EFFECT OF SEED PRIMING ON GERMINATION AND SEEDLING GROWTH OF BLACKGRAM UNDER SALT STRESS CONDITION

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CERTIFICATE

*This is to certify that thesis entitled, "**EFFECT OF SEED PRIMING ON GERMINATION AND SEEDLING GROWTH OF BLACKGRAM UNDER SALT STRESS CONDITION**" submitted to the Institute of Seed Technology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in Agronomy**, embodies the result of a piece of bona fide research work carried out by **Eastik Kanis Fatema**, Registration No. **14-06027** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated:
Place: Dhaka, Bangladesh

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DEDICATED TO MY BELOVED PARENTS AND TEACHERS

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The Author

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ABSTRACT

A laboratory experiment was conducted at the Agronomy lab, Sher-e-Bangla Agricultural University, during the period from December 2019 to March 2020 to investigate the potentiality of seed priming for induction of salt tolerance capability and the pre-sowing seed treatment with water and NaCl on germination behavior of blackgram (BARI Mush-3 and BARI Mush-4) under salt stress conditions. The whole experiment was divided into two experiments. In first work, two blackgram varieties were surface sterilized with 5% sodium hypochlorite (NaOCl) solution, soaked in water and 2 mM and 3 mM NaCl for 6 hours and dry seed used as control. The highest total germination (97.32%), germination index (45.46), coefficient of velocity (21.04), energy of emergence (94.99%) and vigor index (157.00) were obtain from seeds primed in water BARI Mush-3 compare to BARI Mush-4 than all other treatments. In the second experiment, seeds were primed with distilled water and 2 mM and 3 mM NaCl for 6 hours; dry seed used as control and were exposed to 0, 50, 100, 150, and 200 mM NaCl induced salt stress conditions in Petri dishes. Priming with water followed by 2 mM were more effective than the control seed in inducing salt tolerance of blackgram cultivars owing to enhanced germination and growth parameters under salt stress condition. From the results of the study, it was observed that seeds primed with water for 6 hours showed the best result in comparison to 2 mM and 3 mM NaCl primed seed and dry seed.

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LIST OF ACCONYMS AND ABBREVIATIONS

Agric.	Agriculture
Agril.	Agricultural
Anon.	Anonymous
AOSA	Association of Official Seed Analysis
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
^{o}C	Degree centigrate
cm	Centi-meter
CRD	Completely Randomized Design
CV	Coefficient of velocity
Dev.	Development
ed.	Edition
EG	Energy of emergence
Environ.	Environmental
et al.	And others
Expt.	Experimental
GI	Germination index
mM	Milimolar
mg	Milligram
MGT	Mean germination time
ml	Milliliter
mm	Millimeter
mM	Millimole
No.	Number
Sci.	Science
i.e.	*idest* (L), that is
Inst.	Institute
Int.	International
ISTA	International Seed Testing Association

Res.	Research
ROS	Reactive Oxygen Species
j.	Journal
MeJA	Methyl jasm-onate
min	Minute
M.S	Master of Science
DMRT	Duncan's Multiple Range Test
SAU	Sher-e-Bangla Agricultural University
SE	Standard Error
Technol.	Technology
TG	Total germination
VI	Vigour Index
viz	Namely
%	Percentage

CHAPTER I

INTRODUCTION

Pulse crop belongs to grain legume. In Bangladesh various types of pulse crops are grown. Among them lentil, cowpea, blackgram, mungbean, field pea and grass pea are important. Pulses constitute the main source of protein for the people, particularly the poor sections of Bangladesh. These are also the best source of protein for domestic animals. Besides, the crops have the capability to enrich soils through nitrogen fixation (Sharma and Behera, 2009). Pulse protein is rich in lysine that is deficient in rice. According to FAO (2017) recommendation, a minimum intake of pulse by a human should be 80 gm/day, whereas it is 21 g in Bangladesh (BBS, 2018). This is because of fact that national production of the pulses is not adequate to meet our national demand. In Bangladesh, total production of pulses is only 0.38 million ton against 1.3-1.4 million tons requirement. At present, the area under pulse crop is 0.406 million hectare with a production of 0.322 million tons (BBS, 2018), where blackgram is cultivated in the area of 0.108 million ha with production of 0.03 million tons (BBS, 2018).

Blackgram (*Vigna mungo* L.) is one of the most important grain legumes among pulses in Bangladesh. It is widely cultivated in the worldwide for high protein in its seeds. It is highly nutritious and the green pods are eaten as vegetable. Being a legume, it enriches soil health through biological N fixation with rhizobia and it can also break disease cycles and encourage mycorrhizae (Hedley, 2001). A drought resistant crop, it is grown both as a summer and winter crop often in rotation with rice but sometimes in mixed cultivation. Blackgram is cultivated in about 161,000 acres of land in Bangladesh and total annual production is about 50000 m tons. The dried seeds contain approximately 9.7% water, 23.4% protein, 1% fat, 57.3% carbohydrate, 3.8% fiber and 4.8% ash. It is also used as a green manure and cover crop or fodder crop, and as short-lived forage.

Poor crop establishment is a constraint for pulse crop production (Naseem *et al.,* 1997; Rahmianna *et al*., 2000). Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects at physiological, biochemical, and molecular levels, limiting crop productivity. The total worldwide area of land

affected by salinity is about 397.1 million ha (FAO, 2018). It has become a severe threat to ensure food security in the developing world. Increasing salinity had significant impact on food production and more agriculture lands are expected to become salt affected due to climate change effect (Rengasamy, 2006). Soil salinity affects germination by either an osmotic stress or ion toxic effect (Bewley and Black, 1982). Salinity causes a variety of biochemical, physiological and metabolic changes (Xiong and Zhu, 2002), which may result in oxidative stress and affect plant metabolism, performance and thereby the yield (Shafi *et al.,* 2009). Salt and osmotic stresses are also responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al.,* 2001). Soil salinity may affect the germination of seeds either by creating a lower osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the germinating seed (Khajeh-Hosseini et al., 2003). Plant growth and development are regulated by a number of intrinsic and extrinsic factors, which can be modified in various ways. There are different approaches to mitigate the salt hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahloowalia *et al*.,2004), in vitro selection, use of plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H_2O_2) and osmoprotectants as foliar application and seed treatment (Senaratna *et al.,* 2000; Farooq *et al.,* 2009).

For the maintenance of high yield of crops under salt stressed conditions, various research tools are being tried to counteract the effects of salinity. Seed priming treatments are simply applied practices that can reduce the effects of salinity with small inputs of capital and energy. Many such seed priming or invigoration treatments are being used to improve the rate and speed of germination under stressed conditions or with substandard seed lots (Lee and Kim, 2000). Pre-sowing treatments such as priming techniques with different salts, water, and osmoprotectants assist the germination and establishment process in the field.

In fact, this technique is a treatment that applied before germination in a specific environment that seeds are partially hydrated to a point where

germination processes begin but radical emergence does not occur (Dell Aquila and Tritto, 1991; Giri and Schilinger, 2003; Kaur, 2002). On the other hand on seed priming the amount of water absorption is controlled so as necessary metabolic activities occurred for germination but radical emergence is prohibited. Seed priming can be accomplished through different methods such as hydropriming (soaking in DW), osmopriming (soaking in osmotic solutions such as mannitol, PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth inducers (CCC, Ethephon, IAA) (Chiu *et al.,* 2002; Harris *et al.,* 1999; Chivasa *et al.,* 1998). Therefore, seed priming is a technology that enhances rapid emergence (7-10 d) and early establishment of blackgram. Rapid and uniform field emergences are regarded as an essential prerequisite for both irrigated and rain fed conditions to reach the yield potential, quality and ultimately profit in annual crops (Cantliffe *et al.,* 1994). Moreover, it is also important to study more about the performance of on the germination, vigour and other attributes of blackgram. Therefore, the present study on seed priming of blackgram was formulated with the following objectives:

- To evaluate the effect of pre-sowing seed treatment on germination behavior of blackgram in relation to salt tolerance,
- To evaluate the effect of salt concentration on seedling growth of blackgram under saline stress condition, and

CHAPTER II

REVIEW OF LITERATURE

2.1 Blackgram

Blackgram (*Vigna mungo* L.) Hepper commonly known as blackgram or mash, is a grain legume domesticated from V. mungo var. sylvestris (Srivastava *et al.,* 2011). The center of origin of blackgram is in India (Bhosale *et al*., 2013). Blackgram has been distributed mainly tropical to sub-tropical countries. Blackgram is a self-pollinated crop with low percentage of natural out crossing. It belongs to family fabaceae. It is an important pulse crop of many South Asian countries including Bangladesh, Pakistan, India, Nepal, Thailand, Philippines and Korea (Srivastava *et al*., 2011). It is highly nutritious containing easily digestible and good quality protein (24- 26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins. It forms one of the important constituents in the dietary practices of the local population and is affordable due to lower price than that of other pulses. The biological value improves greatly, when wheat or rice is combined with blackgram because complementation of the essential amino acids such as arginine, leucine, lysine, isoleucine, valine and phenylalanine etc. It is cultivated under a wide range of agro-ecological zones mainly under rainfed nature. Among pulses, it is the least researched crop. Although it has been identified as a potential crop in number of countries, but no systematic research information is available except few reports worldwide, whereas in Bangladesh very little study has been reported. It is grown as a sole crop or mixed with sorghum, pearl millet and pigeon pea. It is also cultivated as follow-up crop after rice cultivation (Srivastava *et al*., 2010). Now-a-days the world specially developing countries like Bangladesh facing environmental problems like drought, uneven rainfall, high temperature, salinity problem etc due to global warming. So there have ample chances and crying needs to develop varieties to cope with this type of environmental stresses. Though diseases resistant and few heat sensitive blackgram varieties are released by Bangladesh Agricultural Research Institute (BARI) but still there have no salt tolerant, drought tolerant or other stress tolerant blackgram variety is not released. Enhancing

blackgram yield may be achieved through the use of the fellow lands due to excess salts water deficit and other abiotic stresses.

2.2 Salt stress and Plant Responses

Salt stress explains the presence of soluble salts in excessive amount in the soil which alters plant's normal physiological processes and hinders plant growth, jeopardizing the capacity of agriculture to sustain the burgeoning human population increase (Flowers, T.J 2004; Munns and Tester, 2008). Irrigation water or soil solution with electric conductivity of 4 dsm^{-1} or greater (Cramer, 2002) which is equal to 40 mM NaCl is considered as saline one. Environmental contaminants specifically those containing salt radicals affect crops adversely. Building up of soluble salts in soil column causes serious damage to agricultural production and environmental health. It has been estimated that up to 2050, about 50% of fertile land may be lost due to environmental constraints. In order to cope with the increasing demands of growing population, 38% increase in food production is needed by the year 2025 that would further rise to 50% by the year 2050. There are many factors which are limiting the global demands mainly by physical and chemical degradation of soil due to erosion and salinity (Wild, 2003).

2.2.1 Effect of salinity on seed germination and seedling growth

Germination is one of the most important and vital processes of plant life cycle. It is the determinant of the subsequent growth and yield characteristics of plants. Available literature showed that blackgram seeds tended to germinate at a lower rate and consumed longer time when exposed to salt stress. The reasons underlying this fact are higher concentrations of salt create lower osmotic potential of germination media which hampers the imbibitions of water by seed, creates an imbalance in the normal activities of enzymes responsible for nucleic acid and protein metabolism, causes hormonal imbalance, and deteriorates the food reserves of seed (Hasanuzzaman *et al*. 2013).

Maliwal and Paliwal (1982) observed that germination of all the 42 cultivars of mungbean and black gram was delayed and decreased with an increase in salinity

level. Seedling height also decreased significantly at higher salinity and the salt tolerance limit varied with the cultivar. Win *et al.,* (2014) also suggested that genetically diverse germplasm resistant to salt stresses within *Vigna* genotypes could be of practical value to study the mechanism governing salt tolerance and for the delivery of genetic resources for salinity in breeding program.

The differences between blackgram cultivars in final germination index might be due to the genetically factors and heredity variation similar to the findings of Singh *et al.* (2012).

Sunanda C.H. and Ranganayakulu G.S. (2019) observed that the effects of salt stress on seedling growth (shoot, root length and biomass), accumulation of osmolytes such as proline, glycine betaine, soluble sugars, free amino acids and polyamines were investigated in two Black gram cultivars (Cv. Maruthi and Cv. LBG) where Salt stress resulted reduced seedling growth in all cultivars.

2.2.2 Effect of salt stress on relative water content (RWC)

The plants tend to prolong stress tolerance by using several mechanisms that tend to postpone or tolerate desiccation as reduction of water loss, maintaining of turgor pressure and osmotic potential. High concentration of salts in the root zone (rhizosphere) reduces soil water potential and the availability of water in shoot and root which further influence cellular physiology and metabolic pathways (Lyod *et al*., 1989 and Misra and Dwivedi, 1980). As a result of this reduction of the relative water content, dehydration at cellular level and osmotic stress are observed in blackgram and other crop plants (Munns, 2002). High salt depositions in the soil generate a low water potential zone in the soil making it increasingly difficult for the plant to acquire both water as well as nutrients (Mahajan and Tuteja, 2005). Therefore, salt stress essentially results in a water deficit condition in the plant and takes the form of a physiological drought.

2.2.3 Effect of salinity stress on photosynthetic pigments

Photosynthesis is one of the most promising physiological processes contributing to plant growth and productivity of crops for food. Under salinity stress the reduction in photosynthetic rate is not only attributed to stomata closure leading to a reduction of

intercellular CO_2 concentration, but also to non-stomatal factors finally resulted in reduced quantum yield. It is affected by leaf expansion rate, leaf area, and leaf duration, as well as by photosynthesis and respiration per unit leaf area under salt stress. Inhibition of photosynthesis is also associated with decline in pigment contents resulted from the reduction in leaf area, or due to decrease in leaf organic acid with salinity (Cachorro *et al*., 1993) or due to decrease in PS II electron transport activity (Mishra and Dwivedi, 2004) or due to increase in amylase activity and /or decrease in invertase activity (Promila and Kumar, 2000), or due to les stomatal openings in leaf (Radi *et al.,* 2001). Salt stress directly or indirectly affects the photosynthetic functions by changing the structural organization and physio-chemical properties of thylakoid membranes. Generally, plant water status under stressful environments is maintained by regulating stomatal conductance and transpiration rate Dey *et al., (*2005). High Na^+ levels also lead to reduction in photosynthesis and production of reactive oxygen species. Nafees *et al.,* (2010) reported disturbance in photosynthetic processes due to accumulation of toxic ions, decrease in water and osmotic potential under salinity stress. In other words, photosynthetic capacity in crop plants is the primary component of dry matter productivity. Thus, final biological yield or economical yield can be increased either by increasing rate of photosynthesis finally contribute to the biological yield or economical yield of crop plants (Natr and Lawlor, 2005).

2.2.4 Effect of salinity stress on morphology and growth

The most common salinity effect is a general stunning of plant growth. Literature reported gradual reduction in seed germination, plant height, shoot and root length, dry matter, biomass, root, stem and leaf weights with progressive increase in salinity stress in mungbean plants (Mohamed and El-Kramany. 2005) and also in other crops Yadav *et al.,* (2011). As salt concentration increases above a threshold level both the growth rate and ultimate size of most plant species progressively decreases. Wahid *et al.,* (2007) reported salt induced injury symptom on mungbean such as enhanced chlorosis and necrosis. Not all the plant parts are affected equally due to salinity, top arial growth is often suppressed more that the root growth. It is possible that under salt stress the plant expends more photosynthetic energy in root production in search of water and or

reducing water loss and thus maintains relatively high plant water relations. Growth inhibition by salt stress may be due to the diversion of energy from growth to maintenance that may include the regulation of ion concentration in various organs and within the cell, the synthesis of organic solutes for osmoregulation or protection of macromolecules, and for maintenance of membrane integrity (Greenway and Gibbs, 2003). Due to NaCl, the growth of root, shoot and leaves either increase or decrease. Singh, *et al.,* (2001) reported stunted stem and succulent leaves under salinity stress due to inhibition of cell division and enlargement in the plant's growing point and elongation of the palisade cells caused by toxic Cl^- ions. El-Hendawy *et al.,* (2005) reported that salinity significantly decreased dry matter production. Saha *et al.,* (2010) also reported drastic effect of salinity stress on the roots as compared to shoots, accompanying reductions in length, number of root hairs and branches, while the roots became stout, brittle and brown in color.

2.2.5 Effect of salinity stress on yield characteristics

Soil salinity caused reduction in flowering, and severe yield loss in many crop plants including legumes viz., blackgram, soyabean, mungbean, (Singh and Singh. (2011) and Magda and El-Kramany, 2005).

Mudgal (2004) reported decreased plant growth, delayed flowering, decreased number of flowers, impaired pod-setting resulting in more decrease in number of pods than the seeds in chickpea and pea due to salinity stress. Mass (1986) suggested that salinity may affect pollination and thus decrease seed set and grain yield. Reduction of yield and its component rated under salt stress condition may also be attributed to low production, expansion, senescence and physiologically less active green foliage (Rawson *et al.,* 1988; Schactman and Liu, 1999; Kumar *et al.,* 2003 and Wahid *et al.,* 2007) thus reduced photosynthetic rate might be a supplementary effect. The morphophysiological characteristics also play a crucial role directly or indirectly in the reduction of efficiency per day of plant as well as effective filling period of seed and may lead to decrease the yield of crop. According to Gill, (1979) lengthening the time required for seed filling under salt stress push the plants at seed filling and maturity to high temperature and water stress due to the summer. The effect of both salt

and water stress might lead to shriveled seeds and consequent lower yield. Singh *et al.* (2001) also reported that grain yield reduced because of decrease in dry matter content, leaf size and increase in root: shoot ratio under salt stress. Ahmed, (2009) also reported that reduced yield in mungbean under salt stress may be due to reduced efficiency per day of plant to fill the developing seeds, which may lead to reduced number of seeds/pod or plant and dry matter yield of individual seed.

2.3 Seed priming

Seed priming is a pre-sowing treatment of seed that regulates and increases pre germinative metabolic activity while preventing radicle projection because it indicates completing germination. Seed priming is a controlled hydration activity that stimulates metabolic processes during early germination stage but before radical projection. Stress tolerant variety development is time consuming and economical process. It is wise to develop a technique which is rapid and cost effective against stresses, seed priming may be one of the substitute strategies because it is easy, cheap and effective against various abiotic and biotic stresses (Iqbal and Ashraf, 2007a). In general, seed priming increased germination rate, uniform germination and good seedlings establishment and ultimately better yield (Jisha *et al.,* 2013). Metabolism of energy, osmotic regulation, embryo enlargement, upregulating enzyme activity, and fast cellular defense reaction under abiotic and biotic stresses made seed priming technique as an effective and practical one. Seed priming is one of the most pragmatic and short-term approaches which resists the salt stress and other environmental stress by increasing enzymatic antioxidants activity (Farooq *et al.,* 2008b and Jafar *et al.* 2012). Primed seed improved stand establishment and better yield than non-primed wheat seed. Various seed priming techniques including hydropriming, osmopriming or halo-priming, Chemical priming, Hormone priming, Solid matrix priming etc. are practiced under various abiotic stresses (Jisha *et al.,* 2013 and Paparella *et al.,* 2015). Hydropriming means priming with water under optimal condition with or without aeration (Paparella *et al.,* 2015). Under adverse environmental condition like- salinity, drought, high temperature etc.; hydropriming is one ofthe suitable technique (McDonald, 2000). Osmopriming or halopriming means priming with osmotic solutions like Polyethylene glycol (PEG),

glycine betaine,prolin, ascorbic acid, inorganic salt of sodium, potassium and magnesium (most commonly used NaCl, $NaNO_3$, $MnSO_4$, $MgCl_2$, K_3PO_4 and KNO_3) etc. at low water potential that facilitates the control of water uptake and limit the ROS-mediated oxidative damage **(**Paparella *et al.,* 2015). Priming with phyto-hormone like salicylic acid (SA), abscisic acid (ABA) or gibberellic acid (GA) can regulate biochemical processes during germination and improve antioxidant defense system to cope with environmental stresses (Radhakrishnan and Lee, 2013). Pre-sowing seed treatments improve the performance of seeds under adverse conditions and environmental stresses such as salinity. It has been reported that seed priming has recently been applied to overcome the salt stress problem on agricultural land Tavili *et al.,* (2011). Harris (2004) reported that priming is connected with improved disease resistance in some crops. For instance, in two different seasons in Bangladesh, the damage caused by collar rot (*Sclerotium rolfsii*) was decreased significantly due to seed priming in chickpea Musa *et al.,* (2001). It has been reported that the effects of priming treatment are associated with increased protein synthesis and the repair of membranes. During germination, the water uptake happens in three stages (Bewley 1997).

2.3.1 Effect on germination parameters

2.3.1 (a) Total Germination (%)

Seed priming, a controlled hydration process followed by re-drying is pragmatic approach to counteract the salinity effects in many crops because of its simplicity, low cost and effectiveness (Wahid *et al*., 2007; Afzal *et al*., 2011). It improved the germination percentage and uniformity of growth following reduced emergence time and increased yields are reported in many field crops including rice (Farooq *et al*., 2006b; Afzal *et al*., 2006; Afzal *et al*., 2011). But such enhancements are often found under non-saline conditions (Farooq *et al*., 2006a; 2006b) and few studies are available for alleviation of adverse salinity effects in rice during germination and early seedling growth by seed priming (Xu *et al*., 2011). Patade *et al.* (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. Farhoudi and Sharifzadeh (2006) while

working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions. The higher germination percentage in seeds primed with $CaCl_2$ is according to Ashraf and Rauf (2001) for wheat and Afzal *et al*.(2008b) for maize who reported an increase in germination percentage of plants raised from seeds primed with calcium salt under salinity stress. Short term seed priming with a low NaCl concentration also increases germination rate, field emergence and acquired stress tolerance (Nakaune *et al.*, 2012). Sun *et al*. (2010) also concluded that PEG priming with moderate concentration resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination. It was reported seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition (Demir *et al*., 2006). Aerated hydration treatment of pepper at 250 ^{0}C followed by drying increased germination percentage were reported by (Demir and Okcu, 2004). The final germination percentage of *Melilotus officinalis* was much higher than that of *M. sativa* and *A. adsurgens* at 300 mM NaCl (Wang *et al*., 2009b), and the germination rate in six alfalfa cultivars was also differentially affected by treatments with 200 mM NaCl and 35% PEG (Wang *et al*., 2009a). Vicente *et al*. (2009) has observed varying responses to saline solution of the seeds of three plant species (*Arthrocnemum macrostachyum*, *Juncus acutus* and *Schoenus nigricans*) and different germination recovery of the seeds after submersion in hypersaline solution of different salt types. Seed primed with potassium hydrophosphate (KH_2PO_4) and water improved germination percentage compared to untreated seed treatments. Similarly, Korkmaz and Pill (2003) reported that priming with KH_2PO_4 improved the germination synchrony of low vigour cultivar in lettuce. According to Ghana and Schillinger (2003) seed primed with $KH_2PO_4$and water treatments enhanced germination in wheat under normal condition compared to untreated seed. Basra *et al*. (2003) and Salinas (1996) reported improvement in germination

percent, emergence and seedling stand by using seed priming techniques. In fact priming induces a range of biochemical changes in the seed that required initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation (Ajouri *et al*., 2004). Some previous researcher indicated that some or all process that precede the germination are triggered by priming and persist following the redesiccation of the seed (Asgedom and Becker, 2001). Primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). According to McDonald (2000), primed seeds acquire the potential to rapidly imbibe and revive the seed metabolism thus enhancing the germination rate. Osmopriming with PEG was described as a good technique for improving seed germination of Bromus seeds under salt and drought stress (Tavili *et al*.,2011) and for increasing the germination percentage and seedling vigor of bersim (*Trifolium alexandrinum*) seeds (Rouhi *et al*.,2010). In soybean too, seed priming with PEG was successfully carried out by Khalil *et al*., (2001). Osmopriming with PEG results in strengthening the antioxidant system and increasing the seed germination potential, finally resulting in an increased stress tolerance in germinating seeds of spinach (Chen and Arora, 2011). Osmo conditioning of Italian ryegrass (*Lolium multiflorum*) and sorghum (*Sorghum bicolor*) seeds with 20% PEG-8000 for 2 d at 10°C increased germination percentage, germination rate, seedling establishment and dry matter production under water stress, water logging, cold stress and saline conditions (Hur, 1991). According to Posmyk and Janas (2007), hydropriming and hydropriming along with proline can be used as a safe priming method for improving seed germination and growth of *Vigna radiata* seedlings at low temperature and also allowing fast repair of injuries caused by stress. More uniform germination and emergence were observed in primed seeds on canola (*Brassica compestris*) (Zheng *et al*., 1994), wheat (*Triticum aestivum*) (Nayyar *et al*., 1995) and rice (*Oryza sativa*) (Lee and Kim, 2000; Basra *et al*., 2003) who described improved germination rate and percentage in seeds subjected to

hydropriming and seed hardening for 24 h (Farooq *et al*., 2006b). Coolbear and Grierson (1979) who reported that higher germination rate was a result of higher levels of nucleic acid in primed seeds of tomato cultivars. They indicated that increase in nucleic acid content in primed seeds was due to an enhanced ribonucleic acid (RNA) synthesis during and after priming treatment. Ascorbic acid, another important vitamin is also used for priming due to its antioxidant nature. It has already been proved that a high level of endogenous acrobat is essential to maintain the antioxidant capacity that protects plants from oxidative stress (Zhou *et al*.,2009). Ascorbic acid pretreatment results in improved germination properties of *Agropyron elongatum* under salt stress condition (Tavili *et al*., 2009). ABA priming showed increased rate of germination as compared to non-primed seeds in Indian mustard (Srivastava *et al*., 2010a). The growth regulators IAA and GA_3 were reported to improve germination of pyrethrum seeds under non-saline condition (Bisht *et al*., 2009). Salicylic acid priming in fennel seeds also showed better germination under low water potential (Farahbakhsh, 2012). Moreover, in *Salicornia utahensis*, which is a halophyte, priming with growth regulators like fusicoccin, thiourea, kinetin, and ethephon alleviated the inhibitory effects of salinity on the germination, whereas GA_3, proline, betaine and nitrate had little effect on germination at all salinities (Gul and Khan, 2003). 3% KNO_3 supplemented with 3 lM methyl jasm-onate (MeJA) could promote germination and emergence of dormant *Amaranthus cruentus* L. seeds (Tiryaki *et al*., 2005). More recently, seeds of *Agropyron elongatum* primed with gibberellin (GA) and abscisic acid (ABA) exhibited induced CAT and SOD activities under drought conditions when compared to unprimed seeds (Eisvand *et al*.,2010). In many crops, seed germination and early seedling growth are the most sensitive stages of water limitation and the water deficit may delay the onset and reduce the rate and uniformity of germination, leading to poor crop per dormancy and yield (Demir *et al*., 2006). Therefore, the beneficial effects of priming may be more evident under unfavorable rather than favorable conditions (Parera and Cantliffe 1994). Primed seeds usually exhibit an

increased germination rate, greater germination uniformity, and at times, greater total germination percentage (Basra *et al*., 2005). These attributes have practical agronomic implications, notably under adverse germination conditions (McDonald 2000). Therefore, there is a strong interest in the seed industry to find suitable priming agent(s) that might be used to increase the tolerance of plants under adverse field conditions (Job *et al*., 2000).

2.3.1 (b) Mean germination time (days)

Priming treatments are being used to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such earlier and synchronized emergence often leads to uniform stands and improved yield (Farooq *et al*., 2006b; Afzal *et al*., 2006; Afzal *et al*., 2011). Like germination percentage, prime seeds had lower mean emergence time (MET) compared with non-primed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassan pouraghdam *et al*., 2009; Sivritepe *et al*., 2003). Improved seed invigoration techniques were known to reduce emergence time, accomplish uniform emergence, and give better crop stand in many horticultural and field crops (Ashraf and Foolad 2005). Priming decreased the temperature optimum and ceiling temperature for germination and also helped in advancing the germination time and did not decrease the final percentage emergence (Finch-Savage *et al*., 2004). ‘‘On-farm’’ seed priming (soaking seeds in water prior to sowing) has been shown to be effective in producing early germination, better establishment and increased yields in a wide range of crops in diverse environments (Rashid *et al*., 2006). It had been a common pretreatment that reduces the time between seed sowing until emergence and synchronizes seedling emergence (Parera and Cantliffe 1991). According to Basra *et al*., (1989) priming of corn seed using polyethylene glycol or potassium salt (K_2HPO_4 or KNO_3) resulted in accelerated germination.

Janmohammadi *et al*. (2008) presented hydropriming as a suitable, cheap and easy seed invigoration treatment for inbreed lines of maize, especially when germination is affected by salinity and drought stress. Hydropriming has been shown to result in the earlier germination of desert cacti (Dubrovsky 1996), *Allium porrum* (Ashraf and Bray 1993), pyrethrum (*Tanacetum cinerariifolium*) (Li *et al*.,2011), and coriander (Rithichai *et al*.,2009). Moradi Dezfuli et al., (2008) revealed hydro primed seeds for 36 h had lowest values (T50 and MGT). Osmotic seed priming of maize caryopses resulted in more homogenous and faster seed germination as compared to the control was reported by Fotia, *et al.,* (2008). According to Gray *et al*., (1990) (-0.5 MPa) lowered the mean germination time of seeds of lettuce, carrot and onion. Goobkin (1989) and Ozbingol, *et al*. (1999) also reported that PEG 6000 solution treated tomato seeds germinate faster than untreated seeds and this is due to more rapid water uptake. The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed. Yamauchi and Winn (1996), indicated that seed priming may help in dormancy breakdown possibly by embryo development and leaching of emergence inhibitors which resulted in an earlier start of emergence.

2.3.1 (c) Germination index

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

In addition to better establishment, primed crops grew more vigorously, flowered earlier and yielded higher (Farooq *et al*., 2008). Ruan *et al*., (2002a) had observed that KCl and $CaCl_2$ seed priming had improved germination index of rice. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Dell Aquila and Tritto, 1991; Donaldson *et al*., 2001). Rashid *et al*., (2005) reported that priming enhanced germination, better establishment and increased yields in many diverse environments for a number of crops (Khan *et al*., 2008). Seed priming could enhance sunflower seed germination under the stress conditions was found by Kaya *et al.*, (2006). Bray *et al*., (1989) and Arif *et al*., (2005) who reported that seed priming enhanced germination which may be attributed to repair processes, a buildup of germination metabolites or osmotic adjustments during priming treatment. Maiti *et al.* (2006) also reported that osmotic seed priming of maize caryopses in coper sulphate, zinc sulphate, manganese sulphate, or boric acid induced high levels of seed germination. Hydropriming was found to be the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 h compared to 48 h (Caseiro *et al*., 2004). It improved germination and later growth of different crops species such as in maize, rice, chickpea (Harris *et al*., 2004).

2.3.1 (d) Coefficient of velocity

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

2.3.1 (e) Energy of emergence (%)

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

the germination are trigged by priming and persist following the re-desiccation of the seeds (Asgedom & Becker, 2001). Thus, upon seeding, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). The resulting improved stand established can reportedly increase the drought tolerance, reduce pest damage and increase crop yield in cereals and legumes (Harris *et al*., 1999; Mussa *et al*., 1999; Harris *et al*., 2001; Khan *et al*., 2005). Seed priming stimulates many of the metabolic processes involved in the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions (Cramer, 2002). It has also been reported that seed priming improves emergence, stand establishment, tillering, allometry, grain and straw yields, and harvest index (Farooq *et al*., 2008). Seed priming has been found a double technology to enhance rapid and uniform emergence, and to achieve high vigor and better yields in vegetables and floriculture (Dear man *et al*., 1987; Parera and Cantliffe, 1994; Bruggink *et al*., 1999) and some field crops (Hartz and Caprile 1995; Chiu *et al*., 2006; Giri and Schillinger 2003; Murungu *et al*., 2004; Basra *et al*., 2005; 2006; Kaur *et al*., 2005; Farooq *et al*., 2006 a, b; 2007 a, b). The enhanced phenology in mungbean due to primed seed is associated with faster emergence and reduced germination imbibition periods (Harris *et al*., 1999). It has been declared that priming had been resulted in more germination speed especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Sivritepe*et al*., 2003; Demir Kaya *et al*., 2004; Foti *et al*., 2002). Soybean seed priming is made better seedling emergence and yield improvement (Arif *et al*., 2008). Seed priming techniques such as hydropriming, hardening, osmopriming, osmo hardening, hormonal priming and hydro priming have been used to accelerate emergence more vigorous plants and better drought tolerance in many field crop like wheat (Iqbal and Ashraf, 2007b), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006), cotton (casenve and Toselli, 2007) triticale (Yagmur and Kaydan, 2008). Potassium hydro phosphate (K_2HPO_4),

polyethylene glycol (PEG6000) (Dell Aquila and Taranto, 1986) and potassium chloride (KCl) (Misra and Dwibedi, 1980) have been introduced as the osmoticum which have shown good potential to enhance germination, emergence, growth, and/or grain yield of wheat. Water has also been used successfully as a seed priming medium for wheat (Harris *et al*., 2001). Ghiyasi *et al*., (2008) declared osmopriming of maize (*Zea mays* L.) seeds with polyethylene glycol 8000 (PEG 8000) at -0.5 MPa osmotic potential had improved emergence, grain and biological yields compared with other treatments. The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005). Halopriming with $CaCl_2$ significantly improved emergence and seedling growth in Shaheen Basmati whereas as $CaCl_2$ and KCl proved better in case of Basmati-2000 which could be related to dormancy breakdown of rice seeds due to enhanced seed K and Ca concentration and amylase activity (Farooq *et al*., 2006b). Zheng *et al*. (2002) reported earlier and uniform emergence in rice *(Oryza sativa)* seeds osmoprimed with KCl and $CaCl_2$ and mixed salts under flooded conditions. However, Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon (*Cucumis melo*) seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale *et al*., 2003; Giri and Schillinger, 2003).

2.3.2 Effect on growth parameters

2.3.2 (a) Shoot length (mm)

Priming with KNO_3 can be used to increase watermelon germination (Demir and Mavi, 2004) and in tomato, seed priming with KNO_3 increased germination percentage, germination index, root length, shoot length and seedling fresh weight (Nawaz *et al*., 2011). It was reported that osmo and hydropriming of chickpea seeds with mannitol and water alleviated the adverse

effects of water deficiency and salt stress on seedling growth. The treatment of seeds with water, 2 and 4 % mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions (Kaur *et al*.,2002, 2005). Priming of chickpea seeds with mannitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Previous studies on tomato (Cuartero *et al*., 2006) and melon (Sivritepe *et al*., 2003), showed that seed priming improves seed germination, seedling emergence and growth under saline conditions. Farhoudi and Sharifzadeh (2006) and Sarwar *et al*. (2006) while working with canola and chickpea, respectively, reported salt priming-induced improvement in seed germination, seedling emergence and growth under saline conditions. Priming of seeds with water promoted seedling vigour, yield and crop establishment of chickpea, maize and rice in India (Harris *et.al*., 1999). It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity affects during early seedling growth (Ashraf and Harris, 2004;). Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solution of KCl or potassium sulfate (K_2SO_4) significantly increased plant height, yield attributes, and grain yield in wheat. The beneficial effects of gibberellic acid (GA_3) on germination are well known (Angrish *et al*., 2001; Radi *et al*.,2001; Khan *et al*., 2002). GA_3 (100 mg l-1) applied as pre-sowing treatment resulted in the highest K^+ and Ca^{2+} content in the shoots of both faba beans (*Vicia faba*) and cotton (*Gossypium barbadense*) crops (Harb,1992). Recently, auxin is also used for priming (Akbari *et al*.,2007). In wheat seed germination, auxin treatments increased the hypocotyl length, seedling fresh and dry weight and hypocotyl dry weight (Akbari *et al*.,2007).

2.3.2 (b) Root length (mm)

Seed priming techniques such as hydropriming, hardening, osmo-conditioning, osmo-hardening, and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought

tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006) and cotton (Casenave and Toselli, 2007). ABA-primed seeds of *Brassica napus* exhibited earlier (2–7 days) germination and higher final percent radicle protrusion than non-primed control seeds, under salt (100 mM NaCl) or water stress (20 % PEG 8000) and at a low temperature (8 LC) (Gao *et al*.,2002). Kulkarni and Eshanna (1988) stated that pre-sowing seed treatment with IAA at 10 ppm improved root length, rate of germination, and seedling vigor. Kathiresan *et al*. (1985) also found similar findings and reported maximum root and shoot growth; seedling height and field emergence in sunflower seeds in response to priming with $CaCl_2$. Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells Kaya *et al*. (2006). Osmopriming and hydropriming of wheat seeds may improve germination and emergence (Ashraf and Abu-Shakra, 1978) and may promote vigorous root growth (Carceller and Soriano, 1972). Hydroprimed seeds produced the largest roots, compared to other seed treatments Kathiresan and Gnanarethinam (1985) in sunflower. This means that during priming, seeds would be simultaneously subjected to processes of repair and deterioration and force between the two determined the success or failure of the treatment (McDonald, 2000). Also, important to consider is the toxic effect reported for PEG (Grzesik and Nowek, 1998) and the decrease in oxygen solubility (Welbaum, 1998; Toselli and Casenave, 2002, 2003) that could be responsible for the anoxia damages suggested by Sung and Chang, (1993).

2.3.2 (c) Seedling length (mm)

The increased shoot and root length in primed plants can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduction of lag time between inhibition and radicle emergence (Bradford *et al.,*1990). Treated seeds had stronger embryos that were able to more easily emerge from seeds (Harris *et al.,* 2005). Sekiya and Yano (2009) also found that enhanced root and shoot length of seedlings obtained from P enriched seeds. To contribute to plant

growth and development seed priming has been widely reported technique (Harris *et al.,* 2005). Ajouri *et al.* (2004) reported a stimulation of P and Zn uptake, as well as an improved germination and seedling growth in barley after soaking seeds in water and in solutions containing 5-500 mM P. Hydropriming method has also been used successfully in wheat (Harris *et al*., 2001), in sunflower (Kaya *et al*., 2006), chickpea (Kaur *et al*., 2002) and cotton (Casenave and Toselli, 2007). Moreover, hydropriming increased germination and seedling growth under salt and drought stresses (Kaur *et al*., 2003; Kaya *et al*., 2006; Casenave and Toselli, 2007). Emergence force and seedling growth were strengthened by hydropriming in watermelon seeds Sung and Chiu (1995). Elkoca *et al*. (2007), recommended that hydropriming for 12 h or osmopriming (PEG -0.5 MPa) for 24 h for a better germination of chickpeas under cold soil conditions. Compared to hydropriming, priming with PEG in a proper concentration was found to have a better effect on seed germination and seedling growth under drought stress (Yuan-Yuan *et al*.,2010). PEG is frequently used to simulate drought stress (Chen *et al*., 2010; Farahani *et al*., 2010; He *et al*., 2009; Khajeh-Hosseini *et al*., 2003; Tohidloo and Kruse, 2009; Zhu *et al*., 2006) as an inert osmoticum in germination tests (Dodd and Donovan, 1999) and is a non-penetrating solute (Almansouri *et al*., 2001), which results in osmotic stress that inhibits seed germination through the prevention of water uptake. However, it has been reported that the inhibitory effect of PEG on germination may not be solely related to water imbibition (Almansouri *et al*., 2001). Wang *et al*. (2009a) have observed that the fresh weight and the length of the roots and shoots of two alfalfa cultivars (Xinmu No.1 and Northstar) were significantly inhibited by 35% PEG treatment. For a potential medicinal plant, *Matricaria chamomilla*, both the seed germination rate and seedling growth have been found to be reduced with the PEG mediated increasing osmotic potential of the growth medium (Afzali *et al*., 2006). Rouhi *et al*. (2011) also suggested that different priming techniques (hydro and osmo priming) had a varying effects on germination on each of the four grass species (*Bromus inermis*, *Festuca arundinacea*, *Agropyron*

elongatum and *Festuca ovina*) and the result showed that, for most evaluated germination parameters, osmopriming treatment (with PEG) was more useful technique to reduce abiotic stress than hydropriming treatment. Although priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions (Parera and Cantliffe, 1991), the effectiveness of different priming agents varies under different stresses and different crop species (Iqbal and Ashraf, 2005). Patade *et al.,* (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming induced improvement in seed germination, seedling emergence and growth under saline conditions. Paul and Choudhury (1991) also observed that seed soaking with 0.5 to 1% solutions with KCl or K_2SO_4 significantly increased plant height, grain yield and its components in wheat genotypes. Priming of chickpea seeds with manitol and water improved seedling growth under salt stressed conditions (Kaur *et al.,* 2003). Seed treatment with water and mannitol is also useful under water deficit stress and primed chickpea seeds gave high yield as compared to non-primed seeds (Kaur *et al*., 2002). Musa *et al*. (1999) reported that overnight priming of chickpea seeds gave better crop production in Bangladesh. Priming with H_2O_2 failed to improve emergence and seedling growth in rice cultivars which is inconsistent with Wahid *et al*. (2007) who reported improved salt tolerance in wheat by alleviation of salt stress and oxidative damage by H_2O_2 pre-treatment.

2.3.2 (d) Seedling dry weight (mg)

Harris *et al*. (2004) reported that higher plant dry weight and seed yield following seed priming. The increase in the dry matter and grain yield of mungbean was due to better emergence and better performance per plant (Parera and Cantliffe, 1994). In basil (*Ocimum basilicum* L.) under saline conditions, the seedling vigor, germination percentage and seedling dry weight was found to increase due to hydropriming (Farahani and Maroufi, 2011). Increased plumule dry weight due to osmopriming was reported by

Harris *et al*. (2004). Sivritepe *et al.* (2003) evaluate the effect of salt priming on salt tolerance of melon seedling and reported that total emergence and dry weight were higher in melon seedlings derived from primed seeds and they emerged earlier than non-primed seeds. They also observed that total sugar and proline accumulation and prevented toxic and nutrient deficiency effects of salinity because less Na but more K and especially Ca was accumulated in melon in melon seedlings.

2.3.2 (e) Vigour index

Post-harvest seed enhancement treatments improve germination and seedling vigour (Taylor, 1998). Maiti *et al*. (2009) studied the effect of priming on seedling vigour and productivity of tomato, chilli, cucumber and cabbage during post-rainy seasons demonstrating that priming improved germination and seedling development and yield of these vegetable species. Seed priming significantly improved the germination rate and vigour of the mungbean seedlings (Umair *et al.,* 2010). It is also reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS) (Del Ryo *et al*., 2002). Afzal *et al*. (2008 a) observed that the priming-induced salt tolerance was associated with improved seedling vigour, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants. Seed priming is used for improvement of germination speed, germination vigour, seedling establishment and yield (Talebian *et al*., 2008). Afzal *et al*. (2005) also found that the priming-induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants. Primed crops grew more vigorously, flowered earlier and yielded higher (Farooq *et al*., 2008). This technique used for improvement of germination speed, germination vigour, seedling establishment and yield (Talebian *et al*., 2008; Bodsworth and Bewley, 1981). Harris *et al*. (1999) demonstrated that on farm seed priming (soaking seeds overnight in water) markedly improved establishment and early vigour of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. Similarly,

vigorous early growth is often associated with better yields (Okonwo and Vanderlip, 1985; Austin, 1989; Carter *et al*., 1992). Seed-priming technology has twofold benefits: enhanced, rapid and uniform emergence, with high vigour and better yields in vegetables and floriculture (Bruggink *et al*.,1999) and some field crops (Basra *et al*.,2003; Kaur *et al*.,2005). It has been reported that primed seeds showed better germination pattern and higher vigour level than non- primed (Ruan *et al*., 2002a). It has been also reported invigorated seeds had higher vigour levels (Ruan *et al*., 2002b), which resulted in earlier start of emergence as high vigour seed lots performed better than low vigour ones (Hampton and Tekrony, 1995). Seed priming techniques such as hydropriming, hardening, osmo conditioning, osmo hardening and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better stress tolerance in chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006). Various works have shown that hydropriming of seeds have many advantages as compared to non-primed seeds. Hydropriming has resulted in 3 to 4-fold increases in root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition (Kaur *et al*.,2002). This phenomenon was explained to be due to faster emergence of roots and shoots, more vigorous plants, better drought tolerance under adverse conditions (Amzallag *et al*., 1990; Passam and Kakouriotis,1994; Cayuela *et al*.,1996; Lee-suskoon *et al*.,1998). Fujikura *et al*. (1993) presented hydropriming as a simple and inexpensive method of seed priming and according to Abebe and Modi (2009), it is a very important seed treatment technique for rapid germination and uniform seedling establishment in various grain crops.
Priming of seeds with water promoted seedling vigor, yield and crop establishment of chickpea, maize and rice in India (Harris *et.al.,* 1999). Harris *et al*. (1999) also found that hydropriming enhanced seedling establishment and early vigour of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. The resulting improved stand establishment can reportedly increase drought tolerance, reduce pest damage and increase crop yield (Harris *et al*., 1999). Similarly, vigorous

early growth is often associated with better yields (Okonwo and Vanderlip, 1985; Austin, 1989; Carter *et al*., 1992). Chiu *et al*. (2006) reported that KNO_3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties. Salt priming with KNO_3, is an effective way to improve seed and seedling vigour of sunflower and cucumber (Singh and Rao, 1993; Ghassemi-Golezani and Esmaeilpour,2008). Hydropriming improved the early and vigorous crop establishment in maize (Nagar *et al.,* 1998) and *Heiichrysum bracteatum* L. (Grzesik and Nowak,1998). However, other studies resulted in poor emergence from hydroprimed Kentucky bluegrass seeds under field conditions (Pill and Necker, 2001). However, Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale *et al.,* 2003; Giri and Schillinger, 2003).

CHAPTER III
MATERIALS AND METHODS

The experiment was conducted during the period from December 2019, to April, 2020 to study the effect of halo-priming (NaCl @ 2 mM and 3 mM) and hydro priming induced enhancing salt tolerance capability in Blackgram (*Vigna mungo* L.) under salt stress. The materials and methods describe a short description of the experimental site, climatic condition of the culture room, experimental materials, treatments and design, methods of the study, data collection procedure and data analysis. The detailed materials and methods that were used to conduct the study are presented below under the following headings:

3.1 Description of the experimental site

3.1.1 Location

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

3.1.2 Conditions of laboratory room

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study period of the culture room was 26.2^0C to 33.4^0C, respectively and average minimum and maximum relative humidity was 56% and 84%, respectively.

3.2 Test crops

Two blackgram varieties namely- BARI Mush-3 and BARI Mush-4 were used for this experiment. BARI Mush-3 and BARI Mush-4 was collected from Bangladesh Agricultural Research Institute (BARI). The collected blackgram varieties were free from any visible defects, disease symptoms and insect infestations

and transported to the laboratory of the Department of Agronomy, SAU, Dhaka with careful handling to avoid disease and injury.

3.3 Experimental materials

Different equipment's such as electric balance, petri dish, filter paper, micro pipette, forceps, oven etc. were used for this study.

3.4 Chemicals for seed priming

Salt (NaCl) and distilled water were utilized for osmo and hydro priming. Sodium hypochloride (NaOCl) used as seed treating chemical.

3.5 Experimental treatments and design

The experiment comprises of

(a) Three levels of priming agent concentration *viz.* water, NaCl @ 2mM and 3mM with 6 hrs priming

(b) Five levels of salt stress viz. 0, 50, 100, 150, 200 mM NaCl,

The experiment was laid out in a completely randomized design (CRD) with 3 replications.

3.6 Experimental details

The whole experiment was conducted under two different experiments.

3.6.1. First Experiment

Study on the effect of hydropriming and Halo (NaCl) priming on the germination behavior of two blackgram varieties

3.6.1.1 Weight of seeds

200 g seeds were weighted from the total seed from each of two blackgram variety BARI mush-4 and BARI mush-3 to reduce the unnecessary loss of seeds.

3.6.1.2 Surface treatment

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

3.6.1.3 Treatments

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

Blackgram variety (02)

i. V_1: BARI mush-3

ii. V_2: BARI mush-4

Four types of treatments:

i. T_1 = Seeds without priming (control)

ii. T_2 = Hydropriming (Seeds primed with distilled water)

iii. T_3 = Halopriming (Seeds primed with 2mM NaCl)

iv. T_4 = Halopriming (Seeds primed with 3mM NaCl)

3.6.1.4 Priming solutions

2 mM, 3 mM NaCl solution and distilled water were used as priming solutions.

3.6.1.5 Preparation of priming solutions

a) NaCl Solution (2mM and 3mM)

58.44 mg of NaCl was dissolved in 500 ml of water to prepare 2 mM NaCl solution and 87.66 mg of NaCl was dissolved in 500 ml of water to prepare 3 mM NaCl solution.

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.,* halopriming and hydropriming were applied on both the blackgram varieties. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were primed. For hydropriming seeds of a sub-sample were soaked in distilled water and for halopriming seeds of another sub-sample were divided

into another two subsample and pretreated with NaCl at two levels of concentration of 2 mM and 3 mM for 6 hours. Priming was done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.6.1.7 Germination of seeds

Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter Petri dishes on whatman No.1 filter paper moist with 8 ml of distilled water. Here, whatman No.1 filter paper was used as growth media for germination. Experimental units (60 Petri dishes) were arranged factorially in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept saturated condition with water. Seeds were kept at room temperature 25±1°C under normal light to facilitate germination for 7 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari *et al.,* 2007). Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (7 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75±2°C for 48 hours.

3.6.2 Second experiment

Germination behavior of primed blackgram seed under salt (NaCl) stress condition

3.6.2.1 Weight of seeds

Seeds were weighted 200g from the total seed of BARI Mush-3 for this experiment to reduce the unnecessary loss of seeds.

3.6.2.2 Surface treatment

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

3.6.2.3 Treatments

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

The treatments are as follows:

1. T_1 = Primed (NaCl and water) and non-primed (control) seeds placed without salt (control),
2. T_2 = Primed (NaCl and water) and non-primed (control) seeds placed with 50 mM salt,
3. T_3 = Primed (NaCl and water) and non-primed (control) seeds placed with 100 mM salt,
4. T_4 = Primed (NaCl and water) and non-primed (control) seeds placed with 150 mM salt and
5. T_5 = Primed (NaCl and water) and non-primed (control) seeds placed with 200 mM salt.

3.6.2.4 Priming solutions and time

NaCl solution (2mM and 3mM) and distilled water were used as priming solutions and 6 hours as priming time.

3.6.2.5 Preparation of priming solutions

a) NaCl solutions (2 mM and 3 mM)

58.44 mg of NaCl was dissolved in 500 ml of water to prepare 2 mM NaCl solution and 87.66 mg of NaCl was dissolved in 500 ml of water to prepare 3 mM NaCl solution.

b) Distilled water

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

3.6.2.6 Preparation of stress solutions Salt (Nacl) solutions (50mM, 100mM, 150mM and 200mM)

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

3.6.2.7 Priming technique

Two priming techniques viz., halopriming and hydropriming were applied on BARI mush- 4. The surface sterilized seeds were sub-sampled into three parts. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were primed with priming chemicals. Seeds of a sub-sample were soaked in distilled water for hydropriming and seeds of another sub-sample were pretreated with NaCl for halopriming at a concentration of 2mM and 3mM for 6 hours, respectively. Priming is done in different plastic containers covered with lids to prevent evaporation loss. All seeds were removed from the priming solution at the same time. The primed seeds were rinsed thoroughly with distilled water for three times and dried lightly using blotting paper and finally

air dried near to original weight (Umair *et al*., 2011) in room temperature for 24 hours back to the original moisture level.

3.6.2.8 Germination of seeds

The standard germination test was performed by placing randomly selected 30 seeds in 90-mm-diameter Petri dishes on whatman No.1. Petri dishes containing primed and control seeds were irrigated with solutions of8 ml salt stress levels. Here whatman No.1 filter paper was used as growth media for germination. Experimental units (75 Petri dishes) were arranged factorially in a completely randomized design with five replications. During the test filter papers in the Petri dishes were kept water saturated state. Seeds were kept at room temperature 25±1°C under normal light to facilitate germination for 7 days. Germination was considered to have occurred when radicles were 2 mm long (Akbari, *et al*., 2007). Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 days. The seedlings with short, thick and spiral formed hypocotyls and stunted primary root was considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were excluded during counting. At the end of germination test (7 days), 5 seedlings from each of the treatments were selected randomly and roots and shoots were cut from the cotyledons and were transferred to brown paper. Then these seedlings were dried in an oven at 75±2°C for 48 hours.

3.7 Data recording

Parameters that are measured as follows:

3.7.1 Total germination (TG%)

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

TG (%) = Seeds germinated/ total seeds x 100

3.7.2 Mean germination time (MGT)

Mean germination time (MGT) was calculated according to the equation of Moradi Dezfuli *et al.* (2008).

$MGT = \sum Dn / \sum n$

Where,

n = number of seeds germinated on day D, and

D = number of days counted from the beginning of germination.

3.7.3 Germination index (GI)

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae:

Germination index = Gt/ Tt

Where,

Gt = number of seeds germinated on day t and

Tt = the number of germinated seeds at time Ti.

3.7.4 Coefficient of velocity (CV)

Coefficient of velocity (CV)= (number of germinated seeds per day) is measured according to Kader and Jutzi (2004) formula.

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

Where,

Ti= number of days after sowing and

Ni = number of seeds germinated on ith day.

3.7.5 Energy of emergence (EG %)

Energy of emergence (EG) was recorded on the 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al.,* 2002a). Energy of emergence expressed in percentage.

3.7.6 Shoot (cm), root (cm) and seedling length (cm)

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

3.7.7 Seedling dry weight (mg)

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

3.7.8 Vigour Index (VI)

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

VI= TG (%) x seedlings length (mm)/ 100

Here,

TG = Total germination.

3.8 Statistical analysis

The data obtained for different parameters were statistically analyzed to observed the significant difference among the treatment. The mean value of all the parameters was calculated and analysis of variance was performed. The significance of difference among the treatments means was estimated by the Duncan's Multiple Range Test (DMRT) according to Steel *et al*. (1997) at 5% level of probability.

CHAPTER IV
RESULTS AND DISCUSSION

This chapter comprises presentation and discussion of the results obtained from a study to investigate the effect of seed priming with salt (halopriming) and hydropriming on the enhancement of salt tolerance capability in blackgram (Mushkalai) varieties cv. BARI mush-3 and BARI mush-4. The results of the germination and growth parameters of blackgram as influenced by hydropriming and different concentrations of salt and also tolerance capability in salt stress condition have been presented and discussed in this chapter.

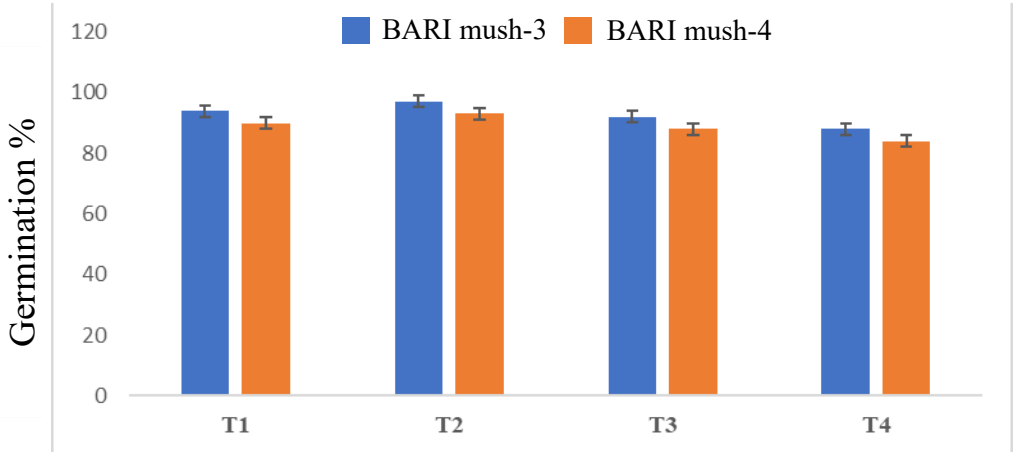
4.1 Experiment 1

Effect of different priming agents on the germination behavior of blackgram

Results obtained from the present study regarding the effects of different concentrations of salt on the germination behavior of mungbean varieties cv. BARI mush- 3 and BARI mush- 4 have been presented, discussed and compared in this chapter. The analytical results have been presented in Figures 1 to 9 and Appendices I to IX.

4.1.1 Total germination

There was no significant variation observed on total germination percentage at T_1 and T_2 treatment for varietal variation but significant variation was observed at T_3 and T_4 treatment (Figure 1 and Appendix I) priming with different concentrations of salt and water. Total germination percentage increased with salt concentration upto 6% and 2% for BARI Mush-3 and BARI mush-4, respectively there after decreased due to increasing concentration of salt. The highest total germination (97.32%) of BARI mush- 3 was observed from T_2 treatment compare to total germination percentage of all other treatments. The lowest germination percentage (84.38%) for BARI Mush-4 was found in T_4 treatment. Total germination of BARI mush- 3 was higher than BARI mush- 4. These findings are consistent of the results of Hasan *et al*., (2019) where they observed the highest germination percentage with water, and in BARI mush- 3.

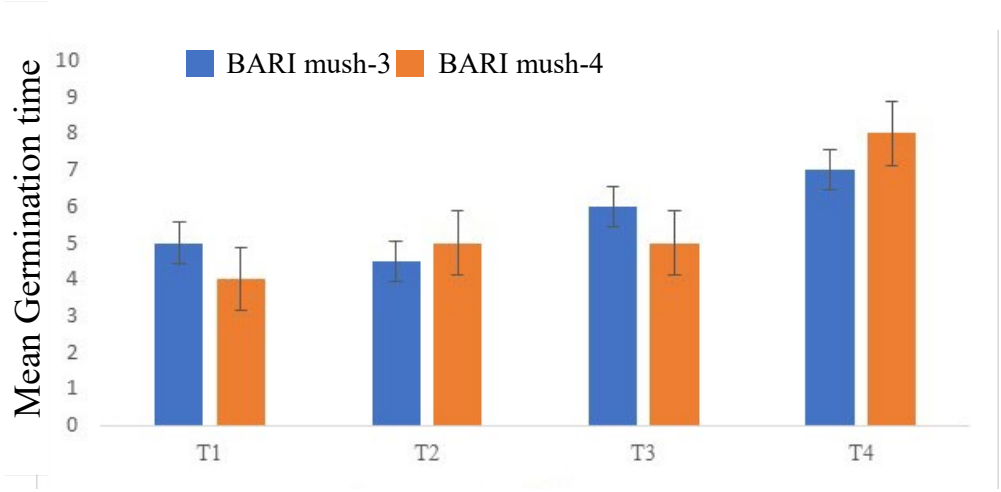


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 1 Effect of different concentrations of priming solution on total germination percentage of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.2 Mean germination time (days)

Different concentrations of salt solutions (Halo priming) and water priming differed significantly in mean germination time of BARI Mush-3 and BARI Mush-4 (Figure 2 and Appendix II). Mean germination time was affected by water priming and different halo priming. Mean germination time increased in T_3 and T_4 treatment for BARI Mush-3 and BARI Mush-4, respectively and decreased in hydroprimed seeds in treatment T_2 and controlled seeds treatment T_1. The longest mean germination time was observed for BARI Mush-4 from T_4 treatment compare to mean germination time of BARI Mush-3 was found at T_1 treatment. The shortest mean germination time (3.5 days) was found in BARI Mush-4 in T_1 treatment compare to mean germination time was found in all other treatments of BARI Mush-3 and BARI Mush-4, respectively. Mean germination time for hydro primed seeds is less than halo primed seeds. Such these positive effects are probably due to stimulatory effects of seed priming on biochemical activities and meiosis during primary stages of germination (Sirritepe *et al*., 2003). However, Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions.

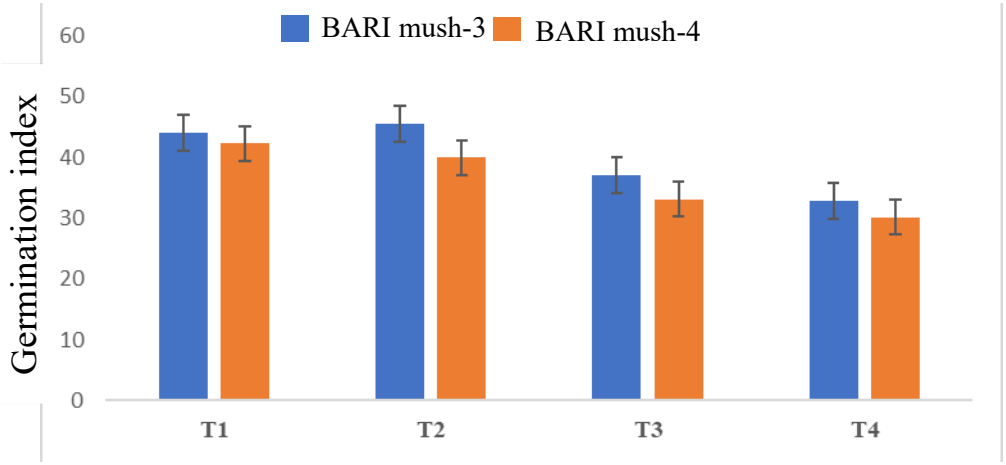


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 2 Effect of different concentrations of priming solution on mean germination time of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.3 Germination index

Germination index of BARI Mush-3 and BARI Mush-4 was influenced by different priming agent (Figure 3) and variance analysis showed that there was significant difference between control (non-primed) and primed seed (Appendix III). Germination index was affected by water priming and different salt concentration induced halo priming. Results reveled that germination index increased in both variety BARI Mush-3 and BARI Mush-4 in T_2 treatment and then decreased in T_3 and T_4 treatment. Highest germination index (45.46) was recorded in T_2 treatment compare to germination index was recorded from T_1, T_3 and T_4 treatment respectively, for BARI Mush-3 and BARI Mush-4, respectively. The lowest germination index (30.11) for BARI Mush-4 was found in T_4 treatment. Germination index of BARI Mush-3 was higher than BARI Mush-4. Rashid *et al*. (2005) concluded that priming of seeds with water before implantation leads to early germination, better establishment and increase of function in some of the crops in unfavorable conditions.

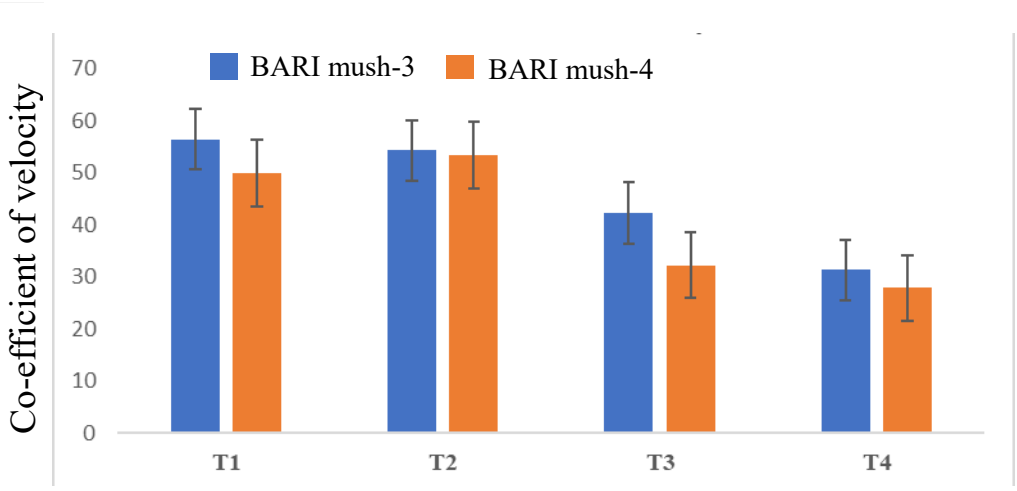


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 3 Effect of different concentrations of priming solution on germination index of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.4 Coefficient of velocity

Significant variation was observed in terms of coefficient of velocity for BARI Mush-3 and BARI Mush-4 due to priming with different salt concentrations (Halopriming) and water (Hydro-priming) (Figure 4). Coefficient of velocity increased with Hydropriming and 2 mM NaCl concentration and decreased gradually with increasing 2% NaCl concentration (Halopriming). The maximum coefficient of velocity was observed in BARI Mush- 3 from T_1 and T_2 treatment compare to coefficient of velocity was observed from T_4 treatment. The minimum coefficient of velocity (19.79) was found for BARI Mush-4 in T_4 treatment. Improving germination and coefficient of velocity in treated fenugreek seeds may be explained by an increase of cell division in the seeds (Gallais *et al*., 2000).

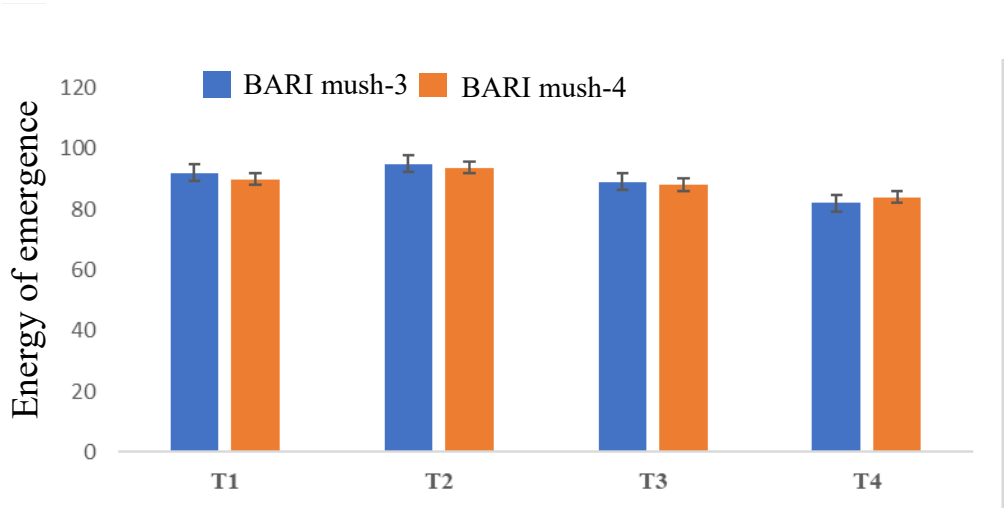


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 4 Effect of different concentrations of priming solution on co-efficient of velocity of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.5 Energy of emergence (%)

Energy of emergence showed no significant variation for BARI Mush-3 and BARI Mush-4 on different concentrations of salt (Halopriming) and water priming except controlled one (Figure 5). Result showed that energy of emergence increased in controlled and hydro-primed seeds of BARI Mush-3 and BARI Mush-4, respectively and decreased slightly in haloprimed seeds. The highest energy of emergence (94.99%) was recorded for BARI Mush-3 in T_1 treatment compare to energy of emergence of other treatments of BARI Mush-3. Same results also found in BARI Mush- 4. Faster emergence rate after priming may be explained by an increased rate of cell division in the root tips as previously found for wheat (*Triticum aestivum*) (Bose and Mishra, 1992; Basra *et al*., 2002) and fine rice (*Oryza sativa*) (Basra *et al*., 2003).

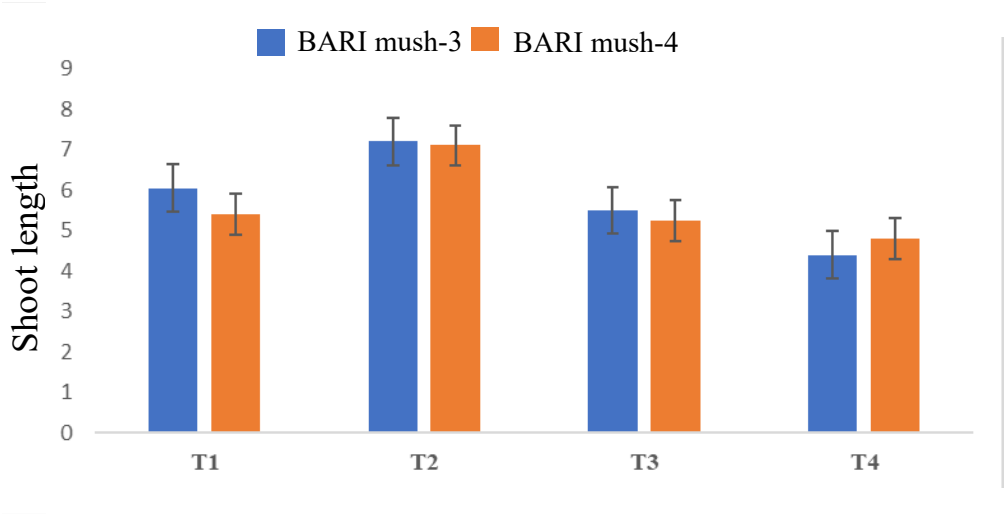


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 5 Effect of different concentrations of priming solution on energy of emergence of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.6 Shoot length (cm)

No significant variation was observed on shoot length among the two varieties (BARI Mush-3 and BARI Mush-4) priming with different concentration of salt and water (Hydropriming) (Appendix VI and Figure 6). Shoot length increase with T_2 and T_3 treatment for BARI Mush-3 and BARI Mush-4, respectively and decrease with the 2% NaCl salt solution (Halo-priming) in T_4 treatment. The maximum shoot length (7.6 cm) was recorded for BARI Mush-3 in T_2 treatment compare to shoot length of was recorded in other treatments. Treated seeds had high germination percentages and quicker germination time. One hypothesis is that benefits of priming can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduces lag time between imbibition and radicle emergence (Bradford *et al*., 1986). Better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins are also some of the basis for enhanced growth (Bray *et al*., 1989). Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds.

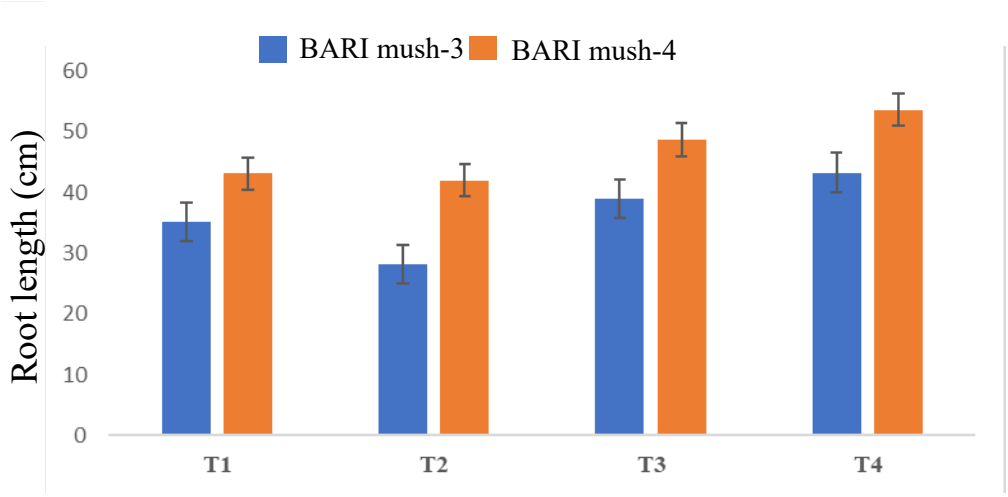


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 6 Effect of different concentrations of priming solution on shoot length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.7 Root length (cm)

Statistically significant variation was recorded in terms of root length of BARI Mush-3 and BARI Mush-4 due to priming with water and different salt concentrations (Appendix VII and Figure 7). Root length was affected by water priming and different halo priming concentration. Root length was minimum with T_1 andT_2 treatment for BARI Mush-3 and BARI Mush-4, respectively and maximum with the 2 mM and 3 mM salt concentration (Halopriming). The maximum root length (5.36 cm) was observed from T_4 treatment for BARI Mush-4 compare to root length was observed from T_1, T_2 and T_3 treatment. The minimum root length (2.82 cm) was found in T_2 treatment for BARI Mush-3. Root length of BARI Mush-4 was higher than BARI Mush-3.

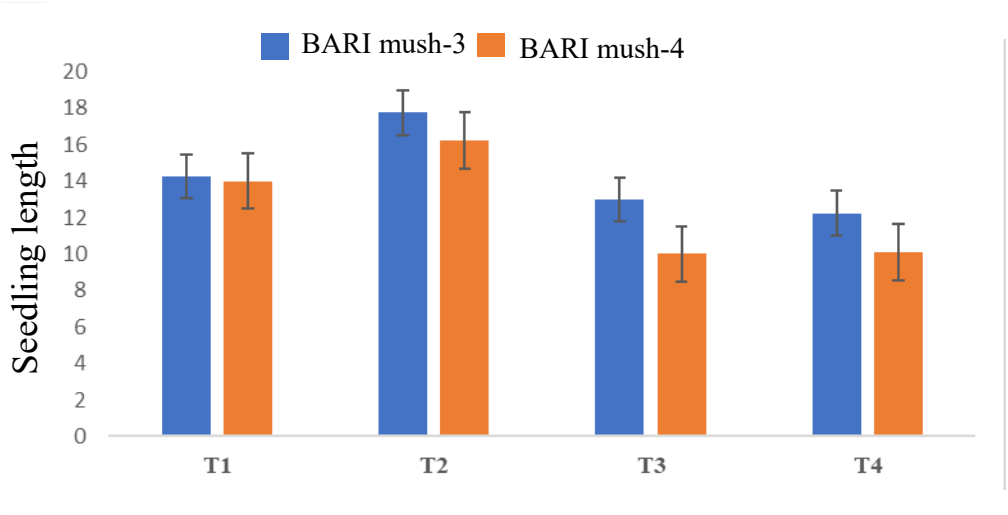


T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 7 Effect of different concentrations of priming solution on root length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.8 Seedling length (cm)

Seedling length of BARI Mush-3 and BARI Mush-4 showed significant variation due to priming with different NaCl concentrations (halopriming) and water (Hydropriming) (Appendix VIII and Figure 8). Seedling length was maximum in T_2 treatment of BARI Mush-3 and BARI Mush-4, respectively compared to all other treatments and decrease in the haloprimed blackgram seeds. The maximum seedling length (17.73 cm) was observed for of BARI Mush-3 from T_2 treatment. Seedling's length of BARI Mush-3 was higher than of BARI Mush-4. Seedling's length was higher in seeds treated with water, mannitol and lower concentration of K_2HPO_4 and KNO_3 as compared to seedlings grown from salt treated Chickpea seed Nighat *et. al* (2006).



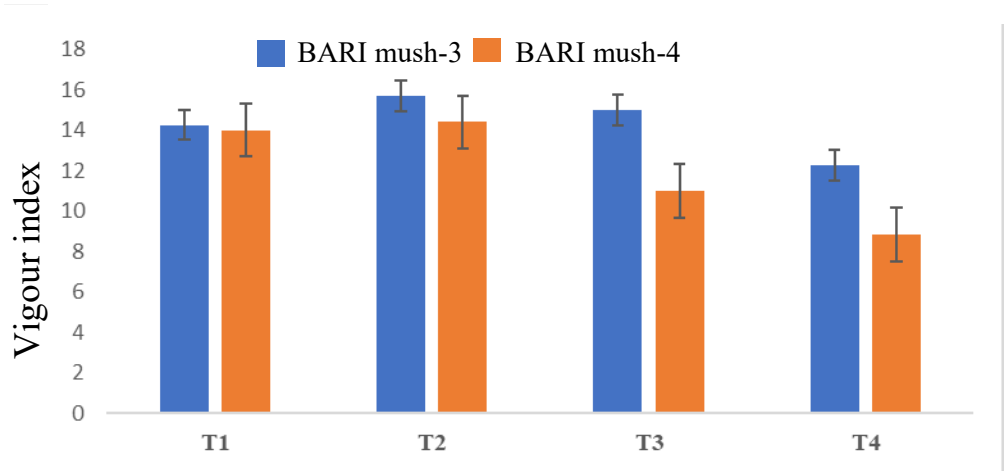
T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 8 Effect of different concentrations of priming solution on seedling length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.1.10 Vigour index

Priming with different concentrations of salt (halopriming) and water (hydropriming) showed significant variation in vigor index of BARI Mush-3 and BARI Mush-4 (Appendix IX and Figure 9). Vigor index was affected by water priming and halopriming. Vigor index increased significantly upto T_2 and T_3 treatment for BARI Mush-3 and BARI Mush-4, respectively and thereafter decreased drastically with the 3 mM salt concentration (Halopriming). The highest vigor index (15.70) of BARI Mush-3 was recorded from T_2 treatment. Grandi *et al*. (1999) found that P enrichment by soaking seeds in 200 mM KH_2PO_4 solution improved the seedlings establishment. The increased vigour of P-enriched seed might be due to increased P content both inside the seeds and on the seed surfaces which leads to better establishment of seedlings (Bolland and Baker 1988; Zhang *et al*., 1990; Thomson and Bolger 1993).

Similarly, the increase in seedling vigour due to salicylic acid may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis decreased catalase and peroxidase levels as recorded in pea seedlings (Srivastava and Dwivedi, 1998).



T_1= Control; T_2=Hydropriming (Seeds primed with Distilled water); T_3=Seeds primed with 2mM NaCl; T_4=Seeds primed with 3mM NaCl

Figure 9. Effect of different concentrations of priming solution on vigour index of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

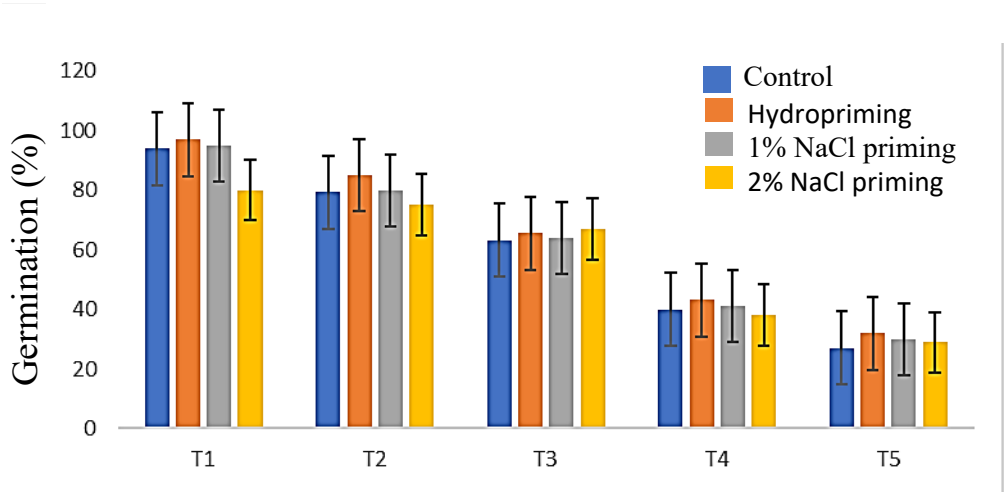
4.2 Experiment 2

Germination behaviour of primed seed (Blackgram) under salt (NaCl) stress condition

4.2. Total germination (%)

Different salinity levels exhibited significant variation in respect of total germination percentage (Figure 10). Result reveled that total germination from both primed and non-primed seeds decreased significantly with increasing salinity level. But total germination of halopriming (2 mM Salt concentration) and water primed (hydropriming) seeds was higher compared to controlled seeds at 0 mM salt concentration and various levels of salt stress whereas hydro primed seed gave the best result. Hydro priming seed had the maximum total germination (79.20 %) followed closely by 2 mM NaCl priming (63.33 %) under controlled conditions. On the other hand, minimum total germination percentage (32.22%) was found in blackgram seeds decreased when salinity of both primed and unprimed treatments increased; but total germination percentage in primed seeds was higher than unprimed seeds in all salinity conditions because water absorption increased in primed seeds and metabolic activities was formed too soon during germination of primed seeds; and consequently, radicle

and plumule appeared sooner (Ascherman Kock *et al*., 1992). Kaya *et al.* 2006 and Khajeh-Hosseini *et al*. 2003 also find that reduction in total germination was significantly lower for non- primed seeds, compared to primed seeds and this may be due to the toxic effects of Na^+ and Cl^- in the process of germination.



T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

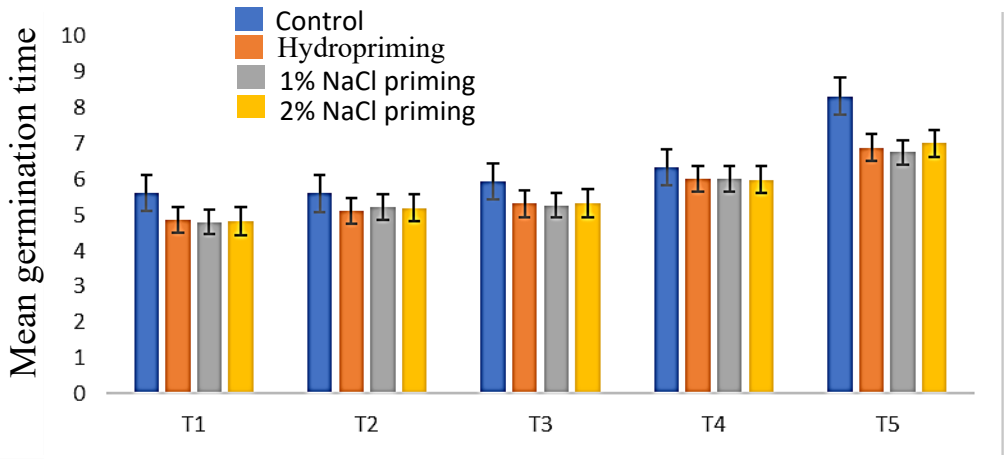
Figure 10. Effect of different salinity levels on total germination percentage of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.2 Mean germination time (days)

Mean germination time of blackgram significantly delayed with the increasing different salinity levels (Figure 11). Results showed that mean germination time increased for both primed (water and salt) and non-primed seed due to increasing salinity levels. But mean germination time of water primed seeds was lower compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas halo primed seeds gave the same trending results. However, hydro primed seeds have lower MGT (4.80 days) which is statistically similar with halo primed (1% and 2% NaCl) seeds (4.86 days) was recorded from the 0 mM salt treatment. The

highest mean germination time (8.32 days) in control treatment was recorded from the 200 mM salt treatment.

Halo primed and water primed blackgram seeds germinated earlier than unprimed ones as it has been reported by Ashraf and Rauf (2001) working with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA.



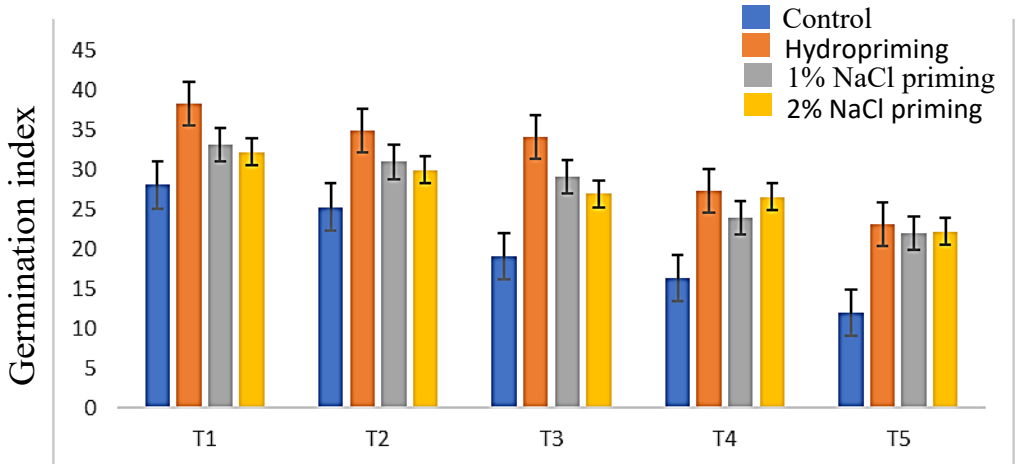
T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 11. Effect of different salinity levels on mean germination time (days) of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.3 Germination index

The results regarding germination index of blackgram in different salinity level (Figure 12) showed that germination index differed significantly with increasing salinity levels. Result revealed that germination index from both primed and non-primed seeds decreased significantly with increasing salinity level. But germination index of hydro-primed and halo primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas hydro primed seed gave the best result. Highest germination index was recorded (38.32) from hydro priming seed in T_1 treatment compare to (33.21), (32.20) and (28.09) halo priming and control, respectively. Minimum germination index (12.02) was found in control at T_5 treatment in non-primed seeds. Intermediate result was obtained from T_2, T_3 and T_4.

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

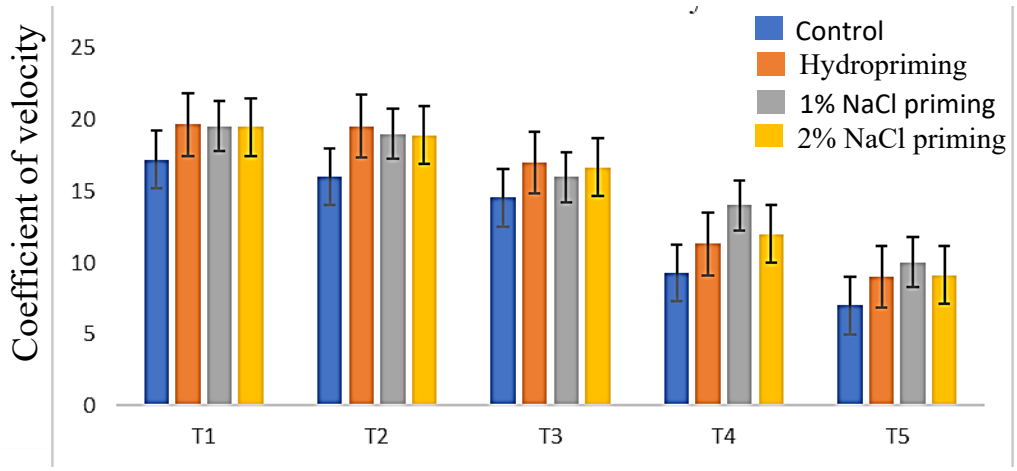


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 12. Effect of different salinity levels on germination index of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.4 Coefficient of velocity

Coefficient of velocity of blackgram showed significant variation among the treatments (Figure 13). Increasing salinity level significantly decreases values of coefficient of velocity. However, this decrease was more pronounced for non-primed seeds than for primed seeds. Coefficient of velocity of halo and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas hydro primed seed gave the best result. Coefficient of velocity was found the highest with hydro priming followed by halo primed seeds (19.65) in T_1 treatment which was statistically similar with (19.54) hydro primed seeds, respectively in T_2 treatment. The maximum coefficient of velocity of germination were found in the low salinity treatment and decreased with increasing salinity. Similar results were reported by Okcu *et al*. (2005).

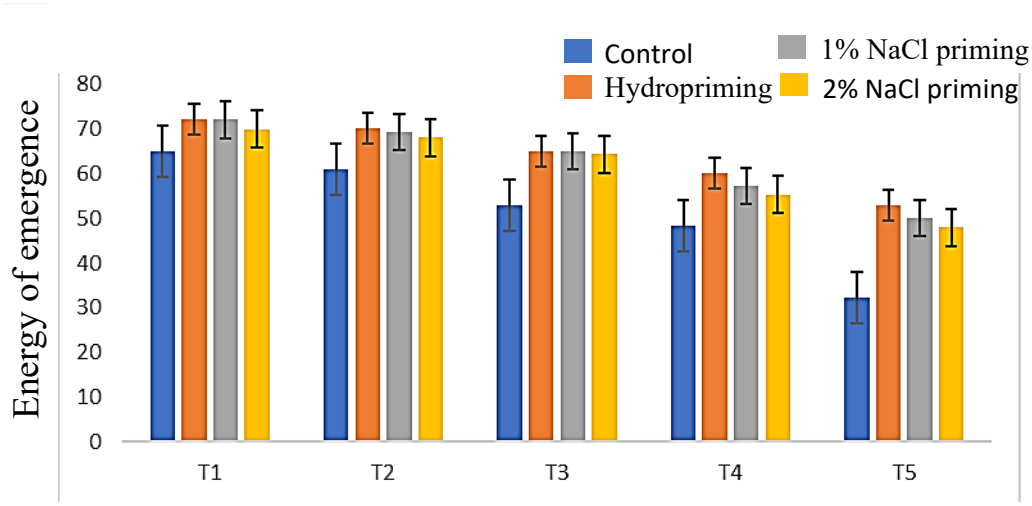


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 13 Effect of different salinity levels on co-efficient of velocity of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.5 Energy of emergence (%)

Energy of emergence was significantly influenced by the different salinity levels (Figure 15). Increasing salinity level significantly decreases values of energy of emergence. However, this decrease was more pronounced for non-primed seeds than for primed seeds specifically hydroprimed seeds. Energy of emergence of halo-primed and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas hydro primed seed gave the best result. In this study, the maximum energy of emergence (72.50 %) was achieved in hydro priming seed from T_1 treatment. The minimum energy of emergence (32.38 %) in control was achieved from T_5 treatment. That is, energy of emergence decreased in halo and water priming solution with increasing salt concentration the decreasing rate is lower in comparison to control seed. The results obtained from T_2, T_3 and T_4 showed intermediate results compared to maximum and minimum energy of emergence. It has been reported that priming had been resulted in more energy of emergence especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Demir Kaya *et al*., 2006; Foti *et al*., 2002).

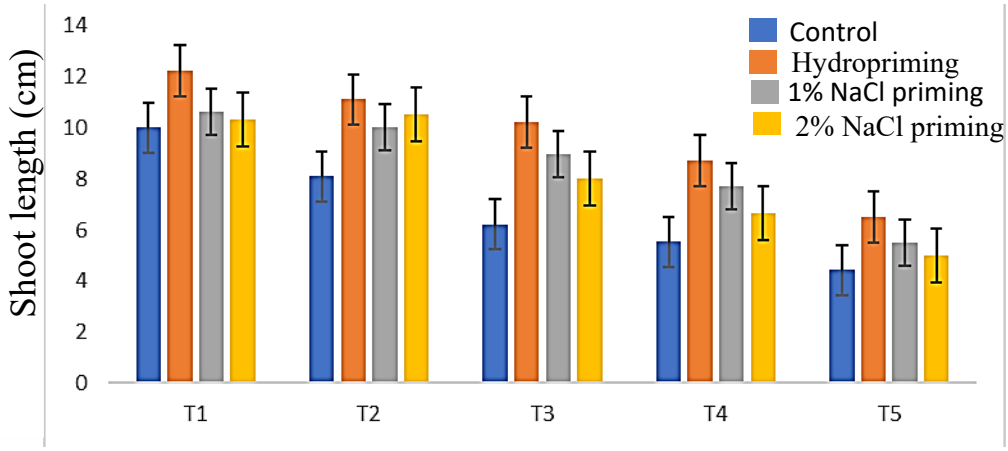


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 14 Effect of different salinity levels on energy of emergence of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.6 Shoot length (cm)

Salinity had a significant inhibitory effect on shoot length (Figure 15) for both primed and non-primed seeds. However, this effect was significantly less pronounced in seedlings priming with water and salt in comparison with control seeds. The highest shoot length (12.22 cm) was observed with hydro priming at T_1 treatment compare to (10.6 cm), (10.2 cm) and (9.55 cm) with salt priming from control seeds, respectively. The lowest shoot length (4.33 cm) from control seeds was found at T_5 treatment. Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita 2000). Similarly, toxic ion accumulation (Na^+ and Cl^-) negatively affect plant metabolism (Grieve and Fujiyama 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim *et al.,* 2008), which could adversely affect seedlings growth.

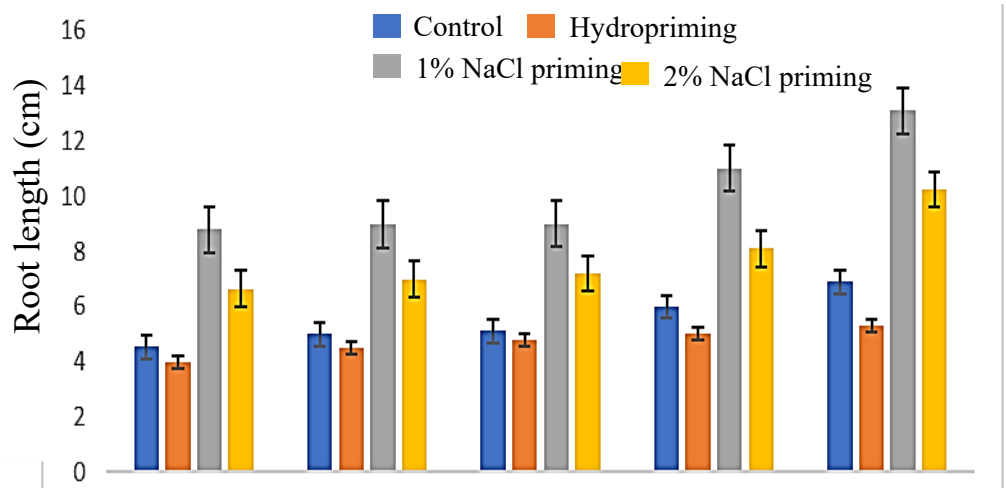


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 15 Effect of different salinity levels on shoot length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.7 Root length (cm)

Root length of mungbean significantly influenced by the different salinity levels (Figure 16). Root length increased with halo priming and hydro priming seeds in comparison to control seeds with increasing salinity levels. The highest root length (8.78 cm) was found with 3 mM NaCl (halo priming) compare to (6.55 cm), (4cm) and (4.54 cm) with 2mM NaCl (halo priming), hydropriming and control seeds, respectively at 0 mM salinity level (T_5) treatment. The lowest root length (3.99 cm) was found in hydroprimed seeds at 200 mM salinity level (T_5) treatment. Significant improvement in root and shoot length may be attributed to earlier germination induced by primed over non-primed seeds (Farooq *et al*.2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds. Present results confirm the findings of Stofella *et al*. (1992), who reported that priming of pepper seeds significantly improved radicle length.

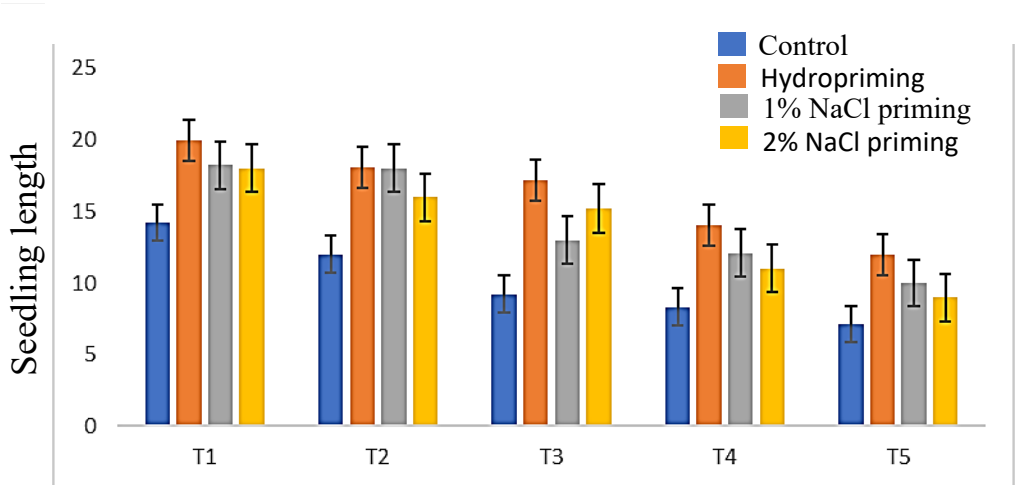


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 16 Effect of different salinity levels on root length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.8 Seedling length (mm)

Significant inhibitory effect was found in seedling length of blackgram with increasing salinity levels (Figure 17) for both primed and non-primed seeds. However, this effect was significantly more pronounced in control seeds in comparison with halo-primed and water primed seeds. Higher the salinity level lesser the seedling growth. The highest seedling length was obtained (19.94 cm) in hydro priming compare to (18.21 cm), (18 cm) in 2 mM, 3 mM NaCl priming (halopriming) and (14.79 cm) in control seeds at 0 mM salt (T_1) treatment. The minimum seedling length (9.55 cm) was found in case of control seeds at 200 mM salt (T_5) treatment. Result indicated that seed priming significantly improved blackgram seedling growth at different salinity level compare to control seed. Similar result was found by Katembe *et al.* (1998) who investigated the effect of seed priming as a method to improve seedling growth of two *Atriplex* species under salt stress.

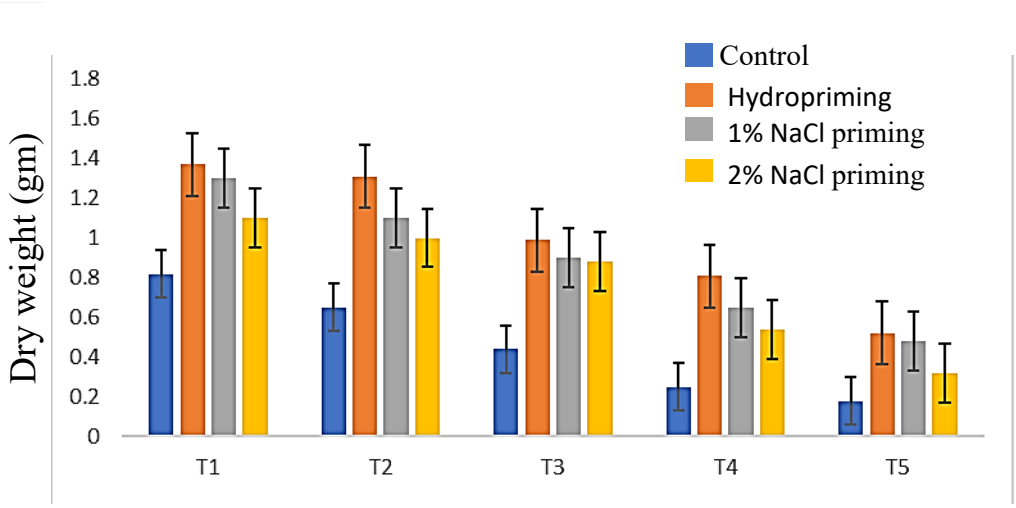


T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 17 Effect of different salinity levels on seedling length of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

4.2.9 Seedling dry weight (gm)

Increasing salinity significantly decreased blackgram seedlings dry weight for both primed and non-primed seed (Figure 18). Result revealed that seedlings dry weight from both primed and non-primed seeds decreased significantly with increasing salinity level. But seedlings dry weight of halo-primed and water primed seeds was higher compared to non-primed seeds at 0 mM salt concentration and various levels of salt stress whereas hydro primed seed gave the best result. Figure 19 shows that the highest dry weight (1.37 g) of seedlings was recorded from hydro priming at 0 mM salt (T_1) treatment. On the other hand, lowest seedlings dry weight (0.818 g) was obtained from control seed at 200 mM salt (T_5) treatment. Seedling dry weight decreased linearly with increasing salinity. The similar results were also obtained by other researchers (Mansour *et al*. 2005). Increased dry weight in primed seeds over the non-primed seeds were also observed by Sivritepe *et al*. (2003) who reported an increase in seedling dry weight in hydro primed melons seeds under saline conditions as compared to the non-primed seeds.



T_1=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed without salt: T_2=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 50 mM salt: T_3=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 100 mM salt: T_4=Primed (Water, 2 mM and 3 mM NaCl) and non-primed (control) seeds placed with 150 mM salt: T_5=Primed (Water, 2 mM and 3 mM NaCl) and nonprimed (control) seeds placed with 200 mM salt.

Figure 18 Effect of different salinity levels on dry weight (gm) of primed and non-primed seeds. Mean (SD) was calculated from three replicates for each treatment. Error bars are significantly different at $P \leq 0.05$ by applying DMRT test

CHAPTER V
SUMMARY AND CONCLUSION

The experiment was conducted at Laboratory of the Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from December, 2019 to March, 2020 to evaluate water and salt induced seed priming on salt tolerance capability in blackgram varieties cv. BARI Mush-3 and BARI Mush-4 under salt stress condition. The whole work was conducted in two different experiments. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Different priming agents such as salt (NaCl) and distilled water were utilized for halo and hydro priming. Sodium hypochlorite used as seed treating chemical.

Priming was done in room temperature and all the primed seeds were removed from the priming solution at the same time. Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter Petri dishes on whatman No.1filter paper and filter paper was moistened with 8 ml of distilled water. Germination was considered to have occurred when radicles were 2 mm long. Germination progress was inspected and data were collected at every 24 h intervals and continued up to 7 days. The abnormal or dead seedlings with short, thick and spiral formed hypocotyls and stunted primary root were excluded during counting. The data on germination parameters of blackgram like total germination percentage, mean germination time, germination index, coefficient of velocity, energy of emergence and growth parameters like plumule length, root length, seedling length, dry weight and vigour index.

The first experiment was carried out to find the effect of hydropriming and halopriming on germination behavior of blackgram varieties cv. BARI Mush-3 and BARI Mush-4 without any stress condition. Two levels of salt such as 2 mM, 3 mM are used for halopriming and water used as hydropriming agent for 6 hours, respectively. The priming treatments were seeds without priming (control) (T_1), seeds primed with distilled water for 6 hours (T_2), seeds primed with 2 mM salt solution for 6 hours (T_3),

seeds primed with 3 mM salt solution for 6 hours (T_4). For BARI Mush-3 and BARI Mush-4, the maximum total germination percentage (97.32 %) and lowest germination percentage (84.38%) was recorded in T_4 treatment; mean germination time was shortening in T_1 treatment (3.5 days) and (4.41 days) in BARI Mush-3 of T_1 and T_2 treatment, highest germination index (45.46) in BARI Mush- 3 in T_2 treatment and (30.11) in BARI Mush-4 in T_4 treatment; maximum coefficient of velocity was observed in BARI Mush- 3 from T_1 and T_2 treatment and minimum coefficient of velocity (19.79) was found for BARI Mush-4 in T_4 treatment; highest energy of emergence (94.99%) was recorded for BARI Mush-3 in T_1 treatment;

The maximum shoot length (7.6 cm) was recorded in BARI Mush-3 in T_2 treatment; maximum and minimum root length (5.36 cm) and (2.82 cm) in T_4 and T_2 treatment, respectively; maximum and minimum seedling length (17.73 cm) and (9.37 cm) in T_2 and T_4 treatment, respectively; and maximum and minimum vigor index (15.70) and (123.30) in T_2 and T_4 treatment, respectively. Variety BARI Mush-4 was not further used as it gives poor result than BARI Mush-3.

In the second experiment germination behavior of primed seed (blackgram) under different salt (NaCl) stress condition was evaluated. Salt solution 2 mM, 3 mM and distilled water were used as priming solutions, 6 hours as priming time and salt stress levels 50 mM, 100 mM, 150 mM and 200 mM were used in this experiment. Primed (halo and water) and non-primed (control) seeds placed without salt (T_1), primed (salt and water) and non-primed (control) seeds placed with 50 mM salt (T_2), primed (salt and water) and non primed (control) seeds placed with 100 mM salt (T_3), primed (salt and water) and non-primed (control) seeds placed with 150 mM salt (T_4) and primed (salt and water) and non-primed (control) seeds placed with 200 mM salt (T_5) were used as treatment.

The maximum total germination percentage (79.20%) in hydroprimed seeds under control condition, germination index (38.32) in T_1 treatment of hydroprimed seeds without any significant varience upto T_3 treatments, coefficient of velocity (19.65) in hydroprimed seeds in T_1 treament without any significance varience upto T_3 treatment, maximum energy of emergence (72.50%) in hydroprimed seeds in T_1 treatment without significance varience with other hydroprimed and haloprimed treatments but

significant varience compared to unprimed seeds, shoot length (12.22 cm), root length (8.78 cm), seedling length (19.94 cm), and seedling dry weight (1.37 g) were found in hydro primed seeds in (T_2) treatment. The maximum mean germination time (8.32 days) was found in control seeds. The minimum total germination percentage (32.22%), germination index (12.02), coefficient of velocity (5.52), energy of emergence (32.38 %), shoot length (4.33 cm), root length (3.99 cm), seedling length (9.55 cm), and seedling dry weight (0.818 g) were found in control seeds at (T_5) treatment.

From the results of the study, it may be concluded that the performance of hydro primed blackgram cv. BARI Mush-3 was better in respect of germination and growth parameters. Priming with distilled water for 6 hours increase the germination behabiour of blackgram seeds. Reduction in germination parameters and seedling growth was more profound in control seeds than primed seeds under salt stress condition. Thus, the priming specifically hydropriming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of salt stress. For this reason, further studies are needed to assess the efficacy of seed priming during the later stages of the culture.

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APPENDICES

Appendix I: Mean square values on different priming agents for total germination percentage of blackgram

BARI Mush-3	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	440.121	3	88.024	3.565	.033
Within Groups	296.282	6	24.690		
Total	736.403	9			
BARI Mush-4	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1854.740	3	370.948	8.014	.002
Within Groups	555.422	6	46.285		
Total	2410.162	9			

Appendix II: Mean square values on different priming agents for mean germination time of blackgram

BARI Mush-3	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6.351	3	1.270	.870	.529
Within Groups	17.527	6	1.461		
Total	23.879	9			
BARI Mush-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.436	3	.087	3.435	.037
Within Groups	.304	6	.025		
Total	.740	9			

Appendix III: Mean square values on different priming agents for germination index of blackgram

BARI Mush-3	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	108.767	3	21.753	5.703	.006
Within Groups	45.772	6	3.814		
Total	154.539	9			
BARI Mush-4	Sum of Squares	Df	Mean Square	F	Sig.
etween Groups	1017.795	3	203.559	25.805	.000
Within Groups	94.661	6	7.888		
Total	1112.456	9			

Appendix IV: Mean square values on different priming agents for coefficient of velocity of blackgram

BARI Mush-3	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.023	3	.605	1.408	.289
Within Groups	5.152	6	.429		
Total	8.174	9			
BARI MUSH-4	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.977	3	.395	9.296	.001
Within Groups	.510	6	.043		
Total	2.487	9			

Appendix V: Mean square values on different priming agents for energy of emergence of blackgram

BARI Mush-3	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	307.809	3	61.562	3.920	.024
Within Groups	188.463	6	15.705		
Total	496.272	9			
BARI Mush-4	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3865.151	3	773.030	21.243	.000
Within Groups	436.680	6	36.390		
Total	4301.830	9			

Appendix VI: Mean square values on different priming agents for shoot length of blackgram

BARI Mush-3	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5080.507	3	1016.101	84.832	.000
Within Groups	143.733	6	11.978		
Total	5224.240	9			
BARI MUSH-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1422.971	3	284.594	4.853	.012
Within Groups	703.707	6	58.642		
Total	2126.678	9			

Appendix VII: Mean square values on different priming agents for root length of blackgram

BARI Mush-3	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	612.896	3	122.579	22.036	.000
Within Groups	66.753	6	5.563		
Total	679.649	9			
BARI MUSH-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	408.224	3	81.645	3.287	.042
Within Groups	298.053	6	24.838		
Total	706.278	9			

Appendix VIII: Mean square values on different priming agents for seedling length of blackgram

BARI Mush-3	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8129.092	3	1625.818	86.009	.000
Within Groups	226.833	6	18.903		
Total	8355.925	9			
BARI MUSH-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2367.060	3	473.412	4.411	.016
Within Groups	1287.840	6	107.320		
Total	3654.900	9			

Appendix IX: Mean square values on different concentrations of NaCl for vigour index of blackgram

BARI Mush-3	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8213.487	3	1642.697	31.657	.000
Within Groups	622.683	6	51.890		
Total	8836.170	9			
BARI MUSH-4	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7563.309	3	1512.662	7.336	.002
Within Groups	2474.271	6	206.189		
Total	10037.580	9			

Appendix X: Mean square values for total germination percentage under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7477.983	19	534.142	47.501	.000
Within Groups	337.346	38	11.245		
Total	7815.329	57			

Appendix XI: Mean square values for mean germination time under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.443	19	.103	2.628	.013
Within Groups	1.176	38	.039		
Total	2.619	57			

Appendix XII: Mean square values for germination index under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2239.651	19	159.975	119.422	.000
Within Groups	40.187	38	1.340		
Total	2279.839	57			

Appendix XIII: Mean square values for coefficient of valocity under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.336	19	1.238	2.517	.017
Within Groups	14.757	38	.492		
Total	32.093	57			

Appendix XIV: Mean square values for energy of emergence under salt stress condition of blackgram

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

Appendix XV: Mean square values for shoot length under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75428.532	19	5387.752	2783.700	.000
Within Groups	58.064	38	1.935		
Total	75486.596	57			

Appendix XVI: Mean square values for root length under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20078.091	19	1434.149	440.907	.000
Within Groups	97.582	38	3.253		
Total	20175.673	57			

Appendix XVII: Mean square values for seedling length under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	169695.776	19	12121.127	2612.889	.000
Within Groups	139.169	38	4.639		
Total	169834.945	57			

Appendix XVIII: Mean square values for seedling dry weight under salt stress condition of blackgram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35977.497	19	2569.821	771.271	.000
Within Groups	99.958	38	3.332		
Total	36077.455	57			