibitory effect, causing a significant decrease in germination. There was an increase in germination up to 12% or a decrease up to 11%, depending on line and treatment. Lines that were positively affected by this method also exhibited increased values for other germination parameters: mean germination time (MGT) and time to 50% germination (T50). Lower values of MGT and T50 were observed in lines which showed a negative response to priming and a decrease in germination, but also a more rapid radicle protrusion, as compared to the control.

Shete *et al*. (2018). Conducted an experiment entitled “Effect of seed priming on yield of soybean [*Glycine max* (L.) Merrill.]”, different priming treatments are given before one day of sowing with seven treatments and three replication such as hydropriming, osmopriming, halopriming and control. The experimental design was randomized block design with three replications and seven treatments such as T_1- Hydro priming for 30 minutes, T_2-Hydro priming for 1 hour, T_3-0.5% KNO_3 (Osmo priming for 30 min), T_4- 1% KNO_3 (Osmo priming for 1 hour), T_5- 0.1% NaCl (Halo priming for 30 min), T_6 -0.2% NaCl (Halo priming for 1 hour), T_7-Control. Results indicated relatively higher mean performance of hydropriming for one hour in yield and yield contributing trades such as days to field emergence, number of pods per plant, seed yield per plant, seed yield per ha, test weight and harvest index.

Mehri (2015) reported about effect of seed priming on seed yield and yield components of soybean (Glycine max var Williams). The experimental design was two factors factorial on basis of randomized complete block design with four replications. The first factor was priming methods (control, $ZnSO_4$, KH_2PO_4, KNO_3 and H_2O) and the second factor was priming duration (control, 6, 12, 18 and 24 hours). Results showed that priming methods and duration increased germination percentage, germination rate, number of pods per plant, seed numbers per pod, 1000-seed weight, biological and seed yields. According to the results of this experiment, seed priming by H_2O_2 with 18 hours had an appropriate performance and could increase seed germination, seed yield and yield components to an acceptable level. Therefore, hydro-priming is a simple, low cost and environmentally friendly technique for improving seed yield in soybean.

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

Tabatabaei (2013) reported that Seed priming with PEG 6000 treatments significantly ($p < 0.01$) affected germination percentage, normality seedling percentage, and germination index. Seed priming with PEG increased germination characteristics as the compared to the unprimed. As ennzyme activity was significantly improved in barley seeds primed as compared to the unprimed. CAT significantly improved in barley seeds primed with PEG as compared to the unprimed. Also APX significantly improved in barley seeds under stress condition. This germination characteristics of primed seeds could be results of increasing the antioxidant profile of treated seeds.

Ahmadvand *et al*. (2012) conducted two laboratory and green house experiments to evaluate effect of seed priming with potassium nitrate on germination and emergence traits of two soybean cultivars cv. Gorgan -3 and cv. Sahar at Bu-Ali Sina University, Iran. They reported that seed priming with KNO_3 caused a significant increase in germination and emergence percentage, radical and plumule length, seedling dry weight, plant height, plant leaf area and plant dry weight. Seed priming led to significant increase of leaf area per plant and leaf area of non-primed seeds was decreased by 78%.

Bassi *et al*. (2011) reported that priming with GA_3@ 50 ppm for 2 hour enhanced emergence, germination and speed of germination in soybean as compared to non-primed seed lots.

A laboratory study and a field experiment was carried out by Golezani *et al*. (2011) to evaluate the effect of priming on seed in vigouration and field performance of soybean. Results showed that pods per plant, seeds per plant and seed yield per plant were significantly enhanced by seed priming particularly with KNO_3. On contrary, seedling emergence percentage and germination time were significantly adversely affected by seed priming (KH_2P0_4 and KNO_3) as compared to nonprime seeds.

Assefa and Hunje (2011) reported that the speed of germination in soybean increased as the priming duration increased from 0 to 14 hours. The germination decreased with increased priming duration beyond 14 hours. In the early stage of germination seeds of a wide variety of plants can be dried back to 10 per cent moisture without loss of viability, but if they are dried after radical emergence (as the duration increases) the seeds are not able to germinate. The priming duration affected the speed of germination more than the final percentage of germination. Significantly higher speed of germination (57.1), root length (16.3 cm), shoot length (13.8 cm) and vigour index (2933) were consistently in favour of 14 hour seed priming duration as compared to lesser and more duration.

Field experiment was conducted by Mahajan *et al*. (2011) at Punjab Agricultural University Ludhiana to enhance the performance of dry direct seeded basmati rice with four seed priming treatments (control, osmo hardening, water hardening and hydro-priming). Crop with hydropriming gave superior performance as compared to other seed priming treatments. Highest grain yield of Pusa Basmati 1121 was obtained with hydro-priming at 60 kg/ha of N application applied in 3 splits. Hydro-primed seeds produced more panicles/m-2 (291), filled grain per panicle (67), 1000 grain weight and spikelet sterility (25.9 g and 21.9 %).

Sharma *et al*. (2010) conducted an experiment at CSK Himachal Pradesh Agricultural University to study the effect of seed priming on late sowed rainfed wheat. The results showed that emergence count was increased with pre sowing hydration of seed with 3% calcium chloride and 15 ppm gibberellic acid as compared to rest of the treatments.

Yari *et al*. (2010) conducted experiment in Iran to evaluate the effect of different seed priming techniques on germination and early growth of two wheat cultivars. Seeds were primed for 12, 24 and 36 hours at different temperature range in four priming media (PEG

20%, KCl 2%, KH_2PO_4 0.5 and KH_2PO_4 1%). They reported that KH_2PO_4 and KCl showed good potential to enhance germination, emergence, growth and grain yield of wheat. It has also been reported that seed priming improves emergence, stand establishment, tillering, grain and straw yields and harvest index in wheat (Farooq *et al.* 2008).

Harris *et al*. (2007) conducted an experiment and reported that seed priming led to better establishment and growth, earlier flowering, increased seed tolerance to adverse environment and higher yield in soybean.

The direct benefits of seed priming were reported by Harris *et al*. (2001) in crops like wheat, rice and maize which included faster emergence, better and uniform stands, less need to re-sow, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield. The indirect benefit reported were earlier sowing of crops, earlier harvesting of crops and increased willingness to use of fertilizer because of reduce risk of crop failure and also reported that water has been used successfully as a seed priming medium for wheat.

A field experiment was conducted by Bassi (2005) to monitor the effect of various seed priming treatments on late sown wheat. Result showed that Gibberellic acid treatment enhanced germination and emergence (94 and 82 %, respectively) as compared to non-primed seed treatment (85 and 77%, respectively).

Raun *et al*. (2002) reported that priming the rice seed with KCl improved its germination index. Greater efficiency of seed priming with KCl is possibly related to the osmotic advantage that K^+ has in improving cell water saturation and that act as co-factor in activities of numerous enzymes (Taiz and Zeiger, 2002).

Park *et al*. (1997) reported that the priming aged seeds of soybean resulted in good germination and stand establishment in the field trials.

Bensen *et al*. (1990) demonstrated that hypocotyls growth rate of soybean crop is directly associated with the amount of GA_3. The enhanced plant height was due to the improved and faster plant emergence in GA_3, KH_2PO_4 and KCl primed seed plots.

Riedell *et al*. (1985) and Maske *et al*. (1997) reported that GA_3 treated soybean seeds recorded better field performance due to its stimulation effect in the formation of enzymes which are important in the early phase of germination which helps for a fast radical protrusion in many field crops.

Beneficial effects of KCl have been reported by Vijayakumar *et al*. (1988) in Okra, Rajandran (1982) in red gram and Basha (1982) in green gram.

According to Graf *et al*. (1987) KH_2PO_4 showed a relatively positive effect presumably because phosphorous reserves in the seed play very important role in the metabolism of germinating seed.

Mishra and Dwibedi (1980) found that seed soaking in 2.5% KCl for 12 hour before sowing increased wheat yield by 15%. KCl and KH_2PO_4 have been introduced as the osmotica which have shown good potential to enhance emergence and germination in wheat.

2.3 Effect of priming under stress condition in different crops

Kumar *et al*. (2017) were conducted an experiment 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 durations that is 6hrs and 12 hrs were evaluated by screening a range of durations and concentrations *viz*., T_0 - Unprimed Control, T_1–Polyethylene glycol (PEG) (for 6hrs & 12hrs), T_2 –Mannitol 4% (6hrs & 12hrs) T_3–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 (gm), seedling dry weight (gm) and vigour index were observed. Among all the osmopriming treatments, osmopriming PEG 6000 was found to be the best osmopriming treatment followed by Mannitol .Among all osmopriming treatments PEG 20% of 12 hrs was more responsive to all treatments moreover osmopriming treatments have more pronounced effect on germination behavior and vigour in chickpea.

Baque *et al*. (2016) were conducted a lab experiment to find out the effect of different levels of drought stress on germination behaviour of BARI Gom 27. Non primed and primed seeds (osmoprimed and hydroprimed) were germinated under 0, 5, 10, 15 and 20% PEG solution induced drought stress conditions. Results showed that wheat seeds primed with 10% PEG and distilled water enhanced germination behavior and seedling growth over nonprimed seeds. The drought tolerant capability of nonprimed and hydroprimed seeds decreased drastically as droughtstress increased, but osmoprimed seeds showed considerable tolerance capability upto stress level induced by 10% PEG then significantly decreased with increasing drought stress. Seeds pre-soaked with 10% PEG and distilled water showed better performance in terms of germination behavior and seedling growth compared to untreated control under drought stress.

Ashagre *et al*. (2014). They reported that increase in PEG 6000 concentrations decreased germination percentage and rate, while shoot and root lengths and shoot fresh and dry weights decreased beyond 60g/L and increased up to 120g/L PEG but further increase in stress negatively influenced cultivars tolerance.

Syaiful *et al*. (2014) were carried out an experiment to evaluate the effects of seed priming with osmoticum Polyethylene glycol (PEG) 8000 in improving tolerance of soybean to drought stress. Green house factorial experiment in a completely randomized design with three replications was conducted. Treatments consisted of five levels of seed priming (dry seeds - untreated, PEG concentration: 0, 100, 200, 300 g L-1 water, respectively) and three levels of drought stress treatments (100 % field capacity, 75% field capacity and 50% field capacity). Results showed that seedling growing from primed seeds differed significantly with respect to plant height increment, shoot/root ratio, chlorophyll content and protein content. However, seed priming with PEG had no effect on relative growth rate (RGR), number of stomata, 100 grains weight and grain yield. Among the various concentration of PEG used, priming with 300 g PEG L^{-1} water significantly increased chlorophyll content and protein content. Drought stress treatment applied significantly affected plant height, shoot-root ratio, chlorophyll and protein content. Seed priming and drought stress treatments proved to be significant with respect to shoot-root ratio, 100 grains weight, protein and chlorophyll content. Seeds treated with 300 g PEG L^{-1} water demonstrated to be superior to the non-primed and all other primed seeds when water stress increased (50% field capacity). The results indicate that seed priming with PEG can improve plant growth in soybean by conferring more resistant seedlings to drought stress.

Arif *et al*. (2014) cited that 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 (nonprimed) 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 was concluded that priming with PEG was much effective but priming with water alone was also better than control.

Miladinov *et al*. (2014) examined the effect of seed lot, sowing date, and priming on germination and vigour of soybean seed. Three different seed lots of an old soybean variety (Lot 1, Lot 2, Lot 3) were tested. There were three sowing dates: 10 April, 20 May and 20 June. KNO_3 (1%), H_2O_2 (0.1%), GA_3 (0.075%) and distilled water were used as primers.
The results showed that soaking the seed prior to sowing, so called priming, positively affected seed germination and vigour in all three lots and with all three sowing dates. The best effect was accomplished with Lot 1 and sowing on the third date. With regards to primers, the best effect was achieved with the application of KNO_3 and H_2O_2 solutions. Research has shown that even soaking the seed in distilled water, which is a simple, cheap, and ecological method of priming, in most cases leads to improvement of germination and vigour of soybean seed.
Soughir *et al.* (2012) conducted a study to develop an optimum protocol for fenugreek and determinate the effect of NaCl seed priming on seed germination. Fenugreek seeds were primed with four concentrations of NaCl as priming media (0, 4, 6 and 8 g/l) for different

durations. Results indicated that different priming concentration of NaCl and duration has significant effects on total germination percentage, mean germination tine, germination index and coefficient of velocity of fenugreek seeds and the best results was obtained with 4 g/l for 36 hour. The result of this experiment showed that under undesirable conditions such as salinity stress, priming with NaCl can prepare a suitable metabolic reaction in seeds and can improved seed germination.

Zokaee-Khosroshahi *et al*. (2014) conducted an experiment to identify morphological changes in young seedlings of 5 Iranian almond species (*Prunus dulcis*, *P. eburnea*, P. *eleagnifolia*, P. *haussknechti*, and P. *scoparia*) under polyethylene glycol-induced drought stress. Drought stress caused a significant reduction in plant growth parameters such as fresh and dry weights of plant organs, leaf number, total leaf area, and leaf relative water content in all almond species. Specific leaf weight also increased significantly in drought-treated plants compared to control. No significant changes in shoot length, individual leaf area, leaf dimension (length and width), or stomatal size and frequency were observed in response to drought treatments. P. *eburnea* had the highest relative water content among the species and showed the smallest decrease in fresh and dry weights of organs and greatest decrease in leaf number and total leaf area (the most reduction in transpiration area) as an adaptive mechanism to drought stress.

Sarkar (2012) was conducted an experiment to study the effect of seed priming under Flooded and Nonflooded Conditions in Rice. Seed priming was done with water and 2% Jamun (*Syzygium cumini*) leaf extract. Seed priming improved seedling establishment under flooding. Acceleration of growth occurred due to seed pretreatment, which resulted longer seedling and greater accumulation of biomass. Seed priming greatly hastened the activities of total amylase and alcohol dehydrogenase in variety Swarna-Sub1 than Swarna. Swarna-Sub1 outperformed Swarna when the plants were cultivated under flooding. Weed biomass decreased significantly under flooding compared to nonflooding condition. Priming had positive effects on yield and yield attributing parameters both under non-flooding and early flooding conditions.

Sadeghi *et al*. (2011) performed an experiment to evaluate the effect of seed Osmopriming by using PEG6000 priming media on germination behavior and seed vigor of soybean (cultivar 033). Seeds were primed with six levels of Poly ethylene glycol (PEG 6000) as priming media (distilled water as control, -0.4, -0.8, -1.2, -1.6 and-2 MPa) for 6, 12, 24 and 48 hours at 25°C. Experimental units were arranged factorial in a completely randomized design with three replications. Dry soybean seeds considered as a control treatment (non primed). Results of variance analysis made clear that different osmotic potential and priming duration had significant effect on germination percentage, mean germination time, germination index, and the time to get 50% germination, seed vigor and electrical conductivity of seeds. Also -1.2 MPa osmotic potential increased germination percentages, germination index and seed vigor meanwhile decreased mean germination time, the time to get 50% germination and electrical conductivity of seeds. Also it was observed that 12 h priming duration had most effect on studied traits as-1.2 MPa osmotic potential treatment. Generally primed seeds showed better condition than control treatment in aspect of studied criteria.

Hamayun *et al*. (2010) investigated the adverse effects of drought stress on growth, yield and endogenous phytohormones of soybean. Polyethylene glycol (PEG) solutions of elevated strength (8% & 16%) were used for drought stress induction. Drought stress period span for two weeks each at pre and post flowering growth stage. They reported that growth and yield attributes of soybean was adversely affected by PEG induced drought stress. Soybean plants were found to be more susceptible to an early drought stress as compared to drought stress at a later growth stage. The level of endogenous growth hormones was also affected by drought stress, as the contents of plant growth promoting hormone (gibberellin) declined, while those of JA and ABA increased under drought stress. It shows that JA and ABA are concerned with plant stress and reaffirms their role in plant resistance to abiotic stress. SA is related to systemic acquired resistance (SAR) of plant and an increase in the quantity of endogenous SA shows that soybean become more susceptible to injuries and pathogens under drought stress.

Eisvand *et al*. (2010) experimented that the effects of hormonal priming on physiological quality and antioxidant enzymes of aged seeds of tall wheatgrass were evaluated under control and drought (-0.5MPa) conditions.Rate of germination, vigour index and growth of root, shoot and seedling were declined by stress conditions. According to the results, hormonal priming improved physiological quality of deteriorated seeds of tall wheatgrass under drought and control conditions. Germination percentage and rate of germination of primed seeds were higher than non-primed seeds under drought condition. 50ppm of auxin increased germination of naturally aged seeds by 18% under drought condition. Likewise, cytokinin treatment resulted in the highest vigour index. Auxin decreased root length and increased number of seminal roots. For other hormones, seed priming by 100ppm of gibberellin, 50ppm of cytokinin, and 50ppm of abscisic acid (ABA) improved seed performance under control and drought conditions.

Yuanyuan *et al*. (2010) conducted an experiment for disclosing the effects of seed priming with water and polyethylene glycol (PEG) on physiological characteristics in rice (Oryza sativa L.), the seeds of 4 rice cultivars were treated with H_2O and different concentrations of PEG before germination. Primed or nonprimed (control) seeds were then germinated under drought stress conditions simulated with PEG in a serious of concentrations. Compared to hydro-priming, priming with PEG in a proper concentration had a better effect on seed germination and seedling growth under drought stress, and the optimal priming concentrations of PEG were 20% for Gangyou 527 (indica hybrid rice) and 10%–15% for Nongken 57 (conventional japonica rice). Even higher concentrations of PEG had negative effects on seed germination. Moderate priming intensity improved metabolism of rice seed, germination indices, seedling quality, and drought tolerance of seedlings under drought stress for all cultivars. However, such effects had limited capability, and severe drought stress inhibited germination and caused damages of rice seedlings. Rice cultivars had significant impact on priming effect, and indica rice showed better performance than japonica rice.

Kaya *et al*. (2006) conducted an experiment to determine the determine factors responsible for germination and early seedling growth due to salt toxicity or osmotic effect and to optimize the best priming treatment for salt and drought stress conditions. The treated seeds (control, KNO_3 and hydropriming) of sunflower (*Helianthus annuus* L.) cultivar Sanbro 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 −1.2MPa. Electrical conductivity (EC) values of the NaCl solutions were 0.0, 6.5, 12.7, 18.4 and 23.5 dSm−1, respectively. 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 −1.2MPa 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.

Misra and Dwivedi (1980) reported positive effect of seed priming with potassium and distilled water on growth, dry matter accumulation, grain and straw yield in 12 wheat varieties under rainfed conditions. In trials with 12 wheat cv. grown under rain-fed conditions, soaking seeds in 2.5% KCl sol. at 300 cm3/500 g seed increased DM accumulation and grain and straw yields by 15%. Soaking seeds in distilled water was less effective than soaking in KCl sol.

CHAPTER III

MATERIALS AND METHODS

The study was conducted at the laboratory to know the effect of polyethylene glycol induced seed priming for enhancing drought tolerance capability in soybean (*Glycine max* L.) under drought stress from 10 March to 2 April, 2018. A short elucidation of the experimental site, climatic condition of the laboratory room, experimental materials, treatments and design, methods of the study, data calculation procedure and data analysis are discussed at this section. Materials and methods that were exercised to carry the study are defined below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

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

3.1.2 Conditions of the laboratory room

Laboratory room temperature and relative humidity were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, and Germany). The average minimum and maximum temperature were 22.4^0 C to 33.2^0 C, respectively and average minimum and maximum relative humidity were 55.56% and 81.20%, respectively.

3.2 Experimented crops

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

3.3 Experimental materials

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

3.4 Chemicals for seed priming

Polyethylene Glycol (PEG 6000) and distilled water were used as priming agent. Polyethylene Glycol (PEG) 6000 was used for induce drought stress. 75% alcohol was used for seed treating.

3.5 Experimental treatments and design

The experiment comprises of

a) Five levels of priming agent concentration *viz*. distilled water, 5%, 10%, 15% and 20% Polyethylene Glycol (PEG) 6000.

b) Six levels of priming time *viz*.0, 3, 6, 9, 12, 15 and 18 hours.

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

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

3.6 Experimental details

The whole experiment was carried out under three different experiments.

3.6.1 First Experiment

Study on the germination behavior of soybean at different concentrations of priming agents (PEG 6000 and Distilled water).

3.6.1.1 Weight of seeds

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

3.6.1.2 Surface treatment

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

3.6.1.3 Treatments

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 =Seeds without priming (control)
2. P_1= Seeds primed with distilled water
3. P_2=Seeds primed with 5% PEG solution
4. P_3=Seeds primed with 10% PEG solution
5. P_4=Seeds primed with 15% PEG solution
6. P_5=Seeds primed with 20% PEG solution

3.6.1.4 Priming solutions

Distilled water, 5%, 10%, 15%, and 20% of PEG solution were utilized as priming solutions.

3.6.1.5 Preparation of priming solutions

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

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

b) Distilled water

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

3.6.1.6 Priming technique

PEG priming (osmopriming) and hydro priming was applied on both the soybean varieties. Surface sterilized seeds were sub-divided into three parts. One for control (unprimed), one for hydro priming and another for PEG priming. For hydro priming seeds were immersed in distilled water and PEG priming seeds were divided into another four sub-group and treated with 5%, 10%, 15%, and 20% PEG respectively for 6 hours. Different plastic pots were used with lids for avoiding evaporation loss. All seeds were taken off form the priming agents at same time after 6 hours. The primed seeds were rinsed with distilled water three times gentlly and removed excess moisture by using tissue paper and finally air dried (Umair *et al*., 2011) in room temperature for 72 hours back the original moisture level.

3.6.1.7 Germination of seeds

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

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

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

3.6.2 Second Experiment

Study on the pre-sowing priming time on the germination behavior of soybean.

3.6.2.1 Weight of seeds

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

3.6.2.2 Surface treatment

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

3.6.2.3 Treatments and

Soybean variety**:** BARI soybean 6

Factor A: Priming of seeds

P_1: Osmopriming (10% PEG)

P_2: Hydropriming (Distill water)

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

T_6= Seeds primed for 18 hours

3.6.2.4 Priming solutions

Distilled water, 10% of PEG solution were utilized as priming solutions.

3.6.2.5 Preparation of priming solutions

a) PEG solutions (10%)

10% PEG solution were prepared by mixing 25g of PEG 6000 at 250 mL distilled water.

b) Distilled water

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

3.6.2.6 Priming technique

Surface sterilized seeds were sub-divided into three parts. One for hydro priming and another for PEG priming. Second parts of seeds were sub-divided into another six parts for six different priming times such as 3, 6, 9, 12, 15 and 18 hours hydro priming and third parts seeds also were divided into another five parts for five different priming times such as 3, 6, 9, 12,15 and 18 hours PEG priming. Different plastic pots were used with lids for avoiding evaporation loss. Seeds were taken off from the priming solution at the required time. The primed seeds were rinsed with distilled water three times gently and removed excess moisture by using tissue paper and finally air dried (Umair *et al*., 2011) in room temperature for 72 hours to back the original moisture level.

3.6.2.7 Germination of seeds

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

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

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

3.6.3 Third Experiment

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

3.6.3.1 Weight of seeds

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

3.6.3.2 Surface treatment

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

3.6.3.3 Treatments

Factor A: Priming:

P_1: Osmopriming for 6 hours with 10% PEG

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

10% of PEG solution and distilled water were used for both variety BARI soybean 5 and BARI soybean 6 for 6 hours priming.

3.6.3.5 Preparation of priming solutions

a) PEG 6000 solutions (10%)

10% PEG were prepared by mixing 25g of PEG at 250 mL distilled water.

b) Distilled water

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

3.6.3.6 Preparation of drought stress solutions

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

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

3.6.3.7 Priming technique

PEG priming and hydro priming was applied on both the soybean varieties. Surface sterilized seeds were sub-divided into three parts. One for control (unprimed), one for hydro priming and another for PEG priming. For hydro priming BARI soybean 5 and BARI soybean 6 were immersed in distilled water 6 hours. For PEG priming BARI soybean 5 and BARI soybean 6 were emerged 10% solution for 6 hours. Different plastic pots were used with lids for avoiding evaporation loss. Seeds were taken off from the priming solution at the required time. The primed seeds were rinsed with distilled water three times gentlely and removed excess moisture by using tissue paper and finally air dried (Umair *et al*., 2011) in room temperature for 72 hours to back the original moisture level.

3.6.3.8 Germination of seeds

Twenty seeds were selected randomly from each treatment of both verity and placed on 120 mm diameter Petri dishes. Where saturated (8 mL distillated water) whatman no.1 filter paper was used as growth media. During the test Petri dishes were kept saturated and placed at room temperature 25°C under normal light to facilitate germination for 8 days. Emergence of 2 mm radical considered for germination occurred (Akbari *et al*., 2007).

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

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

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

3.7 Data recording

Parameters that's were measured as follows

3.7.1 Germination percentage (GP %)

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

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

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

From each treatment 5seedlings 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 and Anderson (1970).

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

Here,

TG = total germination.

3.7.5 Germination coefficient (GC)

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

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

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, Karim and Hamid (2002).

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

3.7.7 Water saturation deficit (WSD)

Water saturation deficit (WSD) was recorded using following formula of Baque, Karim and Hamid (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, karim and Hamid (2002).

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

3.8 Statistical analysis

The data obtained for different parameters were statistically analyzed to observe the significant difference among the treatment. The mean value of all the parameters was calculated and analysis of variance was performed. The significance of difference among the treatments means was estimated by the Least Significance Difference (LSD) at 1% level of probability. A computer software MSTAT-C was used to carry out the statistical analysis. Drawings were made using Excel software.

CHAPTER IV

RESULTS AND DISCUSSIONS

The result of the experiment as influenced by different treatments have been summarized and presented in this chapter with the help of appropriate tables and figures. The results of the statistical analysis were critically perused and the inferences derived from the analysis have been presented in this chapter. Salient experimental results presented in the preceding chapter have been discussed with suitable causes and wherever necessary it has been supported with relevant facts as generated by various scientists working within country and abroad.

4.1 First experiment

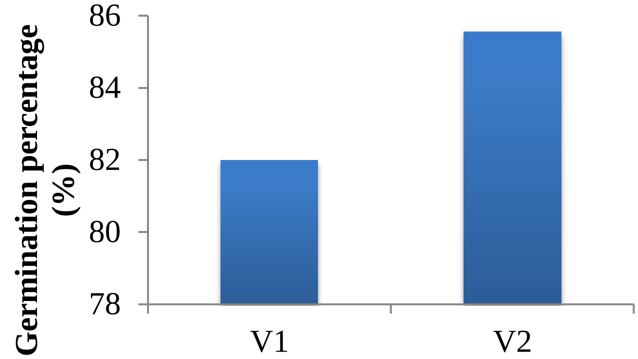
Study on the germination behavior of soybean at different concentrations of priming agents (PEG and distilled water).

Results obtained from the present study regarding the germination behavior of soybean at different concentrations of priming agents have been demonstrated and discussed. The analytical results have been displayed in Figures 1 to 10 and Tables 1 to 3.

4.1.1 Germination percentage (%)

4.1.1.1 Effect of variety

Soybean variety exhibited significant difference in respect of the germination percentage (Figure 1). Between the varieties, BARI soybean 6(V_2) showed the maximum germination percentage (85.56%) and BARI soybean 5 (V_1) showed minimum germination percentage (82%). The variety/genotype Shohag, BARI soybean 6 and BD 2331 is considered as drought tolerant because of their proline, soluble sugar accumulation, chlorophyll content and cell membrane stability (Chowdhury *et al*., 2017).

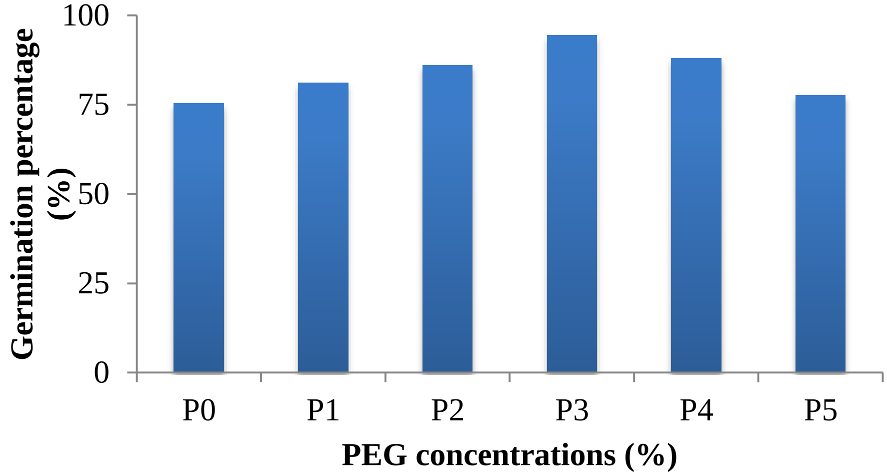


V_1: BARI soybean 5 V_2: BARI soybean 6

Figure 1. Effect of variety on the germination percentage of soybean ($LSD_{0.01}$=3.17)

4.1.1.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of germination percentage (Figure 2). Germination percentage (GP) increases with increasing PEG concentration up to 10% then decreases gradually with increasing PEG concentration. P_3 (primed with 10% PEG concentration) showed the maximum germination percentage (94.43%) and thereafter decrease due to increasing concentration of PEG. The lowest germination percentage was found in P_0. Total germination of BARI soybean 6 is higher than BARI soybean 5. This study was in agreement with the findings of Faijunnahar *et al*. (2017); Ahammad *et al*. (2014) and Abnavi and Ghobadi (2012). Priming with PEG can be used as increasing germination up to certain level. According to Ajouri *et al*. (2004) priming induces several biochemical changes in the seed that required initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibitions and enzymes activation.



P_0 = Seeds without priming (control); P_1 = Seeds primed with O% PEG for 6 hours; P_2 = Seeds primed with 5% PEG for 6 hours, P_3 = Seeds primed with 10% PEG for 6 hours; P_4 = Seeds primed with 15% PEG for 6 hours, P_5 = Seeds primed with 20% PEG for 6 hours

Figure 2. Effect of priming solutions on the germination percentage of soybean ($LSD_{0.01}$=5.48)

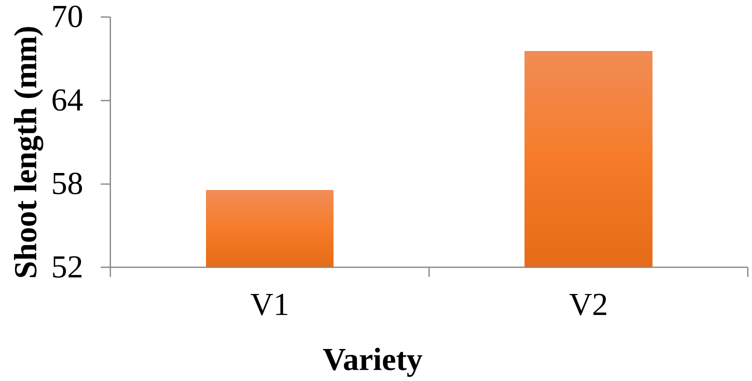
4.1.1.3 Interaction effect of variety and priming solutions

Interaction of variety and priming concentrations showed significant variation on germination percentage of soybean (Table 1). Highest germination percentage was showed in V_2P_3 (95.91%), which was statistically similar with V_2P_4, V_1P_3. Lowest germination percentage was showed in V_1P_0 (72.46%), which is statistically identical with V_1P_5 and statistically similar with V_1P_1, V_2P_0 and V_2P_5. GP was increased up to 10% PEG priming than gradually decrease due to excess concentration which could not be imbibed and BARI soybean 6 has greater genetical make up than BARI soybean 5.

4.1.2 Shoot length

4.1.2.1 Effect of variety

Soybean varieties exhibited significant difference in respect of the shoot length (Figure 3). Among the varieties, BARI soybean 6 (V_2) showed the greater shoot length (67.55 mm) and BARI soybean 5 (V_1) showed smaller shoot length (57.55 mm).

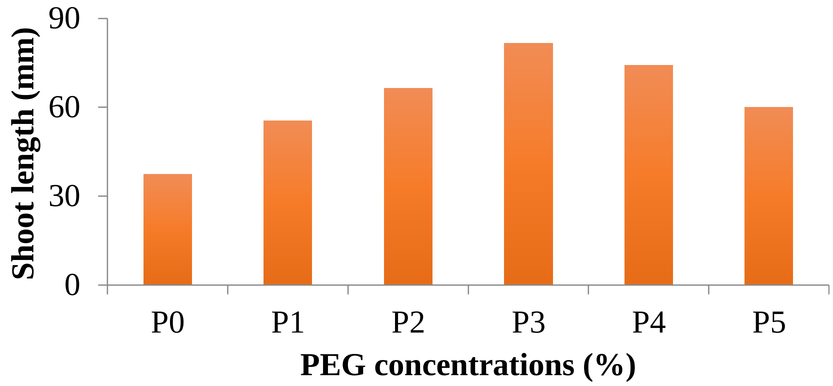


V_1: BARI soybean 5 V_2: BARI soybean 6

Figure 3. Effect of variety on the shoot length of soybean ($LSD_{0.01}$=2.35)

4.1.2.2 Effect of priming solutions

Priming with PEG concentrations and water showed high amount of variation in respect of root length (Figure 4). Root length was slightly affected by hydro priming and different PEG concentration. Shoot length increases with increasing PEG concentration up to 10% then decreases gradually with increasing PEG concentration. P_3 (primed with 10% PEG concentration) showed the greater shoot length (81.64 mm) and thereafter decrease due to increasing concentration of PEG. The lowest root length was found in P_0 (37.48 mm). Root length of BARI soybean 6 was higher than BARI soybean 5.Treated seeds had high germination percentages and quicker germination time. One hypothesis is that benefits of priming can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduces lag time between imbibition and radicle emergence (Bradford *et al*., 1990). Better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins are also some of the basis for enhanced growth (Bray *et al*., 1989). Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted an early initiation of germination in carrot seeds. Kumar *et al*. (2017) experimented on chickpea shows that shoot length has recorded high in case of osmo-primed seeds than that of unprimed seeds. Among different osmo-priming with PEG 20% found to be highest followed by mannitol 4% and control found to be lowest among the treatments.



P_0 = Seeds without priming (control); P_1 = Seeds primed with 0% PEG for 6 hours; P_2 = Seeds primed with 5% PEG for 6 hours, P_3= Seeds primed with 10% PEG for 6 hours; P_4= Seeds primed with 15% PEG for 6 hours, P_5 = Seeds primed with 20% PEG for 6 hours

Figure 4. Effect of priming solutions on the shoot length of soybean ($LSD_{0.01}$=4.07)

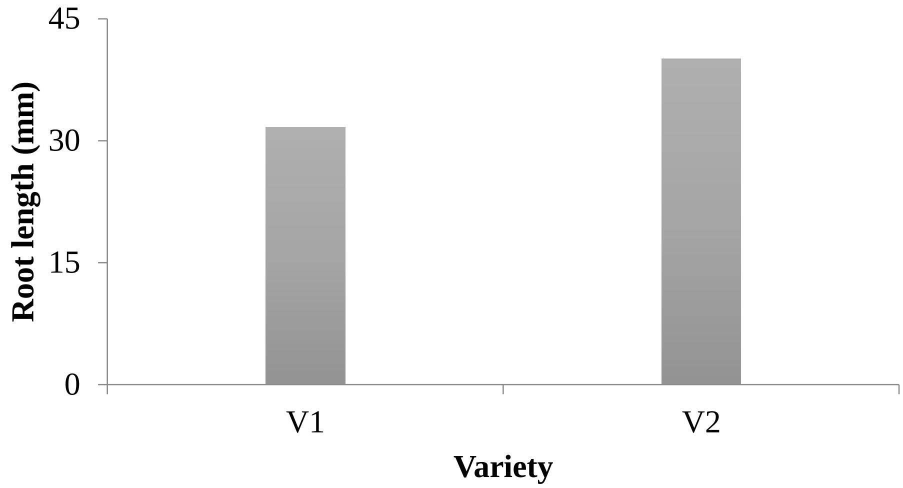
4.1.2.3 Interaction effect of variety and priming solutions

The interaction of variety and priming solutions significantly influenced on shoot length (Table 1). Highest shoot length was showed in V_2P_3 (87.92 mm) which was found from BARI soybean 6 when treated with 10% PEG solution. Lowest shoot length was recorded from V_1P_0 (30.69 mm) when BARI soybean 5 under control. Priming improves physiological activities which leads to better genetic repair for which growth can be improved ultimately shoot length increased. But excess priming concentration is detrimental for seed because of osmotic potential.

4.1.3 Root length

4.1.3.1 Effect of variety

Soybean variety exhibited significant difference in respect of the root length (Figure 5). Between the varieties, BARI soybean 6 (V_2) showed the greater root length (40.12 mm) and BARI soybean 5(V_1) showed smaller root length (31.67 mm). Varietal effect is responsible for successful priming because genetical variation is different in each variety.

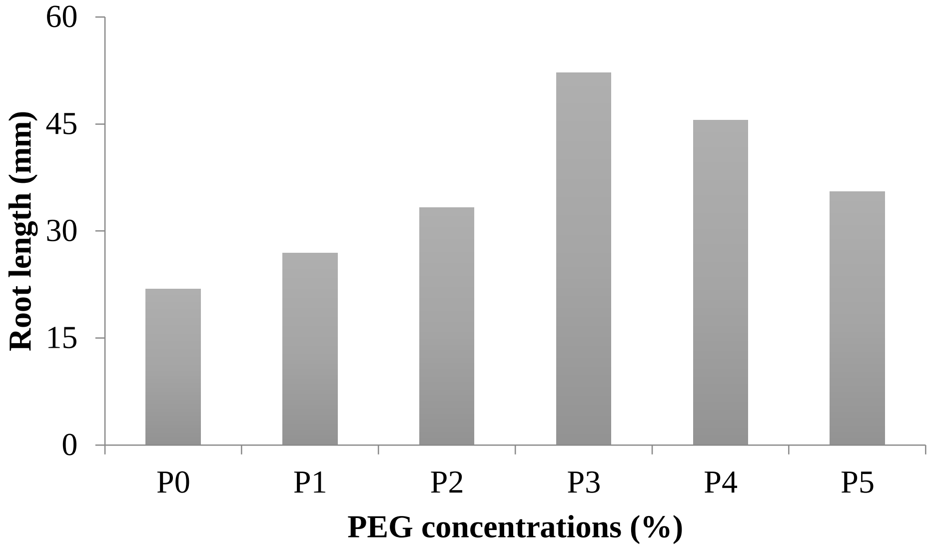


V_1: BARI soybean 5 V_2: BARI soybean 6

Figure 5. Effect of variety on the root length of soybean ($LSD_{0.01}$=1.43)

4.1.3.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of root length (Figure 6). Root length was affected by hydro priming and different PEG concentrations. Root length increases with increasing PEG concentration up to 10% then decreases gradually with increasing PEG concentration. P_3 (primed with 10% PEG concentration) showed the highest root length (52.21mm) and thereafter decrease due to increasing concentration of PEG. The lowest shoot length was found in P_0 (21.90 mm). Root length of BARI soybean 6 was higher than BARI soybean 5. This study is corroborates with the study of previous researcher Sarwar *et al*. (2006) who reported that root length were better when treated with water and mannitol over control. Ahmadvan *et al*. (2012), showed that priming seed with PEG can increase germination capacity, root length, length of plumule, seedling dry weight and plant height than the dry seed. Hopper *et al*. (1979) indicated that in primed seeds because of more water uptake efficiency and faster metabolic activity in term of germination, radicle and plumule initiated faster. Priming may improve germination by enhancing imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells. (Kaya *et al*., 2006).



P_0 = Seeds without priming (control); P_1 = Seeds primed with 0% PEG for 6 hours; P_2 = Seeds primed with 5% PEG for 6 hours, P_3 = Seeds primed with 10% PEG for 6 hours; P_4 = Seeds primed with 15% PEG for 6 hours, P_5 = Seeds primed with 20% PEG for 6 hours

Figure 6. Effect of priming solutions on the root length of soybean ($LSD_{0.01}$=2.47)

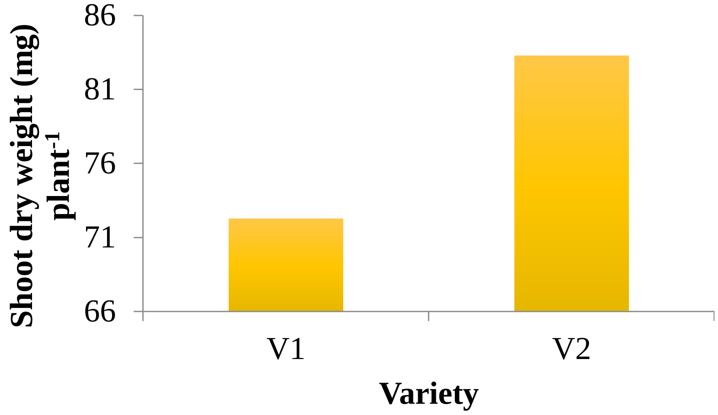
4.1.3.3 Interaction effect of variety and priming solutions

A perusal data revealed (Table 1) that the combined effect of variety and priming solutions significantly affected the root length of both soybean variety. Highest root length was recorded when BARI soybean 6 treated with 10% PEG (V_2P_3) and the value is 57.44mm. Lowest root length (18.82 mm) was recorded from BARI soybean 5 under control (V_1P_0). Priming enhances metabolic activities inside the seed which helps to extending the root system.

4.1.4 Shoot dry weight plant^{-1}

4.1.4.1 Effect of variety

Citation of data regarding shoot dry weight plant^{-1} soybean variety exhibited significant difference. (Figure 7). Between the varieties, BARI soybean 6(V_2) showed the higher shoot dry weight (83.28 mg) and BARI soybean 5(V_1) showed lower shoot dry weight plant^{-1} (72.26 mg).

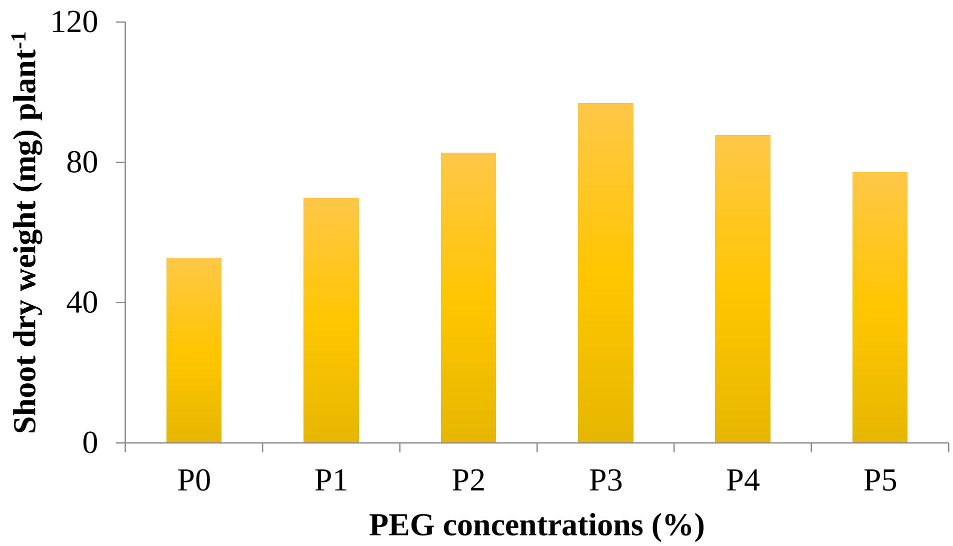


V_1: BARI soybean 5 V_2: BARI soybean 6

Figure 7. Effect of variety on the shoot dry weight plant^{-1} of soybean ($LSD_{0.01}$=2.90)

4.1.4.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of shoot dry weight plant^{-1}(Figure 8). Shoot dry weight is affected by hydropriming and different PEG concentration. Shoot dry weight increases with increasing PEG concentration up to 10% and decreases due to increasing concentration. P_3 (primed with 10% PEG concentration) showed the highest (96.83mg) and thereafter decrease due to increasing concentration of PEG. The lowest shoot dry weight plant^{-1}was found in P_0 (52.68 mg). Shoot dry weight plant^{-1} of BARI soybean 6 was higher than BARI soybean 5. In view of some earlier studies it is now evident that priming of seeds of different crops improved seedling shoot dry weight. These results are in agreement with Harris *et al*. (2004) who reported that higher plant dry weight and seed yield were observed following seed priming. The increase in the dry matter and grain yield of mungbean was due to better emergence and better performance per plant (Parera and Cantliffe, 1994). Increased plumule dry weight due to osmo priming was reported by Harris *et al*. (2004).



P_0 = Seeds without priming (control); P_1 = Seeds primed with 0% PEG for 6 hours; P_2 = Seeds primed with 5% PEG for 6 hours, P_3 = Seeds primed with 10% PEG for 6 hours; P_4 = Seeds primed with 15% PEG for 6 hours, P_5 = Seeds primed with 20% PEG for 6 hours

Figure 8. Effect of priming solutions on the shoot dry weight plant^{-1} of soybean ($LSD_{0.01}$=5.02)

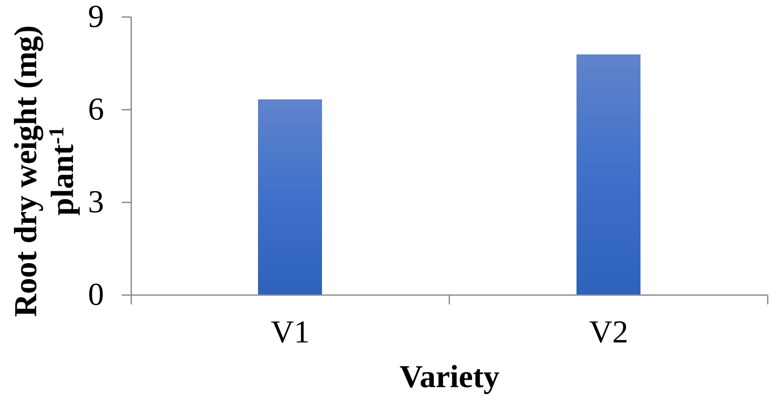
4.1.4.3 Interaction effect of variety and priming solutions

Combination of variety and priming solutions significantly affected the shoot dry weight of both variety of soybean (Table 1). Highest shoot dry weight (99.19 mg) was recorded from V_2P_3 indicated that when BARI soybean 6 treated with 10% PEG which is statistically similar with V_1P_3 and V_2P_4. Lowest shoot dry weight (42.16 (mg) was observed in V_1P_0 (BARI soybean 5 under control). Priming ensures greater emergence, stand establishment and growth for which dry matter content can be increased in primed seedlings.

4.1.5 Root dry weight plant^{-1}

4.1.5.1 Effect of variety

Soybean variety exhibited significant difference in respect of the root dry weight plant^{-1} (Figure 9). Between the varieties, BARI soybean 6 (V_2) showed the higher root dry weight (7.78 mg) and BARI soybean 5 (V_1) showed lower root dry weight plant^{-1} (6.32 mg).

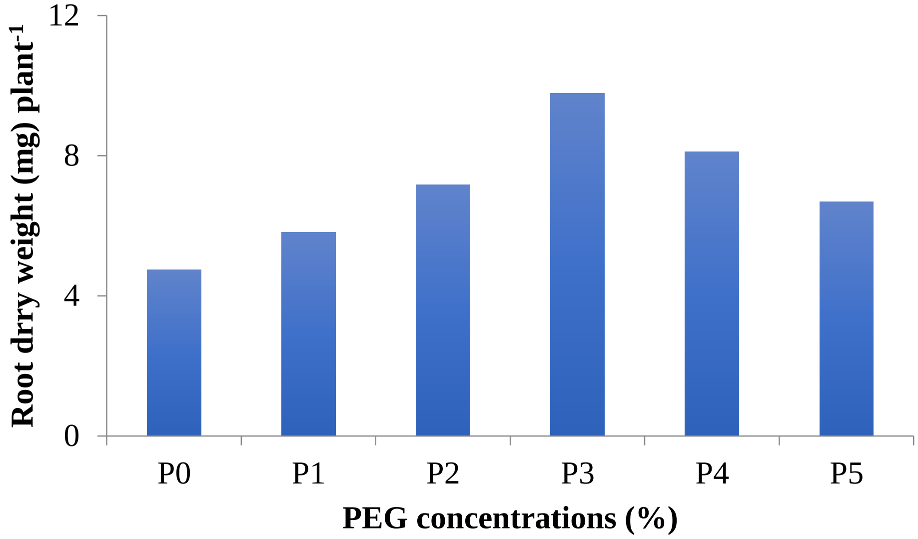


V_1: BARI soybean 5 V_2: BARI soybean 6

Figure 9. Effect of variety on the root dry weight plant^{-1} of soybean ($LSD_{0.01}$=0.31)

4.1.5.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of shoot dry weight plant^{-1} (Figure 8). Root dry weight is affected by hydropriming and different PEG concentration. Root dry weight increases with increasing PEG concentration up to 10% and decreases due to increasing concentration. P_3 (primed with 10% PEG concentration) showed the highest (96.83mg) and thereafter decrease due to increasing concentration of PEG. The lowest shoot dry weight plant^{-1}was found in P_0 (52.68 mg). Shoot dry weight plant^{-1} of BARI soybean 6 was higher than BARI soybean 5. The result of the present study is also supported by the result of previous researchers (Khalil *et al*., 2010; Ghassemi-Golezani *et al*., 2008; Sarwar *et al*., 2006). Khalil *et al*. (2010) observed that dry matter yield increased with each increment of priming. Ghassemi-Golezani *et al*. (2008) showed that hydropriming significantly enhanced root weights and Sarwar *et al*. (2006) reported that root length and biomass of roots and shoots were better when treated with water and mannitol.



P_0 = Seeds without priming (control); P_1 = Seeds primed with O% PEG for 6 hours; P_2 = Seeds primed with 5% PEG for 6 hours, P_3 = Seeds primed with 10% PEG for 6 hours; P_4 = Seeds primed with 15% PEG for 6 hours, P_5 = Seeds primed with 20% PEG for 6 hours

Figure 10. Effect of priming solutions on the root dry weight plant^{-1} of soybean ($LSD_{0.01}$=0.54)

4.1.5.3 Interaction effect of variety and priming solutions

Interaction of variety and priming solution had significant variation in case of root dry weight (Table 1). Highest root dry weight was recorded from V_2P_3 (10.5 mg) when BARI soybean 6 treated with 10% PEG and lowest data was recorded from V_1P_0 (3.94 mg) under control condition of BARI soybean 5. Root dry weight is increased uo to 10% PEG than gradually decreased due to excess concentration cannot be penetrated for lower osmotic potential. This may occur due to in primed seedling contained more biomass and extended size than unprimed one.

According to result of this experiment it can be said that interaction of variety and different concentration of PEG shows significant variation in case of germination percentage, shoot length, root length, shoot dry weight and root dry weight. BARI soybean 6 with 10% PEG showed better performance than any other treatment combination. PEG concentration up tp 10% with BARI soybean 6 were gradually increase in case of above parameters and were gradually decrease more than 10% PEG concentration. Present study under the agreement with Baque *et al*. (2016). They reported that 10% PEG was best for improved germination behavior of wheat.

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

Treatment combinations	Germination percentage (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
V_1P_0	72.46 f	30.69 h	18.82 h	42.16 g	3.94 h
V_1P_1	78.55 ef	50.34 f	24.93 g	63.26 f	5.05 g
V_1P_2	85.23 b-e	64.69 cd	31.20 f	76.13 de	6.58 de
V_1P_3	92.95 ab	75.36 b	46.99 c	94.47 ab	9.04 b
V_1P_4	87.21 b-d	68.41 c	39.83 d	82.77 cd	7.12 cd
V_1P_5	75.63 f	55.84 ef	28.27 fg	74.77 e	6.17 ef
V_2P_0	78.25 ef	44.26 g	24.97 g	63.20 f	5.55 fg
V_2P_1	83.80 c-e	60.64 de	28.92 f	76.01 de	6.58 de
V_2P_2	86.90 b-d	68.18 c	35.35 e	89.22 bc	7.75 c
V_2P_3	95.91 a	87.92 a	57.44 a	99.19 a	10.5 a
V_2P_4	88.76 a-c	79.94 b	51.32 b	92.57 ab	9.10 b
V_2P_5	79.75 d-f	64.32 cd	42.75 d	79.51 de	7.19 cd
$LSD_{(0.01)}$	7.75	5.75	3.50	7.10	0.76
CV (%)	5.45	5.42	5.74	5.38	6.32

V_1P_0: BARI soybean 5 with control; V_1P_1: BARI soybean 5 with 0% PEG; V_1P_2: BARI soybean 5 with 5% PEG; V_1P_3: BARI soybean 5 with 10% PEG; V_1P_4: BARI soybean 5 with 15% PEG; V_1P_5: BARI soybean 5 with 20% PEG; V_2P_0: BARI soybean 6 with control; V_2P_1: BARI soybean 6 with 0% PEG; V_2P_2: BARI soybean 6 with 5% PEG; V_2P_3: BARI soybean 6 with 10% PEG; V_1P_4: BARI soybean 6 with 15% PEG; V_1P_5: BARI soybean 6 with 20% PEG

4.1.6 Relative water content

4.1.6.1 Effect of variety

Relative Water Content could be the perfect indicator of plant hydrologic condition as it denotes the physiological consequences of cellular water deficit. Soybean variety exhibited significant difference in respect of relative water content (Table 2). Among the varieties, BARI soybean 6 (V_2) indicated the higher relative water content (82%) than BARI soybean 5(V_1) which showed relative water content 76.62%.

4.1.6.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of relative water content (Table 2). Relative water content was increased up to 10% PEG and decreased when this concentration increased. P_3 (primed with 10% PEG concentration) showed the highest (92.25%) relative water content. The lowest relative water content was found in P_0 (68.24%). Relative water content of BARI soybean 6 was higher than BARI soybean 5. This result is in agreement with the findings of Faijunnahar *et al*. (2017). Under stress condition, osmo and hydro primed seedling can thrive and provide better water use efficiency thus plant growth not hampered than non-primed seeds Flower *et al*.(1998)0. A similar finding was reported by Sairam *et al*. (2002).

4.1.6.3 Interaction effect of variety and priming solutions

Interaction of variety and different priming concentrations showed significant variation on relative water content of soybean (Table 3). According to analyzed data in the case of relative water content, highest relative water content was showed in V_2P_3 (94.43%) which is statistically similar with V1P3. Lowest relative water content recorded from V_1P_0 (65.39%) which is statistically similar with V_1P_1, V_1P5 and V_2P0. BARI soybean 6 when treated with 10% PEG showed higher relative water content due to higher water use efficiency.

4.1.7 Water saturation deficit

4.1.7.1 Effect of variety

Soybean variety exhibited significant difference in respect of water saturation deficit content (Table 2). Between the varieties, BARI soybean 5 (V_1) showed the higher water saturation deficit (23.42) than BARI soybean 6 (V_2) which showed lower water saturation deficit 18.87.

4.1.7.2 Effect of priming solutions

Priming with PEG concentrations and water showed significant variation in respect of water saturation deficit (Table 2). P_0 (control) showed the highest (31.76) the lowest water saturation deficit was found in P_3 (7.753). The water saturation deficit was maximum at 0% PEG concentration and gradually decreased up to 10% PEG concentration and then steadily increased. This result also support the findings of Faijunnahar *et al*. (2017). Due to lack of defense mechanism, the non-primed seedling failed to uptake enough water necessary for running the physiological process smoothly than the primed seedling. Thus there was a massive water deficit occurred in case of non-primed genotypes than the primed genotypes. A similar result was reported by Baque *et al*. (2002).

4.1.7.3 Interaction effect of variety and priming solutions

Interaction of variety and priming solution significantly affected the water saturation deficit of two soybean varieties were analyzed (Table 3), highest water saturation deficit was found in V_1P_0 (34.61). It is opposite than other parameters. BARI soybean 5 under control showed higher water saturation deficit because of non-primed seed showed lower relative water content than primed one. Lowest was found in V_2P_3 (5.57) when BARI soybean 6 treated with 10% PEG due to higher relative water content.

4.1.8 Water retention capacity

The water retention capacity (WRC) as represented by the ratio of turgid weight: dry weight (TW: DW) illustrates the water holding capacity of a shoot at a particular time. The TW: DW ratio is determined by the cell structures. Seed priming helps to repair damaged cell of seed.

4.1.8.1 Effect of variety on water retention capacity

Soybean variety exhibited significant difference in respect of water retention capacity (Table 2). Between the varieties, BARI soybean 6 (V_2) showed the higher water retention capacity (7.61) than BARI soybean 5(V_1) which showed lower water retention capacity 6.68.

4.1.8.2 Effect of priming solutions on water retention capacity

Priming with PEG concentrations and water showed significant variation in respect of water retention capacity (Table 2). P_3 (primed with 10% PEG concentration) showed the highest (9.00) water retention capacity. The lowest water retention capacity was found in P_0 (5.83) which is statistically identical with P_5 (6.23). This study was in agreement with the findings of Faijunnahar *et al*. (2017); Baque *et al*. (2016) and Rahman, (2014). As priming helps to activate the metabolic enzymes responsible for germination of seed before germination takes place, so the hydro and osmo primed seedlings can uptake more water than the non-primed ones and gained the maximum turgid weight, in consequence, they gained the maximum water retention capacity. Generally higher doses of potassium resulted the maximum relative water content, higher water retention capacity and exudation rate in drought affected wheat. (Baque *et al*., 2002).

4.1.8.3 Interaction effect of variety and priming solutions

The combined effect of variety and priming solutions varied significantly at water retention capacity (Table 3). Highest water retention capacity was found in V_2P_3 (9.57) and lowest was recorded from V_1P_0. They both are statistically different from others. As priming improves water uptake through improving metabolic activity leads to increase water retention capacity. So BARI soybean 6 treated with 10% PEG showed highest result over control.

4.1.9 Coeffiecient of germination

4.1.9.1 Effect of variety on coefficient of germination

Soybean variety exhibited significant difference in respect of coefficient of germination (Table 2). Among the varieties, BARI soybean 6 (V_2) showed the higher coefficient of germination (19.79) than BARI soybean 5 (V_1) which showed lower coefficient of germination 18.18.

4.1.9.2 Effect of priming solutions on coefficient of germination

Priming with PEG concentrations and water showed significant variation in respect of coefficient of germination (Table 2). Coefficient of germination is increased up to 10% of PEG then gradually decreased. P_3 (primed with 10% PEG concentration) showed the highest (22.28) coefficient of germination. The lowest coefficient of germination was found in P_0 (16.64) which is statistically similar with P_1 and P_5.This findings is in agreement with Huns and Sung (1997). He reported that priming increases the antioxidant increment like glutathione and ascorbate which help to increase germination speed by reducing the lipid peroxidation activity, thus it leads to higher germination coefficient in osmoprimed and hydro primed than non-primed.

4.1.9.3 Interaction effect of variety and priming solutions

An appraisal of mean data showed that interaction of variety and priming solutions significantly influenced coefficient of germination (Table 3). Highest value was recorded from V_2P_3 (23.40) which is statistically similar with V_2P_4 and lowest was found in V_1P_0 (15.89) which is statistically similar with V_1P_1, V_1P_5 and V_2P_0. Priming improves antioxidant activity ultimately germination speed is increased, so BARI soybean 6 showed higher performance when primed with 10% PEG.

4.1.10 Vigor index

4.1.10.1 Effect of variety on vigor index

Soybean variety exhibited significant difference in respect of vigor index (Table 2). Among the varieties, BARI soybean 6(V_2) showed the higher vigor index (93.43) than BARI soybean 5(V_1) which showed lower vigor index 84.81. Vigor is an important biological property of seeds, which determines the potential for uniform emergence and development of normal seedlings even under adverse field conditions. Uniformity in crop stand establishment is mandatory in all vegetable crops and can be achieved by improving seed quality. There are different seed vigor enhancement techniques, priming is one of those

4.1.10.2 Effect of priming solutions on vigor index

Priming with PEG concentrations and water showed significant variation in respect of vigor index (Table 2). This parameter increases up to 10% then degrease due to higher concentrations. P_3 (primed with 10% PEG concentration) showed the highest (126.7) vigor index. The lowest vigor index was found in P_0 (45.09). Nascimento and West (1998) mentioned that after seed priming, the minimization of seed coat adherence occurred during the emergence of muskmelon. According to Sadeghi *et al*. (2011) Germination and vigor advancement of soybean plant was occurred due to the reserve mobilization of food material, activation and re-synthesis of some enzymes, DNA and RNA synthesis started during osmotic priming.

Table 2. Effect of variety and 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	76.62 b	23.42 a	6.68 b	18.18 b	74.81 b
V_2	82.00 a	18.87 b	7.61 a	19.79 a	93.43 a
LSD $_{(0.01)}$	3.07	0.92	0.25	0.74	3.13
CV (%)	5.61	6.25	4.95	5.63	5.36
Effect of different priming solutions					
P_0	68.24 e	31.76 a	5.83 d	16.64 d	45.09 f
P_1	72.88 de	27.12 b	6.93 c	17.66 cd	67.06 e
P_2	79.07 c	20.93 d	7.81 b	18.77 c	85.87 c
P_3	92.25 a	7.753 f	9.00 a	22.28 a	126.7 a
P_4	85.08 b	14.92 e	7.04 c	20.76 b	105.5 b
P_5	75.63 cd	24.37 c	6.23 d	17.79 cd	74.49 d
LSD $_{(0.01)}$	5.31	1.59	0.42	1.28	5.41
CV (%)	5.61	6.25	4.95	5.63	5.36

V_1:BARI soybean 5 P_0: Control; P_1:0% PEG; P_2: 5% PEG

V_2:BARI soybean 6 P_3:10% PEG; P_4: 15%PEG; P_5: 20% PEG

4.1.10.3 Interaction effect of variety and priming solutions

Interaction of variety and priming solutions significantly affected the vigor index (Table 3). Highest value (139.4) was recorded from V_2P_3 (BARI soybean 6 primed with 10% PEG) and lowest (35.94) was found in V_1P_0 (BARI soybean 5 with control). Priming presumably allowed some repairs of damaged to membrane caused by deterioration. Priming also causes physiological and biochemical changes in seed during the seed treatments and metabolic activities increases alpha-amylase activity, thus indicating higher vigor index (Lee and Kim, 2000).

Table 3. Interaction effect of variety and 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	65.39 g	34.61 a	5.11 h	15.89 f	35.94 i
V_1P_1	71.77 fg	28.23 bc	6.66 ef	17.19 ef	59.12 gh
V_1P_2	77.20 d-f	22.80 d	7.52 cd	18.19 de	81.79 ef
V_1P_3	90.07 ab	9.93 h	8.44 b	21.15 bc	113.9 b
V_1P_4	83.52 b-d	16.48 f	6.43 fg	19.56 cd	94.41 c
V_1P_5	71.74 fg	28.46 b	5.91 g	17.08 ef	63.66 g
V_2P_0	71.08 fg	28.92 b	6.56 f	17.39 ef	54.24 h
V_2P_1	73.98 ef	26.02 c	7.21 de	18.13 de	75.01 f
V_2P_2	80.93 c-e	19.07 e	8.10 bc	19.35 cd	89.95 cd
V_2P_3	94.43 a	5.57 i	9.57 a	23.40 a	139.4 a
V_2P_4	86.64 bc	13.36 g	7.65 cd	21.96 ab	116.7 b
V_2P_5	79.53 c-e	20.27 e	6.56 f	18.50 de	85.32 de
$LSD_{(0.01)}$	7.50	2.24	0.60	1.81	7.65
CV (%)	5.61	6.25	4.95	5.63	5.36

V_1P_0: BARI soybean 5 with control; V_1P_1: BARI soybean 5 with 0% PEG; V_1P_2: BARI soybean 5 with 5% PEG; V_1P_3: BARI soybean 5 with 10% PEG; V_1P_4: BARI soybean 5 with 15% PEG; V_1P_5: BARI soybean 5 with 20% PEG; V_2P_0: BARI soybean 6 with control; V_2P_1: BARI soybean 6 with 0% PEG; V_2P_2: BARI soybean 6 with 5% PEG; V_2P_3: BARI soybean 6 with 10% PEG; V_1P_4: BARI soybean 6 with 15% PEG; V_1P_5: BARI soybean 6 with 20% PEG.

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

Results obtained from the present study regarding the effects of different priming time of PEG (Polyethylene Glycol) and water on the germination and water relation behavior of soybean (BARI soybean 6) have been presented and discussed from Table 4 to 6 and Figure 11 to 20.

4.2.1 Germination percentage (%)

4.2.1.1 Effect of osmo and hydro priming

Osmo and hydro priming showed significant difference on germination percentage of BARI soybean 6 (Table 4). Osmo priming done with 10% PEG (P_1) showed higher germination percentage (84.34%) than hydropriming (priming with water). Hydropriming (P_2) was showed germination percentage (76.86%). This results is in agreement with Di Girolamo and Barbanti (2012). They reported that through osmopriming water enters seed slowly which due to low water potential of osmotic solutions, allows gradual seed imbibition and activation of early phases of germination but prevents radicle protrusion. Otherwise hydropriming is uncontrolled water uptake by seeds. This is a consequence of free water availability to seeds during hydropriming, so that the rate of water uptake depends only on seed tissue affinity to water (Taylor *et al*., 1998). Moreover, this technique may result in unequal degree of seeds hydration thus leading to lack of simultaneous metabolic activation within seeds followed by unsynchronized emergence (Mcdonald, 2000). Rahman and Nahar (2014) reported that osmoprimed seed with mannitol and PEG respectively showed better effect than hydropriming in case of germination percentage.

4.2.1.2 Effect of priming times:

Priming times (3hr, 6hr, 9hr, 12hr, 15hr and 18hr) showed significant variance on germination percentage of BARI soybean 6 (Table 4). T_2 (6 hr) was found highest germination germination percentage (91.73%) which was statistically similar with T_3. Lowest germination percentage was found in T_1 (71.55%) which is also statistically similar with T_6. This result is agreement with Arif *et al*. (2008) and Varshini *et al*. (2018). They reported that 6hr priming time with different concentration like -1.1 MPa and -0.5 MPa showed better germination percentage in case of soybean and chickpea respectively.

4.2.1.3 Interaction effect of osmo and hydropriming and priming times

Interaction of osmo and hydro priming and priming times showed significant variation on germination percentage of soybean (Table 5). At germination percentage, highest germination percentage was showed in P_1T_2 (94.40%), which was statistically similar with P_1T_3, P_1T_4 and P_2T_2. Lowest germination percentage was showed in P_2T_1 (66.17%), which is statistically similar with P_2T_4, P_2T_5. Primed seeds at appropriate time showed higher germination. Osmopriming with 10% for 6 hr showed best result than hydropriming with distill water fo r 3hr. Short and over priming time reduced the germination percentage.

4.2.2 Shoot length (mm)

4.2.2.1 Effect of osmo and hydro priming

Osmo and hydro priming showed significant difference on shoot length of BARI soybean 6 (Table 4). Osmo priming done with 10% PEG (P_1) showed higher shoot length (78.11 mm) than hydropriming (priming with water). Hydropriming (P_2) was showed shoot length (68.65mm). Baque *et al*. (2016) reported that the highest shoot length of wheat was secured when the seed primed with osmopriming (10% PEG solution). Lee and Kim (2000) revealed that, priming increased the metabolic activities of seed ultimately gained the substantial shoot length.

4.2.2.2 Effect of priming times

Priming times (3hr, 6hr, 9hr, 12hr, 15hr and 18 hr) showed significant variance on shoot length of BARI soybean 6 (Table 4). T_2 (6 hr) was found highest shoot length (89.47 mm) which was statistically identical with T_3. Lowest shoot length was found in T_1 (56.70) which is also statistically identical with T_6. This findings is in line with Faijunnahar *et al*. (2017) and Yari *et al*. (2010). Faijunnahar *et al*. (2017) reported that priming time may beef up the enzymatic activities which results in vigorous plant growth ultimately longer shoot length otherwise over time may leads to aging of seeds for which seeds lose their potentiality to germinate and growth. Yari *et al*. (2010) revealed that maximum shoot length of sunflower is achieved when it was osmoprimed for 24hr.

4.2.2.3 Interaction effect of osmo and hydropriming and priming times

A perusal data revealed the interaction of osmo and hydro priming and priming times significantly affect the shoot length (Table 5). Highest shoot length (91.20 mm) was scored by osmopriming with 10% PEG for 6 hr (P_1T_2) which is statistically similar with P_1T_3, P_2T_2 and P_2T_3, lowest shoot length (45.93mm) was scored by hydropriming with distill water for 3hr (P_2T_1) which is statistiacally identical with P_2T_6. Priming with PEG helps to enhance the enzymatic activities and so plant growth ultimately shoot length can be increased under optimum time of priming but over timing may lower the potentiality of growth.

4.2.3 Root length

4.2.3.1 Effect of osmo and hydro priming

Osmo and hydro priming showed significant difference on root length of BARI soybean 6 (Table 4). Osmo priming done with 10% PEG (P_1) showed higher root length (72.51 mm) than hydropriming (priming with water). Hydro priming (P_2) was showed root length (61.08 mm). Carceller and Soriano (1972) reported that root length of osmo and hydro primed seed exerted the highest length than non-primed seed. Increased root length by osmopriming with PEG was also earlier reported in chickpea (Khadraji *et al*., 2017).

4.2.3.2 Effect of different priming times

Priming times (3hr, 6hr, 9hr, 12hr, 15hr and 18 hr) showed significant variance on root length of BARI soybean 6 (Table 4). T_2 (6 hr) was observed highest root length (82.79 mm). Lowest root length was found in T_1 (54.98 mm) which is statistically identical with T_6. This result is in agreement with Ajirloo *et al*. (2013) revealed that increasing time had greater effect on root length in case of wheat. Nahar *et al*. (2016) reported that maximum root length was obtained when wheat seed primed with 10% PEG for 12 hr.

4.2.3.3 Interaction effect of osmo and hydropriming and priming times

Interaction of osmo and hydro priming and priming times significantly influence the root length (Table 5). Highest root length (84.82 mm) was recorded when osmopriming with 10% PEG for 6 hr (P_1T_2) and lowest root length was showed in P_2T_1 (47.17 mm) when hydroprimed for 3hr which is statistically similar with P_2T_6. . According to Varshini *et al*. (2018) seed priming with -0.5 MPa PEG for 6 hours recorded highest root length (17.05 cm and 16.19 cm in between paper and sand methods, respectively) and 0 MPa for 24 hours recorded lowest root length. Osmopriming is a method of controlled seed priming in which the amount of water available for seed is restricted by regulating the water potential of priming medium (Farahani *et al*., 2011). So appropriate amount of water helps to proper growth then root length also increased.

4.2.4 Shoot dry weight (mg)

4.2.4.1 Effect of osmo and hydro priming

Osmo and hydro priming showed significant difference on shoot dry weight of BARI soybean 6 (Table 4). Osmo priming done with 10% PEG (P_1) showed higher shoot dry weight (75.37 mg) than hydropriming (priming with water). Hydropriming (P_2) showed shoot dry weight (61.18 mg). Nahar *et al*., (2016) concluded that osmopriming with 10% PEG had highest shoot dry weight than osmopromed with 0% PEG (hydropriming).

4.2.4.2 Effect of different priming times

Priming times (3hr, 6hr, 9hr,12hr, 15hr and 18 hr) showed significant variance on shoot dry weight of BARI soybean 6 (Table 4). T_2 (6 hr) was observed highest shoot dry weight (90.07mg). Lowest shoot dry weight was found in T_1 (56.36 mg) which is statistically identical with T_6. When seeds are Osmo-primed for 6hrs, it is significantly affected shoot dry weight and had highest shoot dry weight in wheat (Hamidreza *et al*., 2013). But Moghanibashi *et al*. (2012) showed that, when sunflower seed is hydroprimed for 24 hr shoot dry weight was increased than non-primed seed.

4.2.4.3 Interaction effect of osmo and hydro priming and priming times

Interaction of osmo and hydro priming and priming times significantly affected shoot dry weight (Table 5). Highest shoot dry weight (91.62 mg) was recorded from P_1T_2 when BARI soybean 6 primed with 10% PEG for 6 hr which is statistically similar with P_1T_3 and P_2T_2. Lowest shoot dry weight (53.87 mg) was recorded when hydro primed with 3hr (P_2T_1) which is statistically similar with P_1T_1, P_2T_5 and P_2T_6**.** Priming time helps to increased enzymatic activities of seed which trigger the vigorous plant growth and in consequence increased the shoot dry weight on the contrary over priming deteriorated the seed quality.

4.2.5 Root dry weight (mg)

4.2.5.1 Effect of osmo and hydro priming

Osmo and hydro priming showed significant difference on root dry weight of BARI soybean 6 (Table 4). Osmo priming done with 10% PEG (P_1) showed higher root dry weight (7.94 mg) than hydropriming (priming with water). Hydropriming (P_2) was showed root dry weight (6.28 mg). Baque *et al*. (2016) found that maximum root dry weight was recorded when the seed osmoprimed with 10% PEG solution than hydroprimed seed.

4.2.5.2 Effect of different priming times

Priming times (3hr, 6hr, 9hr,12hr,15hr and 18hr) showed significant variance on root dry weight of BARI soybean 6 (Table 4). T_2 (6hr) was observed highest root dry weight (9.53 mg). Lowest root dry weight was found in T_6 (5.52 mg) which is statistically similar with T_1. Hamidreza *et al*. (2013) revealed that osmopriming for 6hr had highest root dry weight in case of wheat. Faijunnahar *et al*. (2017) reported that seed priming accelerated the enzymatic activities which leads to proliferous root growth and subsequently highest root growth was achieved.

Table 4. Effect of osmo and hydro priming 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	84.34 a	78.11 a	72.51 a	75.37 a	7.94 a
P_2	76.86 b	68.65 b	61.08 b	67.18 b	6.28 b
LSD $_{(0.01)}$	4.37	3.85	3.59	3.99	0.36
CV (%)	5.82	5.62	5.76	6.00	5.49
Effect of different priming times					
T_1	71.55 d	56.70 c	54.98 c	56.36 d	6.08 cd
T_2	91.73 a	89.47 a	82.79 a	90.07 a	9.53 a
T_3	86.20 ab	84.71 a	71.41 b	79.39 b	7.67 b
T_4	79.96 bc	75.87 b	67.00 b	73.87 bc	7.30 b
T_5	79.27 bc	70.86 b	65.90 b	68.08 c	6.56 c
T_6	74.88 cd	62.67 c	58.69 c	59.87 d	5.52 d
LSD $_{(0.01)}$	7.58	6.66	6.21	6.91	0.63
CV (%)	5.82	5.62	5.76	6.00	5.49

P_1: Osmopriming T_1: 3hr; T_2: 6hr; T_3: 9hr

P_2: Hydropriming T_4:12hr; T_5: 15hr; T_6: 18hr

4.2.5.3 Interaction effect of osmo and hydropriming and priming times

Interaction of osmo and hydro priming and priming times significantly influenced the root dry weight of BARI soybean 6 (Table 5). Maximum root dry weight (10.52 mg) was scored by osmopriming with 10% PEG for 6 hrs (P_1T_2) and minimum root dry weight (4.90 mg) was scored by hydropriming for 18 hr (P_2T_6) which is statistically similar with P_2T_1. Priming increases the protein synthesis and carbohydrate accumulation through enhancing the biochemical activities. As dry matter production may be increased leads to increasing root dry weight.

According to result of this experiment it can be expressed that interaction of osmo and hydro priming and different priming times shows significant variation in case of germination percentage, shoot length, root length, shoot dry weight and root dry weight. BARI soybean 6 with 10% PEG for 6hr showed better performance than any other treatment combination. Priming time 6hr of BARI soybean 6 were showed highest in case of above parameters and were gradually decrease more than 6 hr .Present study under the agreement with Nahar *et al*. (2016) and Arif *et al*. (2008). Nahar *et al*. (2016) reported when the seed primed with 10% PEG solution for 12 h showed best result in case of BARI Gom 27. Arif *et al*. (2008) concluded that seed priming duration of 6 h resulted in faster and improved emergence and higher grain yield of soybean.

Table 5. Interaction effect of osmo and hydro priming 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	76.92 cd	67.47 d	62.79 e	58.85 e-g	7.17 cd
P_1T_2	94.40 a	91.20 a	84.82 a	91.62 a	10.52 a
P_1T_3	89.67 ab	85.27 ab	75.56 bc	83.97 a-c	8.28 b
P_1T_4	84.52 a-c	79.79 bc	73.72 bd	79.63 bc	8.27 b
P_1T_5	82.80 bc	73.65 cd	72.17 bd	74.31 cd	7.20 c
P_1T_6	77.71 cd	71.25 cd	65.97 de	63.84 ef	6.15 ef
P_2T_1	66.17 e	45.93 e	47.17 g	53.87 g	4.98 gh
P_2T_2	89.06 ab	87.74 ab	80.76 ab	88.52 ab	8.54 b
P_2T_3	82.73 b-d	84.15 ab	67.27 c-e	74.80 cd	7.03 cde
P_2T_4	75.74 c-e	71.95 cd	60.27 e	68.11 de	6.33 def
P_2T_5	75.40 c-e	68.06 d	59.63 ef	61.86 e-g	5.87 fg
P_2T_6	72.04 de	54.08 e	51.40 fg	55.89 fg	4.90 h
$LSD_{(0.01)}$	10.72	9.42	8.78	9.77	0.89
CV (%)	5.82	5.62	5.76	6.00	5.49

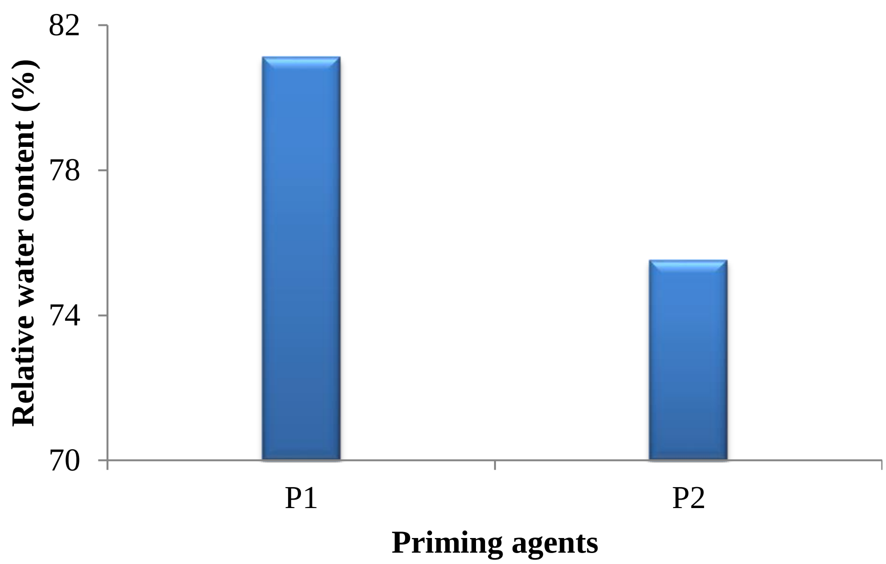
P_1T_1: Osmopriming for 3hr; P_1T_2: Osmopriming for 6hr; P_1T_3: Osmopriming for 9hr; P_1T_4: Osmopriming for 12 hr; P_1T_5: Osmopriming for 15 hr; P_1T_6: Osmopriming for 18 hr; P_2T_1: Hydropriming for 3hr; P_2T_2: Hydropriming for 6hr; P_2T_3: Hydropriming for 9hr; P_2T_4: Hydropriming for 12 hr; P_2T_5: Hydropriming for 15 hr; P_2T_6: Hydropriming for 18 hr

4.2.6 Relative water content

The relative water content (RWC; or 'relati ve turgidity') of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity. RWC provides a measurement of the 'water deficit' of the leaf, and may indicate a degree of stress expressed under drought and heat stress.

4.2.6.1 Effect of osmo and hydro priming

Priming agents showed significant variation in relative water content of BARI soybean 6 when it was analyzed (Figure 11). It was observed that Osmo priming (P_1) showed higher performance (81.12%) than hydro priming P_2 (75.52%). Control hydration of osmo priming gives it better performance than hydro priming.

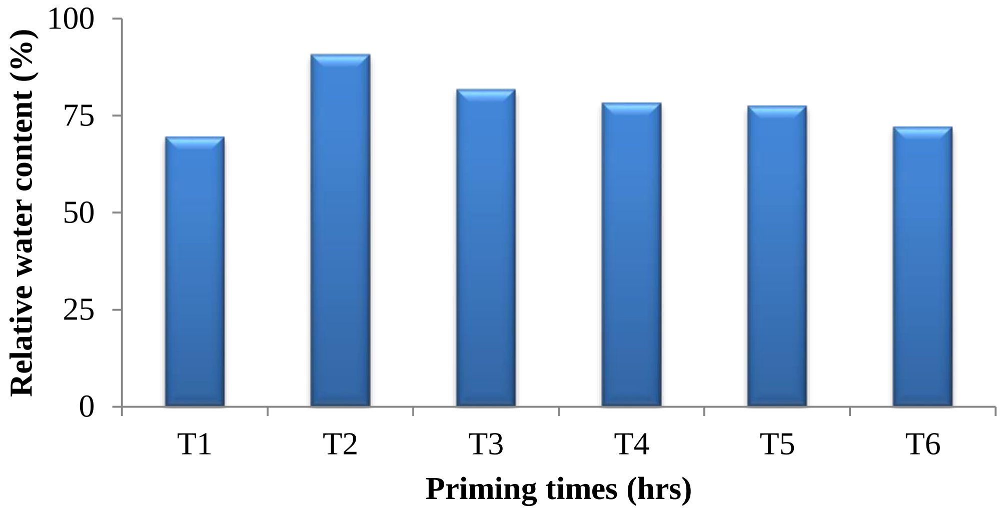


P_1: Osmo priming P_2: Hydropriming

Figure 11. Effect of osmo and hydro priming on the relative water content of soybean ($LSD_{0.01}$=4.09)

4.2.6.2 Effect of different priming times

Priming times (3hr, 6hr, 9hr,12hr, 15hr and 18hr) showed significant variance on relative water content of BARI soybean 6 (Figure 12). T_2 (6hr) showed highest relative water content 90.75%. T_1 (3hr) showed lowest relative water content 69.55 % which is statistically similar with T_6. According to Faijunnahar *et al*., (2017) Priming time helps to accelerated enzymatic activities of seed to facilitate the growth of healthy and vigorous seedling, and so this seedling may have higher relative water content. Over priming time beef up the ageing process of primed seed, produced weak and lean seedling and the ultimate result is lower relative water content.



T_1= Priming for 3 hr; T_2= Priming for 6 hr; T_3= Priming for 9 hr; T_4= Priming for 12 hr; T_5= Priming for 15 hr; T_6= Priming for 18 hr

Figure 12. Effect of priming times on the relative water content of soybean ($LSD_{0.01}$=7.08)

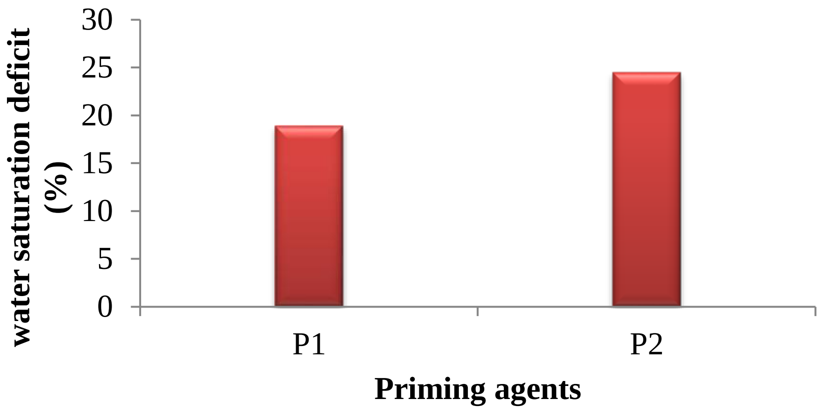
4.2.6.3 Interaction of osmo and hydropriming and priming times

Interaction of osmo and hydro priming and different priming times showed significant variation on relative water content of soybean (Table 6). According to analyzed data in the case of relative water content , highest relative water content was showed in P_1T_2 (92.71%) which is statistically similar with P_1T_3 and P_2T_2. Lowest data recorded from P_2T_1 (65.15%) which is statistically similar with P_1T_1, P_1T_6, P_2T_5 and P_2T_6. Osmoprimed with 10% PEG for 6 hr showed better result because of priming helps to uptake more water ultimately water in plant is higher than non-primed one.

4.2.7 Water saturation deficit

4.2.7.1 Effect of osmo and hydro priming on water saturation deficit

It followed the opposite trend compared to the previously described parameter. Different priming agents showed significant variation in water saturation deficit of BARI soybean 6 when it was analyzed (Figure 13). It was observed that hydro priming (P_1) showed higher performance (24.48) than osmo priming P_2 (18.88).

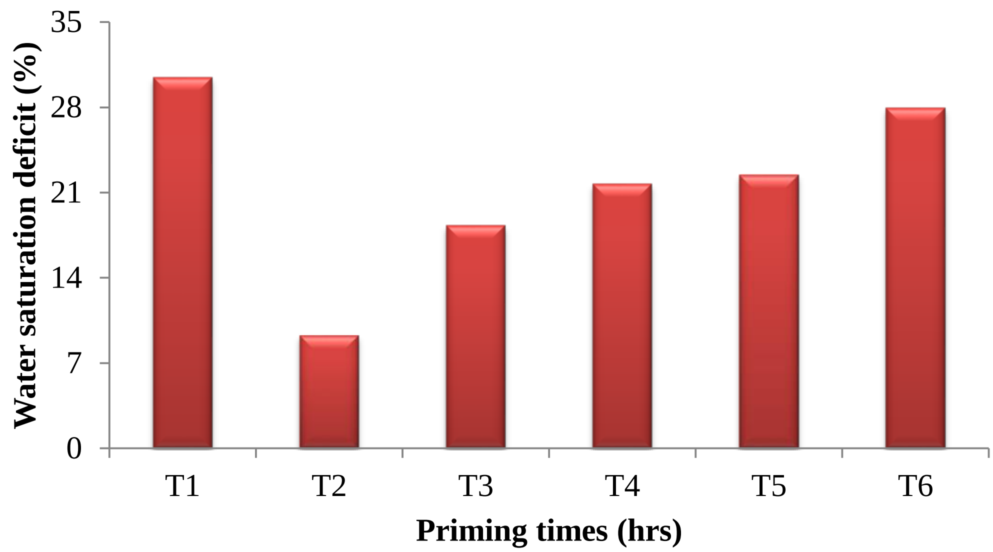


P_1: Osmo priming P_2: Hydropriming

Figure 13. Effect of osmo and hydro priming on the water saturation deficit of soybean ($LSD_{0.01}$=1.14)

4.2.7.2 Effect of different priming times on water saturation deficit

Priming times (3hr, 6hr, 9hr,12hr,15hr and 18hr) showed significant variance on water saturation deficit of BARI soybean 6 (Figure 12). Water saturation deficit is reverse than other parameter.T_1 (3hr) showed highest water saturation deficit 30.45. T_2 (6hr) showed lowest water saturation deficit 9.250. According to Faijunnahar *et al*. (2017) when priming time is low, enzymatic activities are also low, as a result weak and lean seed are produced. Again when over priming time result is same and they are unable to uptake water needed water and so water saturation deficit value is increased.



T_1= Priming for 3 hr; T_2= Priming for 6 hr; T_3= Priming for 9 hr; T_4= Priming for 12 hr;

T_5= Priming for 15 hr; T_6= Priming for 18 hr

Figure 14. Effect of priming times on the water saturation deficit of soybean ($LSD_{0.01}$=1.98)

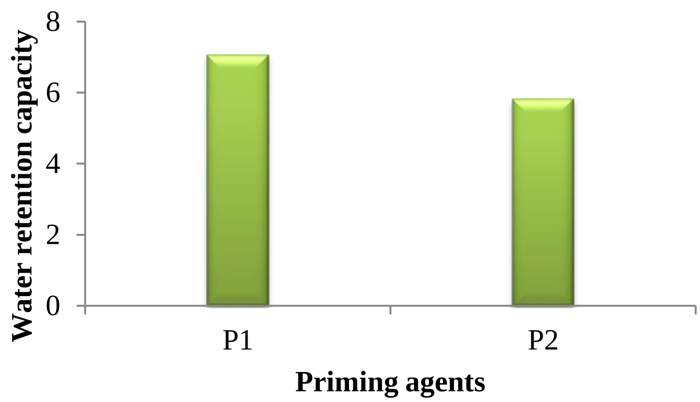
4.2.7.3 Interaction of osmo and hydropriming and priming times

Interaction of osmo and hydropriming and priming times significantly influenced the water saturation deficit of BARI soybean 6 (Table 6). Maximum water saturation deficit (34.85) was found when hydropriming done with 3hr in P_2T_1 which was statistically different from others and minimum was found when osmopriming with 10% PEG for 6 hr in P_1T_2 (7.29). They both are statistically different from others. It is the opposite trend of relative water content. As priming helps to increase relative water content so water saturation deficit should be lower in primed seed.

4.2.8 Water retention capacity

4.2.8.1 Effect of osmo and hydro priming on water retention capacity

Priming agents showed significant variation in water retention capacity of BARI soybean 6 when it was analyzed (Figure 15). It was observed that Osmo priming (P_1) showed higher performance (7.06) than hydro priming P_2 (5.82).

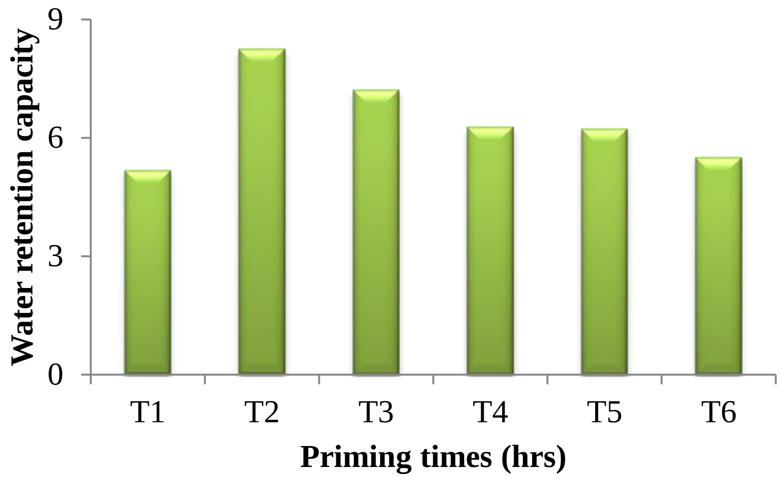


P_1: Osmo priming P_2: Hydropriming

Figure 15. Effect of osmo and hydro priming on the water retention capacity of soybean ($LSD_{0.01}$=0.34)

4.2.8.2 Effect of different priming times on water retention capacity

Priming times (3hr,6hr,9hr,12hr,15hr and 18hr) showed significant variance on water retention capacity of BARI soybean 6 (Figure 16). T_2 (6hr) showed highest water retention capacity 8.248. T_1 (3hr) showed lowest water retention capacity 5.173 which is statistically identical with T_6. Lower and over priming time both have deleterious effect on growth and development of seedling. Vigorous seedling have higher water retention capacity than weak and lean seedlings (Faijunnahar *et al*., 2017).



T_1= Priming for 3 hr; T_2= Priming for 6 hr; T_3= Priming for 9 hr; T_4= Priming for 12 hr;

T_5= Priming for 15 hr; T_6= Priming for 18 hr

Figure 16. Effect of priming times on the water retention capacity of soybean ($LSD_{0.01}$=0.58)

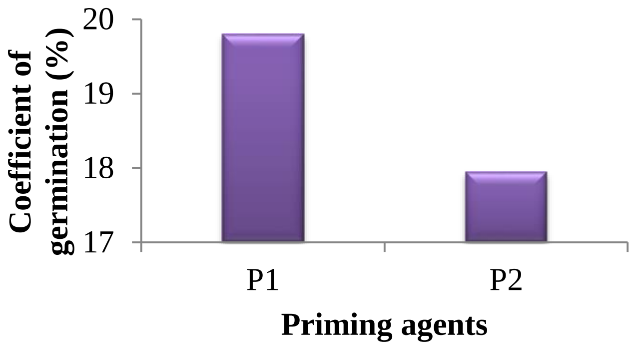
4.2.8.3 Interaction of osmo and hydropriming and priming times

Interaction of osmo and hydro priming and priming times significantly influenced the water retention capacity (Table 6). Highest data was found in P_1T_2 (9.16) and lowest was recorded from P_2T_1 (4.63) which is statistically similar with P_2T_6. Osmopriming with 10% PEG for 6 hr produce vigorous seedling and vigorous seedling has more water retention capacity than hydro priming with 3 hr.

4.2.9 Coefficient of germination

4.2.9.1 Effect of osmo and hydro priming on coefficient of germination

Priming agents showed significant variation in coefficient of germination of BARI soybean 6 when it was analyzed (Figure 17). It was observed that osmo priming (P0) showed higher performance (19.80%) than hydro priming (17.95%). Osmo priming of Italian ryegrass (Lolium multiflorum) and sorghum (Sorghum bicolor) seeds with 20% PEG-8000 for 2 d at 10°C enhanced germination coefficient of wheat genotypes under water stress, waterlogging, cold stress and saline conditions (Hur, 1991).

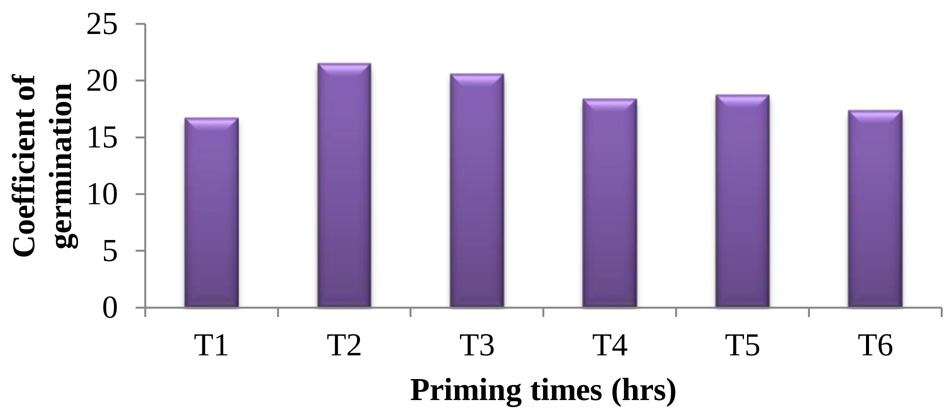


P_1: Osmo priming P_2: Hydropriming

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

4.2.9.2 Effect of priming times on coefficient of germination

Different priming times (3hr,6hr,9hr,12hr,15hr and 18hr) showed significant variance on coefficient of germination capacity of BARI soybean 6 (Figure 18). T_2 (6hr) showed highest coefficient of germination 21.48% which is statistically identical with T_3. T_1 (3hr) showed lowest coefficient of germination which is statistically similar with T_6. 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). Hydropriming for 24 h enhanced germination coefficient of sunflower seed as compared with the control (Moghanbashi *et al*., 2012).



T_1= Priming for 3 hr; T_2= Priming for 6 hr; T_3= Priming for 9 hr; T_4= Priming for 12;

T_5=Priming for 15 hr; T_6= Priming for 18 hr

Figure 18. Effect of priming times on the coefficient of germination of soybean ($LSD_{0.01}$=1.64)

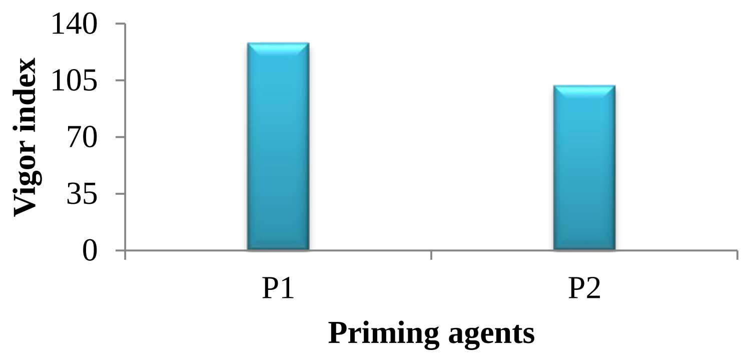
4.2.9.3 Interaction effect of osmo and hydropriming and priming times

Coefficient of germination had significantly affected by interaction of osmo and hydro priming and priming times (Table 6). Maximum value (22.48) was recorded from osmopriming with 10% PEG for 6 hr (P_1T_2) which is statistically similar with P_1T_3 and P_2T_2. Minimum coeffient of germination (15.94) was found in P_2T_1 (Hydroprimed for 3hr) which is statistically identical with P2T5 and P2T6. It was identical with P_1T_1, P_1T_6 and P_2T_4. From this result it can be concluded that lower time and over time both are harmful for priming.

4.2.10 Vigor index

4.2.10.1 Effect of osmo and hydro priming on vigor index

Priming agents showed significant variation in coefficient of germination of BARI soybean 6 when it was analyzed (Figure 19). It was observed that Osmo priming (P0) showed higher performance (127.84) than hydro priming (101.67). It has been reported that primed seeds showed better germination pattern and higher vigor level than non- primed (Ruan *et al.*, 2002).

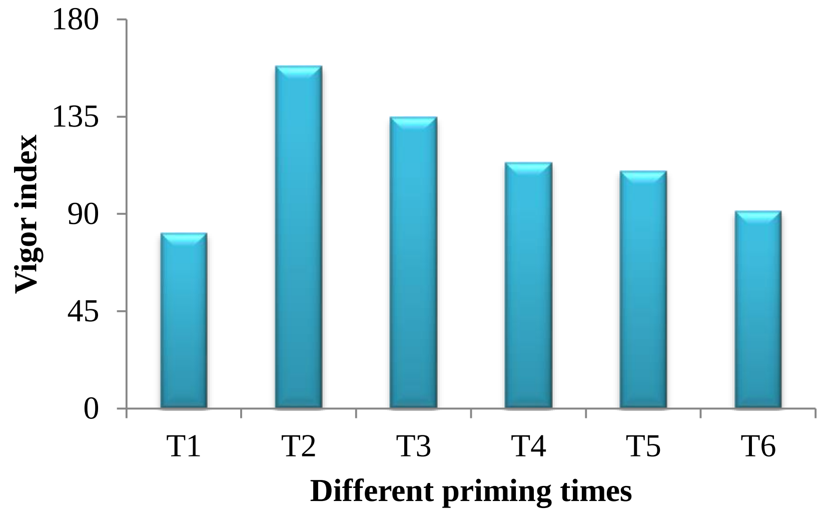


P_1: Osmo priming P_2: Hydropriming

Figure 19. Effect of osmo and hydro priming on the vigor index of soybean ($LSD_{0.01}$=5.83)

4.2.10.2 Effect of different priming times on vigour index

Priming times (3hr, 6hr, 9hr,12hr,15hr and 18hr) showed significant variance on vigour index of BARI soybean 6 (Figure 20). T_2 (6hr) showed highest vigor index 158.2. T_1 (3hr) showed lowest vigor index 80.94. The highest vigor index of BARI Gom 27 was recorded when the seed primed with 10% PEG solution for 12 h (Nahar *et al.*, 2016 and Sadeghi *et al.*, 2011).



T_1= Priming for 3 hr; T_2= Priming for 6 hr; T_3= Priming for 9 hr; T_4= Priming for 12 hr; T_5= Priming for 15 hr; T_6= Priming for 18 hr

Figure 20. Effect of priming times on the vigor index of soybean ($LSD_{0.01}$=10.09)

4.2.10.3 Interaction of osmo and hydropriming and priming times

In case of vigor index of BARI soybean 6 with interaction of different priming agents and priming times, highest analyzed data was recorded from P_1T_2 (166) when osmopriming done with 10% PEG for 6 hr and lowest was found in P_2T_1 (61.74) when hydroprimed for 3hrs. Both are statistically different from others. A double technology named seed priming to accelerate rapid and uniform emergence and to achieve high vigour and better yields in cumin (Nematollahi *et al.*, 2009). Osmopriming helps reserve mobilization through activation of important enzymes thus vigor is increased after germination takes place.

According to the result of this experiment, 6 hr. showed better result than other priming time in case of relative water content, water retention capacity, and germination coefficient and vigor index. But in case of water saturation deficit it shows better in 3hr, totally opposite of other water relation behaviors parameters. This result is in line with Faijunnahar *et al*. (2017). They concluded that all water relation parameter resulted same except water saturation deficit. It follows inverse relation than other parameters.

Table 6. Interaction effect of osmo and hydro priming and 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	73.95 d-f	26.05 c	5.72 ef	17.46 ef	100.1 d
P_1T_2	92.71 a	7.29 g	9.16 a	22.48 a	166.0 a
P_1T_3	84.28 a-c	15.72 e	7.71 b	21.38 ab	144.1 b
P_1T_4	80.94 b-d	19.06 d	6.89 c	19.92 b-d	127.1 c
P_1T_5	80.75 b-d	19.25 d	6.88 c	19.34 b-e	123.1 c
P_1T_6	74.06 d-f	25.94 c	5.97 de	18.20 c-f	106.6 d
P_2T_1	65.15 f	34.85 a	4.63 g	15.94 f	61.74 f
P_2T_2	88.79 ab	11.21 f	7.33 bc	20.48 a-c	150.4 b
P_2T_3	79.16 b-e	20.84 d	6.71 cd	19.82 b-d	125.3 c
P_2T_4	75.66 c-e	24.34 c	5.65 ef	18.12 d-f	100.3 d
P_2T_5	74.31 c-f	25.69 c	5.56 ef	16.89 f	96.27 d
P_2T_6	70.08 ef	29.92 b	5.03 fg	16.47 f	76.08 e
$LSD_{(0.01)}$	10.02	2.80	0.82	2.32	14.27
CV (%)	5.60	5.64	5.61	5.39	5.45

P_1T_1: Osmopriming for 3hr; P_1T_2: Osmopriming for 6hr; P_1T_3: Osmopriming for 9hr; P_1T_4: Osmopriming for 12 hr; P_1T_5: Osmopriming for 15 hr; P_1T_6: Osmopriming for 18 hr; P_2T_1: Hydropriming for 3hr; P_2T_2: Hydropriming for 6hr; P_2T_3: Hydropriming for 9hr; P_2T_4: Hydropriming for 12 hr; P_2T_5: Hydropriming for 15 hr; P_2T_6: Hydropriming for 18 hr.

4.3 Experiment 3: Effect of different level of drought stress on germination and water relation behavior of soybean

Results obtained from the present study regarding the germination behavior and water relation behavior of primed seed under drought (Polyethylene Glycol) stress condition. BARI soybean 6 primed with 10% PEG for 6hr used for this experiment where drought is induced by 0%, 5%, 10%, 15% and 20% PEG. The analytical results have been displayed in Tables 7 to 10.

4.3.1 Germination percentage (%)

4.3.1.1 Effect of osmo and hydro priming

Osmopriming done with 10% PEG and hydropriming showed significant variation in respect of germination percentage. Osmopriming showed higher germination percentage P_1 (85.94%) than hydropriming P_2 (77.95%). This result is in line with Tabatabaei (2013). This germination characteristics of primed seeds could be results of increasing the antioxidant profile of treated seeds. Osmopriming with PEG results in improving the antioxidant enzymes and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach (Chen and Arora, 2011). Beckers and Conrath (2007) concluded that primed seeds have the physiological defence mechanism which help them to activate their enzyme faster or better or both. Osmopriming in the PEG solution allows initiating the membrane repairing systems and metabolic preparation for germination via controlling the water absorption rate of seeds. (Jisha *et al*. 2013).

4.3.1.2 Effect of stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of germination percentage (Table 7). Highest germination percentage was found in T_0 (85.94%) which is statistically identical with T_1 and T_2. Lowest GP was obtained from T_4 (66.02%). This result is in agreement with Almaghrabi (2012) findings who observed significant difference in response to drought stress on germination percentage of wheat cultivars. Reduction in germination with moisture stress is attributed to lower infusibility of water through the seed coat and initial water imbibition of the seed under stress condition and decreased external water potential (Bahrami *et al*. 2012)

4.3.1.3 Interaction effect of osmo and hydropriming and stress concentration

Interaction of osmo and hydro priming and stress concentrations showed significant variation on germination percentage of soybean (Table 8). Highest germination percentage (93.73%) was obtained from osmopriming under control or 0% stress (P_1T_0) which was statistically similar with P_1T_1, P_1T_2, P_2T_0 and P_2T_1. Lowest germination percentage (58.31%) was obtained from hydropriming under 20% stress concentration (P_2T_4) .The combined results of Rajjou *et al*. (2006) revealed several processes potentially affected by Salicylic Acid. This molecule enhanced the reinduction of the late maturation program during early stages of Arabidopsis seed germination, thereby allowing the germinating seeds to reinforce their capacity to mount adaptive responses in environmental water stress. In case of germination percentage drought tolerance was icreased about 7.41% by osmopriming over than hydropriming under 10% PEG stress.

4.3.2 Shoot length (mm)

4.3.2.1 Effect of osmo and hydro priming

Osmo priming (P_1) done with 10% PEG and hydro priming (P_2) showed significant variation in respect of shoot length. Results obtained from table 7, it can be said that osmopriming showed higher shoot length (79.98 mm) than hydropriming (70.79 mm). Ghazari and Zeinali (2003) also observed an increase in shoot and radicle lengths until -0.2Mpa when using PEG 6000. On the contrary, Kaur *et al*. (2002) reported that hydropriming showed three to four fold more growth with respect to root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition.

4.3.2.2 Effect of stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of shoot length (Table 7). With increasing drought stress level shoot length is decreased. Highest shoot length (87.53mm) was found in T_0 which is statistically similar with T_1. Lowest shoot length (58.31%) was obtained from T_4 (20% stress). According to Ashagre *et al*. (2014) Maximum shoot and root length were recorded at

60g/L, but increase in PEG concentration decreased shoot and root length. Radhouane (2007) observed that decreases in the external osmotic potential induced decreased shoot growth.

4.3.2.3 Interaction effect of osmo and hydro priming and stress concentrations

Interaction of osmo and hydro priming and stress concentrations significantly affected the shoot length (Table 8). Highest shoot length (91.18 mm) was obtained from osmopriming under control in P_1T_0 which is statistically similar with P_1T_1, P_1T_2 and P_2T_0 and lowest shoot length was recorded from P_2T_4 (52.06 mm). When stress concentration increases the shoot length decreases. This may occur due to the reduction in water uptake by germinating seed in stress condition resulted in decreases of seedling growth (Alam, 2001).

4.3.3 Root length (mm)

4.3.3.1 Effect of osmo and hydro priming

Osmopriming (P_1) done with 10% PEG and hydropriming (P_2) showed significant variation in respect of root length. Results obtained from table 7 it can be said that P_1 showed higher root length (72.78 mm) than P_2 (58.26 mm). Similar result also have been reported by Boureima *et al*., (2011) who stated that root length increased by 19.94% at 0.5Mpa in comparison with control. The increased shoot and root length with osmopriming treatments may be due to the fact that, osmopriming increased nuclear replication in root and shoot (Salehzade *et al*., 2009).

4.3.3.2 Effect of stress concentrations on root length (mm)

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of root length (Table 7). Highest root length was found in T_0 (80.27 mm) which is statistically similar with T_1. Lowest data was obtained from T_4 (46.16 mm). Moderate drought stress increase root length of pearl millet by 15.8% (Radhoaune, 2008). Badiow *et al*. (2004) reported that the development of root system in response to water deficit suggests that expression of certain genes controlling root formation is stimulated by drought condition. However the reduction of radicle length due to excess moisture stress could be due to a cessation in cellular division and elongation at root level (Ashagre *et al*., 2014). Dodd *et al*. (2008) reported that under water stress, ABA biosynthesis will increase in the root and, successively, the synthesized ABA will be transported to the shoot through the xylem; however, the higher concentration of ABA in root may also limit the function of ethylene which accordingly inhibits the plant growth.

4.3.3.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction effect of osmo and hydropriming and stress concentrations significantly influenced the root length (Table 8). Highest root length (84.93 mm) was recorded from osmopriming under control (P_1T_0) which is statistically similar with P_1T_1 and lowest root length (35.70 mm) was recorded from hydropriming in P_2T_4 Fraser *et al*. (1990) concluded that the reduction in the root length under drought stress may due to an impediment of cell division and elongation leading to Kind tuberization. This tuberization and the lignifications of the root system allow the conditions to become favorable again.

4.3.4 Shoot dry weight (mg)

4.3.4.1 Effect of osmo and hydro priming on shoot dry weight

Osmopriming (P_1) done with 10% PEG and hydropriming (P_2) showed significant variation in respect of shoot dry weight. Results obtained from table 7 it can be said that osmopriming showed higher shoot dry weight (78.11 mg) than hydropriming (70.65 mg). According to Salehzade *et al*. (2009), seedling dry weight (shoot and root dry weight) may increase due to synchronized germination, DNA and RNA synthesis of osmoprimed seed.

4.3.4.2 Effect of different stress concentrations on shoot dry weight

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of shoot dry weight (Table 7). Highest shoot dry weight was found in T_0 (85.42 mg) which is statistically similar with T_1. Lowest data was obtained from T_4 (57.76 mg). Almaghrabi (2012) reported that PEG caused a greater reduction in fresh and dry weights of shoot and root at higher concentration. Zokaee-Khosroshahi *et al*. (2014) reported that the dry weight of organs and subsequently the whole plant decreased as drought stress levels increased. A decrease in dry matter may be due to the considerable reduction of photosynthesis and plant growth (Shao *et al*. 2008).

4.3.4.3 Interaction effect of osmo and hydropriming and stress concentrations

A perusal mean data showed interaction of osmo and hydro priming significantly affected the shoot dry weight when stress condition applied (Table 8). Highest shoot dry weight (87.30 mg) was recorded when osmopriming occurred under control (P_1T_0) which is statistically identical with P_1T_1, P_2T_0 and similar with P_1T_2, P_2T_1. Lowest shoot dry weight (51.43 mg) was recorded when hydropriming done under 20% stress concentration (P_2T_4). Soltani *et al.* (2006) found that reduction in seedling dry weight in response to drought and salinity in wheat cultivars is a consequence of decrease in mobilized seed reserve due to low water uptake by the germinating seeds.

4.3.5 Root dry weight

4.3.5.1 Effect of osmo and hydro priming on root dry weight

Osmopriming (P_1) done with 10% PEG and hydropriming (P_2) showed significant variation in respect of root dry weight. Results obtained from table 7 it can be said that osmopriming showed higher root dry weight (7.73 mg) than hydropriming (6.41 mg). Osmopriming enhanced the enzymatic activities through which biomass production increased ultimately root dry weight is increased.

4.3.4.2 Effect of different stress concentrations on root dry weight

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of root dry weight (Table 7). Highest root dry weight was found in T_0 (9.370 mg). Lowest data was obtained from T_4 (4.56 mg). Increasing stress condition root dry weight is gradually decreases. Arji and Arzani (2000) reported that decreasing root DW under drought conditions may be caused by a decrease in the accumulation of root carbohydrates.

Table 7. Effect of osmo and hydro priming and stress concentration 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	85.94 a	79.98 a	72.78 a	78.11 a	7.73 a
P_2	77.95 b	70.79 b	58.26 b	70.65 b	6.41 b
LSD $_{(0.01)}$	4.61	4.54	4.16	4.26	0.35
CV (%)	5.42	5.80	6.12	5.51	4.79
Effect of different stress concentrations					
T_0	92.15 a	87.53 a	80.27 a	85.42 a	9.37 a
T_1	89.84 a	83.15 ab	74.34 ab	82.47 ab	8.36 b
T_2	85.00 a	77.75 b	67.94 b	76.96 b	7.21 c
T_3	76.70 b	69.99 c	58.89 c	69.27 c	5.85 d
T_4	66.02 c	58.50 d	46.16 d	57.76 d	4.56 e
LSD $_{(0.01)}$	7.30	7.19	6.58	6.73	0.56
CV (%)	5.42	5.80	6.12	5.51	4.79

P_1: Osmopriming P_2: Hydropriming

T_0: Control; T_1: stress with 5% PEG stress; T_2: stress with 10% PEG; T_3: stress with 15% PEG; T_4: stress with 20% PEG

4.3.4.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction effect of osmo and hydropriming and stress concentrations significantly influenced the root dry weight (Table 8). Highest root dry weight (9.950 mg) was recorded from osmopriming under control (P_1T_0) and lowest data was recorded from P_2T_4 (3.97 mg) when hydroprimed seed placed under 20% stress concentration. Under stress condition root dry weight is decreased due to hindrance in enzyme activation after that reduction of biomass occurred.

From this result it can be expressed that Osmoprimed seed showed better performance than hydroprimed seed under lower stress concentration of PEG in case of germination percentage, shoot length, root length, shoot dry weight and root dry weight. In case of germination drought tolerance was increased about 7.41% by osmopriming over than hydropriming under 10% PEG stress. This occurs due to soaking the seed in distilled water had the weakest effect than the other primers, since distilled water has zero osmotic potential, which decreases seed accessibility to water (Rdhan & Yanaht, 1982). This result is in agreement with Ashagre *et al*. (2014), Carbineau and Come (2006) and Ashraf and Foolad (2005). According to Ashagre *et al*. increase in PEG 6000 concentrations decreased germination percentage and rate, while shoot and root lengths and shoot fresh and dry weights decreased beyond 60g/L and increased up to 120g/L PEG but further increase in stress negatively influenced cultivars tolerance. Carbineau and Come (2006) and Ashraf and Foolad (2005) reported that, under stress condition primed seedling able to grow normally without any disturbance. It may be occurs due to priming helps to imbibe seed with restricted amounts of water to allow sufficient hydration and advancement of metabolic processes but preventing germination or loss of desiccation tolerance.

Table 8. Interaction effect of osmo and hydro priming and stress 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	93.73 a	91.18 a	84.93 a	87.30 a	9.95 a
P_1T_1	91.98 ab	87.13 ab	80.29 ab	85.30 a	9.09 b
P_1T_2	88.04 ab	82.35 a-d	74.91 bc	80.32 ab	7.90 c
P_1T_3	82.21 bc	74.29 c-e	67.13 cd	73.52 b-d	6.57 d
P_1T_4	73.73 cd	64.95 e	56.62 ef	64.09 d	5.16 e
P_2T_0	90.57 ab	83.88 a-c	75.61 bc	83.53 a	8.79 b
P_2T_1	87.71 ab	79.18 b-d	68.39 cd	79.64 ab	7.63 c
P_2T_2	81.96 bc	73.14 de	60.98 de	73.61 bc	6.52 d
P_2T_3	71.18 d	65.69 e	50.64 f	65.02 cd	5.14 e
P_2T_4	58.31 e	52.06 f	35.70 g	51.43 e	3.97 f
$LSD_{(0.01)}$	10.32	10.16	9.31	9.52	0.79
CV (%)	5.42	5.80	6.12	5.51	4.79

P_1T_0: Osmo priming under control; P_1T_1: Osmo priming under 5% PEG stress; P_1T_2: Osmo priming under 10% PEG stress; P_1T_3: Osmo priming under 15% PEG stress; P_1T_4: Osmo priming under 20% PEG stress P_2T_0: Hydro priming under control; P_2T_1: Hydro priming under 5% PEG stress; P_2T_2: Hydro priming under 10% PEG stress ; P_2T_3: Hydro priming under 15% PEG stress; P_2T_4: Hydro priming under 20% PEG stress

4.3.6 Relative water content (%)

Relative water content is considered an alternative measurement of water status, and a plant's drought resistance is related to its ability to maintain high RWC in leaves under stress (Faraloni *et al*., 2011).

4.3.6.1 Effect of osmo and hydropriming

Osmopriming (P_1) and hydropriming (P_2) showed significant difference after analysis of variance. P_1 (81.58%) showed higher relative water content than P_2 (73.88%). This result is in consistent with Zhang *et al*. (2015). They reported that priming with PEG resulted in better germination performance and increased RWC in sorghum seedling under various suboptimal soil moisture conditions. This may occur due to physiologically seed priming strengthened the antioxidant activities of APX, CAT, POD, and SOD, as well as compatible solutes including free amino acid, reducing sugar, proline, soluble sugar, and soluble protein contents. As a result, seed priming reduced lipid peroxidation and stabilized the cell membrane, resulting in increased stress tolerance under drought or excessive soil moisture environments.

4.3.6.2 Effect of different stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of relative water content (Table 9). Highest relative water content was found in T_0 (90.32 %) which is statistically similar with T_1. Lowest data was obtained from T_4 (58.55%). This result is in line with Meher *et al*. (2017). According to them increasing drought stress has effect on relative water content because it is decreasing with increasing stress due to hampering absorbing water from soils.. It was also observed the decline of RWC with increased water stress was also observed in pigeon pea plants (Kumar *et al*., 2011).

4.3.6.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction of osmo and hydro priming and different stress concentrations showed significant variation relative water content of soybean (Table 10). According to analyzed data in the case of relative water content, highest relative water content (93.41%) was observed when osmoriming seed placed under control condition (P_1T_0) which is statistically identical with P_1T_1 and similar with P_1T_2, P_2T_0 and P_2T_1. Lowest data recorded from P_2T_4 (52.08%). Lowest relative water content found when hydropriming done under 20% PEG stress. Under 10% PEG stress osmopriming showed 8.96% drought tolerance than hydropriming.

4.3.7 Water saturation deficit (%)

4.3.7.1 Effect of osmo and hydropriming

Osmopriming (P_1) and hydropriming (P_2) showed significant difference after analysis of variance in table 9. P_2 (26.12%) showed higher water saturation deficit than P_1 (18.42%). Osmopriming might help to recover physiological damage and minimize the water saturation deficit. (Baque *et al*., 2002)

4.3.7.2 Effect of different stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of water saturation deficit (Table 9). Highest water saturation deficit was found in T_4 (41.45). Lowest data was obtained from T_0 (9.68). Under stress condition plant cannot uptake water smoothly for running their physiological process, so water deficit is occurred.

4.3.7.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction of osmo and hydropriming and stress concentrations significantly influenced water saturation deficit of BARI soybean 6 when it was analyzed (Table 10). Highest water saturation deficit (47.92) was found in hydropriming under 20% stress concentration (P_2T_4) and lowest (6.59) was found in P_1T_0 when osmopriming done under control condition.They both were statistically different from others. RWC started reducing when stress increasing. WSD is opposite than RWC so when stress increasing WSD is also increased.

4.3.8 Water retention capacity

4.3.8.1 Effect of osmo and hydropriming

Osmopriming (P_1) and hydropriming (P_2) showed significant difference after analysis of variance in table 9. Osmopriming with (P_1) showed higher water retention capacity (6.98) than P_2 (4.78) indicates hydropriming.

4.3.8.2 Effect of different stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of water retention capacity (Table 9). Highest water retention capacity was found in T_0 (7.67) under control . Lowest data was obtained from T_4 (3.71) under 20% stress condition. The water retention capacity of BARI soybean 6 is gradually decreased when stress increased due to under stress condition water uptake is hampered.

4.3.8.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction effect of osmo and hydropriming significantly influenced the water retention capacity (Table 10). Highest data (8.623) was found when osmopriming under control condition (P_1T_0) which is statistically identical with P_1T_1. Lowest was recorded from P_2T_4 (2.593). When hydroprimed seed under 20% stress condition. Osmopriming can remove the adverse effect of stress though improving metabolic activities of plant.

4.3.9 Coefficient of germination

4.3.9.1 Effect of osmo and hydropriming

Osmopriming (P_1) and hydropriming (P_2) showed significant difference after analysis of variance in table 9. Osmopriming (P_1) showed higher coefficient of germination (19.83) and hydropriming (P_2) showed lower CG (15.57).

4.3.9.2 Effect of different stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of coefficient of germination (Table 9). Highest coefficient of germination was found in T_0 (21.19). Lowest data was obtained from T_4 (14.36) which is statistically identical with T_4. CG was decreasing in different extent when drought stress increasing.

4.3.9.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction of osmo and hydropriming stress concentrations significantly influenced the coefficient of germination (Table 10). Highest value (22.78) was recorded from P_1T_0 (osmopriming under control condition) which is statistically similar with P_1T_1. Lowest

(11.71) was found in P_2T_4 (hydropriming under 20% stress) which is statistically identical with P_2T_3. Under 10% PEG stress osmopriming showed 26.05% drought tolerance than hydropriming. Primed with osmoticum (PEG), it reduced the negative impact of drought stress and maintains a improve performance of germination coefficient.

4.3.10 Vigor index

4.3.10.1 Effect of osmo and hydropriming

Osmopriming (P_1) and hydropriming (P_2) showed significant difference after analysis of variance in table 9. P_1 (132.56) showed higher vigor index than P_2 (103.52). Safiatou, (2012) stated that seedling vigour is improved by using seed priming solutions in sorghum and Bambara groundnut. Also, highest seedling vigour was revealed by osmo-priming (Mannitol priming) in Bambara groundnut and by hydro-priming in sorghum. The increase in seedling vigour due to salicylic acid may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis (Karthiresan *et al*., 1984)

4.3.10.2 Effect of different stress concentrations

Different stress concentrations (0%, 5%, 10%, 15% and 20%) showed significant difference in respect of vigor index (Table 9). Highest vigor index was found in T_{0} (154.5) Lowest data was obtained from T_4 (70.29). Zhang *et al*. (2015) revealed that seed priming with PEG was effective in improving seed germination, seedling establishment and vigor index of sorghum under adverse soil moisture conditions. Osmopriming effectively strengthened the antioxidant system and increased osmotic adjustment, likely resulting in increased stress tolerance.

Table 9. Effect of osmo and hydro priming and stress concentration on the water relation behavior of soybean

Treatments	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
Effect of osmo and hydro priming					
P_1	81.58 a	18.42 b	6.98 a	19.83 a	132.56 a
P_2	73.88 b	26.12 a	4.78 b	15.57 b	103.52 b
LSD $_{(0.01)}$	4.53	1.70	0.29	0.96	6.11
CV (%)	5.61	7.34	4.81	5.21	4.98
Effect of different salt concentrations					
T_0	90.32 a	9.68 e	7.67 a	21.19 a	154.5 a
T_1	86.64 ab	13.36 d	7.08 b	19.50 b	141.6 b
T_2	81.09 b	18.91 c	6.13 c	17.65 c	124.2 c
T_3	72.06 c	27.94 b	4.83 d	15.81 d	99.51 d
T_4	58.55 d	41.45 a	3.71 e	14.36 d	70.29 e
LSD $_{(0.01)}$	7.16	2.68	0.46	1.52	9.65
CV (%)	5.61	7.34	4.81	5.21	4.98

P_1: Osmopriming P_2: Hydropriming

T_0: Control; T_1: stress with 5% PEG; T_2: stress with 10% PEG

T_3: stress with 15% PEG T_4: stress with 20% PEG

4.3.10.3 Interaction effect of osmo and hydropriming and stress concentrations

Interaction of osmo and hydropriming and stress concentrations significantly influenced the vigor index under different stress concentrations (Table 10). Highest analyzed data (164.8) was recorded from P_1T_0 when osmopriming done under control which is statistically similar with P1T1 and lowest (51.03) was found in P_2T_4 when hydropriming done under 20% stress concentration. As drought and/or salinity levels increased, vigour reduced but the priming treatments clearly improved the parameter under drought and salinity conditions so these treatments can be used to improve seed performance of sunflower under normal and stress. (Moghanbashi *et al.*, 2013). Under 10% PEG stress osmopriming showed 26.32% drought tolerance than hydropriming. According to the result of this experiment, under 0% stress condition all the characters germination percentage, shoot length, root length, shoot and root dry weight, relative water content, water retention capacity, coefficient of germination and vigor index except water saturation deficit showed best results. In case of water saturation deficit it showed lowest result under 0% stress condition. Osmoprimed seed under 0% stress condition showed better result than hydro primed seed under 0% stress condition in case of above all parameters except water saturation deficit. It's a negative parameters. Which showed best result under 20% stress condition when hydro primed.

Table 10. Interaction effect of osmo and hydro priming and different stress 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	93.41 a	6.59 f	8.62 a	22.78 a	164.8 a
P_1T_1	89.58 a	10.42 e	8.21 a	21.44 ab	153.7 ab
P_1T_2	84.57 a-c	15.43 d	7.30 b	19.69 bc	138.7 cd
P_1T_3	75.29 cd	24.71 c	5.96 c	18.24 cd	116.1 ef
P_1T_4	65.02 e	34.98 b	4.83 d	17.02 de	89.55 g
P_2T_0	87.23 ab	12.77 de	6.72 b	19.61 bc	144.3 bc
P_2T_1	83.69 a-c	16.31 d	5.95 c	17.56 c-e	129.6 de
P_2T_2	77.61 b-d	22.39 c	4.96 d	15.62 e	109.8 f
P_2T_3	68.82 de	31.18 b	3.70 e	13.37 f	82.93 g
P_2T_4	52.08 f	47.92 a	2.59 f	11.71 f	51.03 h
$LSD_{(0.01)}$	10.13	3.80	0.66	2.14	13.65
CV (%)	5.61	7.34	4.81	5.21	4.98

P_1T_0 : Osmo priming under control; P_1T_1: Osmo priming under 5% PEG stress; P_1T_2: Osmo priming under 10% PEG stress ; P_1T_3: Osmo priming under 15% PEG stress; P_1T_4: Osmo priming under 20% PEG stress P_2T_0 : Hydro priming under control; P_2T_1: Hydro priming under 5% PEG stress; P_2T_2: Hydro priming under 10% PEG stress ; P_2T_3: Hydro priming under 15% PEG stress; P_2T_4: Hydro priming under 20% PEG stress.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted under the laboratory condition of the department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka from 10 March to 2 April, 2018 to study the induction of drought tolerance capability of Soybean (*Glycine max* L.) through Polyethylene Glycol and hydro priming. The present studies were conducted with three different experiments those laid out in Completely Randomized Design (CRD) with five replications.

In the 1st experiment, Soybean seeds of BARI Soybean 5 and BARI Soybean 6 were primed with water, 0%, 5%, 10%, 15% and 20% PEG solution. Results revealed that all the characters viz. germination percentage (GP), shoot length, root length, shoot and root dry weight, relative water content, water saturation deficit, water retention capacity, coefficient of germination and vigor index (VI) were significantly influenced by various PEG concentrations. BARI soybean 6 showed better performance than BARI soybean 5 in case of germination, seedling growth and water relation behavior of soybean except water saturation deficit (WSD) at 10% PEG. BARI soybean 6 showed GP (85.56%), root and shoot length (67.55 mm and 40.12 mm respectively), root and shoot dry weight (83.28 mg and 7.78 mg respectively), RWC (82%), WRC (7.61), CG (19.79%) and VI (84.81%). . But in case of water saturation deficit BARI Soybean 5 primed with distill water shows better result.

In the 2nd experiment, BARI Soybean was primed in 3, 6,9, 12,15 and 18 hours under 10% PEG solution and distilled water. Results revealed that all the characters viz. germination percentage (GP), shoot length, root length, shoot and root dry weight, relative water content, water saturation deficit, water retention capacity, coefficient of germination and vigor index (VI) were significantly influenced by different priming time. Osmopriming showed better result than hydropriming. All the parameters increased with increasing priming time up to 6 h then decreased gradually. The highest GP, shoot length, root length, shoot and root dry weight, relative water content, water retention capacity, coefficient of germination and vigor index were obtained from seeds primed with 10% PEG solution for

6 hr .Whereas the highest water saturation deficit was obtained from when seeds were hydroprimed for 3h.

In the 3rd experiment osmo primed and hydroprimed seeds were germinated under 0%, 5%, 10%, 15% and 20% PEG solution induced drought stress conditions. Results revealed that under stress condition osmopriming showed better result than hydroprimed seeds in case of all the characters viz. germination percentage, shoot length, root length, shoot and root dry weight, relative water content, water retention capacity, coefficient of germination and vigor index except water saturation deficit. Under control condition means without stress or 0% stress condition all the characters germination percentage, shoot length, root length, shoot and root dry weight, relative water content, water retention capacity, coefficient of germination and vigor index except water saturation deficit shows best results. In case of water saturation deficit it showed lowest result under 0% stress condition. Drought tolerance capability increased in osmopriming over hydropriming in case of GP (7.41%), RWC (8.96%), CG (26.05%) and VI (26.32%) under 10% PEG stress. Osmoprimed seed under 0% stress condition showed better result than hydroprimed seed under 0% stress condition in case of above all parameters except water saturation deficit. It's a negative parameters. Which showed best result under 20% stress condition when hydroprimed.

Conclusion

Response of soybean varieties was different to pretreatments. Germination behavior and water relation behavior of both varieties gave the best results when seeds treated with 10% PEG solution compared to hydroprimed seeds and decreased gradually with increasing PEG concentration. All the parameters except water saturation deficit gave the best result from seeds pre-treated with 10% PEG solution for 6 h after that decreased gradually. Seeds pre-soaked with 10% PEG showed better performance than hydroprimed seed in terms of germination behavior and water relation behaviours compared under drought stress. So, soybean seed primed with 10% PEG for 6h is considered as best priming concentration and priming time to induce drought tolerance capability of soybean enhancing germination behavior and water relation behavior of soybean than hydropriming.

Recommendation

In this study when seeds primed with 10% PEG for 6hr gave the best results in respect of germination and growth behavior and water relation behavior of soybean. Under drought stress conditions, it is recommended that seeds should be primed with 10% PEG for 6h. It is suggested that further study should be carried out with different priming agent and field experiments should be done in different location with seeds treated with various priming agents and optimum time of priming could be explored before drawing valid conclusions.

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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 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	190.068**	1497.301**	1071.037**	1823.811**	32.355**
Priming solution (B)	5	502.426**	2398.813**	1286.718**	2362.770**	31.163**
Variety (A) X Priming solution (B)	5	8.074**	33.232**	46.606**	94.152**	0.281**
Error	48	20.884	11.500	4.247	17.518	0.199

**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 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	301.504**	310.583**	13.020**	38.930**	5198.332**
Priming solution (B)	5	753.964**	754.007**	13.031**	45.44**	8355.979**
Variety (A) X Priming solution (B)	5	9.983**	11.374**	0.368**	0.866**	92.845**
Error	48	19.562	1.746	0.125	1.142	20.356

**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 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	503.928**	804.290**	1174.319**	604.586**	24.850**
Priming solution (B)	5	326.507**	951.535**	579.988**	946.422**	12.108**
Variety (A) X Priming solution (B)	5	6.517**	98.668**	29.032**	19.959**	0.263**
Error	24	22.018	17.012	14.793	18.312	0.152

**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 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	281.457**	281.401**	13.764**	30.581**	6164.081**
Priming solution (B)	5	339.139**	339.089**	7.728**	20.538**	4803.047**
Variety (A) X Priming solution (B)	5	5.014**	5.017**	0.157**	0.605**	100.396**
Error	24	19.246	1.498	0.130	1.035	39.061

**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 priming agent and stress concentration

Source of variation	df	Mean square of				
		Germination percentage	Shoot length	Root length	Shoot dry weight	Root dry weight
Variety (A)	1	478.961**	633.053**	1579.922**	417.387**	13.108**
Priming solution (B)	4	685.569**	791.410**	1079.954**	744.412**	22.100**
Variety (A) X Priming solution (B)	4	39.428**	7.177**	29.702**	17.056**	0.029**
Error	20	19.721	19.133	16.057	16.779	0.115

**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 stress concentration

Source of variation	df	Mean square of				
		Relative water content	Water saturation deficit	Water retention capacity	Germination Coefficient	Vigor index
Variety (A)	1	443.752**	443.828**	36.278**	136.107**	6325.203**
Priming solution (B)	4	973.762**	973.825**	15.782**	45.220**	6825.068**
Variety (A) X Priming solution (B)	4	13.179**	13.175**	0.044**	1.071**	76.459**
Error	20	19.005	2.670	0.080	0.851	34.536

*Significant at 1% level of significance

NS Non significant

PLATES

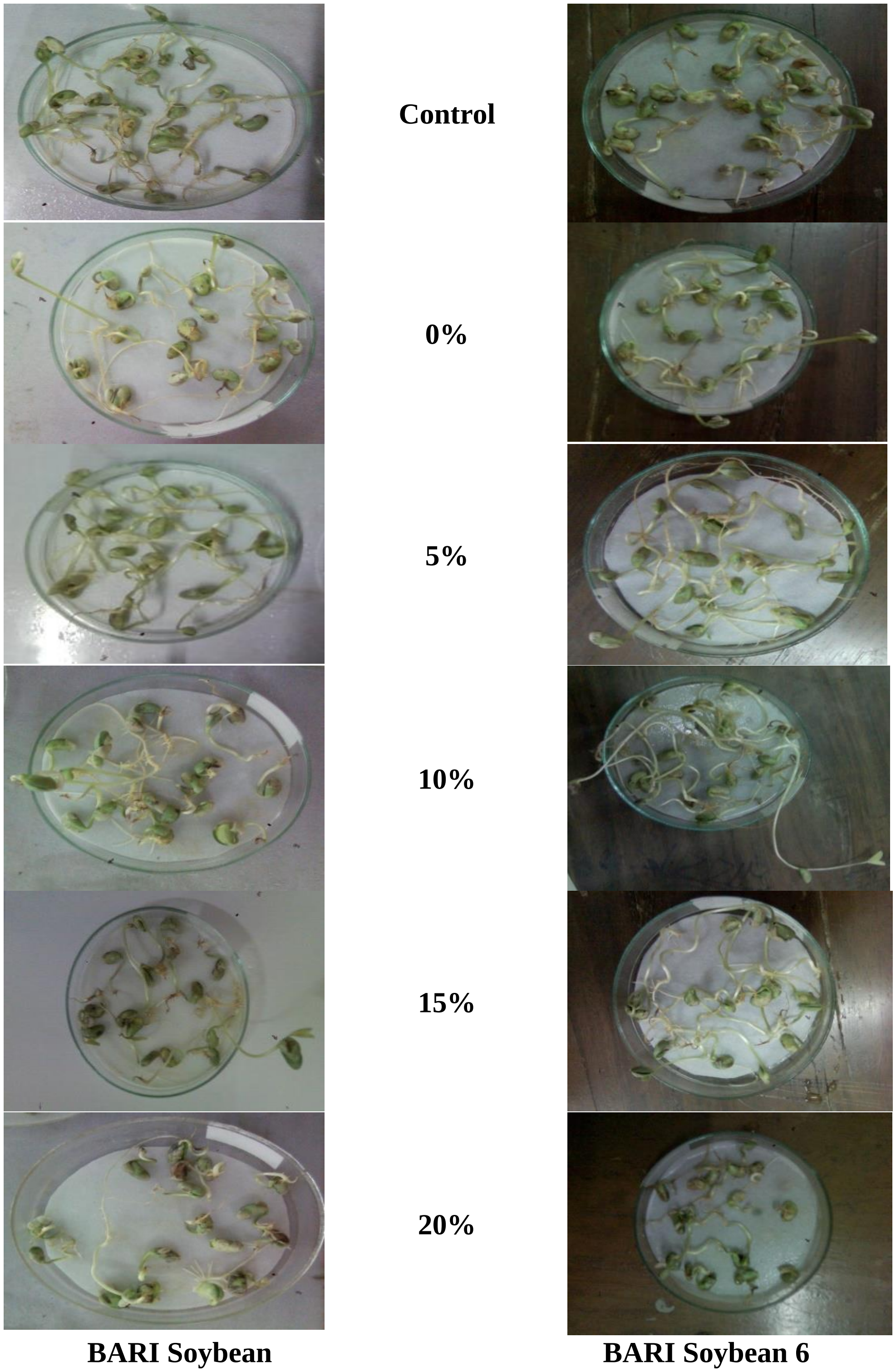

Plate 1: Effect of different concentration of priming solution on germination behavior of soybean varieties (BARI Soybean 5 and BARI Soybean 6)

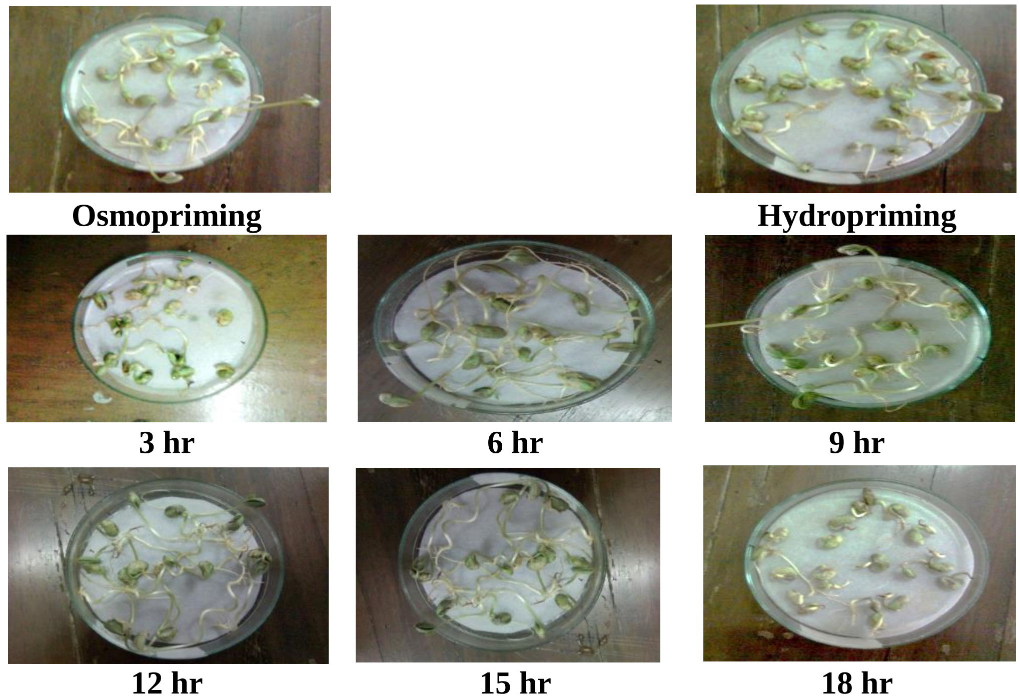

Plate 2: Effect of osmo and hydro priming and priming time on germination behavior of BARI Soybean 6

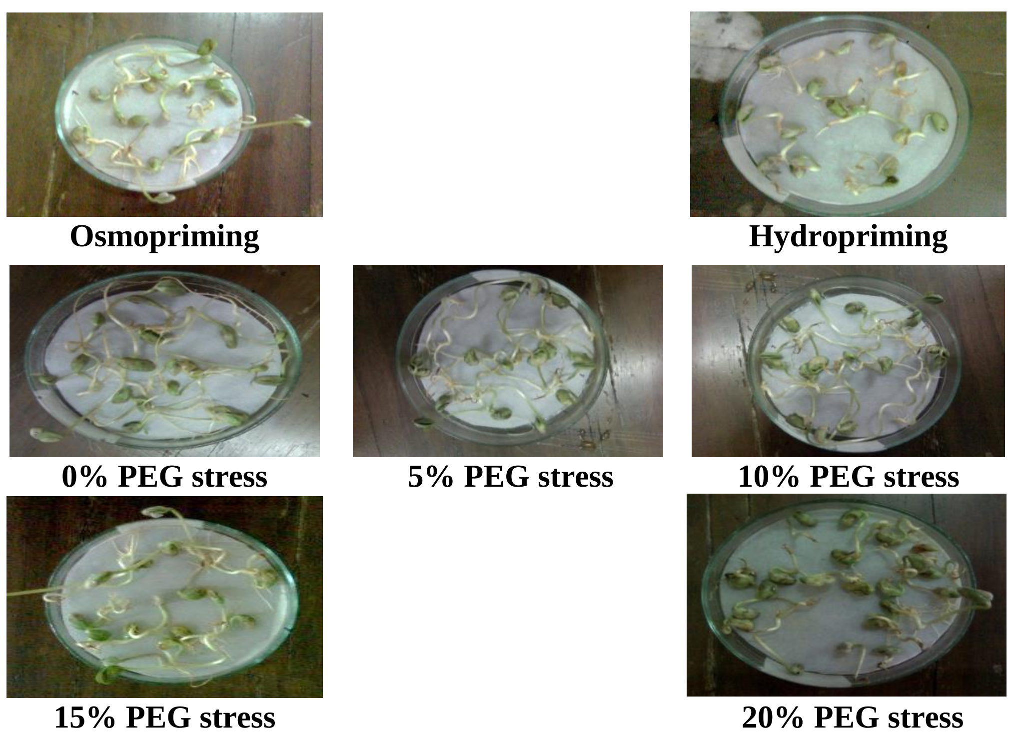

Plate 3: Effect of osmo and hydro priming and stress concentration on germination behavior of BARI Soybean 6