INDUCTION OF DROUGHT TOLERANT CAPABILITY OF MUNGBEAN THROUGH HYDROGEN PEROXIDE (H_2O_2) AND HYDROPRIMING

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CERTIFICATE

This is to certify that the thesis entitled **"INDUCTION OF DROUGHT TOLERANT CAPABILITY OF MUNGBEAN THROUGH HYDROGEN PEROXIDE (H_2O_2) AND HYDROPRIMING''** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.)** in **AGRONOMY**, embodies the results of a piece of *bona fide* research work carried out by **SYEADA JANNATUL FERDUSH SHILA**, Registration No. **12-05013**, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

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

Dated: June, 2018
Dhaka, Bangladesh

Asst. Prof. Marjana Yeasmin
Supervisor

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ABSTRACT

An experiment was conducted at the Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 during the period from July 2017 to September 2017. Three different experiments were conducted in a Completely Randomized Design (CRD) with five replications. Two mungbean varieties namely - BARI Mung 6 and BINA Moog 8 were used as test crops while H_2O_2 and distilled water were used for osmo and hydro priming. Polyethylene Glycol (PEG) was used to induce drought stress. Data on germination rate (GR), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), relative water content (RWC), water saturation deficit (WSD), water retention capacity (WRC), coefficient of velocity of germination (CV) and vigor index (VI) were recorded. The first experiment was carried out to find out the effect of different concentrations of H_2O_2 on germination and growth behavior of two mungbean varieties (BARI Mung 6 and BINA Moog 8). It was found that BARI Mung 6 showed the highest GR (90.31%), SL (145.10 mm), RL (91.25 mm), SDW (64.24 mg), RDW (23.23 mg), RWC (89.25%), WRC (21.81), CV (21.48) and VI (214.20) when the seeds were primed with 2% H_2O_2 and the lowest from BINA Moog 8 with 8% H_2O_2. The second experiment was carried out to optimize the priming time on the germination and growth behavior of BARI Mung 6. It was found that priming with 2% H_2O_2 for 6 hours gave the highest GR (90.21%), SL (139.90 mm), RL (78.29 mm), SDW (59.86 mg), RDW (20.89 mg), RWC (89.17%), WRC (20.72), CV (20.78) and VI (197.40) and the lowest from 2% H_2O_2 for 15 hours. In the third experiment, germination and growth behavior of primed seeds of BARI Mung 6 under 5 different concentrations of PEG i.e. 0%, 5%, 10%, 15% and 20% were evaluated. It was observed that highest GR (91.12%), SL (132.30 mm), RL (90.02 mm), SDW (65.40 mg), RDW (22.94 mg), RWC (88.40%), WRC (20.85), CV (21.11) and VI (202.80) were obtained from primed seeds placed without drought stress. But under drought stress, the highest GR (88.21%), SL (128.20 mm), RL (85.09 mm), SDW (61.32 mg), RDW (20.74 mg), RWC (85.48%), WRC (19.60), CV (19.80) and VI (188.50) were achieved from primed seeds placed with 5% PEG and the lowest from 20% PEG. There were a slow reduction observed with the increasing of drought concentration from 0 to 20% PEG. The result of the study suggest that 2% H_2O_2 with 6 hours priming time effectively alleviated the adverse effect of drought stress.

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
^{0}C	Degree Celsius
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
BINA	Bangladesh Institute of Nuclear Agriculture
CRD	Completely Randomized Design
CV%	Percentage of Coefficient of Variance
e.g.	As for example
et al.	and others
i.e.	that is
kg ha^{-1}	kg per hectare
LSD	Least Significant Difference
mm	Millimeter
mg	Milligram
SAU	Sher-e-Bangla Agricultural University

LIST OF PLATES

CHAPTER 1

INTRODUCTION

Mungbean (*Vigna radiata* L.) is an important legume crop in Bangladesh belonging to the family Fabaceae. It is an excellent source of vegetable protein and edible grain of mungbean is characterized by good digestibility, flavor, high protein content and absence of any flatulence effects (Ahmed *et al*., 2008). The seeds of mungbean are rich in carbohydrate (59.9 mg), protein (24.5 mg), fat (1.2 mg), energy (348 kcal), minerals (8.5 mg), calcium (75 mg), thiamin (0.72 mg), beta-carotene (49 µg) and riboflavin (0.15 µg)/100 g (BARI, 2008). In Bangladesh, it ranks 3rd in terms of protein content and 4th in terms of both acreage and production (MoA, 2014). In Bangladesh per capita intake of pulse is 7.92 g/day which is much lower than the recommendation of FAO, who recommended a minimum intake of pulse by a human should be 80 g/day (FAO, 2013). At present, the area under pulse crop is 0.406 million hectare with a production of 0.322 million tons (BBS, 2013), where mungbean is cultivated in the area of 0.108 million ha land with production of 0.03 million tons (BBS, 2014).

Plant growth and productivity are affected by nature's wrath in the form of various abiotic stress factors. Plants are frequently exposed to the plethora of stress 2 conditions such as salt, drought, oxidative stress and others. All these stress factors are a means for plants and prevent them from reaching their full genetic potential and limit the crop productivity worldwide. Among the abiotic stresses, drought is a major limiting factor for crop productivity all over the world. Drought affects almost every aspect of the physiology and biochemistry of plants which in turn significantly reduces yield (Parida and Das, 2005). Plants create some defense mechanism within itself during the stress condition as a result yield of crops reduce but helps to increase the seed quality. Though stress this positive impact on seed, but it is not good for seed germination especially for drought primed stress. And for this reason seed priming is considered as a promising approach to increase stress tolerance capacity of crop plants including drought (Quamruzzaman *et al.*, 2016).

There are many strategies which have been adopted to overcome the negative effects of drought. A good strategy is the selection of cultivars and species tolerant to drought condition (Pavlousek, 2011). However, an alternative strategy for the possibilities to overcome drought stress is by seed pre-sowing treatments (Ghiyasi *et al.*, 2008).

Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows the seed to imbibe water to proceed to the first stage of germination, but prevents radical protrusion through the seed coat (Yari *et al*., 2012). In mungbean, 4 hours and 8 hours primed seeds showed significant difference in germination percentage and seed moisture percentage over non-primed seeds (Saha *et al.*, 2006). It improves seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination and emergence of different crops (Powell *et al.*, 2000). This also helps seedlings to grow under stress conditions (Ashraf and Foolad, 2005).

Seed priming can be accomplished through different methods such as hydropriming (soaking in distilled water), osmopriming (soaking in osmotic solutions such as PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth inducers (Ethephon and indole acetic acid) (Capron *et al.*, 2000). The beneficial effects of priming may be more evident under unfavorable rather than favorable conditions (Parera and Cantliffe, 1994).

Seed priming is the application of natural or synthetic compounds to seed before germination. Seed priming increases the physiological and biochemical processes necessary for enhancing seed germination and associated traits. Seeds are hydrated to a point that germination processes start but radicle does not emerge out (Bradford, 1986). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. Thus the seeds were prevented from absorbing enough water for radicle protrusion, and suspending the seeds in the lag phase (Taylor *et al.*, 1998). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera and Cantliffe, 1994).These effects of priming are associated with repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes (McDonald, 2000). Priming also enhances the activities of anti-oxidative enzymes in treated seeds (Wang *et al.*, 2003).

Osmopriming or halopriming is an easy, low cost and low risk method which is considered as a common strategy for increasing the rate and uniformity of germination, emergence of seeds and qualitative and quantitative improvement of crop under undesirably environmental conditions and could increase tolerance of plants against salinity stress (Guzmán and Olave, 2004; Iqbal *et al*., 2006). Osmotic potential of polyethylene glycol solution increases the rate, uniformity and percentage of germination of various primed seeds compared with unprimed ones and seed priming in osmotic solution increases the water amount being absorbed by the seed and eventually boosts the rate of germination of the seeds and the growth of the radicle and shoot (Michel and Kaufmann, 1973). Hydropriming improves seedling establishment and vigor of crop plants which in turn yields accelerated growth, flowering, maturity and yield. Hydropriming raises the tolerance of plants against dryness and decreases the damages caused by pests due to the accelerated emergence of seedlings out of soil (Harris *et al*., 1999).

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

Objectives:

- To evaluate the effect of seed priming on germination behavior of mungbean
- To optimize the priming time of the priming chemical on germination behavior of mungbean and
- To evaluate the effect of seed priming on germination and vigor of mungbean under drought stress

CHAPTER II

REVIEW OF LITERATURE

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

2.1 Seed Priming:

Seed priming is a technique of seed enhancement which improves germination or seedling growth and rate of uniformity of the seedling establishment (Taylor *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).

2.2 Effect of seed priming

Singh and Agrawal (1977) found out that wheat which seeds were treated with DW for 12h increased nitrogen uptake for 11 kg/ha.

Misra and Dwivedi (1980) reported that seed soaking in 2.5% KCl for 12 hours before sowing increased wheat grain yield for 15%.

Kulkarni and Eshanna (1988) stated that pre-sowing seed treatment with IAA at 10 ppm improved root length, rate of germination, and seedling vigor.

Paul and Choudhury (1991) observed that seed soaking with 0.5 to 1% solutions with KCl or K_2SO_4 significantly increased plant height, grain yield and its components in wheat genotypes.

Parera and Cantliffe (1994) seed priming is a technology that enhances rapid (7-10 d) emergence and early establishment of wheat. Rapid and uniform field emergence is an essential prerequisite at two irrigated and rainfall conditions to reach the yield potential, quality, and ultimately profit in annual crops. Seed priming has been common pretreatment that reduces the time between seed sowing until emergence and synchronizes seedling emergence.

Foliar application of TRIA has been known to regulate various physiochemical process under both normal and stressful environments in different crop species such as in *Erythrina variegata* L. seedling (Muthuchelian *et al*., 1997), soybean (Krishnan and Kumari, 2008), sweet basil (Borowski and Blamowski, 2009), common duckweed (Kilic *et al*., 2010), maize (Ertani *et al*., 2013), sunflower (Aziz *et al*., 2013), canola (Zulfiqar and Shahbaz, 2013) and wheat (Perveen *et al*., 2014).

Seed priming can be accomplished through different methods such as hydro-priming (soaking in DW), osmopriming (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).

Several investigations confirmed that seed priming has many benefits including early and rapid emergence, stand establishment, higher water use efficiency, deeper roots, increasing in root growth, uniformity in emergence, germination in wide range of temperature, break of seed dormancy, initiation of reproductive organs, better competition with weed, early flowering and maturity, resistance to environmental stresses (such as drought and salinity) and diseases (*Sclerotium rolfsii* L.), higher grain yield in wheat (*Triticum aestivum* L.) (Ghana and Schillinger, 2003), corn (*Zea mays* L.) (Subedi and Ma, 2005) canola (*Brassica napus* L.) (Farhoudi and Sharifzadeh, 2006), pearl millet (*Pennisetum glaucum* L.), chickpea (*Cicer arietinum* L.), rice (*Oryza sativa* L.) (Harris *et al*., 1999) lettuce (*Lactuca sativa* L.) (Cantliffe *et al*., 1984) is reported from field and laboratory studies. Inversely, longevity of primed seed can be decreased (Bruggink *et al*., 1999).

El Tayeb (2006) reported that the organic compounds such as proline and glycinebetaine are known as osmoprotectants that play significant role in the osmotic regulation, protection of membranes and stability of enzymes.

Beckers and Conrath (2007) reported that priming (pre-treatment of seeds or plants by exposure to stressor or chemical compounds, making them more tolerant to later stress events) is potentially an important mechanism of induced resistance in plants against biotic stresses.

Lambers *et al*. (2008) reported that Drought reduces crop yield more than any other abiotic stress. Drought reduces turgor pressure and causes wilting of cells leading to

reduction in the cell expansion and growth (Aslam *et al*., 2014; Srivastava and Srivastava, 2014).

Sadeghi *et al*. (2011) reported that lowest electrical conductivity (EC) value indicate the best priming time. Greater membrane integrity in primed seed was also reported by Afzal *et al*. (2002) for hybrid maize and Rudrapal and Naukamura (1998) for eggplant and radish.

Filippou *et al.* (2012); Hossain and Fujita (2013) showed that priming can modulate abiotic stress tolerance.

Aymen and Cherif (2013) reported that with increasing salinity, emergence traits (total emergence, mean emergence time), growth parameters (plant height, shoot fresh and dry weight) and mineral contents (K^+ and Ca^{2+}) decreased, but to a less degree in primed seeds. At different salinity levels, primed seeds possessed higher emergence and growth rate than control.

Abiotic stresses alter hormonal balance to modulate growth in plants. However, exogenous application of plant growth regulators could regulate hormonal balance to induce stress tolerance such as in spring wheat when under salt stress (Iqbal and Ashraf, 2013).

Dalil (2014) reported that during seed priming in medicinal plants seeds are partially hydrated, so that pre-germinative metabolic activities proceed, while radicle protrusion was prevented, then were dried back to the original moisture level. Primed seeds are physiologically closer to germination and growth after planting than unprimed seeds.

Meriem *et al.* (2014) conducted an experiment to investigate the interactive effect of salinity and seed priming on coriander. The experiment was carried out in completely randomized design with three replications consisting of four coriander genotypes (Tunisian cv, Algerian cv, Syrian cv and Egyptian cv) at two seed conditions (seed priming with 4 g L^{-1} NaCl for 12h or no seed priming). Results showed that seed priming and salinity had significantly ($p<0.05$) affected all the parameters under study. Seed priming with NaCl had diminished the negative impact of salt stress in all cultivars and primed plants showed better response to salinity compared to unprimed plants.

The exogenously applied TRIA maintained water homeostasis through increased uptake of water, essential nutrients, and accumulation/synthesis of compatible organic

compounds in wheat (Perveen *et al*., 2014). Furthermore, foliar application of TRIA at different growth stages stimulated growth in ginger (Singh *et al*., 2011), wheat (Ries, 1991) and chickpea (Singh *et al*., 1991).

Teng *et al.* (2014) stated that the initial exposure to chemical priming agents (such as H_2O_2, ABA, NO, SA etc.) renders plants more tolerant to abiotic stresses.

Peifang *et al*. (2015) stated that drought-induced oxidative stress oxidizes various macromolecules such as proteins, lipids and nucleic acid and damages cell membranes However, plants have adapted antioxidative defense system comprising of enzymatic antioxidants and some low-molecular weight antioxidants to cope with drought-mediated oxidative stress (El-Beltagi and Mohamed, 2013).

2.3 Effect of hydrogen peroxide in plants

Orozco-Cárdenas *et al.* (2001) stated that hydrogen peroxide is being investigated for its role as a second messenger in the induction of several defense genes in response to biotic stresses such as pathogen infection and wounding.

Dat *et al.* (1998) suggested that H_2O_2 accumulation induces thermo tolerance.

Munné-Bosch *et al.* (2001) reported that H_2O_2 accumulates in senescing leaves of drought-susceptible plants exposed to drought stress.

Sairam *et al.* (2002) and Kathiresan *et al.* (2006) reported that hydrogen peroxide (H_2O_2) is one of the main chemicals which are induced to elevate in plants by biotic and abiotic stresses. Higher levels of H_2O_2 usually result in toxicity to cellular membrane system and damages to plant cells.

Kovtun *et al.* (2000) and Hung *et al.* (2005) stated that H_2O_2 directly regulates the expression of numerous genes involved in plant defense and the related pathways such as antioxidant enzymes, defense proteins and transcription factors. Hence, H_2O_2 signaling functions importantly in plant growth and development and defense against environmental stresses.

Murphy *et al.* (2002) reported that exogenous application of H_2O_2 increases chilling tolerance by enhancing the glutathione level of mungbean seedlings.

Azevedo Neto *et al.* (2005) reported that the addition of H_2O_2 to the nutrient solution induces salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize as an acclimation response.

Hossain *et al.* (2015) reported that exogenous H_2O_2 can enhance abiotic stress tolerance either by modulating ROS detoxification or regulating multiple stress-responsive pathways and gene expression.

Ishibashi *et al.* (2011) showed that exogenous application of H_2O_2 promotes the up-regulation of stress responsive genes and increases drought stress tolerance.

Terzi *et al.* (2014) found that exogenous H_2O_2 pretreatment induced osmotic stress tolerance by increasing soluble sugar, proline and polyamine levels in maize (*Zea mays* L.) seedlings.

Liu *et al.* (2003) reported that H_2O_2 pre-treatment of two cucumber varieties improved osmotic stress resistance by activating antioxidant system.

Abass and Mohamed (2011) studied the effects of H_2O_2 pre-treatment on the seeds of common bean seedlings (*Phaseolus vulgaris* L.) and analyzed drought tolerance in seedlings.

Petrov and Van Breusegem (2012) stated that the signaling role of H_2O_2 is well established, particularly with reference to plant processes like stress acclimation, antioxidative defense, cell wall cross-linking, stomatal behavior, phytoalexin production, regulation of the cell cycle, and photosynthesis. So, the toxicity or danger associated with H_2O_2 on one hand and signaling cascades on other make it a versatile molecule whose concentration needs to be tightly controlled within plant cells.

Gechev and Hille (2005) reported that at higher concentrations H_2O_2 causes oxidative damage to important cellular metabolites, whereas at lower concentrations it initiates cell signaling.

Azevedo Neto *et al.* (2005) reported that the pre-treatment with an appropriate level of H_2O_2 can enhance abiotic stress tolerance through the modulation of multiple physiological processes, such as photosynthesis, and by modulating multiple stress-responsive pathways, such as the ROS and methyl glyoxal (MG) detoxification pathways.

Hossain and Fujita (2013) observed that the exogenous application of H_2O_2 can induce tolerance to salinity, drought, chilling, and high temperatures, and heavy metal stress, all of which cause elevated H_2O_2 production.

Uchida *et al.* (2002) studied the effects of H_2O_2 and nitric oxide (NO) pre-treatments on oxidative stress in rice (*Oryza sativa*) plants under salt or heat stress. Their results showed that seedlings treated with low concentrations (<10 μM) of H_2O_2 or NO resulted in greener leaves and a higher photosynthetic activity than that of the control plants under conditions of salt or heat stress. It was also shown that pre-treatment induced increases in ROS scavenging enzyme activities and increased expression of genes encoding pyrroline-5-carboxylate synthase, sucrose- phosphate synthase, and the small heat shock protein 26.

Azevedo Neto *et al.* (2005) found that supplementation of the nutrient solution with H_2O_2 induced salt tolerance in maize plants, by enhancing antioxidant metabolism and reducing lipid peroxidation in both leaves and roots.

Wahid *et al.* (2007) reported that exogenous H_2O_2 improved salinity tolerance in *Triticum aestivum* when seeds were soaked in H_2O_2 (1-120 μM, 8h) and subsequently grown in saline conditions (150 mM NaCl). H_2O_2 levels in the seedlings, arising from H_2O_2-treated seeds were markedly lower when grown under saline conditions than control seedlings from seeds not treated with H_2O_2 and also exhibited better photosynthetic capacity. These results suggest that seedlings from H_2O_2-treated seeds had more effective antioxidant systems than found in untreated controls.

Fedina *et al.* (2009) reported that *Hordeum vulgare* seedlings pre-treated with H_2O_2 (1 and 5mM) had higher rates of CO_2 fixation and lower malondialdehyde (MDA) and H_2O_2 contents, following exposure to 150 mM NaCl for 4 and 7 days, when compared with seedlings subjected to NaCl stress only.

Gondim *et al.* (2010) evaluated the roles of H_2O_2 on the growth and acclimation of maize (*Zea mays*) triple hybrid (BRS3003) seedlings exposed to salinity stress, with three consecutive studies. In the first studies, H_2O_2 accelerated the percentage germination of seeds at 100 mM, but not at 500 mM H_2O_2. In second study, pre-treatment of seeds with H_2O_2 caused an up-regulation of APX and CAT activities after 30 h. In contrast, GPX activity was lower in seeds primed with H_2O_2 for 12, 24, 30, 36, and 42 h as compared with the seeds primed with water only. The activity of SOD was

not affected by pre-treatment of seeds with H_2O_2, except for the 24 h pre-treatment. In the third experiment, seeds were pre-treated by soaking in 100 mM H_2O_2 for 36 h, or in distilled water (DW), and then grown in a culture solution with or without salt stress (80 mM NaCl). Their findings showed that priming of seeds with H_2O_2 increased seedling tolerance to salinity, with seedlings demonstrating improved growth rates. The differences in the levels of antioxidant enzyme activities detailed above may explain the higher salinity tolerance of seedlings from seeds pre-treated with H_2O_2.

Li *et al.* (2011) reported that exogenously applied H_2O_2 (0.05 μM) reduced the MDA content, enhanced the GSH content and increased the activities of APX, CAT, SOD, and POD in wheat seedlings under salt stress.

Hameed *et al.* (2012) indicating that cellular defense antioxidant mechanisms are enhanced by the exogenous application of H_2O_2. Up-regulation of the activities of CAT and SOD following the exogenous application of H_2O_2 (0.5 mM) was also observed in oat (*Avena sativa*) plants under salt stress (Xu *et al*., 2008).

Gondim *et al.* (2012) found that foliar H_2O_2 priming was effective in minimizing salt stress in maize and analysis of the antioxidant enzymes CAT, GPOX, APX, and SOD revealed that the H_2O_2 foliar spray increased the activities of all of these enzymes. CAT was found to be the most highly responsive of the above enzymes to H_2O_2, with high activities observed (48 h) after treatment, while GPX and APX responded much later (240 h after treatment). Lower MDA levels were also detected in maize plants with higher CAT activities, which may have resulted from the H_2O_2 detoxifying function of this enzyme.

Gondim *et al.* (2013) studied the influence of exogenous H_2O_2 application on AsA and GSH metabolism, relative chlorophyll content, relative water content (RWC), and gas exchange in *Zea mays* grown under salinity. Photosynthesis and transpiration, stomatal conductance, and intercellular CO_2 concentrations all declined in plants under salt stress; however, the negative impact of salt stress was not as great in plants sprayed with H_2O_2. In addition, H_2O_2-sprayed plants had higher RWCs, relative chlorophyll contents and lower leaf H_2O_2 accumulation, which correlated positively with improved gas exchange, compared with control plants under conditions of NaCl stress. The non-enzymatic antioxidants AsA and GSH did not appear to play any obvious roles as ROS scavengers.

Ashfaque *et al.* (2014) conducted an experiment to study the role of H_2O_2 played in mitigating salt stress in wheat (*Triticum aestivum* L.) plants. Treatment of plants with H_2O_2 positively influenced plant growth under saline and non-saline conditions. The application of 50 or 100 μM H_2O_2 reduced the severity of salt stress, with reductions in both Na^+ and Cl^- ion levels and an increase in proline content and in N assimilation. Improved water relations, increased levels of photosynthetic pigments and greater growth rates were also observed in H_2O_2 under salt stress when compared with untreated plants. Under non-saline conditions application of H_2O_2 also improved all the parameters detailed above. Treatment with 100 μM H_2O_2 provided maximal protection for wheat plants grown under non- saline conditions and also alleviated the effects of salt stress in plants grown under saline conditions.

Sathiyaraj *et al.* (2014) found that *Panax ginseng* seedlings treated with 100 μM H_2O_2 for 2 days showed enhanced salinity tolerance and increased activities of APX, CAT, and guaiacol peroxidase. Other oxidative parameters such as MDA levels and endogenous H_2O_2 and O_2^- levels were lower in H_2O_2 treated salt-stressed seedlings. Seedling dry weight, and chlorophyll and carotenoid contents were also greater in H_2O_2 treated seedlings than in untreated controls, when seedlings were subjected to salt stress.

De Carvalho (2013) stated that drought stress is widely thought to induce oxidative stress by increasing the levels of H_2O_2 and singlet oxygen.

Jing *et al.* (2009) investigated the capacity of H_2O_2 priming to promote drought tolerance in Cucumber plants. Drought stress resulted in cucumber plants with round chloroplasts, and indistinct chloroplast membranes and thylakoids. While H_2O_2 priming did not change chloroplast ultra-structure, priming did increase the activities of the antioxidant enzymes SOD, CAT, GPOX, APX, DHAR, GR, and the levels of AsA and GSH, resulting in lower levels of MDA, H_2O_2 and O_2^-.

Ishibashi *et al.* (2011) showed that spraying plants with H_2O_2 could alleviate the symptoms of drought stress in soybean. The RWC content, photosynthetic rate and stomatal conductance of drought-stressed leaves in plants sprayed with H_2O_2 were all higher than in leaves sprayed with DW. In contrast to spraying with DW, spraying with H_2O_2 caused an increase in the expression of *galactinol synthase (GolS)* and *d-myo-inositol 3-phosphate synthase 2 (GmMIPS2)* genes, which are responsible for the

synthesis of oligosaccharides. These findings indicated that H_2O_2 spraying enabled soybean plants to avoid drought stress by helping to maintain leaf water levels, and that leaf water retention was probably due to increased oligosaccharide biosynthesis rather than rapid stomatal closure.

Abbas and Mohamed (2011) studied the effects of priming seeds with H_2O_2 on the drought tolerance of common bean seedlings (*Phaseolus vulgaris* L.). A significant decrease in plant growth parameters, photosynthetic pigments, and the total carbohydrate content was observed in response to drought stress. In contrast, a significant increase in compatible solutes, polyamine and antioxidant levels, and abscisic acid (ABA) contents were observed in plants in response to drought stress. H_2O_2-priming of seeds enhanced all of the above parameters in seedlings grown under drought conditions when compared with the seedlings of water-treated seeds.

Liao *et al.* (2012) studied the beneficial roles of exogenous NO and H_2O_2 in marigold (*Tagetes erecta* L.) adventitious root formation in response to drought. NO or H_2O_2 treatment reduced the damage to mesophyll cell ultra-structure caused by drought stress. NO or H_2O_2 treatment also increased leaf chlorophyll contents, chlorophyll fluorescence parameters, and hypocotyl soluble carbohydrate and protein contents, while reducing starch contents. These findings demonstrate that NO or H_2O_2 can protect mesophyll cell ultra-structure from damage, improve the photosynthetic performance of leaves and mitigate the negative effects of drought stress, by enhancing nitrogen and carbohydrate accumulation.

Hossain and Fujita (2013) examined the potential biochemical mechanisms of H_2O_2 priming-induced drought tolerance in mustard (*Brassica juncea* L.) seedlings by investigating ROS scavenging and MG metabolism. Eight-day-old seedlings were pre-treated with a low concentration (50 μM) of H_2O_2 for 24 h prior to the imposition of drought stress for 48 h. H_2O_2 priming enhanced cell membrane stability in leaf tissues under drought stress, by reducing tissue MDA contents. The levels of endogenous H_2O_2, in H_2O_2 pre-treated, drought stressed-seedlings were markedly lower than that of seedlings subjected to drought stress without H_2O_2 pre-treatment. Lower activities of APX, CAT, and GlyII were observed in response to drought stress, whereas DHAR, GPX, and GlyI activities significantly increased. AsA, GSH, and GSSG levels increased significantly, whereas the GSH/GSSG ratio decreased in drought-stressed

seedlings. Surprisingly, H_2O_2 pre-treated drought-stressed seedlings maintained significantly higher APX, GR, CAT, GST, and GlyII activities, as well as a higher GSH/GSSG ratio compared with seedlings under drought only. These results show that H_2O_2 priming can activate both ROS and MG detoxification pathways and modulate the tolerance of seedlings to water deficit.

Ashraf *et al.* (2014) investigated the beneficial roles of exogenous H_2O_2 on drought stress tolerance in maize. Maize seedlings were pre-treated with different concentrations of H_2O_2 and grown under conditions of water stress. Higher germination percentages were found in seeds soaked in 140 mM H_2O_2. Drought led to a sharp decrease in photosynthetic pigments, whereas the levels of H_2O_2, lipid peroxidation and AsA increased. The activities of CAT, SOD, and POX rapidly increased. Importantly, the 140 mM H_2O_2 treatment reduced photosynthetic pigment degradation and lipid peroxidation and increased the activities of antioxidant enzymes and AsA levels. The beneficial influence of exogenous H_2O_2 treatments have also been observed in plants under osmotic stress.

Liu *et al*. (2010) studied the effects of exogenous H_2O_2 on osmotic stress-induced alterations in the ultra-structures of chloroplasts and mitochondria in two cucumber (*Cucumis sativus* L.) varieties. Osmotic stress caused the degradation of chloroplast and mitochondrial membranes in both cucumber genotypes and increased MDA levels. Osmotic stress and exogenous H_2O_2 both increased MnSOD, GPX, CAT, GPOX, APX, GR, MDHAR, DAHR activities and levels of the antioxidants AsA and GSH. The combined effects of osmotic stress and exogenous H_2O_2 resulted in the highest antioxidant levels in both cucumber ecotypes.

Liu *et al*. (2010) proposed that pre-treatment with H_2O_2 increased antioxidant levels in the leaves of cucumbers, thereby decreasing MDA levels, and protecting the ultrastructure of most chloroplasts and mitochondria in plants under osmotic stress.

Terzi *et al.* (2014) found that exogenous H_2O_2 (10 mM) pre-treatment induced osmotic stress tolerance in maize (*Zea mays* L.) seedlings. H_2O_2 treatment caused a decrease in MDA levels and stomatal conductance, whereas an increase in endogenous H_2O_2, leaf water potential, ABA concentration, and metabolite levels, including soluble sugars, proline, and polyamines were observed. Osmotic stress caused a decline in leaf water potential and stomatal conductance, but the levels of MDA, H_2O_2, metabolite levels and

the ABA content increased. Importantly, H_2O_2 pre-treated osmotical stressed seedlings showed improved water status and stomatal conductance, as well as accumulation of MDA, H_2O_2, ABA, and metabolites. These results demonstrate that H_2O_2 pre-treatment induces osmotic stress tolerance by increasing soluble sugar, proline and polyamine levels.

Prasad *et al.* (1994) reported that addition of H_2O_2 modulated chilling tolerance, due to a transient increase in H_2O_2-activated acclimation mechanisms. The authors suggested that H_2O_2 has dual effects on maize plants during acclimation to chilling; it serves as a signal to induce the synthesis of ROS-scavenging enzymes, and in non-acclimated seedlings it accumulates to higher levels and acts as a destructive agent. Additionally, it was reported that both H_2O_2 and SA could mediate the induction of protective mechanisms against abiotic stresses. SA pre-treatment induced an increase in H_2O_2 concentrations that in turn triggers an increase in antioxidant enzyme activities and eventually leads to higher tolerance to chilling stress in maize seedlings.

Yu *et al.* (2003) showed that a transient oxidative shock, induced by exogenous H_2O_2, effectively increased chilling tolerance in mungbean (*Vigna radiata* L. cv. V3327) seedlings. Seedlings pre-treated with 200 mM H_2O_2 had increased survival rates (from 30 to 70%) and lowered EL (86 to 21%). Importantly, the endogenous level of H_2O_2 was not affected by exogenous application of H_2O_2. Surprisingly, exogenous H_2O_2 repressed the stimulation of ROS detoxifying enzymes APX and CAT; however, GSH levels increased significantly under both chilling and control conditions. Pre-treatment of mungbean plants with both ABA and H_2O_2 showed no synergistic effect on GSH content. The authors concluded that H_2O_2-mediated chilling tolerance in mungbean plants might be mediated by an increase in GSH content that is independent of ABA.

Hung *et al.* (2007) showed that H_2O_2 pre-treatment induced chilling tolerance in chilling sensitive mungbean seedlings (*V. radiata* L. cv Tainan Number 5). Seedlings pre-treated with 200 mM H_2O_2 or cold-acclimated (10^0C for 48 h in the light) showed lower electrolyte leakage (EL) compared to seedlings subjected to chilling stress (4^0C for 36 h) without H_2O_2 treatment or cold acclimation. Chilling tolerance induced by H_2O_2 appeared to depend on the accumulation of GSH, as tolerance could be reversed by pre-treatment with buthionine sulfoximine (BSO). In contrast, tolerance induced by cold- acclimation was neither accompanied by the accumulation of GSH nor reversed

by BSO, suggesting that there are at least two independent mechanisms for developing chilling tolerance.

2.4 Effect of seed priming on germination parameters

2.4.1 Total Germination (%)

Afzal *et al*. (2011) and Wahid *et al*. (2007) stated that seed priming, a controlled hydration process followed by re-drying is pragmatic approach to counteract the salinity effects in many crops because of its simplicity, low cost and effectiveness.

Afzal *et al*. (2011); Afzal *et al*. (2006) and Farooq *et al*. (2006b) reported that improved germination percentage and uniformity of growth following reduction in emergence time and increased yields in many field crops including rice due to seed priming.

Sun *et al*. (2010) reported that priming with PEG at moderate concentration resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination.

Demir Kaya *et al*. (2006) reported that seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition.

Demir and Okcu (2004) observed aerated hydration treatment of pepper at 250^0C followed by drying increased germination percentage.

Wang *et al*. (2009b) observed the final germination percentage of *Melilotus officinalis* was much higher than that of *M. sativa* and *A. adsurgens* at 300 mM NaCl and the germination rate in six alfalfa cultivars was also differentially affected by treatments with 200 mM NaCl and 35% PEG (Wang *et al*., 2009a).

Korkmaz and Pill (2003) reported that potassium di hydrogen phosphate (KH_2PO_4) priming improved the germination synchrony of low vigor cultivar in lettuce. Seed primed with potassium di hydrogen phosphate (KH_2PO_4) and water improved germination percentage compared to untreated seed treatments.

Ghana and Schillinger (2003) stated that KH_2PO_4 seed priming and water treatments enhanced germination in wheat under normal condition compared to untreated seed.

Salinas (1996) and Basra *et al*. (2003) reported that seed priming techniques improve germination percent, emergence and seedling stand.

Ajouri *et al*. (2004) stated that priming induces a range of biochemical changes in the seed that required initiating the germination process i.e. breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation.

Rowse (1995) reported that primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage and a reduction in the inherent physiological heterogeneity in germination.

McDonald (2000) stated that primed seeds acquire the potential to rapidly imbibe and revive the seed metabolism thus enhancing the germination rate. Khalil *et al*. (2001) carried out successful seed priming with PEG in soybean.

Chen and Arora (2011) stated that PEG induced osmopriming results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach.

Hur (1991) reported that osmo priming with 20% PEG-8000 for 2d at 10^0C of seeds of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions.

Posmyk and Janas (2007) stated that in *Vigna radiata*, hydropriming along with proline and hydro priming can be used as a safe priming method for improving seed germination and growth of seedlings at low temperature and also allowing fast repair of injuries caused by stress.

Zheng *et al*. (2002) observed more uniform germination and emergence in primed seeds on canola (*B. campestris*), wheat (*Triticum aestivum*) (Nayyar *et al*., 1995) and rice (*Oryza sativa*) (Lee and Kim, 2000; Basra *et al*., 2003) who described improved germination rate and percentage in seeds subjected to hydro priming and seed hardening for 24 hours (Farooq *et al*., 2006b).

Coolbear and Grierson (1979) reported that in seeds of tomato cultivars, seed priming resulted in higher germination rate as a result of higher levels of nucleic acid. They

stated that increase in nucleic acid content in primed seeds was due to an enhanced ribonucleic acid (RNA) synthesis during and after priming treatment.

Zhou *et al*. (2009) reported that ascorbic acid as another important vitamin used for priming due to its antioxidant nature. It has already been proved that a high level of endogenous acrobat is essential to maintain the antioxidant capacity that protects plants from oxidative stress.

Srivastava *et al*. (2010) observed increased rate of germination as compared to non-primed seeds in Indian mustard due to ABA priming.

Farahbakhsh (2012) observed that fennel seeds showed better germination under low water potential due to priming with salicylic acid.

Gul and Khan (2003) observed that priming with growth regulators like fusicoccin, thiourea, kinetin, and ethephon alleviated the inhibitory effects of salinity on the germination in *Salicornia utahensis* (a halophyte); whereas GA_3, proline, betaine and nitrate had little effect on germination at all salinities.

Eisvand *et al*. (2010) reported that seeds primed with gibberellin (GA) and abscissic acid (ABA) of *Agropyron elongatum* exhibited induced CAT and SOD activities under drought conditions when compared to unprimed seeds.

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

Parera and Cantliffe (1994) stated that the beneficial effects of priming is more evident under unfavorable rather than favorable conditions.

Saha *et al*. (2006) reported that seed priming of mungbean for 4 hours and 8 hours showed significant difference in germination percentage and seed moisture percentage over non-primed seeds.

Lee and Kim (2000) observed that increase in germination rate by 10-15% through increasing total sugar content and α-amylase activity in aged rice seeds due to hardening (150 g seeds soaked in 500 mL water for 18 and 24 hours).

Basra *et al*. (2005) stated that primed seeds usually exhibit an increased germination rate, greater germination uniformity, and at times, greater total germination percentage.

2.4.2 Mean germination time (days)

Farooq *et al*. (2006b); Afzal *et al*. (2006) and Afzal *et al*. (2011) reported that priming is used to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such earlier and synchronized emergence often leads to uniform stands and improved yield. Like germination percentage, prime seeds had lower mean emergence time (MET) compared with non-primed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassanpouraghdam *et al*., 2009 and Sivritepe *et al*., 2003).

Ashraf and Foolad (2005) stated that seed priming techniques reduce emergence time, accomplish uniform emergence and give better crop stand in many horticultural and field crops.

Finch-Savage *et al*. (2004) observed that seed priming decreased the optimum temperature and ceiling temperature for germination and also helped in advancing the germination time and did not decrease the final percentage emergence.

Rashid *et al*. (2006) reported that on-farm seed priming (soaking seeds in water prior to sowing) showed effective results in producing early germination, better establishment and increased yields in a wide range of crops in diverse environments. It had been a common pretreatment that reduces the time between seed sowing until emergence and synchronizes seedling emergence (Parera and Cantliffe, 1994).

Basra *et al*. (1989) stated that corn seed primed with polyethylene glycol (PEG) or potassium salt (KH_2PO_4 or KNO_3) resulted in accelerated germination.

Janmohammadi *et al*. (2008) presented hydro priming as a suitable, cheap and easy seed invigoration treatment for inbreed lines of maize, especially when germination is affected by salinity and drought stress.

Dubrovsky (1996) reported that hydro priming has been shown to result in the earlier germination of desert cacti, *Allium porrum* (Ashraf and Bray, 1993), pyrethrum

(*Tanacetum cinerariifolium*) (Li *et al*., 2011), and coriander (Rithichai *et al*., 2009). Moradi Dezfuli *et al*. (2008) revealed hydro primed seeds for 36 h had lowest values (T50 and MGT).

Fotia *et al*. (2008) reported that maize caryopses resulted in more homogenous and faster seed germination as compared to the control due to osmotic seed priming.

Gray *et al*. (1990) reported that (-0.5 MPa) lowered the mean germination time of seeds of lettuce, carrot and onion.

Goobkin (1989) and Ozbingol *et al*. (1999) reported that tomato seeds treated with PEG 6000 solution germinate faster than untreated seeds and this is due to more rapid water uptake.

Arif (2005) stated that primed seed emerge early due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed.

Yamauchi and Winn (1996) reported that seed priming techniques help in dormancy breakdown possibly by embryo development and leaching of emergence inhibitors which resulted in an earlier start of emergence.

2.4.3 Germination index

Farooq *et al*. (2008) concluded that in addition to better establishment, primed crops grew more vigorously, flowered earlier and yielded higher.

Ruan *et al*. (2002) observed that seed priming with KCl and $CaCl_2$ had improved germination index of rice.

Dell Aquila and Tritto (1991); Donaldson *et al*. (2001) concluded that seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops particularly seeds of vegetables and small seeded grasses.

Rashid *et al*. (2006) reported that priming enhanced germination, better establishment and increased yields in many diverse environments for a number of crops.

Arif *et al*. (2008) reported that seed priming enhanced germination which may be attributed to repair processes, a build-up of germination metabolites or osmotic adjustments during priming treatment.

Maiti *et al*. (2006) reported that osmotic priming of maize caryopses in copper sulphate, zinc sulphate, manganese sulphate or boric acid induced high levels of seed germination.

Caseiro *et al*. (2004) stated that hydro priming was found to be the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 hours compared to 48 hrs.

Harris *et al*. (1999) concluded that seed priming improved germination and later growth of different crops species such as in maize, rice and chickpea.

2.4.4 Coefficient of velocity

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

2.4.5 Energy of emergence (%)

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

Asgedom and Becker (2001) concluded that processes that precede the germination are trigged by priming and persist following the re-desiccation of the seeds. Thus upon seeding, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995).

Harris *et al*. (1999); Mussa *et al*. (1999); Harris *et al*. (2000) and Khan *et al*. (2005) reported that seed priming resulted in improved stand established which can reportedly increase the drought tolerance, reduce pest damage and increase crop yield in cereals and legumes.

Cramer (2002) stated that priming of seed stimulates many of the metabolic processes involved in the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously and perform better in adverse conditions.

Farooq *et al*. (2008) reported that priming of seed improves emergence, stand establishment, tillering, allometry, grain and straw yields, and harvest index.

Seed priming has been found a double technology to enhance rapid and uniform emergence, and to achieve high vigor and better yields in vegetables and floriculture (Dearman *et al*., 1987; Parera and Cantliffe, 1994; Bruggink *et al*., 1999) and some field crops (Hartz and Caprile 1995; Chiu *et al*., 2002; Murungu *et al*., 2004; Basra *et al*., 2005; 2006; Kaur *et al*., 2005; Farooq *et al*., 2006a).

Harris *et al*. (1999) reported that enhanced phenology in mungbean due to primed seed is associated with faster emergence and reduced germination imbibition periods.

Sivritepe *et al*. (2003); Demir Kaya *et al*. (2006) and Foti *et al*. (2002) reported that seed priming resulted in more germination speed especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon.

Iqbal and Ashraf (2007) stated that seed priming techniques such as hydro priming, hardening, osmo-priming, osmo hardening, hormonal priming and hydro priming have been used to accelerate emergence more vigorous plants and better drought tolerance in many field crop like wheat, chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006), cotton (Casenave and Toselli, 2007), triticale (Yagmur and Kaydan, 2008).

Dell Aquila and Taranto (1986); Misra and Dwibedi (1980) stated that potassium di-hydrogen phosphate (KH_2PO_4), polyethylene glycol (PEG 6000) and potassium chloride (KCl) have been introduced as the osmoticum which have shown good potential to enhance germination, emergence, growth, and/or grain yield of wheat.

Harris *et al*. (2001) reported that water has also been used successfully as a seed priming medium for wheat.

Ghiyasi *et al*. (2008) reported that seeds of maize (*Zea mays* L.) osmo primed with polyethylene glycol (PEG 8000) at -0.5 MPa osmotic potential had improved emergence, grain and biological yields compared with other treatments. The probable reason for early emergence of the primed seed may be due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005).

Farooq *et al*. (2006b) observed that halo priming with $CaCl_2$ significantly improved emergence and seedling growth in Shaheen Basmati whereas as $CaCl_2$ and KCl proved better in case of Basmati-2000 which could be related to dormancy breakdown of rice seeds due to enhanced seed K and Ca concentration and amylase activity.

Zheng *et al*. (2002) observed earlier and uniform emergence in rice (*Oryza sativa* L.) seeds osmoprimed with KCl and $CaCl_2$ and mixed salts under flooded conditions.

Nascimento and West (1999) observed early germination of primed seeds in muskmelon (*Cucumis melo*) but not recorded any improvement in the growth of seedlings under laboratory conditions.

2.5 Effect on growth parameters

2.5.1 Shoot length (mm)

Demir and Mavi (2004) reported that seeds of watermelon primed with KNO_3 can be used to increase germination; and in tomato, seed priming with KNO_3 increased germination percentage, germination index, root length, shoot length and seedling fresh weight (Nawaz *et al*., 2011).

Kaur *et al*. (2005) reported that seeds of chickpea alleviated the adverse effects of water deficiency and salt stress on seedling growth upon osmo and hydro primed with mannitol and water. The treatment of seeds with water, 2% and 4 % mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions.

Akbari *et al*. (2007) reported that auxin treatments increased the hypocotyl length, seedling fresh and dry weight and hypocotyl dry weight in wheat seed germination.

Kaur *et al*. (2002) stated that hydro priming has resulted in 3 to 4 fold increases in root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition.

2.5.2 Root length (mm)

Iqbal and Ashraf (2007) stated that seed priming techniques such as hydro priming, hardening, osmopriming, osmo hardening, hormonal priming and hydro priming have been used to accelerate emergence more vigorous plants and better drought tolerance

in many field crop like wheat, chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007).

Gao *et al*., 2002) reported that ABA-primed seeds of *Brassica napus* exhibited earlier (2-7 days) germination and higher final percent radicle protrusion than non-primed control seeds under salt (100 mM NaCl) or water stress (20% PEG 8000) and at a low temperature (8^0C).

Kulkarni and Eshanna (1988) found that seed priming with IAA at 10 ppm improved root length, rate of germination, and seedling vigor.

Kathiresan *et al*. (1984) reported that sunflower seeds in response to priming with $CaCl_2$ resulted in maximum root and shoot growth; seedling height and field emergence.

Kaya *et al*. (2006) stated that seed priming improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells.

Ashraf and Abu-Shakra (1978) observed that wheat seeds may improve germination and emergence (and may promote vigorous root growth) due to osmo priming and hydro priming (Carceller and Soriano, 1972).

Kathiresan and Gnanarethinam (1985) found that on hydro priming, sunflower seeds produced the largest roots compared to other seed treatments. This means that during priming, seeds would be simultaneously subjected to processes of repair and deterioration and force between the two determined the success or failure of the treatment (McDonald, 2000).

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

Harris *et al*. (2004) found that higher plant dry weight and seed yield following seed priming.

Parera and Cantliffe (1994) reported that the dry matter and yield of mungbean increased due to better emergence and better performance per plant.

Harris *et al*. (2004) reported that osmo priming increased plumule dry weight.

2.5.4 Vigor index

Umair *et al*. (2010) reported that germination rate and vigor of the mungbean seedlings improved significantly due to seed priming.

Maiti *et al*. (2009) observed the effect of priming on seedling vigor and productivity of tomato, chilli, cucumber and cabbage during post-rainy seasons and concluded that priming improved germination and seedling development and yield of these vegetable species.

Farooq *et al*. (2008) reported that crops grew more vigorously, flowered earlier and yielded higher which are primed.

Del Ryo *et al*. (2002) reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS).

Talebian *et al*. (2008); Bodsworth and Bewley (1981) reported that seed priming improve germination speed, germination vigour, seedling establishment and yield.

Harris *et al*. (1999) demonstrated that on-farm seed priming (soaking seeds overnight in water) markedly improved establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields.

Ruan *et al*. (2002) reported that primed seeds showed better germination pattern and higher vigor level than non- primed.

Fujikura *et al*. (1993) presented hydropriming as a simple and inexpensive method of seed priming and a very important seed treatment technique for rapid germination and uniform seedling establishment in various grain crops.

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

Harris *et al*. (1999) reported that seedling vigor, yield and crop establishment of chickpea, maize and rice promoted due to hydro priming. They also found that hydro priming enhanced seedling establishment and early vigor of upland rice, maize and

chickpea, resulting in faster development, earlier flowering and maturity and higher yields. The resulting improved stand establishment can reportedly increase drought tolerance, reduce pest damage and increase crop yield.

Chiu *et al*. (2006) reported that KNO_3 effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower varieties.

Brocklehurst *et al*. (1984) reported that osmopriming with KNO_3 improved the rate and generally improved the uniformity of seedling emergence in leek, sorghum (Moradi and Younesi, 2009) and tomato (Heydecker *et al*., 1973; Ozbingol *et al*., 1998).

Singh and Rao (1993); Ghassemi-Golezani and Esmaeilpour (2008) stated that seed and seedling vigor of sunflower and cucumber improve due to salt priming with KNO_3.

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

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

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

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during July 2017 to September 2017 to study the effect of H_2O_2 and hydro priming for enhancing drought tolerance capability in mungbean (*Vigna radiata* L.) under drought stress. The detailed materials and methods that were used to conduct the study are presented below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

The experiment was conducted at the Agronomy Laboratory, Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207. It was located in 23º41' N latitude and 90º22' E longitude at an altitude of 8.6 meter above the sea level.

3.1.2 Laboratory environment

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

3.2 Test crops

Two mungbean varieties namely BARI Mung 6 and BINA Moog 8 were used in this experiment. Mungbean varieties were collected from Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA). The collected mungbean varieties were free from visible defects, disease symptoms and insect infestations.

3.3 Experimental materials

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

3.4 Chemicals for seed priming

Different priming chemicals such as Hydrogen peroxide (H_2O_2) and distilled water were utilized for osmo and hydro priming of mungbean seeds. Polyethylene Glycol (PEG) was needed to induce drought stress and 75% ethyl alcohol was used as seed treating chemical.

3.5 Experimental design and treatments

The experiment comprised of

(a) Six levels of priming agent concentration viz. non primed (control), water primed (hydropriming), 2%, 4%, 6% and 8% hydrogen peroxide (H_2O_2)

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

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

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

3.6 Experimental details

The whole study was accomplished by conducting three different experiments.

3.6.1 Experiment 1

Evaluation of the effect of different concentrations of H_2O_2 on mungbean seed germination and seedling growth

3.6.1.1 Weight of seeds

200 g seeds were weighted from the total seed from each of two mungbean varieties. Remaining seeds were stored in poly bag and preserved in refrigerator.

3.6.1.2 Surface treatment

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

3.6.1.3 Treatments

Factor A - Mungbean varieties

(i) BARI Mung 6

(ii) BINA Moog 8

Factor B - Priming solution

(i) T_0 = Seeds without priming (control)

(ii) T_1 = Seeds primed with distilled water

(iii) T_2 = Seeds without 2% H_2O_2

(iv) T_3 = Seeds without 4% H_2O_2

(v) T_4 = Seeds without 6% H_2O_2

(vi) T_5 = Seeds without 8% H_2O_2

3.6.1.4 Priming solution

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

3.6.1.5 Preparation of priming solution

(a) Hydrogen peroxide solution (2%, 4%, 6% and 8%)

5 ml of hydrogen peroxide was dissolved in 250 ml water to prepare 2% solution of hydrogen peroxide. Similarly 10 ml, 15 ml and 20 ml hydrogen peroxide was dissolved in 250 ml of water to prepare 4%, 6% and 8% solution of hydrogen peroxide respectively.

(b) Distilled water

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

3.6.1.6 Priming technique

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

3.6.1.7 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 120 mm diameter Petri dishes on Whatman No.1 filter paper moistened with 8 ml of distilled water. Here, Whatman No.1 filter papers were used as growth media for germination. Experimental units (30 Petri dishes for each variety) were arranged in completely randomized design with five replications. During the test, filter papers in the Petri dishes were kept saturated condition with water. Seeds were kept at room temperature 25°C under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari *et al*., 2007).

Germination progress was inspected and data were collected at every 24 hrs interval and continued up to 8 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (8 days), 6 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75°C for 48 hours.

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

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

Relative water content (WRC) (%) = {(Fresh weight - Dry weight) / (Turgid weight - Dry weight) × 100 (Smart, 1974)

Water saturation deficit (WSD) (%) = 100 - Relative water content (Sangakkara *et al*., 1996)

Water retention capacity (WRC) = Turgid weight / Dry weight (Sangakkara *et al*., 1996).

3.6.2 Experiment 2

Optimization of priming time for the germination, seedling growth and water relation behavior of mungbean

3.6.2.1 Weight of seeds

200 g seeds were weighted from the total seed of mungbean (BARI Mung 6). Remaining seeds were stored in poly bag and preserved in refrigerator.

3.6.2.2 Surface treatment

Seeds were initially treated with 75% solution of ethyl alcohol for 5 minutes for surface sterilization. The sterilized seeds were rinsed for 2 minutes with distilled water thrice

to remove the residual alcohol from the seed surface. Seeds were then dried in room temperature to regain their normal weight.

3.6.2.3 Treatments

Mungbean variety - BARI Mung 6

Factor A - Priming of seeds

(i) P_O = Osmopriming (2% H_2O_2)

(ii) P_H = Hydropriming (Distilled water)

Factor B - Priming time

(i) T_1 = 0 hour

(ii) T_2 = 3 hours

(iii) T_3 = 6 hours

(iv) T_4 = 9 hours

(v) T_5 = 12 hours

(vi) T_6 = 15 hours

3.6.2.4 Priming technique

For chemical priming the sample of seeds were divided into five sub-sample and pre-treated with H_2O_2 for 0, 3, 6, 9, 12 and 15 hours. Priming was done in different plastic containers covered with lids to prevent evaporation loss. Seeds were removed from the priming solution at the required time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight in room temperature for 24 hours back to the original moisture level (Umair *et al*., 2011).

3.6.3 Experiment 3

Evaluation of the effect of seed priming on germination and vigor of mungbean under drought stress

3.6.3.1 Weight of seeds

200 g seeds were weighted from the total seeds of mungbean (BARI Mung 6).

3.6.3.2 Surface treatment

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

3.6.3.3 Treatments

Mungbean variety - BARI Mung 6

Factor A - Priming of seeds

(i) P_O = Osmopriming for 6 hours

(ii) P_H = Hydropriming (Distilled water)

Factor B - Drought stress level

(i) T_0 = Primed seeds placed without drought (control)

(ii) T_1 = Primed seeds placed with 5% PEG

(iii) T_2 = Primed seeds placed with 10% PEG

(iv) T_3 = Primed seeds placed with 15% PEG

(v) T_4 = Primed seeds placed with 20% PEG

3.6.3.4 Priming solution and time

2% H_2O_2 solution, distilled water and 6 hours priming time were used to test drought stress.

3.6.3.5 Preparation of priming solution

(a) Hydrogen peroxide solution (2%)

5 ml of hydrogen peroxide was dissolved in 250 ml water to prepare 2% solution of hydrogen peroxide.

(b) Distilled water

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

3.6.3.6 Preparation of stress solution

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

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

3.6.3.7 Priming technique

Two priming techniques viz. osmo priming and hydro priming were applied on both mungbean varieties. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (non-primed) and the other two sub-samples were primed with priming chemicals. For hydro priming seeds of a sub-sample were soaked in distilled water and for osmo priming seeds of another sub-sample were pretreated with hydrogen peroxide at a concentration of 2% for 6 hours. Priming was

done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight in room temperature for 24 hours to the regain original moisture level (Umair *et al*., 2011).

3.6.3.8 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 120 mm diameter Petri dishes on Whatman No.1 filter paper moist with 8 ml of distilled water. Here, Whatman No.1 filter papers were used as growth media for germination. Experimental units (30 Petri dishes for each variety) were arranged in completely randomized design with five replications. During the test, filter papers in the Petri dishes were kept saturated with water. Seeds were kept at room temperature (25°C) under normal light to facilitate germination for 8 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari *et al*., 2007). Germination progress was inspected and data were collected at every 24 hours interval and continued up to 8 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (8 days), 6 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75°C for 48 hours.

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

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

Relative water content (WRC) (%) = {(Fresh weight - Dry weight) / (Turgid weight - Dry weight) × 100 (Smart, 1974)

Water saturation deficit (WSD) (%) = 100 - Relative water content (Sangakkara *et al*., 1996)

Water retention capacity (WRC) = Turgid weight / Dry weight (Sangakkara *et al*., 1996).

3.7 Data recording

The following parameters were measured:

3.7.1 Germination rate (%)

3.7.2 Shoot length (mm)

3.7.3 Root length (mm)

3.7.4 Shoot dry weight (mg)

3.7.5 Root dry weight (mg)

3.7.6 Relative water content (%)

3.7.7 Water saturation deficit (%)

3.7.8 Water retention capacity

3.7.9 Coefficient of velocity of germination

3.7.10 Vigor index

3.8 Procedure of data recording

3.8.1 Germination rate (%)

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

GR (%) = (Number of germinated seed / Total number of seed set for germination) × 100 (Othman *et al.*, 2006)

3.8.2 Shoot length (mm) and root length (mm)

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

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

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

3.8.4 Relative water content (%)

Relative water content was measured using following formula:

Relative water content (WRC) (%) = {(Fresh weight - Dry weight) / (Turgid weight - Dry weight) × 100 (Smart, 1974)

3.8.5 Water saturation deficit (%)

Water saturation deficit was recorded using following formula:

Water saturation deficit (WSD) (%) = 100 - Relative water content (Sangakkara *et al*., 1996)

3.8.6 Water retention capacity

Water retention capacity was measured using following formula:

Water retention capacity (WRC) = Turgid weight / Dry weight (Sangakkara *et al*., 1996).

3.8.7 Coefficient of velocity of germination

Coefficient of velocity (CV) = (number of germinated seeds per day) is measured according to the method described by Scott *et al.* (1984).

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

Where,

Ti= number of days after sowing and

Ni = number of seeds germinated on i^{th} day

3.8.8 Vigor index

Vigor index was calculated using following formula:

Vigor index = {Total germination × seedling length $_{(mm)}$} / 100 (Abdul-Baki and Anderson, 1973).

3.9 Statistical analysis

Data recorded for different parameters were compiled and tabulated in proper form for statistical analysis. CRD analysis was done for statistical test. The data were analyzed using “Analysis of Variance (ANOVA)” technique with the help of computer package programme “MSTAT-C” and mean difference among the treatments were adjudged with Least Significant Difference (LSD) test as described by Gomez and Gomez (1984).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter comprises presentation and discussion of the results obtained from the experiment on enhancement of drought tolerance in mungbean (*Vigna radiata* L.) through osmo and hydropriming in two mungbean varieties cv. BARI Mung 6 and BINA Moog 8. The results on the germination and growth parameters of mungbean as influenced by different concentrations of priming agent (H_2O_2) and priming time in drought stress condition have been presented and discussed in this chapter.

4.1 Experiment 1

Evaluation of the effect of different concentrations of H_2O_2 on mungbean seed germination and seedling growth

Results obtained from the present study regarding the effects of different concentrations of H_2O_2 on the germination rate and seedling growth of mungbean (BARI Mung 6 and BINA Moog 8) has been presented, discussed and compared in this chapter. The analytical results have been presented in Figure 1 to 10 and Table 1 to 3.

4.1.1 Germination rate (%)

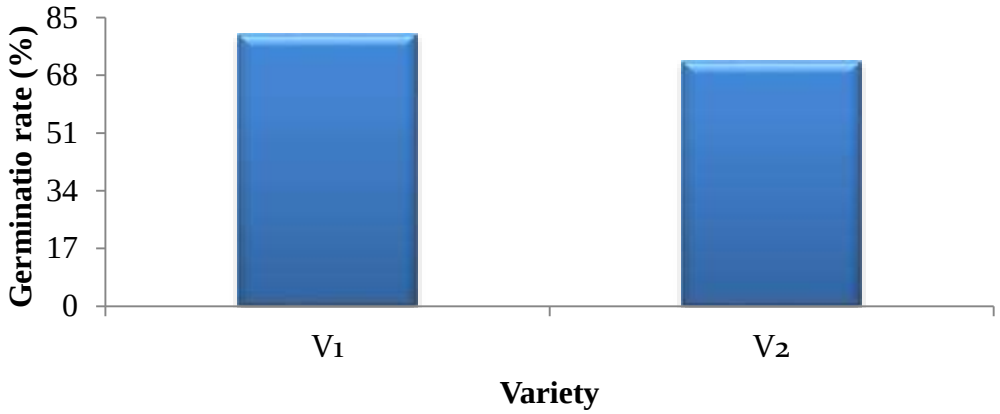
Effect of variety

Significant variation was observed on germination rate of mungbean seeds primed with water and different concentrations of H_2O_2 including control treatment. Results indicated that, between two varieties, V_1 (BARI Mung 6) showed the highest rate of germination (80.14%) and V_2 (BINA Moog 8) showed the lowest (72.19%) (Figure 1). Liheng *et al*. (2009) reported that seed priming with hydrogen peroxide solution stimulated germination and seed vigor since it improved the activity of peroxidase enzyme.

Effect of priming concentration

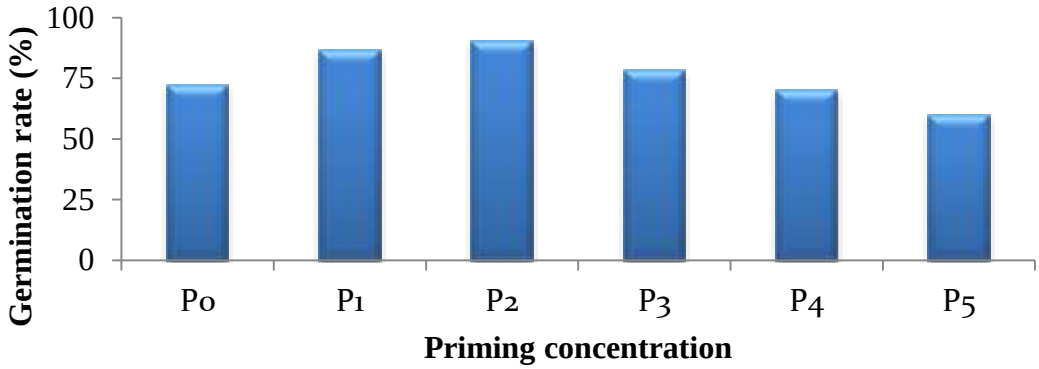
Data revealed that significant variation was observed on germination rate of primed seeds due to difference in priming concentrations. Among different priming concentrations, the highest germination rate (90.31%) was recorded in P_2 (2% H_2O_2 concentration) which was statistically similar with P_1 (86.50%) primed with water and the lowest (59.99%) was recorded in P_5 (8% H_2O_2 concentration) (Figure 2). Germination rate increased with H_2O_2 concentration up to 2% thereafter decreased due to increasing concentration of H_2O_2. Hydrogen peroxide (H_2O_2) was able to promote germination (Christophe *et al*., 2008) or formation and development of adventitious

roots (Li *et al*., 2009). Basra *et al*. (2003) and Farooq *et al*. (2006b) observed that germination was improved by seed priming technique.



V_1 = BARI Mung 6, V_2 = BINA Moog 8

Figure 1. **Effect of variety on the germination rate of mungbean ($LSD_{0.01}$ = 2.40)**



P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Figure 2. Effect of priming concentrations on the germination rate of mungbean ($LSD_{0.01}$ = 5.53)

Interaction effect of variety and priming concentration

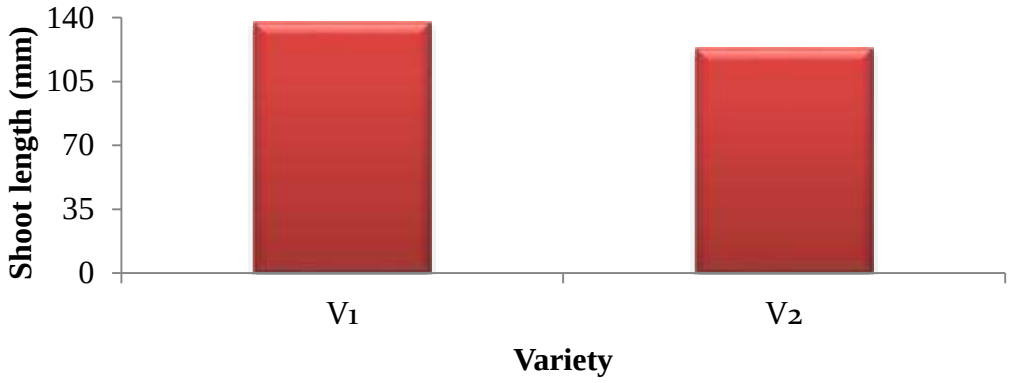
Interaction effect of variety and different priming concentrations on the germination rate of primed seeds showed significant variation (Table 1). The highest germination rate (95.29%) was recorded in V_1P_2 which was statistically similar with V_1P_1 (90.15%) and the lowest (54.27%) in V_2P_5.

4.1.2 Shoot length (mm)

Effect of variety

Significant variation was observed on shoot length among the test varieties primed with water and H_2O_2 at different concentrations including control treatment. Between two

varieties, V_1 (BARI Mung 6) showed the highest shoot length (137.40 mm) and V_2 (BINA Moog 8) showed the lowest (122.83 mm) (Figure 3).

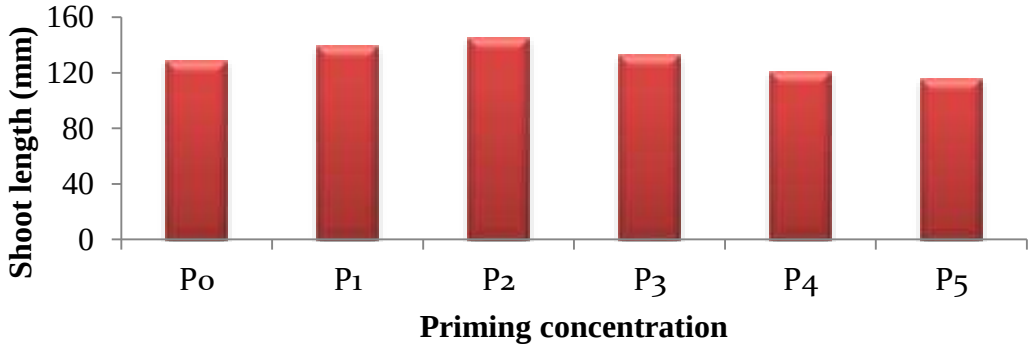


V_1 = BARI Mung 6, V_2 = BINA Moog 8

Figure 3. Effect of variety on the shoot length of mungbean ($LSD_{0.01}$ = 3.77)

Effect of priming concentration

Data revealed that significant variation was observed on shoot length of primed seeds due to difference in priming concentrations. Among different priming concentrations, the highest shoot length (145.10 mm) was recorded in P_2 (2% H_2O_2 concentration) which was statistically similar with P_1 (138.90 mm) primed with water and the lowest (115.2 mm) was recorded in P_5 (8% H_2O_2 concentration) (Figure 4). Gray and Steckel (1983) concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds.



P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Figure 4. Effect of priming concentrations on the shoot length of mungbean ($LSD_{0.01}$ = 8.69)

Interaction effect of variety and priming concentration

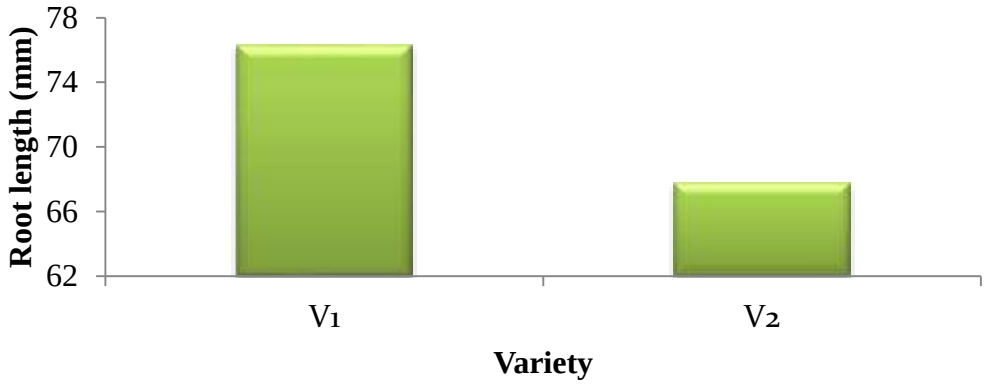
Interaction effect of variety and different priming concentrations on the shoot length of primed seeds showed significant variation (Table 1). The highest shoot length (151.80

mm) was recorded in V_1P_2 which was statistically similar with V_1P_1 (146.90 mm) and the lowest (108.90) in V_2P_5 which was statistically similar with V_2P_4 (111.30 mm).

4.1.3 Root length (mm)

Effect of variety

Root length of mungbean was significantly affected by different H_2O_2 concentrations. Root length of mungbean increased up to 2% H_2O_2 and there was a gradual decreased with the increasing H_2O_2 concentration. Between two varieties, the maximum root length (76.29 mm) was recorded in V_1 (BARI Mung 6) and the minimum (67.74 mm) was recorded inV_2 (BINA Moog 8) (Figure 5).

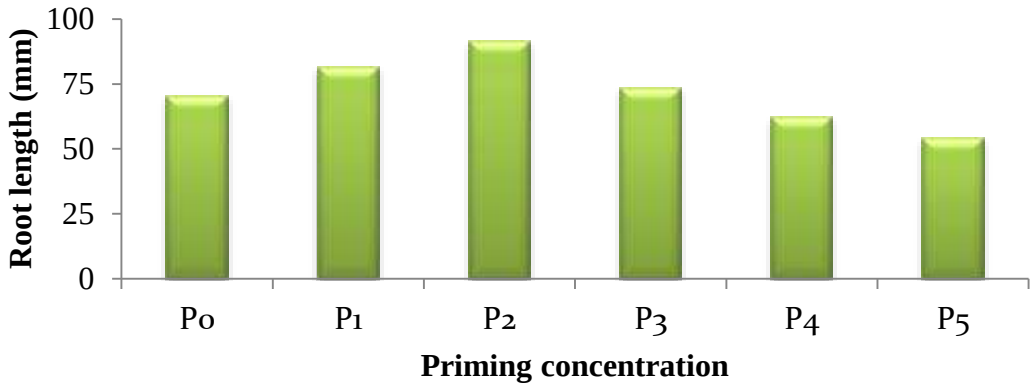


V_1 = BARI Mung 6, V_2 = BINA Moog 8

Figure 5. Effect of variety on the root length of mungbean ($LSD_{0.01}$ = 2.19)

Effect of priming concentration

Data revealed that significant variation was observed on root length of primed seeds due to difference in priming concentrations. Among different priming concentrations, the highest root length (91.25 mm) was recorded in P_2 (2% H_2O_2 concentration) and the lowest (53.89 mm) was recorded in P_5 (8% H_2O_2 concentration) (Figure 6). Earlier study conducted by Ashraf and Abushakra (1978) revealed that priming of wheat seed in osmoticum or water might improve germination, emergence and aggrandize vigorous root growth as a consequence root length of chemical and hydro primed seed exerted the highest length over non-primed seeds.



P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Figure 6. Effect of priming concentrations on the root length of mungbean ($LSD_{0.01}$ = 5.06)

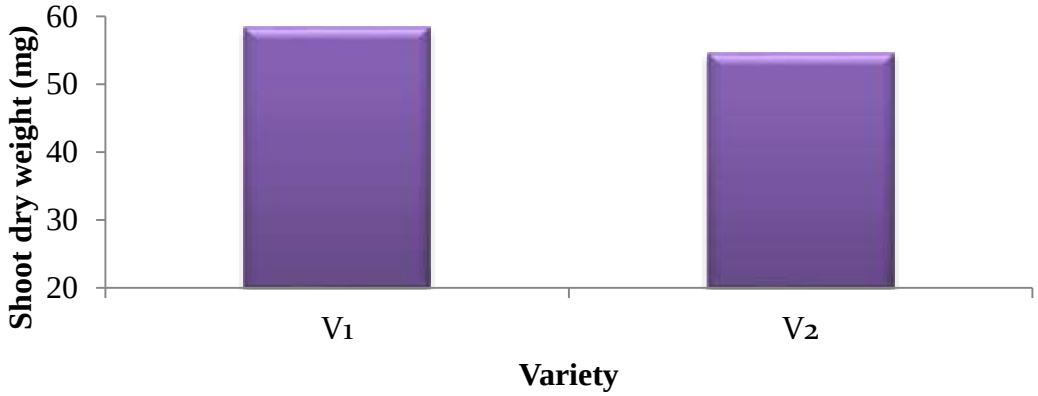
Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the root length of primed seeds showed significant variation (Table 1). The highest root length (93.98 mm) was recorded in V_1P_2 which was statistically similar with V_2P_2 (88.53 mm) and the lowest (48.63 mm) in V_2P_5.

4.1.4 Shoot dry weight (mg)

Effect of variety

Shoot dry weight of mungbean varieties was significantly varied by different concentrations of H_2O_2 and water priming including control. Between two varieties, the highest shoot dry weight (58.38 mg) was scored by V_1 (BARI Mung 6) and the minimum (54.55 mg) was recorded in V_2 (BINA Moog 8) (Figure 7).

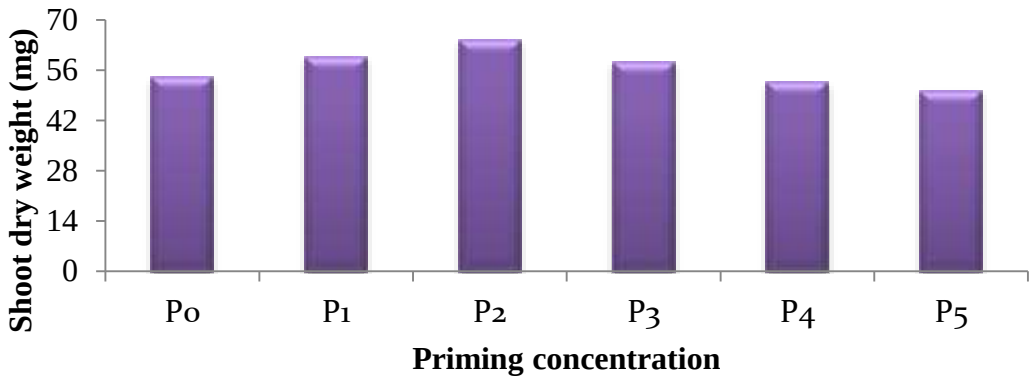


V_1 = BARI Mung 6, V_2 = BINA Moog 8

Figure 7. Effect of variety on the shoot dry weight of mungbean ($LSD_{0.01}$ = 1.64)

Effect of priming concentration

Data revealed that significant variation was observed on shoot dry weight of primed seeds due to difference in priming concentrations. Among different priming concentrations, the highest shoot dry weight (64.24 mg) was recorded in P_2 (2% H_2O_2 concentration) and the lowest (50.15 mg) was recorded in P_5 (8% H_2O_2 concentration) (Figure 8). Sarwar *et al*. (2006) reported that shoot length and biomass of shoots were increased when treated with water and H_2O_2. Basra *et al*. (2005) and Iqbal and Ashraf (2007) also observed that seed priming improved shoot dry weight significantly compared to nonprimed seeds in wheat.



P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Figure 8. Effect of priming concentrations on the shoot dry weight of mungbean ($LSD_{0.01}$ = 3.79)

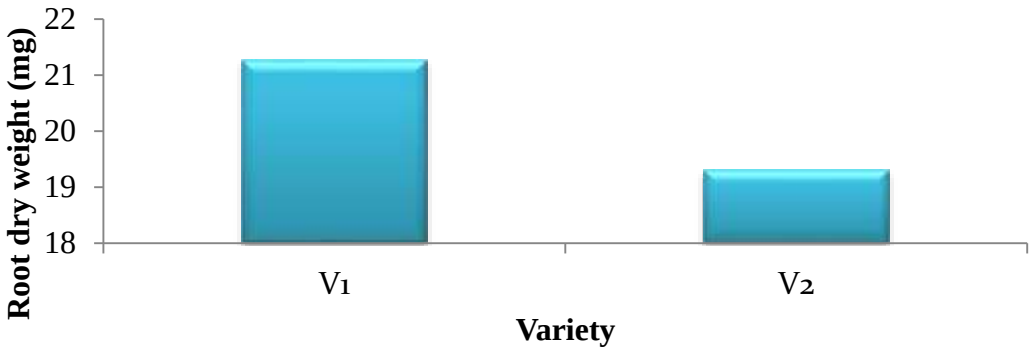
Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the shoot dry weight of primed seeds showed significant variation (Table 1). The highest shoot dry weight (66.43 mg) was recorded in V_1P_2 which was statistically similar with V_1P_1 (61.91 mg) and V_2P_2 (62.04 mg) while, the lowest (47.46 mg) in V_2P_5 which was statistically similar with V_2P_4 (50.76 mg) and V_2P_0 (52.37 mg).

4.1.5 Root dry weight (mg)

Effect of variety

Statistically significant variation was found in case of root dry weight of two varieties of mungbean due to priming with water and different concentrations of H_2O_2 including control treatment. Between two varieties, V_1 (BARI Mung 6) showed the highest root dry weight (21.76 mg) and V_2 (BINA Moog 8) showed the minimum (19.80 mg) (Figure 9).

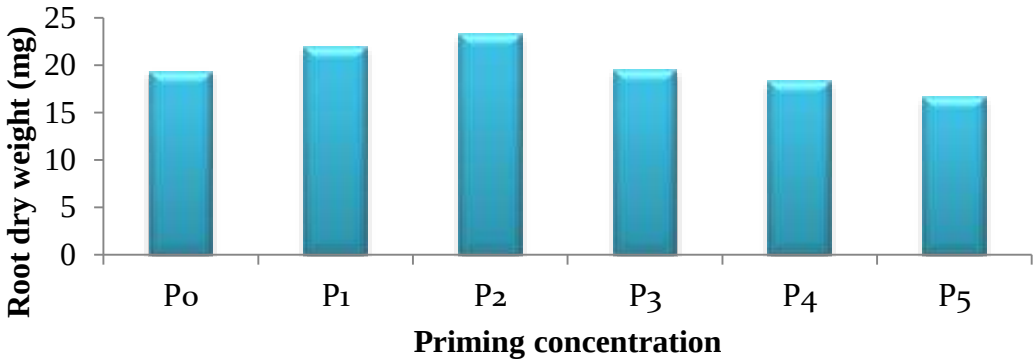


V_1 = BARI Mung 6, V_2 = BINA Moog 8

Figure 9. Effect of variety on the root dry weight of mungbean ($LSD_{0.01}$ = 0.63)

Effect of priming concentration

Data revealed that significant variation was observed on root dry weight of primed seeds due to difference in priming concentrations. Among different priming concentrations, the highest root dry weight (23.23 mg) was recorded in P_2 (2% H_2O_2 concentration) which was statistically similar with P_1 (21.82 mg) primed with water and the lowest (16.60 mg) was recorded in P_5 (8% H_2O_2 concentration) (Figure 10). Similar findings were reported by Pill and Necker (2001) who reported that seed priming resulted in greater seedling dry weights compared to non-primed seeds.



P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Figure 10. Effect of priming concentrations on the root dry weight of mungbean ($LSD_{0.01}$ = 1.46)

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the root dry weight of primed seeds showed significant variation (Table 1). The highest root dry weight

(24.06 mg) was recorded in V_1P_2 which was statistically similar with V_1P_1 (22.45 mg) and V_2P_2 (22.41 mg) while, the lowest (15.43 mg) in V_2P_5.

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

Treatment combinations	Germination rate (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
V_1P_0	75.41 d-f	136.10 bc	74.88 c	55.52 c-e	20.90 bc
V_1P_1	90.15 ab	146.90 ab	86.35 b	61.91 ab	22.45 ab
V_1P_2	95.29 a	151.80 a	93.98 a	66.43 a	24.06 a
V_1P_3	80.66 c-e	138.70 bc	75.92 c	59.24 bc	20.47 b-d
V_1P_4	73.65 e-g	129.40 cd	67.46 d	54.37 c-e	18.89 c-e
V_1P_5	65.70 h	121.50 de	59.15 ef	52.83 de	17.78 e
V_2P_0	68.50 f-h	120.60 d-f	65.65 de	52.37 d-f	17.66 e
V_2P_1	82.86 b-d	130.90 cd	76.45 c	57.23 b-d	21.18 b
V_2P_2	85.33 bc	138.60 bc	88.53 ab	62.04 ab	22.41 ab
V_2P_3	75.62 d-f	126.80 cd	70.43 cd	57.45 b-d	18.50 de
V_2P_4	66.55 gh	111.30 ef	56.74 f	50.76 ef	17.65 e
V_2P_5	54.27 i	108.90 f	48.63 g	47.46 f	15.43 f
LSD $_{(0.01)}$	7.82	12.29	7.15	5.37	2.07
CV (%)	6.05	5.57	5.85	5.60	6.17

V_1 = BARI Mung 6, V_2 = BINA Moog 8

P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

4.1.6 Relative water content (%)

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. A wide range of statistical difference was observed in case of relative water content of mungbean varieties under different H_2O_2 concentrations (Table 2). Corresponding water content followed the similar trend as the previous parameters of mungbean varieties. Between two varieties, V_1 (BARI Mung 6) showed the highest relative water content (78.15%) and V_2 (BINA Moog 8) showed the lowest (72.27%).

Effect of priming concentration

Data revealed that significant variation was observed on relative water content of primed seeds due to difference in priming concentrations (Table 2). Among different priming concentrations, the highest relative water content (89.25%) was recorded in P_2 (2% H_2O_2 concentration) which was statistically similar with P_1 (84.67%) primed with water and the lowest relative water content (56.38%) was recorded in P_5 (8% H_2O_2 concentration). Mouradi *et al.* (2016) reported that alfalfa plants raised from primed seeds maintained high ($P < 0.001$) relative water content values, compared to those raised from unprimed seeds.

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the relative water content of primed seeds showed significant variation (Table 3). The highest relative water content (93.14%) was recorded in V_1P_2 which was statistically similar with V_1P_1 (88.72%) and the lowest (52.40%) in V_2P_5.

4.1.7 Water saturation deficit (%)

Effect of variety

Water saturation deficit of mungbean varieties showed statistically significant variation due to different concentrations of H_2O_2 and water priming including control. It followed the opposite trend compared to the previously described parameters (Table 2). Between two varieties, the highest water saturation deficit (27.73%) was recorded from V_2 (BINA Moog 8) and the lowest (21.85%) was recorded in V_1 (BARI Mung 6).

Effect of priming concentration

Data revealed that significant variation was observed on water saturation deficit of primed seeds due to difference in priming concentrations (Table 2). Among different priming concentrations, the highest water saturation deficit (43.62%) was recorded in P_5 (8% H_2O_2 concentration) and the lowest (10.75%) in P_2 (2% H_2O_2 concentration). Due to lack of defense mechanism, the non-primed seedlings failed to uptake enough water necessary for running the physiological processes smoothly compared to primed seedlings. As a result, excessive amount of water deficit occurred in non-primed seeds than the primed ones. Similar results were observed by Ali *et al.* (2013) who reported that seed priming improves irrigation water use efficiency and resulted lower water saturation deficit.

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the water saturation deficit of primed seeds showed significant variation (Table 3). The highest water saturation deficit (47.60%) was recorded in V_2P_5 and the lowest (6.86%) in V_1P_2.

4.1.8 Water retention capacity

Effect of variety

Water retention capacity of mungbean varieties was influenced significantly by different H_2O_2 concentrations (Table 2). Between two varieties, the maximum water retention capacity (18.19) was recorded in V_1 (BARI Mung 6) and the lowest (15.28) in V_2 (BINA Moog 8).

Effect of priming concentration

Data revealed that significant variation was observed on water retention capacity of primed seeds due to difference in priming concentrations (Table 2). Among different priming concentrations, the highest water retention capacity (21.81) was recorded in P_2 (2% H_2O_2 concentration) and the lowest (9.60) was recorded in P_5 (8% H_2O_2 concentration). Priming helps to activate the metabolic enzymes which are responsible for germination of seed. As a result, primed seeds can uptake more water than the non-primed one and gained the maximum turgid weight and consequently attained the maximum water retention capacity. It is also supported by the finding of Ali *et al.* (2013) who found that seed priming improves irrigation water use efficiency which helps to increase higher water retention capacity.

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the water retention capacity of primed seeds showed significant variation (Table 3). The highest water retention capacity (23.41) was recorded in V_1P_2 and the lowest (8.37) in V_2P_5.

4.1.9 Coefficient of velocity of germination

Effect of variety

Coefficient of velocity of germination of mungbean varieties was significantly affected by different H_2O_2 concentrations. Coefficient of velocity of germination increased in 2% H_2O_2 and there was a gradual decline with the increasing H_2O_2 concentration (Table 2). Between two varieties, the maximum coefficient of velocity of germination (17.59) was scored by V_1 (BARI Mung 6) and the minimum coefficient of velocity of germination (15.55) was recorded in V_2 (BINA Moog 8).

Effect of priming concentration

Data revealed that significant variation was observed on coefficient of velocity of germination of primed seeds due to difference in priming concentrations (Table 2). Among different priming concentrations, the highest coefficient of velocity of germination (21.48) was recorded in P_2 (2% H_2O_2 concentration) and the lowest (9.56) was recorded in P_5 (8% H_2O_2 concentration) (Table 2). Huns and Sung (1997) observed that seed priming resulted from anti-oxidant increment as glutathione and ascorbate in the seed. These enzymes trigger germination speed via reduction of lipid peroxidation activity; as a result coefficient of velocity of germination was higher in primed seed compare to that of non-primed one.

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the coefficient of velocity of germination of primed seeds showed significant variation (Table 3). The highest coefficient of velocity of germination (22.43) was recorded in V_1P_2 which was statistically similar with V_1P_1 (20.84) and the lowest (8.48) in V_2P_5.

4.1.10 Vigor index

Effect of variety

Statistically significant variation was found in case of vigor index of two varieties of mungbean due to priming with water and different concentrations of H_2O_2 including control treatment (Table 2). Between two varieties, the maximum vigor index (173.63) was accounted from V_1 (BARI Mung 6) and the minimum (139.87) was achieved in V_2 (BINA Moog 8).

Effect of priming concentration

Data revealed that significant variation was observed on vigor index of germination of primed seeds due to difference in priming concentrations (Table 2). Among different priming concentrations, the highest vigor index (214.20) was recorded in P_2 (2% H_2O_2 concentration) and the lowest (102.10) was recorded in P_5 (8% H_2O_2 concentration) (Table 2). Kilic and Kahraman (2016) reported similar results who reported that H_2O_2 had positive effect on germination index and vigor index under different concentration in barley.

Interaction effect of variety and priming concentration

Interaction effect of variety and different priming concentrations on the vigor index of primed seeds showed significant variation (Table 3). The highest vigor index (234.50) was recorded in V_1P_2 and the lowest (85.35) in V_2P_5.

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

Treatments	Relative water content (%)	Water saturation deficit (%)	Water retention capacity	Coefficient of germination	Vigor index
Effect of variety					
V_1	78.15 a	21.85 b	18.19 a	17.59 a	173.63 a
V_2	72.27 b	27.73 a	15.28 b	15.55 b	139.87 b
LSD $_{(0.01)}$	2.15	0.81	0.48	0.55	5.06
CV (%)	5.51	6.28	5.55	6.42	6.21
Effect of concentrations					
P_0	72.89 bc	27.11 c	15.88 d	15.72 d	143.50 d
P_1	84.67 a	15.33 e	20.00 b	19.90 b	191.20 b
P_2	89.25 a	10.75 f	21.81 a	21.48 a	214.20 a
P_3	77.33 b	22.67 d	18.16 c	17.81 c	161.10 c
P_4	70.76 c	29.24 b	14.96 d	14.97 d	128.40 e
P_5	56.38 d	43.62 a	9.60 e	9.56 e	102.10 f
LSD $_{(0.01)}$	4.97	1.87	1.11	1.28	11.67
CV (%)	5.51	6.28	5.55	6.42	6.21

V_1 = BARI Mung 6, V_2 = BINA Moog 8

P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

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

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
V_1P_0	74.31 d-f	25.69 e	17.61 de	16.60 fg	159.30 de
V_1P_1	88.72 ab	11.28 h	21.17 b	20.84 ab	210.70 b
V_1P_2	93.14 a	6.86 i	23.41 a	22.43 a	234.50 a
V_1P_3	79.65 c-e	20.35 f	20.03 bc	18.49 de	173.20 d
V_1P_4	72.72 ef	27.28 de	16.14 e	16.55 fg	145.20 e
V_1P_5	60.37 g	39.63 b	10.82 g	10.64 i	118.90 f
V_2P_0	71.47 f	28.53 d	14.16 f	14.83 gh	127.60 f
V_2P_1	80.62 cd	19.38 f	18.83 cd	18.96 cd	171.80 d
V_2P_2	85.36 bc	14.64 g	20.22 bc	20.53 bc	193.90 c
V_2P_3	75.00 d-f	25.00 e	16.29 e	17.13 ef	148.90 e
V_2P_4	68.79 f	31.21 c	13.77 f	13.39 h	111.70 f
V_2P_5	52.40 h	47.60 a	8.37 h	8.48 j	85.35 g
LSD $_{(0.01)}$	7.03	2.64	1.57	1.81	16.51
CV (%)	5.51	6.28	5.55	6.42	6.21

V_1 = BARI Mung 6, V_2 = BINA Moog 8

P_0 = Control, P_1 = Water, P_2 = 2% H_2O_2, P_3 = 4% H_2O_2, P_4 = 6% H_2O_2, P_5 = 8% H_2O_2

Achievement from the first experiment

From the first experiment, BARI Mung 6 with 2% H_2O_2 gave the best result. So, BARI Mung 6 with 2% H_2O_2 was used for the next experiment to evaluate best priming duration.

4.2 Experiment 2

Optimization of priming time for the germination, seedling growth and water relation behavior of mungbean

Results obtained from the present study regarding the effects of different priming time of 2% H_2O_2 on the germination, seedling growth and water relation behavior of BARI Mung 6 has been presented, discussed and compared in this chapter. The analytical results have been presented in Table 4 to 6 and Figures 11 to 20.

4.2.1 Germination rate (%)

Effect of priming technique

Priming technique and time with 2% H_2O_2 showed significant influence on germination rate of BARI Mung 6 (Table 4). Between two priming techniques, the highest germination rate (82.03%) was recorded in P_O (primed with H_2O_2) and the lowest (77.22%) was obtained in P_H (primed with water).

Effect of priming time

Results showed that among different priming time, the highest germination rate (90.21%) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and lowest (69.29%) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Table 4).

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the germination rate of primed seeds showed significant variation (Table 5). The highest germination rate (93.35%) was recorded in P_OT_3 which was statistically similar with P_OT_4 (86.22%) and P_HT_3 (87.07%) while, the lowest (66.75%) in P_HT_6.

4.2.2 Shoot length (mm)

Effect of priming technique

Shoot length of BARI Mung 6 was significantly affected by different priming time (Table 4). Shoot length increased with increasing priming time up to 6 hours and then gradually decreased. Between two priming techniques, the highest shoot length (128.57 mm) was recorded in P_O (primed with H_2O_2) and the lowest (119.35 mm) was obtained in P_H (primed with water).

Effect of priming time

Results showed that among different priming time, the highest shoot length (139.90 mm) was recorded from T_3 (2% H_2O_2 concentration for 6 hours priming) which was statistically similar (133.00 mm) with T_4 (2% H_2O_2 concentration for 9 hours priming) and the lowest shoot length (110.60 mm) was recorded from T_6 (2% H_2O_2 concentration

for 15 hours priming) (Table 4). Priming time increases enzymatic activities of seed and triggers the vigorous plant growth and in consequence up to 9 hours and then it decreases gradually. Over priming facilitate ageing of seeds. Afzal *et al.* (2007) observed that the maximum shoot length (28 cm) was scored by hydro priming seeds followed by 24 hours of chilling.

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the shoot length of primed seeds showed significant variation (Table 5). The highest shoot length (143.80 mm) was recorded in P_OT_3 which was statistically similar with P_OT_4 (138.20 mm) and P_HT_3 (136.00 mm) while, the lowest (106.00 mm) in P_HT_6 was statistically similar with P_HT_2 (115.20 mm), P_HT_1 (109.50 mm), P_OT_6 (115.20 mm) and P_OT_1 (116.30 mm).

4.2.3 Root length (mm)

Effect of priming technique

Significant variation was found in case of root length of BARI Mung 6 due to difference in priming duration (Table 4). Between two priming techniques, the highest root length (71.04 mm) was recorded in P_O (primed with H_2O_2) and the lowest (59.96 mm) was obtained in P_H (primed with water).

Effect of priming time

Results showed that among different priming time, the highest root length (78.29 mm) was recorded in T_3 (2% H_2O_2 solution for 6 hours priming) which was statistically similar (70.39 mm) with T_4 (2% H_2O_2 solution for 9 hours priming) and the lowest root length (55.45 mm) was recorded in T_6 (2% H_2O_2 solution for 15 hours priming) (Table 4). The root length of seedlings obtained from primed seeds increased significantly compared to unprimed seeds. Maximum aboveground biomass and photosynthetic pigments were recorded by Nouman *et al.* (2014) when the seeds were hydro primed (12 hours) but maximum root length and number of roots were found in MLE (*Moringa* leaf extract) primed (12 hours) *Moringa olifera* seeds.

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the root length of primed seeds showed significant variation (Table 5). The highest root length (84.61 mm) was recorded in P_OT_3 which was statistically similar with P_OT_4 (78.08 mm), P_HT_3 (71.98 mm) and P_OT_2 (71.93 mm) while, the lowest (50.52 mm) in P_HT_6 which

was statistically similar with P_HT_5 (52.46 mm), P_HT_4 (62.70 mm), P_HT_1 (55.59 mm), P_OT_6 (60.37 mm) and P_OT_1 (61.28 mm).

4.2.4 Shoot dry weight (mg)

Effect of priming technique

Shoot dry weight of BARI Mung 6 was significantly affected by priming time (Table 4). Between two priming techniques, the highest shoot dry weight (55.21 mg) was recorded in P_O (primed with H_2O_2) and the lowest (51.17 mg) was obtained in P_H (primed with water).

Effect of priming time

Results showed that among different priming time, the highest shoot dry weight (59.86 mg) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) which was statistically similar (55.04 mg) with T_4 (2% H_2O_2 concentration for 9 hours priming) and the lowest (46.57 mg) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Table 4). Increased plumule dry weight due to osmo priming was reported by Harris *et al.* (2004).

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the shoot dry weight of primed seeds showed significant variation (Table 5). The highest shoot dry weight (61.52 mg) was recorded in P_OT_3 which was statistically similar with P_OT_1 (52.57 mg), P_OT_2 (56.24 mg), P_OT_4 (57.41 mg), P_OT_5 (53.91 mg), P_HT_2 (54.73 mg), P_HT_3 (58.19 mg) and P_HT_4 (52.66 mg) while, the lowest (43.54 mg) in P_HT_6 which was statistically similar with P_HT_5 (47.96 mg), P_HT_1 (49.90 mg) and P_OT_6 (49.61 mg).

4.2.5 Root dry weight (mg)

Effect of priming technique

Priming technique and duration showed significant influence on root dry weight of BARI Mung 6 (Table 4). Between two priming techniques, the highest root dry weight (18.89 mg) was recorded in P_O (primed with H_2O_2) and the lowest (16.48 mg) was obtained in P_H (primed with water).

Effect of priming time

Results showed that among different priming time, the highest root dry weight (20.89 mg) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest (14.86 mg) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Table 4).

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the root dry weight of primed seeds showed significant variation (Table 5). The highest root dry weight (22.48 mg) was recorded in P_OT_3 and the lowest (12.99 mg) in P_HT_6.

Table 4. Effect of osmo and hydropriming and priming time on the germination and growth behavior of BARI Mung 6

Treatments	Germination rate (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
Effect of osmo and hydropriming					
P_O	82.03 a	128.57 a	71.04 a	55.21 a	18.89 a
P_H	77.22 b	119.35 b	59.96 b	51.17 b	16.48 b
LSD $_{(0.01)}$	2.25	3.79	4.23	2.94	0.56
CV (%)	5.42	5.88	12.43	10.64	6.04
Effect of priming time					
T_1	76.66 c	112.90 de	58.44 cd	51.23 bc	16.76 c
T_2	80.96 bc	121.6 0 cd	68.20 bc	55.49 ab	18.55 b
T_3	90.21 a	139.90 a	78.29 a	59.86 a	20.89 a
T_4	83.75 b	133.00 ab	70.39 ab	55.04 ab	18.68 b
T_5	76.87 c	125.80 bc	62.19 b-d	50.94 bc	16.36 c
T_6	69.29 d	110.60 e	55.45 d	46.57 c	14.86 d
LSD $_{(0.01)}$	5.18	8.75	9.77	6.79	1.28
CV (%)	5.42	5.88	12.43	10.64	6.04

P_O = Osmopriming, P_H = Hydropriming

T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Table 5. Interaction effect of osmo and hydropriming and priming time on the germination and growth behavior of BARI Mung 6

Treatment combinations	Germination rate (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
P_OT_1	78.00 d-f	116.30 d-f	61.28 c-e	52.57 a-d	17.69 cd
P_OT_2	83.26 b-d	128.10 b-d	71.93 a-c	56.24 a-c	19.85 b
P_OT_3	93.35 a	143.80 a	84.61 a	61.52 a	22.48 a
P_OT_4	86.22 a-c	138.20 ab	78.08 ab	57.41 a-c	19.45 bc
P_OT_5	79.50 c-e	129.80 bc	69.93 bc	53.91 a-c	17.13 de
P_OT_6	71.83 fg	115.20 ef	60.37 c-e	49.61 b-d	16.74 de
P_HT_1	75.33 ef	109.50 ef	55.59 de	49.90 b-d	15.84 e
P_HT_2	78.66 d-f	115.20 ef	64.47 b-d	54.73 a-c	17.24 de
P_HT_3	87.07 ab	136.00 ab	71.98 a-c	58.19 ab	19.29 bc
P_HT_4	81.27 b-e	127.70 b-d	62.70 c-e	52.66 a-d	17.90 cd
P_HT_5	74.25 ef	121.80 c-e	54.46 de	47.96 cd	15.60 e
P_HT_6	66.75 g	106.0 f	50.52 e	43.54 d	12.99 f
$LSD_{(0.01)}$	7.33	12.37	13.81	9.60	1.81
CV (%)	5.42	5.88	12.43	10.64	6.04

P_O = Osmopriming, P_H = Hydropriming

T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

4.2.6 Relative water content (%)

Effect of priming technique

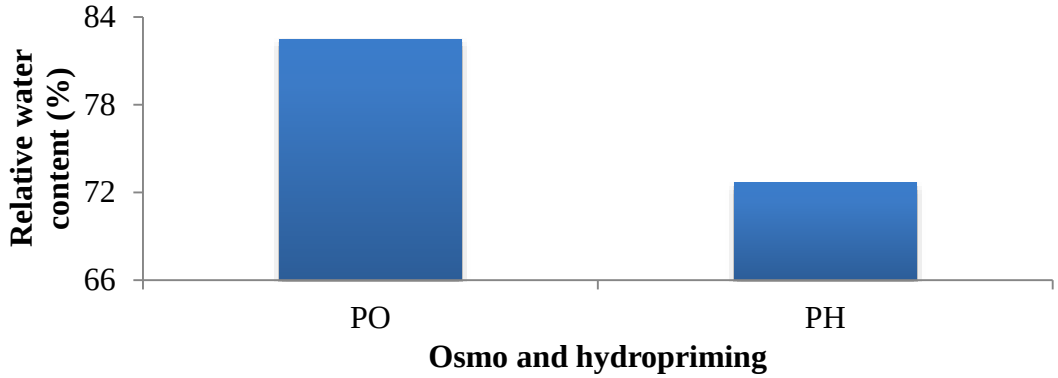
Relative water content of BARI Mung 6 was significantly affected by priming duration. Between two priming techniques, the highest relative water content (82.48%) was recorded in P_O (primed with H_2O_2) and the lowest (72.67%) was obtained in P_H (primed with water) (Figure 11).

Effect of priming time

Results showed that among different priming time, the highest relative water content (89.17%) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest (61.29%) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Figure 12).

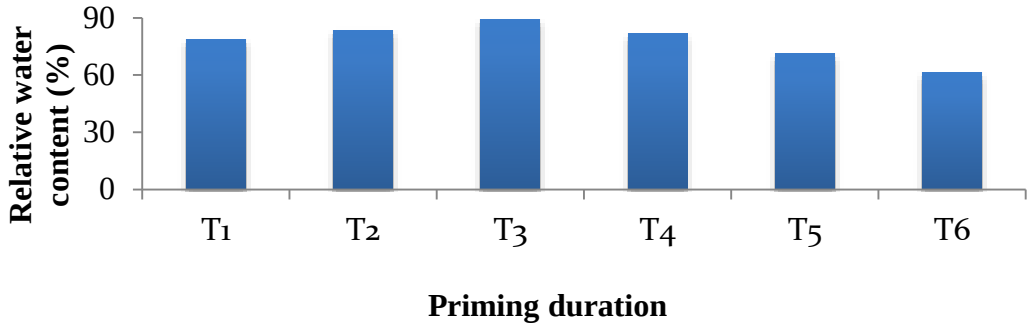
Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the relative water content of primed seeds showed significant variation (Table 6). The highest relative water content (94.95%) was recorded in P_OT_3 and the lowest (55.80%) in P_HT_6.



P_O = Osmopriming, P_H = Hydropriming

Figure 11. Effect of osmo and hydropriming on the relative water content of BARI Mung 6 ($LSD_{0.01}$ = 2.26)



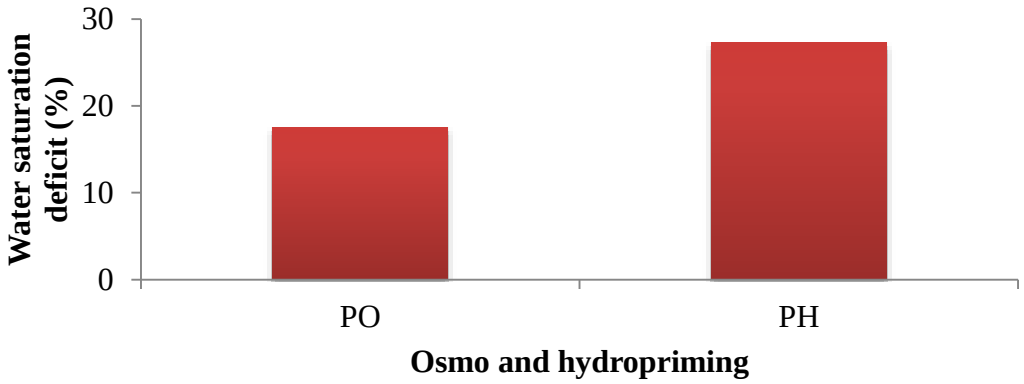
T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Figure 12. Effect of priming duration on the relative water content of BARI Mung 6 ($LSD_{0.01}$ = 5.21)

4.2.7 Water saturation deficit (%)

Effect of priming technique

The trend of water saturation deficit was quite opposite compare to the trend of rest of the parameters. i. e. water saturation deficit decreased with the increasing priming duration and gradually increased with increasing priming time. Between two priming techniques, the highest water saturation deficit (27.30%) was recorded in P_H (primed with water) and the lowest (17.45%) was obtained in P_O (primed with H_2O_2) (Figure 13).

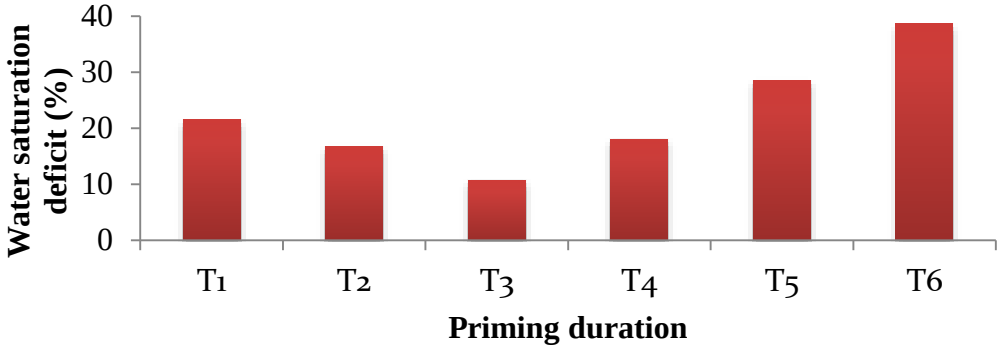


P_O = Osmopriming, P_H = Hydropriming

Figure 13. Effect of osmo and hydropriming on the water saturation deficit of BARI Mung 6 ($LSD_{0.01}$ = 0.83)

Effect of priming time

Results showed that among different priming time, highest water saturation deficit (38.71%) was recorded in T_3 (2% H_2O_2 concentration for 15 hours priming) and the lowest (10.73%) was recorded in T_6 (2% H_2O_2 concentration for 6 hours priming) (Figure 14).



T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Figure 14. Effect of priming duration on the water saturation deficit of BARI Mung 6 ($LSD_{0.01}$ = 1.91)

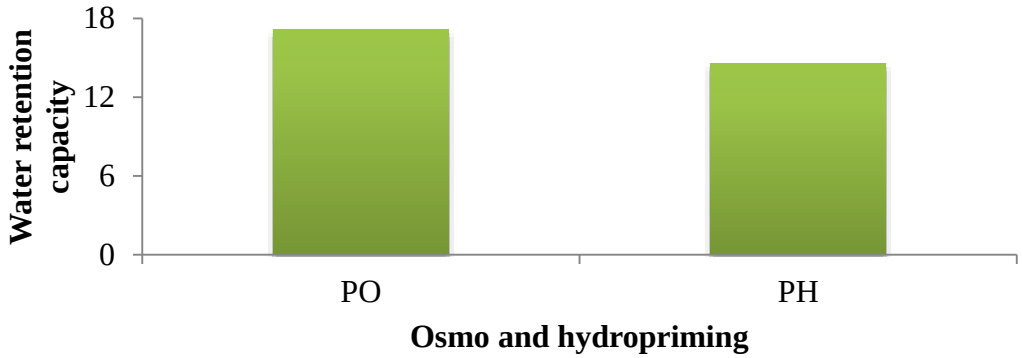
Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the water saturation deficit of primed seeds showed significant variation (Table 6). The highest water saturation deficit (44.20%) was recorded in P_HT_6 and the lowest (5.05%) in P_OT_3.

4.2.8 Water retention capacity

Effect of priming technique

Statistically significant variation was found in case of water retention capacity of BARI Mung 6 due to priming technique and duration. Between two priming techniques, the highest water retention capacity (17.17) was recorded in P_O (primed with H_2O_2) and the lowest (14.58) was obtained in P_H (primed with water) (Figure 15).

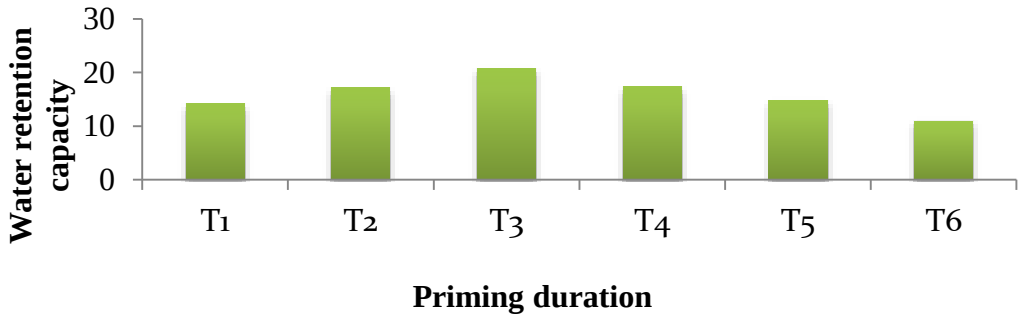


P_O = Osmopriming, P_H = Hydropriming

Figure 15. Effect of osmo and hydropriming on the water retention capacity of BARI Mung 6 ($LSD_{0.01}$ = 0.48)

Effect of priming time

Results showed that among different priming time, the highest water retention capacity (20.72) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest (10.94) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Figure 16). Wahid and Ghazanfar (2006) reported that H_2O_2 treatment improved the water relations of salinity-treated seedlings by turgor maintenance, which was comparable to the water control of seedlings.



T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Figure 16. Effect of priming duration on the water retention capacity of BARI Mung 6 ($LSD_{0.01}$ = 1.12)

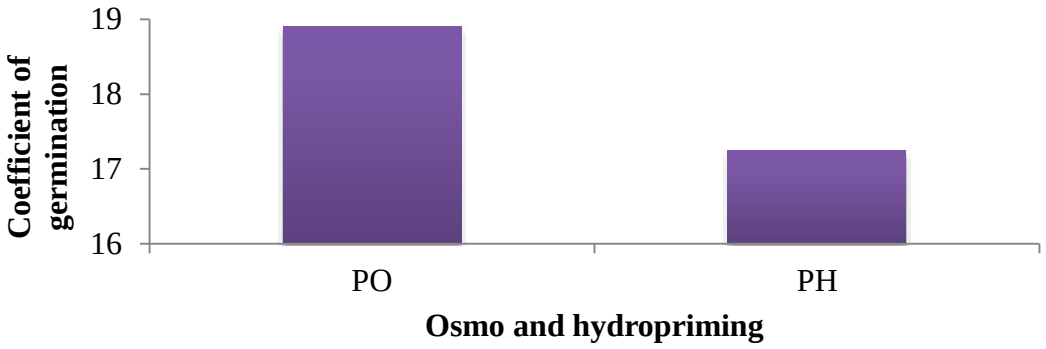
Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the water retention capacity of primed seeds showed significant variation (Table 6). The highest water retention capacity (21.79) was recorded in P_OT_3 and the lowest (9.56) in P_HT_6.

4.2.9 Coefficient of velocity of germination

Effect of priming technique

Significant difference was observed on coefficient in terms of velocity of germination due to different priming duration. Between two priming techniques, the highest coefficient of velocity of germination (18.40) was recorded in P_O (primed with H_2O_2) and the lowest (16.75) was obtained in P_H (primed with water) (Figure 17).

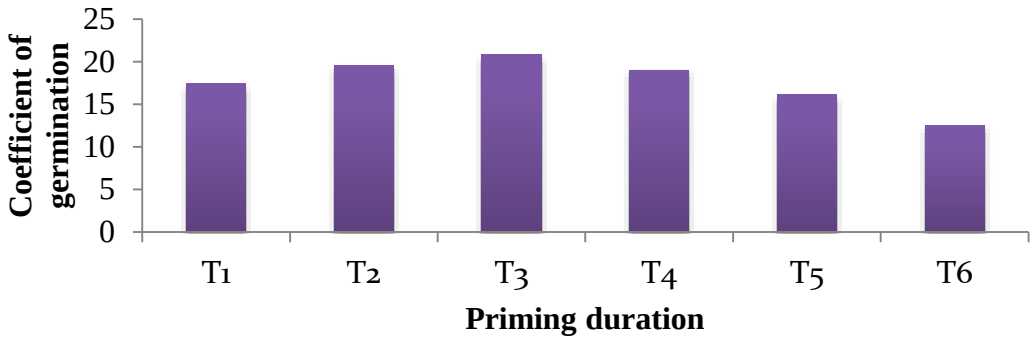


P_O = Osmopriming, P_H = Hydropriming

Figure 17. Effect of osmo and hydropriming on the coefficient of germination of BARI Mung 6 ($LSD_{0.01}$ = 0.57)

Effect of priming time

Results showed that among different priming time, the highest coefficient of velocity of germination (20.78) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest (12.50) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Figure 18). Time of priming significantly influence the coefficient of velocity of germination and the finding of many researcher support this. Omid and Farzad (2012) found that in mountain rye (*Secale montanum*) highest coefficient of velocity of germination was attained from concentration of 9 bar PEG for 24 hours at 10^0C and highest coefficient of velocity of germination was attained from hydro priming for 8 hours at 10^0C as it was increased compared to the non-primed one.



T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Figure 18. Effect of priming duration on the coefficient of germination of BARI Mung 6 ($LSD_{0.01}$ = 1.31)

Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the coefficient of velocity of germination of primed seeds showed significant variation (Table 6). The highest coefficient of velocity of germination (21.60) was recorded in P_OT_3 which was statistically similar with P_OT_2 (20.45) and P_HT_3 (19.97) while, the lowest (11.51) in P_HT_6.

4.2.10 Vigor index

Effect of priming technique

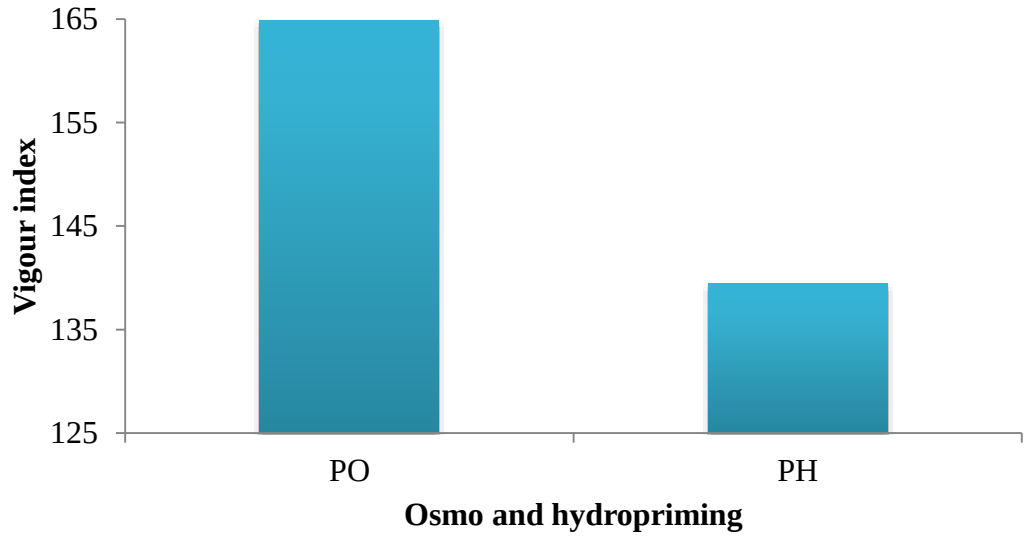
Priming technique and duration showed significant influence on vigor index of BARI Mung 6. Between two priming techniques, the highest vigor index (164.92) was recorded in P_O (primed with H_2O_2) and the lowest (139.49) was obtained in P_H (primed with water) (Figure 19).

Effect of priming time

Results showed that among different priming time, the highest vigor index (197.40) was recorded in T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest (115.30) was recorded in T_6 (2% H_2O_2 concentration for 15 hours priming) (Figure 20).

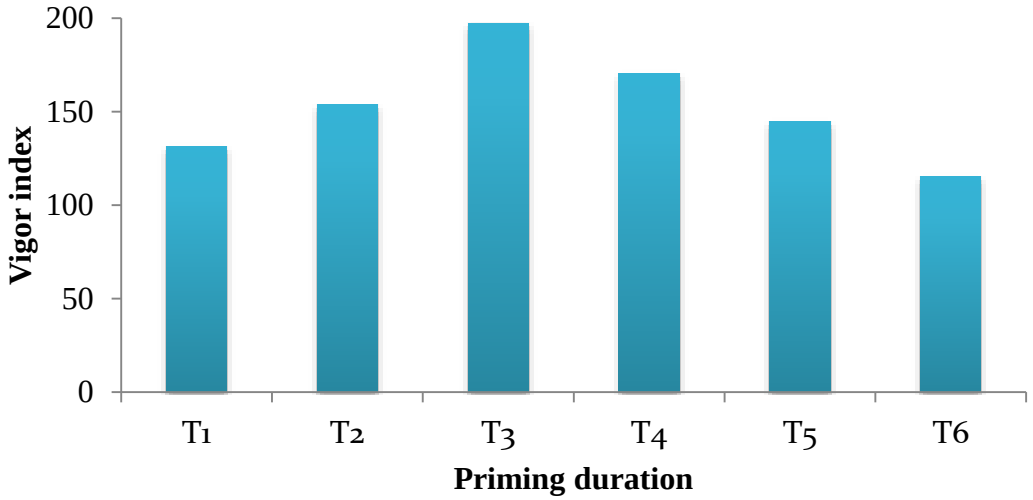
Interaction effect of osmo and hydropriming and priming time

Interaction effect of osmo and hydropriming and different priming time on the vigor index of primed seeds showed significant variation (Table 6). The highest vigor index (213.20) was recorded in P_OT_3 and the lowest (104.50) in P_HT_6.



P_O = Osmopriming, P_H = Hydropriming

Figure 19. Effect of osmo and hydropriming on the vigor index of BARI Mung 6 ($LSD_{0.01}$ = 4.62)



T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Figure 20. Effect of priming duration on the vigor index of BARI Mung 6 ($LSD_{0.01}$ = 10.65)

Table 6. Interaction effect of osmo and hydropriming and priming time on the water relation behavior of BARI Mung 6

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
P_OT_1	81.82 b-d	18.18 e	15.65 c	18.30 c-e	138.90 ef
P_OT_2	87.54 b	12.46 f	18.92 b	20.45 ab	166.40 c
P_OT_3	94.95 a	5.05 g	21.79 a	21.60 a	213.20 a
P_OT_4	86.35 b	13.25 f	18.31 b	19.51 bc	186.20 b
P_OT_5	77.43 cd	22.57 cd	16.00 c	17.02 def	158.70 c
P_OT_6	66.79 e	33.21 b	12.31 d	13.49 g	126.10 f
P_HT_1	75.12 d	24.88 c	12.68 d	16.55 ef	124.10 f
P_HT_2	78.89 cd	21.11 d	15.52 c	18.63 b-d	141.20 de
P_HT_3	83.39 bc	16.41 e	19.64 b	19.97 a-c	181.70 b
P_HT_4	77.14 cd	22.86 cd	16.57 c	18.50 cd	154.80 cd
P_HT_5	65.68 e	34.32 b	13.52 d	15.31 fg	130.60 ef
P_HT_6	55.80 f	44.20 a	9.560 e	11.51 h	104.50 g
LSD $_{(0.01)}$	7.37	2.70	1.58	1.85	15.07
CV (%)	5.60	7.11	5.87	6.21	5.84

P_O = Osmopriming, P_H = Hydropriming

T_1 = 0 hour, T_2 = 3 hours, T_3 = 6 hours, T_4 = 9 hours, T_5 = 12 hours, T_6 = 15 hours

Achievement from the second experiment

From the second experiment, BARI Mung 6 primed with 2% H_2O_2 solution for 6 hours gave the best result. So, 2% H_2O_2 solution for 6 hours priming time was used for the next experiment to evaluate best result under drought stress condition.

4.3 Experiment 3

Evaluation of the effect of seed priming on germination and vigor of mungbean under drought stress

This experiment was conducted under laboratory condition. Mungbean variety (BARI Mung 6) was primed in 2% H_2O_2 for 6 hours. Dry seeds were used as control and was exposed to 0, 5%, 10%, 15% and 20% PEG (Polyethylene Glycol) induced drought stress conditions in petri dishes. The results have been presented separately in Table 7 to 10 under the following headings:

4.3.1 Germination rate (%)

Effect of priming technique

Different PEG levels revealed significant variation in respect of germination rate (Table 7). Result indicated that the rate of germination of primed seeds decreased significantly with increasing PEG level. Between two priming techniques, the highest germination rate (84.44%) was recorded in P_O (primed with H_2O_2) and the lowest (78.36%) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest germination rate (91.12%) was observed in T_0 (no PEG) being followed (88.21%) by T_1 (5% PEG) and (83.84%) from T_2 (10% PEG) while, the lowest (68.04%) in T_4 (20% PEG) (Table 7). Razaji *et al.* (2014) reported that priming resulted improvement in germination components and enzymes activity of rapeseed on drought stress condition and boost the resistance of rapeseed to drought stress. Compared to hydro priming, priming with PEG in a proper concentration had better effect on seed germination under drought stress although such effects had limited capability and severe drought stress inhibited germination (Sun *et al.*, 2010).

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the germination rate of primed seeds showed significant variation (Table 8). The highest germination rate (94.46%) was recorded in P_OT_0 which was statistically similar with P_OT_1 (91.98%), P_OT_2 (86.96%), P_HT_0 (87.77%) and P_HT_1 (84.44%) while, the lowest (64.44%) in P_HT_4 which was statistically similar with P_HT_3 (74.44%) and P_OT_4 (71.63%).

4.3.2 Shoot length (mm)

Effect of priming technique

Shoot length of BARI Mung 6 was significantly influenced by different drought stress levels (Table 7). Data revealed that the shoot length from primed seeds decreased significantly with increasing drought stress level. Between two priming techniques, the highest shoot length (121.84 mm) was recorded in P_O (primed with H_2O_2) and the lowest (110.10 mm) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest shoot length (132.30 mm) was observed in T_0 (no PEG) being followed (128.20 mm) by T_1 (5% PEG) while the lowest (91.80 mm) was obtained in T_4 (20% PEG) (Table 7).

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the shoot length of primed seeds showed significant variation (Table 8). The highest shoot length (137.20 mm) was recorded in P_OT_0 which was statistically similar with P_OT_1 (132.60 mm), P_OT_2 (125.40 mm), P_HT_0 (127.50 mm) and P_HT_1 (123.70 mm) while, the lowest (85.14 mm) in P_HT_4 which was statistically similar with P_HT_3 (100.30 mm) and P_OT_4 (98.45 mm).

4.3.3 Root length (mm)

Effect of priming technique

Root length of BARI Mung 6 was significantly influenced by different drought stress levels (Table 7). Data indicated that the root length from primed seeds decreased significantly with increasing drought stress level. Between two priming techniques, the highest root length (81.90 mm) was recorded in P_O (primed with H_2O_2) and the lowest (70.41 mm) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest root length (90.02 mm) was observed in T_0 (no PEG) being followed (85.09 mm) by T_1 (5% PEG) while the lowest (55.36 mm) was obtained in T_4 (20% PEG) (Table 7). Similar results were reported by Farooq *et al.* (2005) who reported that significant improvement in root and shoot length may be attributed to earlier germination induced by primed seeds over non-primed ones, which resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the root length of primed seeds showed significant variation (Table 8). The highest root length (93.07 mm) was recorded in P_OT_0 which was statistically similar with P_OT_1 (89.42 mm) and P_HT_o (86.98 mm) while, the lowest (45.92 mm) in P_HT_4.

4.3.4 Shoot dry weight (mg)

Effect of priming technique

Drought stress level had significant influence on shoot dry weight of BARI Mung 6 (Table 7). Between two priming techniques, the highest shoot dry weight (58.01 mg) was recorded in P_O (primed with H_2O_2) and the lowest (52.36 mg) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest shoot dry weight (65.40 mg) was observed in T_0 (no PEG) being followed (61.32 mm) by T_1 (5% PEG) while the lowest (42.36 mg) was obtained in T_4 (20% PEG) (Table 7). Umair *et al.* (2010) found that priming treatments increased the dry matter yield of shoot as well as root as compare to control.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the shoot dry weight of primed seeds showed significant variation (Table 8). The highest shoot dry weight (67.86 mg) was recorded in P_OT_0 which was statistically similar with P_OT_1 (64.72 mg) and P_HT_o (62.93 mg) while, the lowest (39.96 mg) in P_HT_4 which was statistically similar with P_HT_3 (47.55 mg) and P_OT_4 (44.47 mg).

4.3.5 Root dry weight (mg)

Effect of priming technique

Drought stress level had significant influence on root dry weight of BARI Mung 6 (Table 7). Between two priming techniques, the highest root dry weight (18.70 mg) was recorded in P_O (primed with H_2O_2) and the lowest (15.60 mg) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest root dry weight (22.94 mg) was observed in T_0 (no PEG) being followed (20.74 mg) by T_1 (5% PEG) while the lowest (10.00 mg) was obtained in T_4 (20% PEG) (Table 7). Umair *et al.* (2010) found that priming treatments increased the dry matter yield of shoot as well as root as compare to control.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the root dry weight of primed seeds showed significant variation (Table 8). The highest root dry weight (24.72 mg) was recorded in P_OT_0 which was statistically similar with P_OT_1 (22.53 mg) and the lowest (8.52 mg) in P_HT_4.

Table 7. Effect of osmo and hydropriming and different PEG concentrations on the germination and growth behavior of BARI Mung 6

Treatments	Germination rate (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
Effect of osmo and hydropriming					
P_O	84.44 a	121.84 a	81.09 a	58.01 a	18.70 a
P_H	78.36 b	110.10 b	70.41 b	52.36 b	15.60 b
LSD $_{(0.01)}$	3.41	5.00	3.25	2.59	0.76
CV (%)	5.5	5.66	5.63	6.17	5.79
Effect of different PEG concentrations					
T_0	91.12 a	132.3 a	90.02 a	65.40 a	22.94 a
T_1	88.21 a	128.2 ab	85.09 ab	61.32 ab	20.74 b
T_2	83.84 a	119.6 b	78.27 b	56.67 b	18.03 c
T_3	75.81 b	107.9 c	70.01 c	50.15 c	14.03 d
T_4	68.04 c	91.80 d	55.36 d	42.36 d	10.00 e
LSD $_{(0.01)}$	7.36	10.78	7.01	5.59	1.63
CV (%)	5.5	5.66	5.63	6.17	5.79

P_O = Osmopriming, P_H = Hydropriming

T_0 = No PEG, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG, T_4 = 20% PEG

Table 8. Interaction effect of osmo and hydropriming and different PEG concentrations on the germination and growth behavior of BARI Mung 6

Treatment combinations	Germination rate (%)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)
P_OT_0	94.46 a	137.20 a	93.07 a	67.86 a	24.72 a
P_OT_1	91.98 a	132.60 a	89.42 ab	64.72 ab	22.53 ab
P_OT_2	86.96 a-c	125.40 ab	83.09 bc	59.93 bc	19.57 c
P_OT_3	77.19 c-e	115.50 b	75.08 c	52.75 cd	15.19 d
P_OT_4	71.63 ef	98.45 d	64.80 d	44.77 e	11.47 e
P_HT_0	87.77 ab	127.50 ab	86.98 ab	62.93 ab	21.16 bc
P_HT_1	84.44 a-d	123.70 ab	80.75 bc	57.93 bc	18.95 c
P_HT_2	80.72 b-e	113.90 bc	73.44 cd	53.41 cd	16.49 d
P_HT_3	74.44 d-f	100.30 cd	64.93 d	47.55 de	12.87 e
P_HT_4	64.44 f	85.14 d	45.92 e	39.96 e	8.52 f
$LSD_{(0.01)}$	10.41	15.24	9.91	7.91	2.31
CV (%)	5.5	5.66	5.63	6.17	5.79

P_O = Osmopriming, P_H = Hydropriming

T_0 = No PEG, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG, T_4 = 20% PEG

4.3.6 Relative water content (%)

Effect of priming technique

Relative water content of BARI Mung 6 was significantly affected by different drought stress level (Table 9). There was a gradual decrease in RWC with increasing the drought stress level. Between two priming techniques, the highest relative water content (80.69%) was recorded in P_O (primed with H_2O_2) and the lowest relative water content (71.76%) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest relative water content (88.40%) was observed in T_0 (no PEG) being followed (85.48%) by T_1 (5% PEG) while the lowest relative water content (58.76%) was obtained from T_4 (20% PEG) (Table 9). Baque *et al*. (2002) reported that higher doses of potassium in drought affected wheat generally showed the maximum relative water content, higher water retention capacity and exudation rate.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the relative water content of primed seeds showed significant variation (Table 10). The highest relative water content (91.61%) was recorded in P_OT_0 which was statistically similar with P_OT_1 (88.82%), P_OT_2 (82.60%), P_HT_0 (85.19%) and P_HT_1 (82.15%) while, the lowest (52.61%) in P_HT_4.

4.3.7 Water saturation deficit (%)

Effect of priming technique

A wide range of statistical difference was observed due to difference in drought stress level (Table 9). Water saturation deficit increased with increased drought stress level. Between two priming techniques, the highest water saturation deficit (28.24%) was recorded in P_H (primed with water) and the lowest (19.32%) was obtained in P_O (primed with H_2O_2).

Effect of PEG concentration

Under drought stress, the highest water saturation deficit (41.24%) was observed in T_4 (20% PEG) being followed (14.52%) by T_1 (5% PEG) while the lowest (11.60%) was obtained in T_0 (no PEG) (Table 9).

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the water saturation deficit of primed seeds showed significant variation (Table 10). The highest water saturation deficit (47.39%) was recorded in P_HT_4 and the lowest (8.39%) in P_OT_0 which was statistically similar with P_OT_1 (11.18%).

4.3.8 Water retention capacity

Effect of priming technique

Significant difference was found in terms of water retention capacity of BARI Mung 6 due to difference in drought stress level (Table 9). Between two priming techniques, the highest water retention capacity (17.53) was recorded in P_O (primed with H_2O_2) and the lowest (14.26) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest water retention capacity (20.85) was observed in T_0 (no PEG) being followed (19.60) by T_1 (5% PEG) while the lowest (9.38) was obtained in T_4 (20% PEG) (Table 9). Noticeably, the relative increment of WRC due to priming the seeds with hydrogen peroxide was greater in seedling grown under severe water stress condition than that in the plants grown under both control and mild stress

condition. Perhaps the better maintenance of cell structure due to application of high level of potassium was partly responsible for the relatively high WRC at water stressed plants than the control plants (Sangakkara *et al*., 1996).

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the water retention capacity of primed seeds showed significant variation (Table 10). The highest water retention capacity (22.42) was recorded in P_OT_0 which was statistically similar with P_OT_1 (21.37) and the lowest (8.34) in P_HT_4 which was statistically similar with P_OT_4 (10.42).

Table 9. Effect of osmo and hydropriming and different PEG concentrations on the water relation behavior of BARI Mung 6

Treatments	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
Effect of osmo and hydropriming					
P_O	80.69 a	19.32 b	17.53 a	18.66 a	173.33 a
P_H	71.76 b	28.24 a	14.26 b	15.92 b	143.90 b
LSD $_{(0.01)}$	3.25	1.08	0.73	0.79	6.35
CV (%)	5.6	5.96	6.06	6.03	5.25
Effect of different PEG concentrations					
T_0	88.40 a	11.60 e	20.85 a	21.11 a	202.80 a
T_1	85.48 ab	14.52 d	19.60 a	19.80 ab	188.50 b
T_2	79.22 b	20.78 c	16.48 b	18.19 b	166.10 c
T_3	69.25 c	30.75 b	13.16 c	15.31 c	135.00 d
T_4	58.76 d	41.24 a	9.38 d	12.04 d	100.70 e
LSD $_{(0.01)}$	7.02	2.33	1.58	1.71	13.69
CV (%)	5.6	5.96	6.06	6.03	5.25

P_O = Osmopriming, P_H = Hydropriming

T_0 = No PEG, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG, T_4 = 20% PEG

4.3.9 Coefficient of velocity of germination

Effect of priming technique

Primed seed showed significant difference on coefficient of velocity of germination of BARI Mung 6 under different drought stress level (Table 9). Between two priming techniques, the highest coefficient of velocity of germination (18.66) was recorded in P_O (primed with H_2O_2) and the lowest (15.92) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest coefficient of velocity of germination (21.11) was observed in T_0 (no PEG) being followed (19.80) by T_1 (5% PEG) while the lowest (12.04) was obtained in T_4 (20% PEG) (Table 9). Asaduzzaman (2014) reported that the maximum coefficient of velocity of germination were found in the low PEG level and decreased with increasing PEG concentration.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the coefficient of velocity of germination of primed seeds showed significant variation (Table 10). The highest coefficient of velocity of germination (22.21) was recorded in P_OT_0 which was statistically similar with P_OT_1 (21.09) and P_HT_0 (20.01) while, the lowest (10.54) in P_HT_4.

4.3.10 Vigor index

Effect of priming technique

Statistically significant difference was observed in case of vigor index of BARI Mung 6 under different drought stress level (Table 9). Between two priming techniques, the highest vigor index (173.33) was recorded in P_O (primed with H_2O_2) and the lowest vigor index (143.90) was obtained in P_H (primed with water).

Effect of PEG concentration

Under drought stress, the highest vigor index (202.80) was observed in T_0 (no PEG) being followed (188.50) by T_1 (5% PEG) while the lowest vigor index (100.70) was obtained in T_4 (20% PEG) (Table 9). Similar findings were reported by Ruan *et al*. (2002) who reported that primed rice seeds showed higher vigor index than non-primed ones.

Interaction effect of osmo and hydropriming and PEG concentration

Interaction effect of osmo and hydropriming and different PEG concentrations on the vigor index of primed seeds showed significant variation (Table 10). The highest vigor

index (217.40) was recorded in P_OT_0 which was statistically similar with P_OT_1 (204.40) and the lowest (84.64) in P_HT_4.

Table 10. Interaction effect of osmo and hydropriming and different PEG concentrations on the water relation behavior of BARI Mung 6

Treatment combinations	Relative water content (%)	Water saturation deficit	Water retention capacity	Coefficient of germination	Vigor index
P_OT_0	91.61 a	8.39 e	22.42 a	22.21 a	217.40 a
P_OT_1	88.82 a	11.18 e	21.37 ab	21.09 ab	204.40 ab
P_OT_2	82.60 ab	17.40 d	18.51 c	19.76 bc	181.20 c
P_OT_3	75.49 b	24.51 c	14.93 d	16.72 d	147.00 d
P_OT_4	64.91 c	35.09 b	10.42 ef	13.53 e	116.70 e
P_HT_0	85.19 ab	14.81 d	19.29 bc	20.01 a-c	188.10 bc
P_HT_1	82.15 ab	17.85 d	17.83 c	18.51 cd	172.60 c
P_HT_2	75.84 b	24.16 c	14.44 d	16.62 d	151.00 d
P_HT_3	63.02 c	36.98 b	11.39 e	13.89 e	123.00 e
P_HT_4	52.61 d	47.39 a	8.34 f	10.54 f	84.64 f
$LSD_{(0.01)}$	9.92	3.29	2.24	2.42	19.35
CV (%)	5.6	5.96	6.06	6.03	5.25

P_O = Osmopriming, P_H = Hydropriming

T_0 = No PEG, T_1 = 5% PEG, T_2 = 10% PEG, T_3 = 15% PEG, T_4 = 20% PEG

Achievement from the third experiment

From the third experiment, seeds primed with H_2O_2 under non-stressed condition (no PEG) gave the best result being followed by T_1 (5% PEG) while the lowest was obtained in T_4 (20% PEG).

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted at the Laboratory of Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during July 2017 to September 2017 to study the effect of H_2O_2 and hydropriming for enhancing drought tolerance capability in mungbean (*Vigna radiata* L.) under drought stress. The whole study was conducted in three different experiments. The experiments were laid out in a Completely Randomized Design (CRD) with five replications. Two mungbean varieties cv. BARI Mung 6 and BINA Moog 8 were used as test crops. Different priming chemicals such as H_2O_2 and distilled water were utilized for osmopriming and hydro priming while PEG was used to induce drought stress. Priming was done in room temperature and all the primed seeds were removed from the priming solution at the same time. Thirty seeds from each of the treatments were selected randomly and placed in 120 mm diameter petri dishes on Whatman No.1 filter papers and filter papers were moistened with 8 ml of distilled water. Germination was considered to have occurred when radicles were 2 mm long. Germination progress was examined and data were collected at every 24 hours intervals and continued up to 10 days. The abnormal or dead seedlings were excluded during counting. The data recorded on germination rate (GR), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), relative water content (RWC), water saturation deficit (WSD), water retention capacity (WRC), coefficient of velocity of germination (CV) and vigor index (VI). Data were analyzed using a computer software MSTAT-C. The significance of difference among the treatments means was estimated by the LSD at 1% level of probability.

Experiment I

The first experiment was carried out to find the effect of different concentration of H_2O_2 on germination and growth behavior of two mungbean varieties (BARI Mung 6 and BINA Moog 8) without any stress condition. Four levels of H_2O_2 such as 2%, 4%, 6% and 8% were used for osmopriming and distilled water used as hydro priming agent for 24 hours respectively. Between two varieties, V_1 (BARI Mung 6) gave the best results on studied parameters. Results revealed that V_1 (BARI Mung 6) showed the highest GR (80.14%), SL (137.40 mm), RL (76.29 mm), SDW (58.38 mg), RDW (20.76 mg), RWC (78.15%), WRC (18.19), CV (17.59) and VI (173.63) and V_2 (BINA Moog 8) showed the lowest GR (72.19%), SL (122.83 mm), RL (67.74 mm), SDW (54.55 mg), RDW

(18.80 mg), RWC (72.27%), WRC (15.28), CV (15.55) and VI (139.87). Among the priming concentrations, the highest GR (90.31%), SL (145.10 mm), RL (91.25 mm), SDW (64.24 mg), RDW (23.23 mg), RWC (89.25%), WRC (21.81), CV (21.48) and VI (214.20) were recorded from P_2 (2% H_2O_2 concentration); whereas, the lowest GR (59.99%), SL (115.20 mm), RL (53.89 mm), SDW (50.15 mg), RDW (16.60 mg), RWC (56.38%), WRC (9.59), CV (9.56) and VI (102.10) were recorded from P_5 (8% H_2O_2 concentration). In interaction, the highest result were recorded from V_1P_2 and the lowest from V_2P_5.

Experiment II

The second experiment was conducted to optimization of priming time on the germination and growth behavior of BARI Mung 6. Mungbean variety (BARI Mung 6) without any stress condition was considered. Six different priming times such as 0, 3, 6, 9, 12 and 15 hours for osmopriming were used using 2% H_2O_2 concentration. Between two priming techniques, the highest GR (82.03%), SL (128.57 mm), RL (71.04 mm), SDW (55.21 mg), RDW (18.89 mg), RWC (82.48%), WRC (17.17), CV (18.40) and VI (164.92) were recorded from P_O (primed with H_2O_2) and the lowest GR (77.22%), SL (119.35 mm), RL (59.96 mm), SDW (51.17 mg), RDW (16.48 mg), RWC (72.67%), WRC (14.58), CV (16.75) and VI (139.49) were obtained from P_H (primed with water). Among different priming times, the highest GR (90.21%), SL (139.90 mm), RL (78.29 mm), SDW (59.86 mg), RDW (20.89 mg), RWC (89.17%), WRC (20.72), CV (20.78) and VI (197.40) were recorded from T_3 (2% H_2O_2 concentration for 6 hours priming) and the lowest GR (69.29%), SL (110.60 mm), RL (55.45 mm), SDW (46.57 mg), RDW (14.86 mg), RWC (61.29%), WRC (10.94), CV (12.50) and VI (115.30) were recorded from T_6 (2% H_2O_2 concentration for 15 hours priming). In interaction, the highest result were recorded from P_OT_3 and the lowest from P_HT_6.

Experiment III

In the third experiment germination and growth behavior of primed seeds of BARI Mung 6 with and without drought (PEG) stress condition was evaluated. H_2O_2 concentration of 2% was used as priming solutions and 6 hours as priming time and drought stress levels; without drought (no PEG), 5%, 10%, 15% and 20% PEG were used in this experiment. Between two priming techniques, the highest GR (84.44%), SL (121.84 mm), RL (81.09 mm), SDW (58.01 mg), RDW (18.70 mg), RWC (80.69%),

WRC (17.53), CV (18.66) and VI (173.33) were recorded from P_O (primed with H_2O_2 concentration for 6 hours priming) and the lowest GR (78.36%), SL (110.10 mm), RL (70.41 mm), SDW (52.36 mg), RDW (15.60 mg), RWC (71.76%), WRC (14.26), CV (15.92) and VI (143.90) were obtained from P_H (primed with water for 6 hours priming). Under drought stress, the highest GR (91.12%), SL (132.30 mm), RL (90.02 mm), SDW (65.40 mg), RDW (22.94 mg), RWC (88.40%), WRC (20.85), CV (21.11) and VI (202.80) were observed from T_0 (no PEG) and after that the second highest GR (88.21%), SL (128.20 mm), RL (85.09 mm), SDW (61.32 mg), RDW (20.74 mg), RWC (85.48%), WRC (19.60), CV (19.80) and VI (188.50) were in T_1 (5% PEG); whereas, the lowest GR (68.04%), SL (91.80 mm), RL (55.36 mm), SDW (42.36 mg), RDW (10.00 mg), RWC (58.76%), WRC (9.38), CV (12.04) and VI (100.70) were obtained from T_4 (20% PEG). In interaction, the highest result were recorded from P_OT_0 and the lowest from P_HT_4.

Considering the findings of the present experiment following conclusions and recommendations may be drawn:

- Performance of BARI Mung 6 was better in terms of germination and growth parameters when seeds were primed with 2% H_2O_2 concentration for 6 hours.
- Seeds placed under 5% PEG drought stress condition showed more or less similar performance to seeds placed with no drought stress (0% PEG) but seeds placed under 20% PEG stress condition showed very poor performance in all aspects.
- Seed priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of drought stress.
- Further studies are needed to assess the efficacy of seed priming during the later stages of the crop.
- More research should be conducted to evaluate the field performance of priming technique.

CHAPTER VI

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APPENDICES

Appendix I. Monthly records of temperature, rainfall, and relative humidity of the experiment site during the period of July 2017 to September 2017

Year	Month	Air Temperature (^{0}C)			Relative humidity (%)	Rainfall (mm)	Sunshine (Hour)
		Maximum	Minimum	Mean			
2017	July	29.8	19.5	24.65	70.4	0.0	235.4
2017	September	25.3	16.9	21.1	68.3	0.0	232.9

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka

Appendix II. Mean square value on different concentrations of H_2O_2 on germination percentage (%) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	6271.486	1254.297**	58.9849
Error	48	1020.707	21.265	

**Significant at 1% level of significance

Appendix III. Mean square value on different concentrations of H_2O_2 on shoot length (mm) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	6303.278	1260.656**	24.0181
Error	48	2519.408	52.488	

**Significant at 1% level of significance

Appendix IV. Mean square value on different concentrations of H_2O_2 on root length (mm) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	8894.552	1778.910**	100.1323
Error	48	852.749	17.766	

**Significant at 1% level of significance

Appendix V. Mean square value on different concentrations of H_2O_2 on shoot dry weight (mg) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	1350.348	270.070**	26.9934
Error	48	480.240	10.005	

**Significant at 1% level of significance

Appendix VI. Mean square value on different concentrations of H_2O_2 on root dry weight (mg) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	287.693	57.539**	38.6818
Error	48	71.399	1.487	

**Significant at 1% level of significance

Appendix VII. Mean square value on different concentrations of H_2O_2 on relative water content (%) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	6706.827	1341.365**	78.2061
Error	48	823.280	17.152	

**Significant at 1% level of significance

Appendix VIII. Mean square value on different concentrations of H_2O_2 on water saturation deficit (%) of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	6706.546	1341.309**	553.1150
Error	48	116.400	2.425	

**Significant at 1% level of significance

Appendix IX. Mean square value on different concentrations of H_2O_2 on water retention capacity of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	933.457	186.691^{NS}	216.7549
Error	48	41.342	0.861	

NS - Non Significant

Appendix X. Mean square value on different concentrations of H_2O_2 on coefficient of velocity of germination of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	891.544	178.309**	157.5075
Error	48	54.339	1.132	

**Significant at 1% level of significance

Appendix XI. Mean square value on different concentrations of H_2O_2 on vigor index of mungbean varieties

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	84640.297	16928.059**	178.7753
Error	48	4545.074	94.689	

**Significant at 1% level of significance

Appendix XII. Mean square value for germination percentage (%) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	2539.272	507.854**	27.2199
Error	48	895.557	18.657	

**Significant at 1% level of significance

Appendix XIII. Mean square value for shoot length (mm) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	6453.525	1290.705**	24.2806
Error	48	2551.581	53.158	

**Significant at 1% level of significance

Appendix XIV. Mean square value for root length (mm) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	3567.967	713.593**	10.7604
Error	48	3183.208	66.317	

**Significant at 1% level of significance

Appendix XV. Mean square value for shoot dry weight (mg) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	1058.007	201.601**	6.6127
Error	48	1535.960	31.999	

**Significant at 1% level of significance

Appendix XVI. Mean square value for root dry weight (mg) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	225.324	45.065**	39.4515
Error	48	54.829	1.142	

**Significant at 1% level of significance

Appendix XVII. Mean square value for relative water content (%) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	4857.455	971.491**	51.4092
Error	48	907.066	18.897	

**Significant at 1% level of significance

Appendix XVIII. Mean square value for water saturation deficit (%) of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	4897.675	979.535**	386.8948
Error	48	121.526	2.532	

**Significant at 1% level of significance

Appendix XIX. Mean square value for water retention capacity of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	562.336	112.467^{NS}	129.4283
Error	48	41.710	0.869	

NS - Non Significant

Appendix XX. Mean square value for coefficient of velocity of germination of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	439.875	87.975**	73.9638
Error	48	57.093	1.189	

**Significant at 1% level of significance

Appendix XXI. Mean square value for vigor index of BARI Mung 6 on different priming time

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	5	42331.452	8466.290**	107.3223
Error	48	3786.555	78.887	

**Significant at 1% level of significance

Appendix XXII. Mean square value for germination percentage (%) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	2139.060	534.765**	26.6601
Error	20	401.173	20.059	

**Significant at 1% level of significance

Appendix XXIII. Mean square value for shoot length (mm) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	6474.887	1618.722**	37.6161
Error	20	860.655	43.033	

**Significant at 1% level of significance

Appendix XXIV. Mean square value for root length (mm) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	4476.075	1119.019**	61.4981
Error	20	363.920	18.196	

**Significant at 1% level of significance

Appendix XXV. Mean square value for shoot dry weight (mg) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	2003.126	500.781**	43.1978
Error	20	231.855	11.593	

**Significant at 1% level of significance

Appendix XXVI. Mean square value for root dry weight (mg) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	648.538	162.135^{NS}	164.5374
Error	20	19.708	0.985	

NS - Non Significant

Appendix XXVII. Mean square value for relative water content (%) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	3579.193	894.798**	49.0604
Error	20	364.774	18.239	

**Significant at 1% level of significance

Appendix XXVIII. Mean square value for water saturation deficit (%) of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	3579.253	894.813**	445.7151
Error	20	40.152	2.008	

**Significant at 1% level of significance

Appendix XXIX. Mean square value for water retention capacity of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	531.156	132.789^{NS}	143.3686
Error	20	18.524	0.926	

NS - Non Significant

Appendix XXX. Mean square value for coefficient of velocity of germination of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	319.320	79.830**	73.5582
Error	20	21.705	1.085	

**Significant at 1% level of significance

Appendix XXXI. Mean square value for vigor index of BARI Mung 6 under different level of drought stress

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Treatment	4	40901.590	10225.397**	147.3320
Error	20	1388.076	69.404	

**Significant at 1% level of significance

PLATES

Plate 1: Seedlings from non-primed seeds

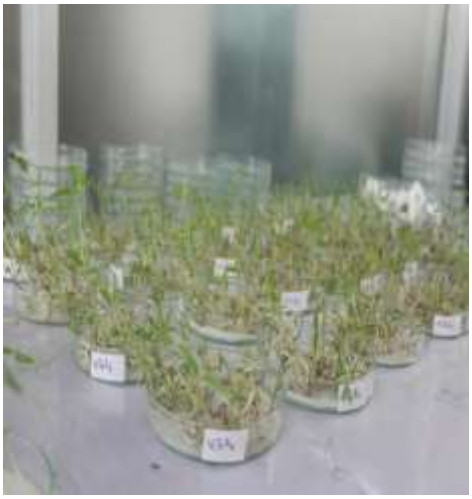

Plate 2: Seedlings from primed seeds

Plate 3: Seedlings from seed primed for 6 hours

Plate 4: Seedlings from seed primed for 15 hours

Plate 5: Seedlings without drought

Plate 6: Seedlings with 5%PEG

Plate 7: Seedlings with 10%PEG

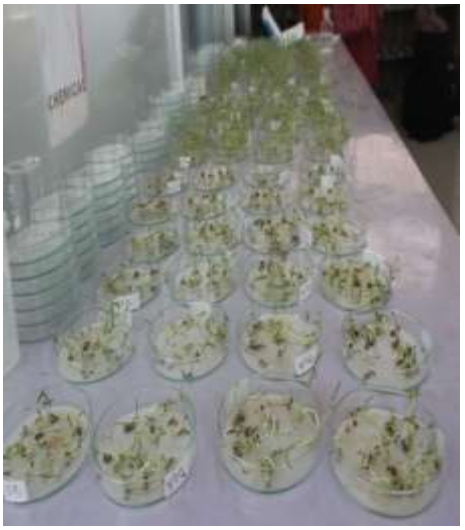

Plate 8: Seedlings with 20%PEG