IMPROVEMENT OF GERMINATION AND SEEDLING GROWTH OF RICE THROUGH PRIMING UNDER SALT STRESS CONDITION

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CERTIFICATE

This is to certify that thesis entitled, "***IMPROVEMENT OF GERMINATION AND SEEDLING GROWTH OF RICE THROUGH PRIMING UNDER SALT STRESS CONDITION"*** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN AGRONOMY**, embodies the result of a piece of bona-fide research work carried out by WASIA JAHAN BADHAN, Registration no. 08-03125 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.

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ABSTRACT

An experiment was conducted at the Agronomy laboratory of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from July, 2013 to December, 2013 to improve germination and seedling growth of rice through seed priming under salt stress condition. The experiment was laid out in a completely randomized design (CRD) with five replications. BRRI dhan28 (V_1, salt sensitive) and BRRI dhan47 (V_2, salt tolerant) were used in this experiment. The treatments comprised five levels of salt concentration (0, 50, 100, 150 and 200 mM NaCl) and priming times (6, 9, 12, 15 and 18 hr). Results revealed that germination percentage (GP) 97.75%, germination index (GI) 40.95, seedling length (SL) 143.3 and vigour index (VI) 144.90 were improved by seed priming. Seeds primed with 100 mM NaCl gave the better result compared to non-primed seeds in V_1 varieties. Seeds primed with 150 mM NaCl gave the better result in terms of germination behavior and seedling growth compared to untreated control in V_2 varieties. Then seeds of V_2 was primed in 0, 6, 9, 12, 15, 18 hrs with 150 mM NaCl solution and distilled water. Priming time 15 hours showed the best result in V_2 varieties when seeds primed with 150 mM NaCl (GP, GI, SL,VI, SL, RL) compared to water priming and untreated control. Finally V_1 primed with water and 100 mM NaCl and V_2 primed with distilled water and150 mM NaCl solution for 15hrs. Dry seed used as control and was exposed to 0, 50, 100, 150 and 200 mM NaCl induced salt stress conditions in Petri dishes. In both varieties (V_1 and V_2) when the salt stress was increased at the same time GP, GI, VI and SL was decreased. The highest GP, GI, VI and SL were detected in the treatments of control and lowest GP, GI, VI and SL were detected in the treatments primed with 200 mM NaCl.

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Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

Rice is the most important human food, eaten by more than half of the world's population every day. In Asia, where 90% of rice is consumed, ensuring there is enough affordable rice for everyone, or rice security, is equivalent to food security (IRRI, 2013). It is the grain with the second-highest worldwide production, after corn. Bangladesh is the fourth highest rice (*Oryza sativa* L.) producing country in the world (FAO, 2013).

In Bangladesh, there are approximately 2.85 million ha of coastal soils which occur in the southern parts of the Ganges tidal floodplain, in the young Meghna estuarine floodplain and in tidal areas of the Chittagong coastal plain and offshore islands (Brammer, 1978). The total saline area forms one third of the 9 million hectares of total national cultivated area in Bangladesh (ABSPII, 2006). Saline soils can be managed by large scale irrigation and drainage schemes and by chemical treatments of soil, but the scale of the problem renders these solutions too costly (Gregorio *et al.*, 2002). One of such efforts is to cultivate rice with elevated level of salt tolerance on salt affected marginal lands. Today, the most economic and sustained way to overcome the problem of salt stress is to develop salt tolerant varieties.

Rice is the most suited crop for saline soils because it can tolerate standing water, which is necessary for reclamation of saline soils. Soils are considered saline if they contain soluble salts in quantities sufficient to interfere with the growth of most crop species. Salinity is a major threat to crop productivity in the southern and south-western part of Bangladesh, where it is developed due to frequent flood by sea water of the Bay of Bengal and on the other hand introduction of irrigation with saline waters. Saline soil covers our earth's surface, estimated to be from 400 to 950 million ha.

However, Rice is a salt sensitive crop. Salinity affects rice productivity during pollination, resulting decrease seed setting and grain yield. (Elouaer and Hamach, 2012). It also affects seed germination, seedling growth of several crop species. It has been reported that water uptake during the imbibitions phase decreases and salinity induces an excessive absorption of toxic ions in the seed (Murillo-Amador *et al*., 2002). Several techniques have been used to improve salt tolerance capability of plants. Priming is an effective technique that improves germination of several crops under salt stress. This approach has also proven its effectiveness to improve crop establishment in saline soil (Ashraf and Rouf, 2001). It is the most important physiological enhancement method to increase stress tolerance capacity of crop plants. Seed priming can be accomplished through different methods such as hydro-priming (soaking in DW), osmo-priming (soaking in osmotic solutions such as PEG, potassium salts, e.g., KCl, K_2SO_4) and plant growth regulators such as ABA, GA_3, IAA etc. (Capron *et al.*, 2000; Chiu *et al*., 2002; Harris *et al.*, 1999; Chivasa *et al.*, 1998).

Seed priming can be a low cost solution for poor farmers, it is reliable and simply adopted and also low risk intervention for ensuring fast germination (Harris 2004; Iqbal and Ashraf 2005; Bakare and Ukwungwu 2009; Tavili *et al*. 2011). Priming has been utilized to accelerate synchronized seed germination, encourage vigorous seedling establishment, and stimulate vegetative growth and crop yield in many field crops (Patade *et al*., 2009). For exemple, maize, rice and wheat (Farooq *et al*. 2008; Moosavi *et al*. 2009; Zhao *et al*., 2009; and Golezani *et al*. 2010). Moreover, seed priming has been effectively confirmed to enhance germination percentage and rate, and emergence percentage and rate mainly in vegetable seeds and small seeded grasses (Heydecker and Coolbear 1977; Bradford 1986; Sadeghi *et al*. 2011; Tavili *et al.* 2011). Furthermore, seed priming has been shown to enhance vigorous root growth regions of low soil water potential. In addition, Moosavi *et al*. (2009) reported that pre-sowing seed treatments improve the performance of seeds under adverse conditions and environmental stresses such as salinity seed treatments improve the performance of seeds under adverse conditions and environmental stresses such as salinity.

With these facts in mind, the resent study was under taken with the following objectives:

i. To understand germination behavior and seedling growth of rice under different priming treatments
ii. To explore suitable and cost effective priming chemical for inducing salt tolerance capability in rice
iii. To optimize priming time for enhancing salt tolerance capability under salt stress

Chapter 2
Review of literature

CHAPTER 2

REVIEW OF LITERATURE

Rice is the most important food of the tropical country. Most of the areas of the southern part of Bangladesh are affected by salt condition and farmers cannot cultivate rice in the salt affected area due to lack of efficient salt tolerant variety or lack of proper management strategy. The findings from this review will help to cultivate rice under salt affected areas. As a result rice production area will be increased and ultimately yield will be increased.

2.1 Effect of Priming

Plants are exposed to any number of potentially adverse environmental conditions such as water deficit, high salinity, extreme temperature, submergence, etc. These abiotic stresses adversely affect the plant growth and productivity. Nowadays various strategies are employed to generate plants that can withstand these stresses. In recent years, seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. Seed priming is the induction of a particular physiological state in plants by the treatment of natural and synthetic compounds to the seeds before germination. In plant defense, priming is defined as a physiological process by which a plant prepares to respond to imminent abiotic stress more quickly or aggressively. Moreover, plants raised from primed seeds showed sturdy and quick cellular defense response against abiotic stresses. Priming for enhanced resistance to abiotic stress obviously is operating via various pathways involved in different metabolic processes. The seedlings emerging from primed seeds showed early and uniform germination. Moreover, the overall growth of plants is enhanced due to the seed-priming treatments. The main objective of this review is to provide an overview of various crops in which seed priming is practiced and about various seed-priming methods and its effects (Jisha, *et al.* 2013).

The effects of priming on seed germination properties have been well documented. High potential in improving field emergence and ensures early flowering and harvest under stress condition especially in dry areas and under drought stress. 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. Priming led to an increased solubilization of seed storage proteins like the betasubunit of the 11-S globulin and reduction in lipid peroxidation and enhanced antioxidative activity in seeds . Afzal *et al.* (2008) observed that the priming-induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K+ and Ca2+ and decreased Na+ accumulation in wheat plants. 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

2.2 Effect of Priming concentration

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 longterm treatment periods using solutuins 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 hydro-priming, while higher concentrations of PEG had negative effects on seed germination

The concentration of the priming agent (osmotic potential) is also a critical factor. Afzal *et al*. (2007a) studied the effect of halopriming with 10, 25 or 50 mM NaCl and $CaCl_2$ in wheat (*Triticum aestivum* cv. Auqab-2000) under saline conditions. The results showed that most priming agents were not effective in improving germination and seedling estabishment under salt stress. However, primed seeds with 25 and 50 mM of $CaCl_2$ significantly reduced the mean germination time and significantly increased the shoot length, and fresh and dry weight of seedlings more than all priming treatments. The shoot and root length, and fresh and dry weight of shoots and roots of seedlings obtained from primed seeds were increased significantly compared to unprimed seeds. This improvement as affected by priming is supported by the findings of Basra *et al*. (2005), Rafiq *et al*. (2006), Afzal *et al*. (2006, 2007, 2008), Iqbal and Ashraf (2007) with wheat. Afzal *et al*. (2008) reported that the growth of wheat increased significantly by using 50 mM $CaCl_2$ as seed priming treatment in saline medium. Moreover, Rafiq *et al*. (2006) reported that priming with CaC_2 significantly enhanced shoot and root length under both saline and non-saline conditions.

2.3 Effect of Priming agent

There are many priming agents that can be used but the outcome may be different. Thus there is no specific agent for each cultivar. Afzal *et al*. (2008) studied the effect of three halopriming agents on the growth of wheat under saline conditions and reported that $CaCl_2$ and $CaSO_4$ decreased the negative effect of salinity on the growth of wheat more effectively than NaCl. Moreover, it has been reported that germination percentage and germination rate of two wheat cultivars were improved under non-saline and saline conditions due to the application of $CaCl_2$ followed by hydropriming (Afzal *et al*. 2006). Furthermore, Farooq *et al*.(2005) studied the effect of three priming agents mainly halopriming with KNO_3 or NaCl and osmopriming with PEG 8000 on four tomato cultivars and the results showed that KNO_3 was the most effective agent for improving germination and seedling growth

2.4 Effect of priming duration

Soaking period has been reported to be one of the key factors of determining the effectiveness of priming (Giri and Schillinger 2003; Subedi and Ma 2005; Dezfuli *et al.*, 2008). Yari, Aghaalikani, and Khazaei (2010) studied the effect of priming duration on germination and early growth of two wheat cultivars (Azar-2 and Sardari 101). Seeds were primed for 12 , 24 and 36 h in several priming agents (PEG 10%, PEG 20%, KCl 2%, KCl 4%, KH_2PO_4 0.5%, KH_2PO_4 1% and distilled water). The results showed that the greatest germination percentage was recorded in cv. Azar-2 when the seed were primed with PEG 20% for 12 h and the greatest stem length was obtained with seeds primed with PEG 10% for 24 h. Moreover, Yari *et al*. (2012) tested the effect of soaking three wheat cultivars (Fajer, Sherodi and Taram) for 12, 24 and 36 h in 0.5 and 1% $CaCl_2$ priming solutions. They concluded that soaking for 24 h was suitable for all three cultivars in terms of increasing the germination percentage. Many studies concluded that the duration of priming is different amoung the species and cultivars, for example, Rashid *et al*. (2006) reported that the best priming duration for soaking barley seeds was between 12 and 16 h. Furthermore, the best duration for maize and rice was 18 h (Harris *et al*., 1999).

Rashid *et al.* (2006) reported that hydropriming increased the germination rate, enhanced yields and provided good establishment of barley under saline and non-saline soils.

Golezani *et al.* (2008) claimed that hydropriming can enhance seedling emergence rate, emergence percentage and seedling establishment of lentil (*Lens culinaris* Medik.) as well as increase the length of roots. Hydropriming by soaking in water for 12, 24, 36 or 48 h improved seed germination and increased maximum length of the radicle of two genotypes of maize (*Zea mays* L.) including B73 and MO17 (Dezfuli, Sharif-Zadeh, and Janmohammadi 2008). Numerous research efforts have concluded that treating crop seeds in water prior to sowing can enhance the resistance of crops to salinity (Zheng *et al.* 2002). By soaking maize in water, the resistance of seeds to salinity has been improved and gave better germination percentage compared with the control (Ashraf and Rauf, 2001). Afzal *et al.* (2007b) studied the effects of several priming treatments (hydropriming, matriconditioning, chilling, osmopriming and hardening) on wheat (*Triticum aestivum* L.). They found that the maximum emergence percentage (61%) was obtained from hydropriming seeds followed by 24 h chilling. Also the maximum shoot length (18 cm) and root length (28 cm) were recorded by hydropriming seeds followed by 24 h of chilling.

2.5 Germination percentage

2.5.1 Enhancement of germination percentage and rate

Seed priming is a pre-sowing strategy for improving seed performance during germination and field performance. During priming seeds are partially hydrated so that pre-germination activities proceed but radical protrusion will be prevented. Thus, when seeds are sown in the field, they can germinate and emerge sooner. Drought and salinity are two main factors limiting crops growth and development worldwide. In this paper the potential effects of priming for improving seed performance under environmental stress conditions are discussed (Kazemi and Eskandari, 2012).

Salinity affect germination and seedling growth of several crop species, many techniques are used to improve tolerance and development of plants. Priming is an effective technique that improves germination of several vegetables crop under saline condition. That’s why, this experiment was carried to study the effect of seed priming with 5 g/L NaCl and KCl on germination and seedlings growth of safflower (*Carthamus tinctorius*) exposed to five levels of salinity (0, 5, 10, 15 and 20 g/L) (Elouaer and Hannachi, 2012).

Asl and Taheri (2012) studied the effect of different solutions of seed priming at the amount of activities of some cotton anti oxidative enzymes the factorial experiment in completely randomized block design with three replications was conducted. Cotton seeds controlled by priming solutions (control, 2% kcl, HH2PO2 %1, KNO3 30 gr/lit 6000 PEG %10) in 200c. the analyze of data presents that the amount of catalyse, peroxidase, ascorbat peroxidase , controlled seeds with PEG solution in 12 and 24 hours except superoxid dismutase in 36 hours and at KCL solutions was obtained at the most amount.

Yari *et al*. (2010) evaluate the effect of different seed priming techniques on germination and early growth of two wheat cultivars (Azar-2 and Sardari 101) an experiment was conducted at the Seed and Plant Certification and Registration Research Institute, Karaj, Iran. Seeds were primed for 12, 24 and 36 hours at three temperature (20, 23 and 28°C) in seven priming media (Poly ethylene glycol (PEG_{6000}) 10%, PEG 20%, KCl 2%, KCl 4%, KH_2PO_4 0.5%, KH_2PO_4 1% and distilled water as control). Maximum seed germination percentage in cv.Azar-2 was observed when the seeds primed by PEG 20% for 12h and at 20°C. The most stem length was obtained for seeds osmoprimed with PEG 10% for 24h. Osmoprimed seeds with PEG 20% for 24h produced maximum radicle length of cv. Sardari. Maximum vigor index (VI) of cv. Azar-2 was obtained from seeds primed with KH_2PO_4 0.5% while the lowest germination percent, speed of germination and VI were observed in seeds which subjected to KCl 4% solution. Speed of germination was improved when the seed soaked water and PEG 10%. The most germination percent, VI and speed of germination were observed on 12h. Altogether 20°C treatment had better effects than other temperatures on germination attributes and seedling parameters. None of the interaction between seed priming media × cultivar × temperature and seed priming media × cultivar × priming duration showed the beneficial effects on germination index, speed of germination, dry weight and seedling height.

Golezani *et al.* (2008) conducted in RCB design in 2006 and 2007 at the Research Farm of the University of Tabriz, Iran, to evaluate the effects of hydro and osmo - priming (PEG: Polyethylene glycol 6000 at -0.8MPa) on seed germination and field emergence of lentil. Analysis of variance for laboratory data showed that hydropriming significantly improved germination rate and root weights, compared to other seed treatments. However germination percentage for seeds primed with water and PEG were statistically similar, but higher than those for unprimed seeds. Over all, hydropriming treatment was comparatively superior in laboratory tests. Invigoration of lentil seeds by hydropriming resulted in higher seedling emergence in the field, compared to control and seed priming with PEG. Seedling emergence rate was also enhanced by priming seed with water. Thus, hydropriming could be used as a simple method for improving seed germination and seedling emergence of lentil in the field.

Effects of seed priming treatments with 0.5% KH_2PO_4 (w/v) solution and water were determined on germination and seedling characters of hexaploid triticale (Triticosecale Witm., cv. Presto) in different osmotic potential of NaCl and PEG solutions. Drought and salt osmotic stress conditions were separately created by using PEG 6000 and NaCl, respectively, at different osmotic potentials (-0.45, - 0.77, -1.03 and -1.44 MPa and control). At the equivalent osmotic potential, the effects of PEG 6000 were more harmful than NaCl on germination and seedling stage. Germination percentage and seedling growth and also relative water content (RWC, %) decreased with the decrease in osmotic potential of PEG 6000 and NaCl. But root-to-shoot length ratios increased with the effects of osmotic stress of PEG 6000 and NaCl. Despite the negative effects of two stress conditions, the two priming treatments were effective in improving germination percentage and seedling growth in Presto. But seed primed treatment was effective at the lowest osmotic potentials;

therefore, seedling growth survived at the highest concentrations. Consequently, the effect of hydropriming is very pronounced particularly in improving germination and seedling growth in low stress (Mehmet and Diğdem, 2008).

Seed priming has been shown to be beneficial to germination and emergence of some crop species grown under saline conditions. For instance, Ashraf and Rauf (2001) conducted an experiment to determine whether salt tolerance could be induced in maize (cv. C-17) at germination stage by soaking of seeds for 8 h in distilled water or in 200 meq/l of Nacl, KCl, $CaCl_2.2H_2O$. They observed that all priming agents were effective in allevitingnthen the adverse effects of salt stress on maize at the germination stage. $CaCl_2.2H_2O$ proved to be more effective since the seeds primed with this salt had significantly higher final germination and rate of germination. In contrast, XiaoFang *et al.* (2000) primed seeds of *Gossypium hirsutum* in Pix (DPC) [mepiquat] and $CaCl_2$ solutions and found decreased seed germination and seedling emergence with the degree of inhibition increasing with increased concentration of the seed treatment chemical. Osmotic priming of melon seeds with 18 dS m^{-1} Nacl solution for 3 days at 20°C increased salt tolerance in seedlings under saline conditions (Sivritepe *et al*. 2003).Total emergence and dry weights were higher in melon seedlings derived from primed (P) seeds and they emerged earlier than non-primed (NP) seeds. Moreover seeds from NP group could tolerate up to 9.0 dS m^{-1} NaCl salinity, while the total emergence values of P groups in cvs. Hasanbey and Kirkagae did not decrease below 50% at 13.5 and 18.0 dS m^{-1}, respectively. In another experiment with melons they determine the optimum concentration of priming agent (NaCl) and found that priming with 0.1% NaCl increased the salt resistance in melon cultivars (Sivritepe *et al*. 2003) that was clear from total germination and mean germination time, tolerance ratio and tolerance index parameters.

The effects of seed priming with 6M Nacl solution were investigated in tomato cv. Pera plants exposed to 70 and 140 mM NaCl nutrient solutions from 11 to

60 days after sowing (Cayuela *et al.* 1996). Tomato seedling emerged earlier from primed seeds than from non-primed seeds. Similarly, other inorganic salts or even PEG also accelerated germination (Haigh and Barlow, 1987; Muhyddin and wiebe, 1989). Zheng *et al.* (1998) determined the effects of prehydration and redrying treatment on canola cv. Tobin seed germination and seedling emergence at 10° C subjected to either water or salt stress. Prehydration and redrying partially overcame the inhibitory effects of both low water potential and salts on seed germination and seedling emergence at 10°C. Salt concentrations inhibiting 50% emergence were higher for treated seeds than control seeds. Neither treated nor control seeds produced seedlings which emerged if the soil water content was lower than 9% or when the soil was continuously irrigated with salt solution of 100 mmol kg^{-1} of Na_2SO_4 , they suggested that the pre-hydration and re-drying treatment did not lower the base water potentials at which seedling emergence could occur.

Al-Humaid (2002) studied the effect of seed priming on germination and seedling growth of Beruda grass under saline conditions and noted that seed priming followed by immediate sowing improved the final germination rat index (CGRI) was improved by seed priming followed by immediate sowing in almost all saline solution treatments. Generally, seeds primed for 3 days and immediately placed in the growth medium exhibited the highest values of seed germination, CGRI and plimule growth. He also indicated that the osmoconditioning of Bermuda grass seeds with PEG followed by immediate sowing improved seed germination and seedling growth under saline conditions. Osmotic priming also increased seed vigour and emergence under saline conditions (Muhyaddin and Wiebe, 1989). ChangZheng *et al*. (2002) investigated the effect of seed priming with mixed-salt solution on the germination by determining the percent germination and germination index under salt stress (0.58 % NaCl). They found that germination of the primed seeds was significantly more rapid than the control under salt stress. Similarly, $CaCl_2$ and $ZnSO_4$ pretreatments increased germination rate and percentage in

Sudan grass (*Sorghum sudanense*) grown under saline conditions (Ismaeil *et al.* 1993).

Mitigative effects of hydration-dehydration treatment on salt stress-induced injuries to tomato seed germination were investigated (JunMin *et al*. 2000). They found that injuries induced by salt stress were all significantly mitigated by hydration-dehydration pretreatment of seeds. In contrast, Rehman *et al*. (1998) hardened seeds of *Acacia* (*A. bivenosa, A. coriacea, A. elata, A. farnesiana, A. nilotica, A. salicina, A. saligna, A. senegal, A. tortilis and A. tunida)* by soaking in distilled water (DW) for 10 h and dried before germination. Depending on species final germination in DW was either reduced or unaffected by hardening while germination in NaCl, at the LD_{50} concentration for each species was reduced unaffected or increased. Similarly germination rate was reduced unaffected or increased in DW and NaCl by hardening. Three different pregermination treatments (pre-hydration, osmoconditioning and pre-hydration/dehydration cycles) were tested on *Senna madranthera* seeds at 18°C for 6 and 8 h, and 27°C for 8h (Cassaro-Silva. 2000). He observed higher germination rate compared with control but found no differences among the treatments tested. Moreover, presoaking seed treatments (hydration-dehydration, $CuSO_4$ at 0.1 mg L^{-1}, $ZnSO_4$ at 0.1 mg L^{-1} and proline at 50, 100 and 200 mg L^{-1}) had varied effects in alleviating subsequent salinity stress in subabul [*Leucaena leuconeephala (lam.)* de Wit] as shown by various germination parameters such as germination relative index germination value and vigour index. Higher mobilization values of presoaked seeds were associated with greater vigour and length of shoots and roots of germinated seeds.

Pujol *et al.* (2000) determined the osmotic potential that enforced dormancy in the majority of seeds by ecposing seeds of four halophytes to iso-osmotic stress with NaCl, $MgCl_2$, $MgSO_4$, and Na_2SO_4. They found the factor responsible for the dormancy of seeds to be the decrease in osmotic potential. Such osmotic treatments promoted an increase in the rate of recovery germination which in halophytes doubled their rate of germination compared to distilled water controls. Similarly, primed seeds of asparagus (Evans and Pill, 1989), carrot, celery and onion (Brocklhurst and Dearman, 1983) showed slow germination when the seeds were redried after priming and this can be attributed to the increased time needed for seed reimbibition. Pill *et al.* (1991) also observed a higher percentage of germination in tomato and asparagus from primed seeds than untreated seeds. In the saline seedbed, they found increased final emergence percentage from asparagus seeds provided they were not subsequently dried after priming but had no effect on the percentage emergence of tomato seeds. Similarly, seeds of *Acacia coriacra* and *A. tortilis* were soaked for 10 h in distilled water (DW) or in 10 or 50 nM CaCl2.Ca(NO3)2, KCl or KNO3 (Rehman *et al*., 1998). The seeds were the air-dried and germination of *A. tortilis* in NaCl solution. Treatment with calcium (Ca) or potassium (K) salt did not give a significantly greater final germination than treatment with DW, but the rate of germination both in DW and NaCl solution increased with all treatments. They suggested treatment with DW and drying a rapid and simple method for improving germination in saline conditions because such a strategy may aid the establishment of species that are susceptible to salinity at germination but more tolerant at later growth stages.

2.5.2 Effect of plant growth regulator

The depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones such as gibberedllic acid or kinetin (Debez *et al*. 2001) and there are many reports that presoaking seeds with optimal concentrations of phytohormones (Ungar, 1977, Bozcuk, 1981; Khan and Ungar, 1985) increased the germination rate and emergence under saline conditions. For example, alleviation of salinity stress by GA3, Kin and IAA on seed germination of *Brassica campestris* L. was reported. They found that growth regulators significantly increase germination under salt stress compared with controls receiving no growth regulators. GA_3 was more effective than kinetin or IAA. Sangkuk *et al.* (1997) treated four cultivars of rice with higher concentrations of ABA and kinetin and observed their alleviating effect on

NaCl injury during rice germination. Kinetin treatment increased germination except in cv. Dasanbyeo. However, some studies showed that ABA also accelerated germination (Haigh and Barlow, 1987; Muhyaddin1989; Finch-Savage and McQuistan, 1991).

Germination of *Lulium multiflorum* seeds under irrigation with saline water at concentrations of 2000, 4000 and 6000 mgL^{-1} and presoaking in indloleacetic acid (IAA) (10 and 20 mgL^{-1}) and gebberellic acid (GA3) (100 and 300 mgL^{-1}) in addition to fresh water (control) were studied (El-Din. 1998). Germination reached 100% after 7 days from the presoaked grains in IAA (10 mgL^{-1}) and after 5 days from the presoaked grains in GA3 (300 mgL^{-1}). In pot trials, *Medicago varia* seeds soaked in 0-50 mgL^{-1} BA were watered with 0-50% NaCl solution and *Medicago varia* seeds soaked in 0-20 mgL^{-1} kinetin were watered with 0-3% NaCl solution (Kachel, 1993). BA slightly reduced germination of *Medicago varia* under non-saline conditions, but increased it from 55.33% to 62.67-73.67% when 5% NaCl was applied and reduced the inhibition of seedling growth caused by NaCl, *Medicago varia* failed to germinate when 1-3% NaCl was applied without growth regulators. Soaking in 1.0 or 10.0 mgL^{-1} kinetin increased germination to 21.33-30.33% at 1% NaCl but had little or no effect at higher salinity levels.

Growth promoting hormone (kinetin) promoted germination of three plant species (tomato, barley and cotton) by alleviating the osmotically-induced dormancy by NaCl (Bozcuk, 1981). Similarly, germination studies with lettuce (Kaufman and Ross, 1970) also indicated that kinetin can alleviate dormancy induced by osmotic stress in seeds. Khan and Ungar (1985) determined the germination of desert forb (*Zygophyllum simplex* L.) under various salinity, proline, betaine, GA and Kinetin treatments. Gebberellic acid (0.3 and 3 mM) and kinetin (0.05 and 0.5 mM) substantially alleviated both innate as well as salinity induced seed dormancy. At higher salinity (125 mM), low concentrations of kinetin (0.05 mM) and high concentrations of GA (3mM) were more effective. GA3 completely alleviated the effect of salinity at all concentrations used. Similarly, Rizvi *et al.* (2001) concluded that priming of

Hordeum vulgare seeds in aqueous solutions (10^{-6} *M*) of gibberellic acid and /or kinetin for 6, 12 and 24 h improved percent germination of the grains irrespective of the hormone and the soaking duration. However, the best response was generated in the grains soaked in gibberellic acid for 12 h where germination increased by 156% over control.

The effects of GA3, kinetin, ABA and CCC (chlormequat chloride) were studied on seed germination of maize seedlings under salt stress (Lin,1985). Seed presoaking with 500 mg L^{-1} GA3 improved germination vigour, while 1000 mg L^{-1} GA3 or 10 mg L^{-1} CCC

Promoted seed germination and germination rate. Reda *et al*. (1998) soaked the seeds of tobacco cv. Cocker -139 in solutions of 20 or 100 mg L^{-1} gibberellic acid (GA3), 10 or 50 mg L^{-1} IAA or 20 or 100 mg L^{-1} boric acid, the exposed to 0, 200, 400, 600, 1600, 3200 or 6400 mg L^{-1} NaCl. Germination percentage generally decreased with increasing salinity but boric acid treatments, especially at 100 mg L^{-1}, were the most effective at counteracting the effects of NaCl up to 1600 mg L^{-1}. *Robinia pseudoacacia* seeds treated with 250 mg L^{-1} triazole for 48h after sterilization in sodium hyponitrite solution improved seedling salt tolerance. Presoaking seeds in 10^{-2} m*M* putrescine also alleviated the adverse effect of NaCl during germination and early seedling growth of *Atropa belladonna* (Ali, 2000).

Seed germination is a major factor limiting the establishment of plants under saline conditions (Al-karaki, 2001). However, it is possible to induce salt resistance at the germination stage in wheat by pretreatment with salt solution. For example, increasing the Ca^{2-} and K^{+} concentrations in seeds of wheat (Chaudhrui and Wiebe, 1968, Idris and Aslam, 1975) improved germination in NaCl solutions. In another study, treatment of wheat seed with 5% KH_2PO_4 solution accelerated seedling emergence, and increased germination percentage whereas seed treatment with 0.25% $CaCl_2$. 2.5 % NaCl or $Ca(OH)_2$ saturated solution was not found effective (Bhati and Rathore, 1986). Chaudhuri and Wiebe (1968) observed the effect of pre-soaking of seeds in different inorganic salts on the germination of wheat under saline conditions and found that soaking seeds in calcium chloride was better than soaking them in sodium chloride solutions. This induction of resistance against sodium by calcium salts apperars to be based on the well known phenomenon of antagonism between monovalent and diavalent cations which has been observed .

Hydropriming is also suggested to increase germination and growth in some crops under saline conditions. For example, Roy and Srivastava (1999) soaked seeds of wheat cv. UP 262 in water, sodium benaoate (50 mg L^{-1}), calcium chloride (100 mg L^{-1}) or ascorbic acid (50 mg L^{-1}) and then germinated at salinities of 0.35 (control), 4, 9, 12 or 16 dS/m. they found that all the seed treatments decreased the negative effects of pre sowing seed treatment at the germination and early seedling stages of wheat and found that treatment with calcium (Ca) or potassium(K) did not lead to a significantly higher rate for final germination than the DW treatment. Similarly, Ashraf and Iram (2002) found no significant effect of water soaking on the germination of wheat cv. SARC-1 under salt stress. They further concluded that neither varying soaking time periods or varying concentrations of different salts proved to be effective in inducing salt tolerance in the two spring wheat cultivars with respect to germination percentage and rate of germination.

Presoaking seeds with optimal concentrations of phytohormones improves growth and yield of some crop species under saline conditions. For instance, the effects of presoaking wheat seeds in 10, 20, 50 and 100 mg L^{-1} each of GA3, IBA, IAA and NAA at 0, 3, 6 and 9 atm osmotic pressure on seedling development were observed (Darra *et al*. 1973). Auxins had little influence on emergence at 0 atm but promoted emergence at 3, 6 and 9 atm. IAA and α-naphthalene acetic acid (NAA) at 50 mg L^{-1} produced maximum shoot length, whereas GA_3 and indolebutyric acid (IBA) gave better results to 100 mg L^{-1}.
Kabar,(1991) soaked seeds of *Brassica campestris* in 50 or 100 mg L^{-1} solutions of the growth regulators (GA3, Kin, or IAA) for 2h and then

germinated in 0-5% NaCl solutions. They resulted that all growth regulators significantly increased germination under salt stress compared with controls receiving no growth regulators and GA3 was more effective than kinetin or IAA. Similarly, seed germination was assessed in different species including wheat in a range of NaCl concentrations after 2 h soaking in 0.5 m*M* kinetin, 1.0 m*M* GA3, or a combination of both growth regulators. He found that GA3 was most effective in increasing germination in osmotically stressed gramineae seeds and kinetin more effective in dicotylendons. Coleoptile emergence in monocotyledons was increased by GA3 whereas hypocotyl emergence in dicotyledons was generally greatest after treatment with kinetin alone.

Gibberellic acid is known to have favourable effects on salt resistance (Nieman and Bernstein, 1959). In a pot experiment, Angrish *et al*. (2001) concluded that pre-sowing seed soaking treatments with 0.4 mM gibberellic acid, 0.4 mM kinetin and 0.4 mM gibberellic acid, 0.4 mM kinetin alleviated the deleterious effects of chloride and sulfate salinity in wheat cultivars. Similarly, pre-soaking wheat (Sakha 92) seeds in gibberellic acid (GA3) increased the germination potential especially at moderate salinization levels. On the other hand, abscisic acid (ABA) did not increase the germination potential of tested seeds at all salinization levels.

Excessive sodium (Na+) and chloride (Cl-) concentrations in rice shoots reduce seedlings growth. In a hydroponic study, we investigated the role of abscisic acid (ABA) seed pre-treatment on growth of salt-stressed rice, in the presence and/or absence of silicate (Si). Caryopses of rice IR4630 (salt tolerant) and IR15324 (salt sensitive) were soaked in an ABA solution (10-5 M) for 24 h, and the plants were then allowed to grow in a medium without or with silicate (Si, 0 or 3 mM). Twenty-one-day-old plants were salinised with NaCl (50 mM).We found the application ABA+Si the most effective treatment for improving plant growth under saline conditions. Si treatment alone or with ABA significantly increased the plant total dry biomass under saline or non-saline conditions, whereas, the effect of ABA seed pre-treatment was significant only on total dry biomass of salt sensitive genotype IR15324. Under saline conditions, ABA, Si and ABA+Si application ameliorated plant growth via suppression of Na+ accumulation in shoots and lowering down Na+/K+ ratios in both the rice genotypes. In addition, treatment with Si alone or with ABA significantly reduced Na+ concentrations in the leaf blades and sheaths, increased net assimilation rate and stomatal conductance of salt affected rice seedlings. We concluded that silicon can be applied as a nutrient for rice under saline or non-saline conditions. Furthermore, seed soaking with ABA has a potential for enhancing salt tolerance, particularly in the sensitive rice genotypes (Gurmani, *et al*. 2013).

Afzal *et al.* (2012) investigated the potential of seed priming for induction of salt tolerance in rice. The seeds of two fine aromatic rice *cv.* Shaheen Basmati (salt tolerant) and Basmati-2000 (salt sensitive) were soaked in aerated solutions of CaCl2 (Ψs=-1.25 MPa), KCl (Ψs=-1.25 MPa), and H_2O_2 (50 mM) each for 36 h. Seeds soaked in distilled water and non-primed seeds were taken as control for comparison. Two experiments were conducted *in vitro* and primed seeds were exposed to 0, 40 and 80 mM NaCl in petri dishes and sand culture during germination and emergence respectively. Priming with $CaCl_2$ followed by KCl were more effective in inducing salt tolerance of both rice cultivars owing to enhanced germination capacity, speed of germination, seedling length and dry weight in saline medium. Incremental salinity reduced total chlorophyll percentage (TCP) by two fold in both cultivars but higher TCP was found at all salinity levels from $CaCl_2$ primed raised seedlings in salt tolerant cultivar. Decreased leaf Na+ accumulation and increased K+ uptake were found in $CaCl_2$ primed seedlings at moderate salinity in both cultivars but effect was more pronounced in Shaheen Basmati. Delayed germination and seedling growth with H_2O_2 pre-treatment indicates sensitivity of H_2O_2 primed seeds to salts and might be the result of higher Na+ uptake and less chlorophyll contents in rice seedlings. Nonetheless, enhanced germination and seedling growth, high Na+/K+ exclusion ndicates Shaheen Basmati tolerance to salinity than Basmati-2000 and seed priming with $CaCl_2$ seems more promising in induction of salinity tolerance in fine aromatic rice.

2.6 Vigour index

Osmopriming of seeds with NaCl nullify the adverse effects of salt stress. Curiously under salt stress, the seedling of tomato emerges earlier in NaCl-primed seeds than the non-primed seeds.

Similarly hydro-priming improved germination and later growth of different crops species such as in maize, rice, chickpea (Harris *et al.*, 1999). This technique has been employed to increase germination rate and seedling vigour in several vegetable crops. On the other hand, there is a general consensus that priming decreases longevity (Taylor *et al*., 1998).

Molecular markers have been identified for priming of seeds in chilli (Lanteri *et al*., 2000). It is reported that aerated hydration treatment of pepper at 250 C followed by drying increased germination percentage (Demir and Okcu, 2004). Different seed enhancement and priming techniques have been documented by Maiti *et al.*, (2006). 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. They used hydropriming, halopriming, and osmopriming. It is observed that few priming technique improved seedling emergence, seedling vigour as well as agronomic traits including yield of the crop species. These findings clearly demonstrated that in all the crops studied the priming technique improved growth and yield of all the crops showing variations among treatments. The present work concentrated to study the effect of priming of tomato and chilli crops during the rainy season of 2012 at Hyderabad, Andhra Pradesh, India.

2.7 Dry matter production

Phenology and dry matter are important traits being affected by seed priming and soil phosphorus (P_2O_5) application. Wheat variety Saleem-2000 was primed in 0.1, 0.2 and 0.3% P_2O_5 solutions and water for 10 hours. Unprimed treatment was included as control. Four levels of P_2O_5 (0, 25, 50 and 75 kg P2O5 ha-1) were applied to soil. Priming enhanced days to emergence, anthesis and increased dry matter (DM) production compared with non primed (control). Seed primed with 0.3% P2O5 solution took less time to anthesis (110 days). DM yield increased with each increment of priming and maximum DM yield (6051 kg ha-1) was obtained from seeds primed in 0.2% P_2O_5 solution. Priming had non significant effect on spike length, spike m-2 and days to maturity. Water primed seed took less time to emergence (16 days). Soil P_2O_5 application enhanced days to heading, anthesis, maturity and increased DM yield, while days to emergence, spike m-2 and spike length were not affected. Earlier heading, anthesis, maturity and highest DM yield was recorded at 75 kg P_2O_5 ha-1. Priming with 0.2% P_2O_5 solution and 50 kg P2O5 ha-1 soil application are the best for obtaining maximum DM and enhancement of phenology (Khalil *et al*., 2010)

Chapter 3

Materials and Methods

CHAPTER 3
MATERIALS AND METHODS

This chapter presents a brief description about experimental period, site description, crop or planting materials, treatments, experimental design and layout, data collection and statistical analysis.

3.1 Location and period

The experiment was conducted at the Agronomy lab of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from July to December, 2013.

3.2 Experimental design

The experiment was laid in a completely randomize design (CRD) with five replications having variety and NaCl concentration.

3.3 Material

3.3.1 Plant material

BRRI dhan28 and BRRI dhan47 seed were collected from Bangladesh Rice Research Institute (BRRI) Joydebpur, Gazipur.

Description of Variety

Two rice varieties (BRRI dhan28 and BRRI dhan47) were used as variety

Variety

1. BRRI dhan28 (salt sensitive)-V_1 and
2. BRRI dhan47 (salt tolarance)-V_2

BRRI dhan28: BRRI dhan28 variety is grown in boro season . This variety was released by BRRI in 1994. Grain colour is white and medium slender in size. The cultivar matures at 140 days of planting. It attains a plant height 90-100 cm. The cultivar gives an average yield of 6.00 t ha^{-1}.

BRRI dhan47: Growing season of BRRI dhan 47 is boro. This variety was released by BRRI in 2007. Medium in size and bold in shape.Growth duration is 150-155 days. It attains a plant height 100-105 cm. The cultivar gives an average yield of 6.00 t ha^{-1}.

3.3.2 Instruments

Petri dish, filter paper (Filter no-102) size-12.5cm, distilled water, growth chamber, electric balance etc.

3.4 Chemicals

1. NaCl
2. Sodium hypochloride

3.5 Preparation of NaCl concentration

50 mM NaCl concentration , 100 mM NaCl concentration, 150 mM NaCl concentration and 200 m M NaCl concentration were prepared by 0.731 g NaCl, 1.463 g NaCl, 2.18g NaCl and 2.98 g NaCl dissolved in 250 ml water respectively. Dry seed used as control.

3.6 Steps of the experiment

This experiment was completed in three steps. In the 1st step found out the best concentration, 2nd step found out the best time and 3rd step found out the best result.

3.7 1st experiment: Optimization of priming solution

3.7.1. Treatments

Two Rice variety

i. V1:BRRI dhan28 (salt sensitive)

ii. V2:BRRI dhan47 (salt tolarance)

Priming treatment

1. 0 mM NaCl -T_0 (No priming)
2. Water- T_1
3. 50mM NaCl -T_2
4. 100mM NaCl -T_3
5. 150 mM NaCl- T_4
6. 200 mM NaCl –T_5

3.7.2 Preparation of seed

At first healthy seeds were collected from the seed of BRRI dhan28 and BRRI dhan47. Then they are sterilized by sodium hypochloride in 5% NaCl concentration. Then seeds were dried in air .Then seeds were weighted for primed with NaCl concentration (0, 50, 100, 150, 200 mM) and dry seeds were used as control.

3.7.3 Priming of seed

Then BRRI dhan 47 and BRRI dhan28 were primed with 0 mM, 50 mM , 100 mM, 150 mM, 200 mM concentration respectively at 12 hours and found out the best priming concentration of BRRI dhan28 and BRRI47. After priming seeds were washed with distilled water and dried in air.

3.7.4 Preparation of Petri dish

Petri dises were washed in distilled water and sterilized by sodium hypochloride in 5%. Fillter paper were set in Petri dishes. Petri dishes were irrigated with water.

3.7.5 Placement of seed

30 seeds were placed in each Petri dish.

3.7.6 Counting of seed germination

Seed germination was recorded daily up to 14 th days after the start of the experiment. A seed was considered germinated when the radicle emerged by about 2 mm in lenth. (Akbari *et al.*, 2007). Generally seeds were germinated after 3 days of place seed.

3.7.7 Collection of data

After 14 th days data were collected and found out the best priming concentration.

Parameters measured in this experiment were:

1. **Germination (%)**

   Germination measured in the seventh day using the formula $=\frac{Total\ Number\ of\ germinated\ seeds}{Total\ seed} \times 100$

2. **Germination index (GI)**

   Germination index was worked out according to the equation of Kader and Jutzi (2004).

   GI=$\sum TiNi$

   Ti= Number of day after sowing and Ni= Number of germinated seed in the day.

3. **Seedling height:** Seedling height (mm) was measured from the root base to the tip of the longest leaf at the time of uprooting of seedlings.
4. **Dry weight**: seedling dry weight was determined after placing sseedlings, after the 7th day in oven to dry at 70°C for 48 hrs.
5. **Vigour index (VI)**: vigour index was worked out byusing the formula of Abdul baki and Aderson (1970).

$$VI=\frac{Germination\ (\%)\times seedlings\ length}{100}$$

3.8 2nd experiment: Optimization of priming time

3.8.1 Treatments

Rice variety:

BRRI dhan47 was used as plant material

Priming time (hr)

1. 0 hr
2. 6 hr
3. 9 hr
4. 12 hr
5. 15 hr
6. 18 hr

3.8.2 Preparation of seed

In this experiment only BRRI dhan47 was used. At first BRRI dhan47 were collected from the seed store. Then they were sterilized by sodium hypochloride in 5% concentration. Then seeds were dried. Then seeds were weighted for primed with NaCl con (150 ml) which was best found the 1st experiment. Dry seeds were used as control.

3.8.3 Priming technique

Then BRRI dhan47 were separately primed with 150 ml NaCl concentration and water for 6, 9,12, 15 and 18 hours respectively and found out the best time. After priming seeds were washed with distilled water and dried in air.

3.8.4 Preparation of Petri dish

Petri dises were washed in distilled water and sterilized by sodium hypochloride in 5% concentration. Fillter paper were set in petri dishes. Petri dishes were irrigated with water.

3.8.5 Placement of seed

30 seeds were placed in each Petri dish.

3.8.6 Counting of seed germination

Seeds germination was recorded daily up to 14th days after the start of the experiment. A seed was considered germinated when the radicle emerged by about 2 mm in lenth. (Akbari *et al*., 2007). Generally seeds were germinated after 3 days of place seed.

3.8.7 Collection of data

After 14 th days data were collected and found out the best priming time 15 hours.

Parameters measured in this experiment were:

1. **Germination (%)**

Germination measured in the seventh day using the formula $= \frac{Total\ Number\ of\ germinated\ seeds}{Total\ seed} \times 100$

2. **Germination index (GI)**

Germination index was worked out according to the equation of Kader and Jutzi (2004).

GI=$\sum TiNi$

Ti= Number of day after sowing and Ni= Number of germinated seed in the day.

3. **Seedling height:** Seedling height (mm) was measured from the root base to the tip of the longest leaf at the time of uprooting of seedlings.
4. **Plumue length (mm)**
5. **Radicle length(mm)**
6. **Dry weight**: seedling dry weight was determined after placing sseedlings, after the 7th day in oven to dry at 70°C for 48 hrs.
7. **Vigour index (VI)**: vigour index was worked out byusing the formula of Abdul Baki and Aderson (1970).

$$VI=\frac{Germination\ \ (\%)\times seedlings\ \ lengt\,h}{100}$$

3.9 3rd experiment :

3.9.1 Treatment of the Experiment

Rice variety:

i. V1: BRRI dhan28 (salt sensitive)

ii. V2: BRRI dhan47 (salt tolarance)

Treatments:

i. T_0= Seeds placed without salt (control),

ii. T_1= Seeds placed with 50 mM salt,

iii. T_2= Seeds placed with 100 mM salt,

iv. T_3= Seeds placed with 150 mM salt and

v. T_4= Seeds placed with 200 mM salt.

3.9.2 Preparation of seed

At first good seeds were collected from the seed store . Then they are sterilized by sodium hypochloride in 5% con. then seeds are dried in air temperature. Then seeds were weighted .

3.9.3 Priming technique

Seed of BRRI dhan47 primed with 150 mM NaCl concentration which was the best found in 1st experiment and water at 15 hours and dry seeds were used as control. Then seed of BRRI dhan28 primed with 150 mM NaCl concentration which was the best found the 1st experiment and water at 15 hours and dry seeds were used as control. Find out the best result and then seeds were redried.

3.9.4 Preparation of Petri dish

Petri dishes were washed in distilled water and sterilized by sodium hypochloride in 5% .Fillter paper were set in petri dishes. Hydro priming seeds were placed in Petri dishes .Petri dishes were irrigated with five different saline solution consisted of 0, 50,100, 150, 200 mM NaCl concentration .Then salt primed seeds were placed in Petri dishes .Petri dishes were irrigated with five different saline solution consisted of 0, 50,100, 150, 200 mM NaCl concentration .Then dry seeds were placed in petri dishes .Petri dishes were irrigated with five different saline solution consisted of 0, 50,100, 150, 200 mM NaCl concentration.

3.9.5 Placement of seed

30 seeds were placed in each Petri dish.

3.9.6 Counting of seed germination

Seeds germination was recorded daily up to 14 days after the start of the experiment. A seed was considered germinated when the radicle emerged by about 2 mm in lenth. Generally seeds were germinated after 3 days of place seed.

3.9.7 Collection of data

After 14th days of start of experiment data were collected and found out the best time.

Parameters measured in this experiment were:

1. **Germination (%)**

   Germination measured in the seventh day using the formula $=\frac{Total\ Number\ of\ germinated\ seeds}{Total\ seed} \times 100$

2. **Germination index (GI)**

Germination index was worked out according to the equation of Kader and Jutzi (2004).

GI=$\sum TiNi$

Ti= Number of day after sowing and Ni= Number of germinated seed in the day.

3. **Seedling height:** Seedling height (mm) was measured from the root base to the tip of the longest leaf at the time of uprooting of seedlings.

4. **Shoot length(mm)**
5. **Root length(mm)**
6. **Dry weight**: seedling dry weight was determined after placing sseedlings, after the 7th day in oven to dry at 70°C for 48 hrs.
7. **Vigour index (VI)**: vigour index was worked out byusing the formula of Abdul baki and Aderson (1970).

$$VI=\frac{Germination\ (\%)\times seedlings\ length}{100}$$

3.10 Statistical analysis

The collected data were analyzed statistically following CRD design by MSTAT-C computer package programme. The treatment means were compared by Least Significance Differences (LSD) used as and when necessary.

Chapter 4

Results and Discussion

CHAPTER 4
RESULTS AND DISCUSSION

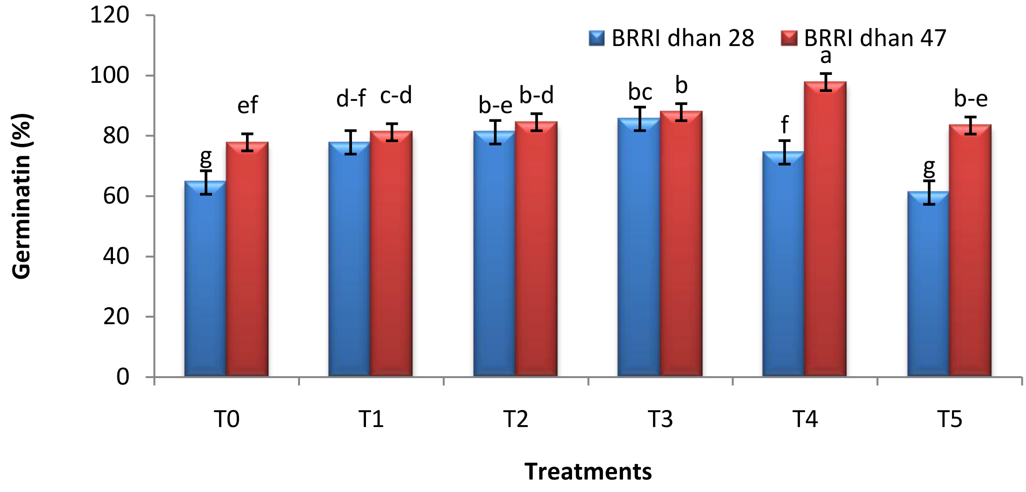
Three separate experiments were performed for the seed priming of the rice. The overall objective of the present study has been to develop a system for the seed priming of rice. The results of these experiments were presented and discussed in this chapter with Figure (1-40).

4.1 Effect of priming solution

This experiment was conducted under laboratory condition to find out the best NaCl concentration. The results are presented separately under different headings below.

4.1.1 Germination Percentage

Seed germination percentage was significantly influenced by NaCl or water concentration. The highest seed germination percentage (97.75 %) observed from BRRI dhan47 with T_4 tretment and lowest result with T_0 treatment. But variety BRRI dhan28 with T_3 gave the best result compared to non primed seeds. The lowest seed germination percentage (64.44%) was observed in BRRI dhan28 with T_0 treatment which was statistically similer with T_5 treatment. BRRI dhan47 always gave best result than BRRI dhan28 (Fig. 1). It has been reported that priming with KNO_3 or NaCl and PEG 8000 on four tomato cultivars , KNO_3 was the most effective for improving germination and seedling growth (Farooq *et al*. 2005). It has been reported that, Na-Cl priming generally requires treatment periods using solutuins 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 seed priming with PEG resulted in higher tolerance to drought stress than hydro-priming, while higher concentrations of PEG had negative effects on seed germination.

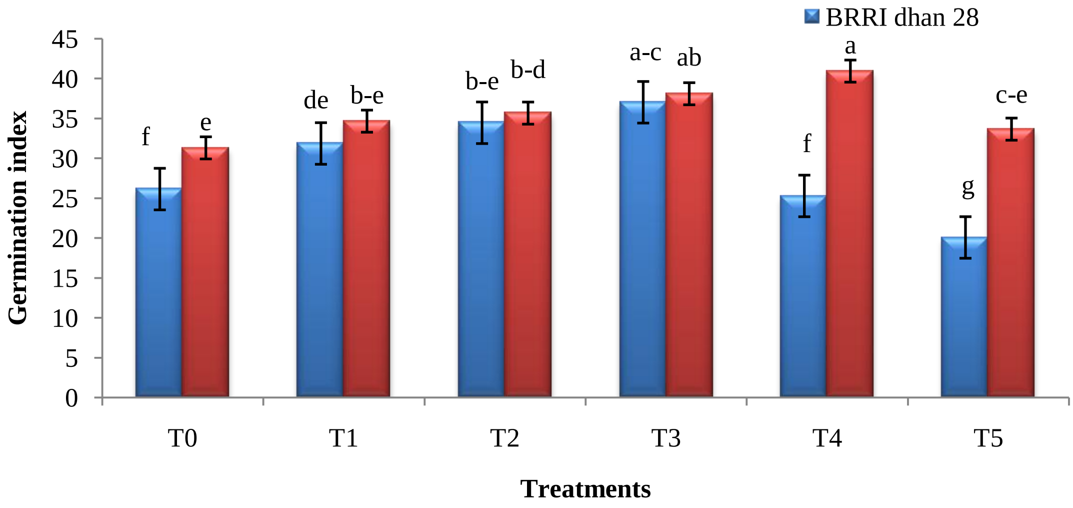


T_0= 0 mM NaCl, T_1= water, T_2= 50 mM NaCl, T_3= 100 mM NaCl, T_4=150 mM NaCl,T_5= 200 mM NaCl

Figure 1. Effect of different priming level on germination percentage of rice seed ($LSD_{0.05}$ = 6.271)

4.1.2 Germination index

Seed germination index was significantly influenced by NaCl or water concentration. The highest seed germination index (40.95) was observed from BRRI dhan47 when primed with T_4 treatment which was statistically similar with T_3 treatment in BRRI dhan47 and BRRI dhan28 and T_5 gave the lowest result. But variety BRRI dhan28 primed with T_3 treatment gave the best germination index (37.04) compared to T_0 treatment which was statistically similler with T_3 in BRRI dhan47. The lowest germination index (26.15) was observed from non primed of BRRI dhan28 with non primed seed. BRRI dhan47 always gave best result than BRRI dhan28 (Fig.2). NaCl and KCl priming increases the germination parameters (total germination, mean germination time, germination index, coefficient of velocity) and growth parameters (radicle and seedling length, fresh and dry weight and Vigour Index) of safflower, as compared with non primed seeds, under saline condition (Elouaer and Hamach, 2012).

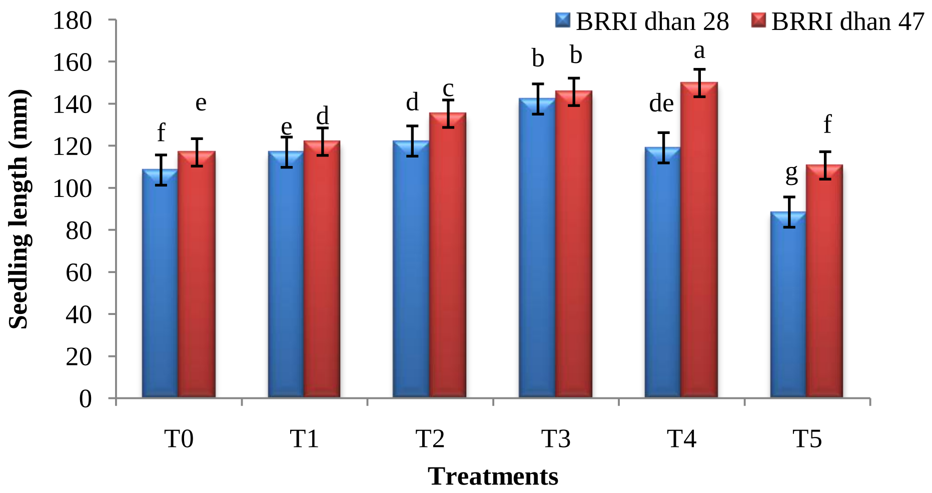


T_0= 0 mM NaCl, T_1= water, T_2= 50 mM NaCl, T_3= 100 mM NaCl, T_4=150 mM NaCl,T_5= 200 mM NaCl

Figure 2. Effect of different priming level on germination index of rice (LSD $_{0.05}$)= 3.906

4.1.3 Seedling length

NaCl or water priming significantly influenced on seedling length. The highest seedling length (149.9 mm) was observed from BRRI dhan47 when primed with T_4 and T_5 gave the lowest result in BRRI dhan47. BRRI dhan28 primed with T_3 showed highest seedling length (142.3 mm) compared to non primed seeds. The lowest seedling lenth (108.5mm) was observed in BRRI dhan28 with control the lowest seedling lenth (26.15 mm) was observed in BRRI dhan28 with non primed seed. BRRI dhan47 always gave the best result than BRRI dhan28 (Fig. 3). Priming techniques improved seedling vigour, growth and yield of tomato and chilli although varieties showed variation in response to different treatments (Maiti *et al*, 2013). Especially halopriming increased speed of emergence, seedling vigour index, root length and shoot length over hydropriming in tomato and chilli. 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 comparison with seedlings obtained from non-primed seeds under 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)



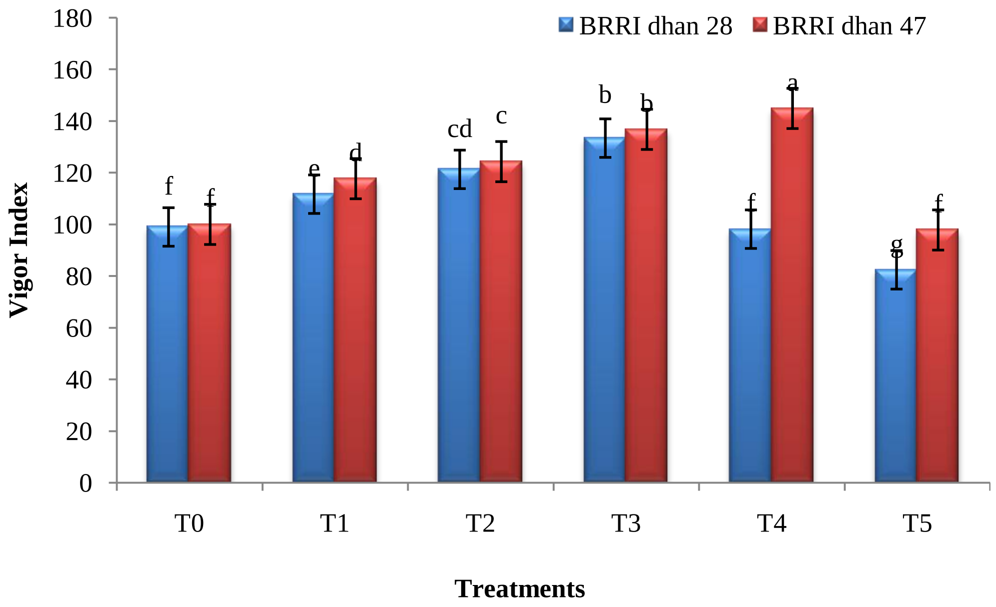
T_0= 0 mM NaCl, T_1= water, T_2= 50 mM NaCl, T_3= 100 mM NaCl, T_4=150 mM NaCl,T_5= 200 mM NaCl

Figure 3. Effect of different priming level on seedling length of rice seed (LSD $_{0.05}$= 4.049)

4.1.4 Vigour index

Seed vigour index was significantly influenced by NaCl/water concentration. The highest seed vigour index (144.90) was observed from BRRI dhan47 in T_4 treatment and T_5 gave the lowest result. But variety BRRI dhan28 with T_3 gave the best seed vigour index (133.4) compared to non primed seeds. The lowest seed vigour index (99.00) was observed in BRRI dhan28 with non primed seed. BRRI dhan47 always gave best result than BRRI dhan28 (Fig. 4).

Harris *et al*. (1999) observed that hydro-priming technique has been employed to increase germination rate and seedling vigour in several vegetable crops. On the other hand, there is a general consensus that priming decreases longevity (Taylor *et al*., 1998). Maiti *et al*. (2009) reported that priming improved germination and seedling development and yield of various vegetable species. It was observed that few priming technique improved seedling emergence, seedling vigour as well as agronomic traits including yield of the crop .It has been concluded that seed priming with NaCl showed improved germination and seedling vigor by dormancy breakdown as compared to control (Farooq *et al.*, 2005).

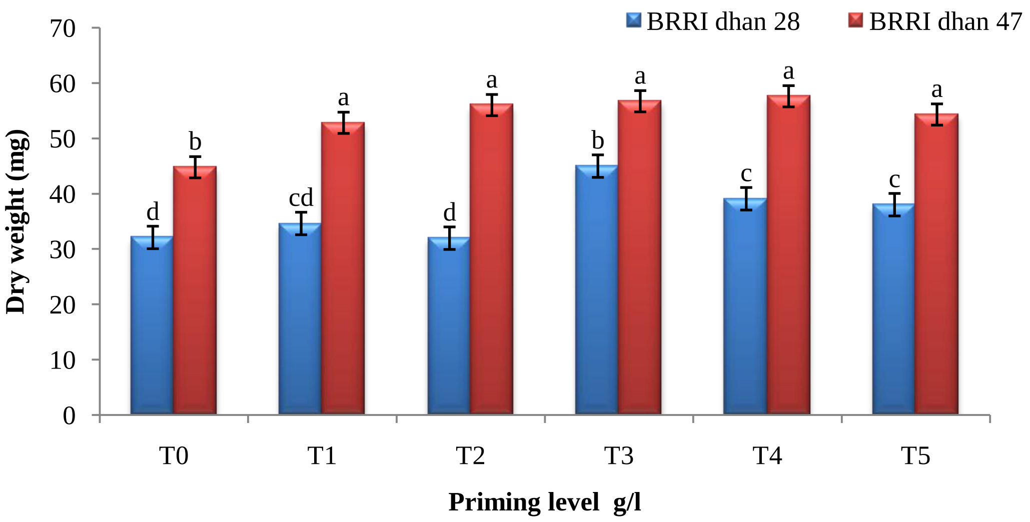


T_0= 0 mM NaCl, T_1= water, T_2= 50 mM NaCl, T_3= 100 mM NaCl, T_4=150 mM NaCl,T_5= 200 mM NaCl

Figure 4. Effect of different priming level on vigour index of rice seed (LSD $_{0.05}$= 4.420)

4.1.5 Dry weight

Dry weight of seedling was significantly influenced by priming solution. The highest dry weight (57.73mg) was observed from BRRI dhan47 with T_4 treatment which was statistically similler with T_1, T_2, T_3 and T_5 treatment. BRRI dhan28 with T_4 treatment gave the highest dry weight (45.00 mg) compared to non primed seeds. The lowest seed dry weight (32.10mg) was observed in BRRI dhan28 with non primed seed which was statistically similar with water and T_2 in BRRI dhan28. BRRI dhan47 always gave best result than BRRI dhan28 (Fig. 5). Khalil *et al*., (2010) was observed that dry weight of yield increased with each increment of priming levels and maximum dry weight of yield was obtained from seeds primed in 0.2% P_2O_5 solution. Zhang *et al*., (1993) also observed that the increase in dry weight of yield due to priming might be due to better early seedling growth and plant nutrition.



T_0= 0 mM NaCl, T_1= water, T_2= 50 mM NaCl, T_3= 100 mM NaCl, T_4=150 mM NaCl,T_5= 200 mM NaCl

Figure 5. Effect of different priming level on dry weight of rice seed (LSD $_{0.05}$= 4.093)

4.2 Optimization of the best priming time

This experiment was conducted under laboratory condition to find out the best priming time. BRRI dhan47 were separately primed with 150 mM NaCl concentration and water for 6, 9,12, 15 and 18 hours respectively. Soaking period has been reported to be one of the key factors of determining the effectiveness of priming (Giri and Schillinger 2003., Subedi and Ma 2005., Dezfuli *et al*., 2008). The results are presented separately under different headings below.

4.2.1 Germination Percentage

Seed germination percentage was significantly influenced by different priming time of primed with 150 mM NaCl . The highest seed germination percentage (85.55%) was observed from priming time 15hr. The lowest seed germination percentage (65.66%) was observed from priming time 6hr (Fig. 6).

Seed germination percentage was significantly influenced by different priming time of primed with water. The highest seed germination percentage (82.22%) was observed from priming time 15hr. The lowest seed germination percentage (58.88%) was observed from priming time 0 hr (Fig.7). Hydropriming by soaking in water for 12, 24, 36 or 48 h improved seed germination of two genotypes of maize (*Zea mays* L.) including B_{73} and MO_{17} (Dezfuli *et al*., 2008).

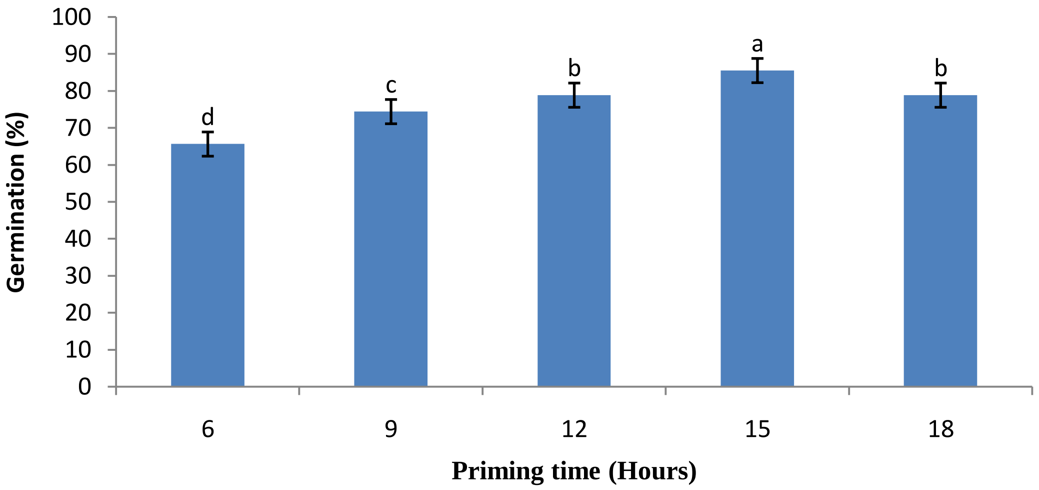


Figure 6. Effect of different priming time on germination percentage of rice seeds (primed with 150mM NaCl) (LSD $_{0.05}$= 3.84)

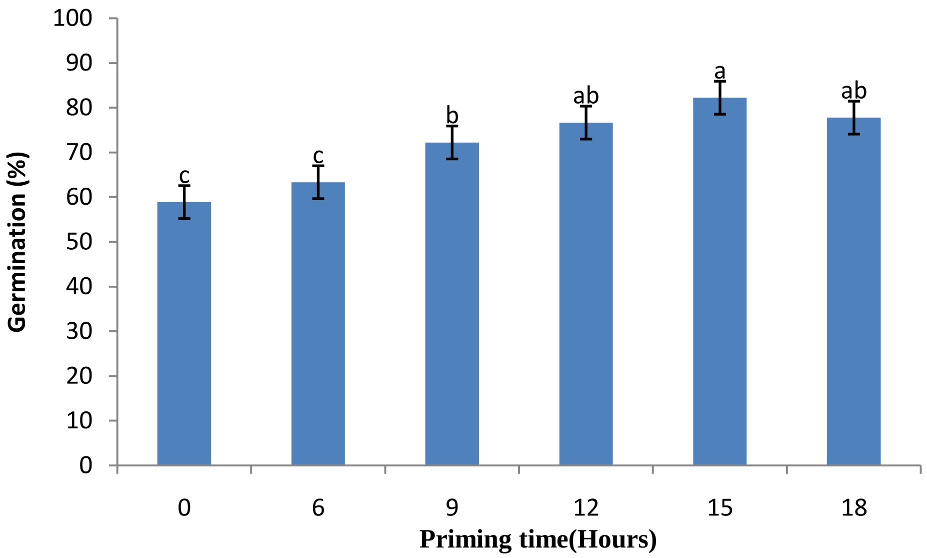


Figure 7. Effect of different priming time on germination percentage of rice seeds (primed with water) (LSD $_{0.05}$ = 5.716)

4.2.2 Germination index

Seed germination index was significantly influenced by different priming time primed with 150 mM NaCl concentration. The highest seed germination index (49.22) was observed from priming time 15hr. The lowest seed germination index (36.00) was observed from priming time 6hr (Fig. 8).

Seed germination index was significantly influenced by different priming time of seed primed with water concentration. The highest seed germination index (43.81) observed from priming time 15hr. The lowest seed germination index (30.43) was observed from priming time 0hr (Fig. 9).

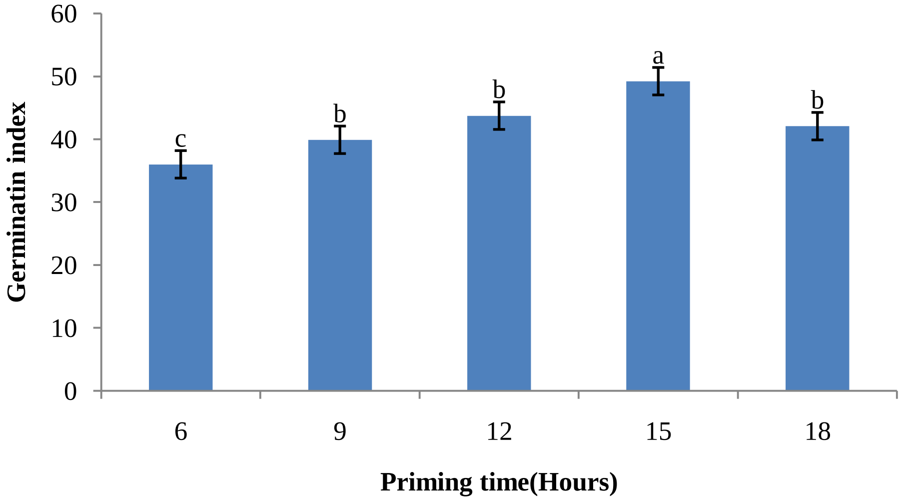


Figure 8. Effect of different priming time on germination index of rice seeds primed with 150mM NaCl (LSD $_{0.05}$ = 3.769)

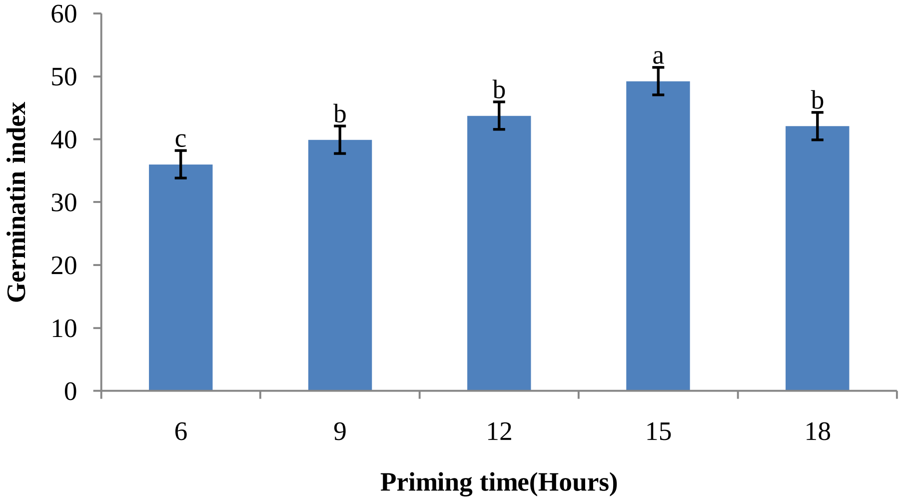


Figure 9. Effect of different priming time on germination index of rice seeds primed with water (LSD $_{0.05}$ = 4.569)

4.2.3 Seedling length

Priming time significantly influeced on the seedling length when primed with NaCl. The highest seedling length (94. 48mm) was observed from priming time 15hr which was statistically similar with 9 hr and 12 hr. The lowest seedling length (76.50 mm) was observed in priming time 6hr (Fig. 10).

Priming time was significantly effect on seedling length when primed with water. The highest seedling length (100.00 mm) was observed from priming time 15hr. The lowest seedling length (51.56 mm) was observed in priming time 0 hr (Fig. 11). It has been reported that rice cultivers Shaheen Basmati and Basmati-2000 were soaked in $CaCl_2$, KCl and H_2O_2 for 36 h , increased seedling length and dry weight in saline medium (Afzal *et al.*, 2012).

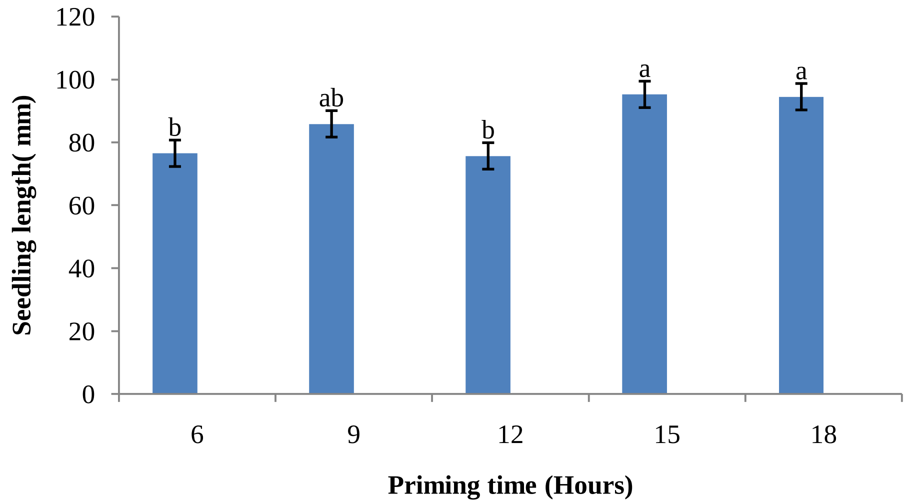


Figure 10. Effect of different priming time. on seedling length rice seeds (primed with 150 mM NaCl) (LSD $_{0.05}$= 6.763)

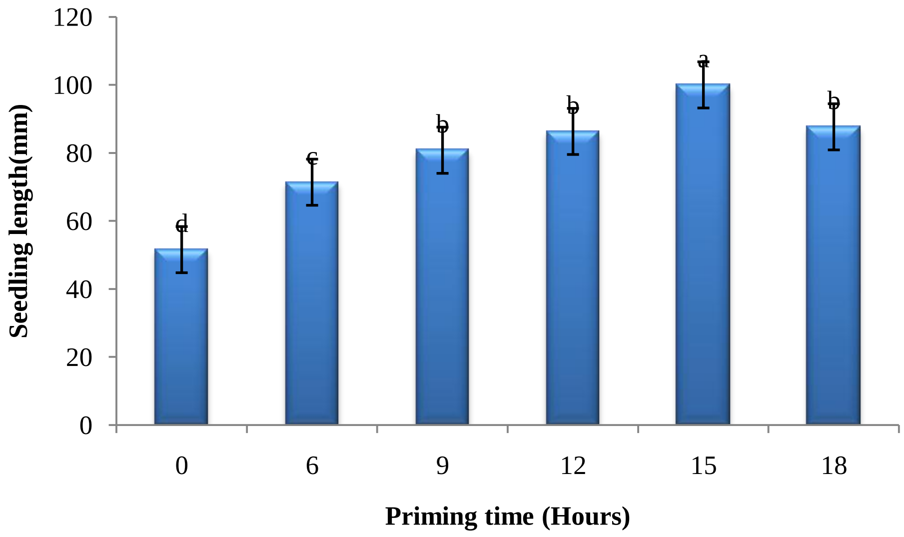


Figure 11. Effect of different priming time on seedling lenth of rice seeds (primed with water) (LSD $_{0.05}$= 5.14)

4.2.4 Vigour index

Seed vigour index was significantly influenced by priming time when seed primed with 150 mM NaCl. The highest vigour index (82.44) was observed from priming time 15hr. The lowest seed vigour index (50.61) was observed in priming time 6hr (Fig. 12).

Seed vigour index was also significantly influenced by priming time when seed primed with water concentration. The highest vigour index (69.00) observed from priming time 15hr. The lowest seed vigour index (34.98) was observed in priming time 0hr which was statistically similer with 6 hr priod of priming time (Fig. 13).

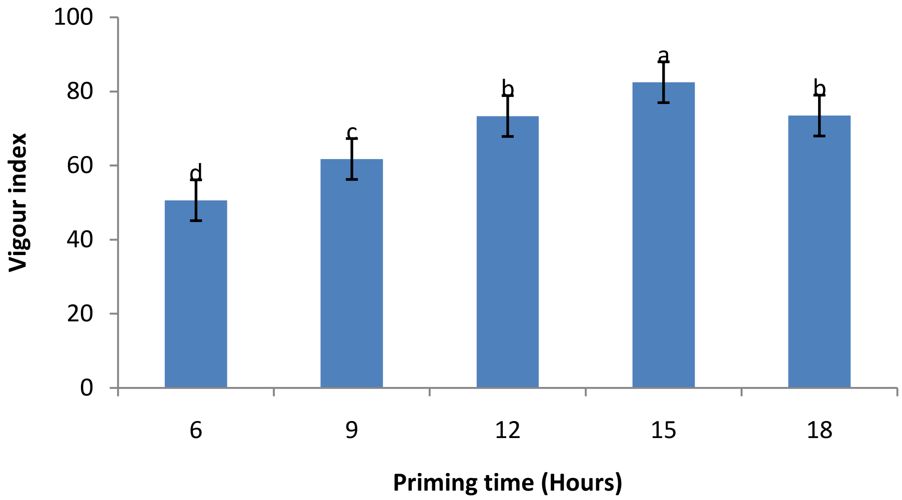


Figure 12. Effect of different priming time on vigour index of rice seeds (primed with 150mM NaCl) (LSD $_{0.05}$= 4.489)

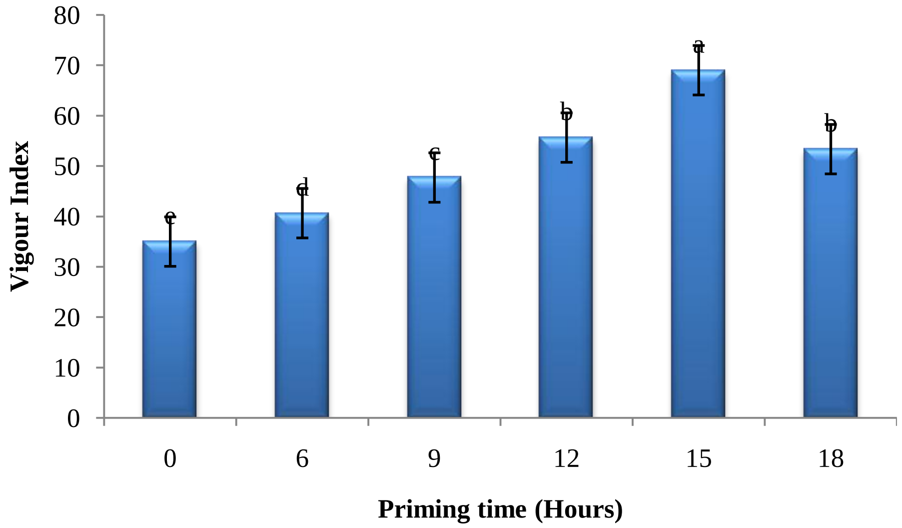


Figure 13. Effect of different priming time on vigour index of rice seeds (primed with water) (LSD $_{0.05}$= 5.315)

4.2.5 Dry weight

Dry weight of seedling was significantly influenced by priming time when seed primed with 150 ml NaCl solution. The highest dry weight (39.22 mg) was observed from priming time 15hr. The lowest seed dry weight (25.99 mg) was observed in priming time 6hr priod.(Fig. 14).

Water priming was also significantly influenced dry weight of seedling . The highest dry weight (32.73 mg) observed from priming time 15hr. The lowest seed dry weight (19.00 mg) was observed in priming time 0hr which was statistically similer with 6 hr.(Fig. 15). It has been reported that rice cultivers Shaheen Basmati and Basmati-2000 were soaked in $CaCl_2$, KCl and H_2O_2 for 36 h , increased seedling length and dry weight in saline medium (Afzal, *et al.*, 2012).

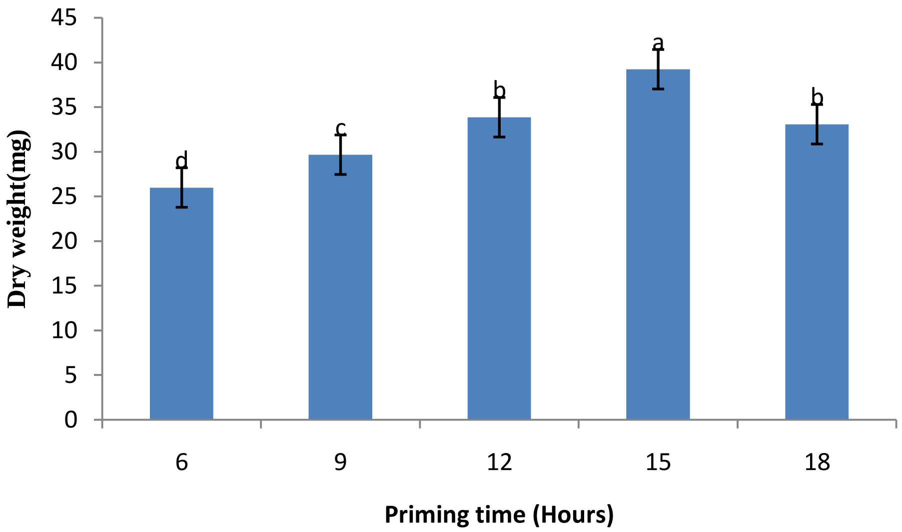


Figure 14. Effect of different priming time on dry weight of rice seeds (primed with 150 mM NaCl) (LSD $_{0.05}$= 3.072)

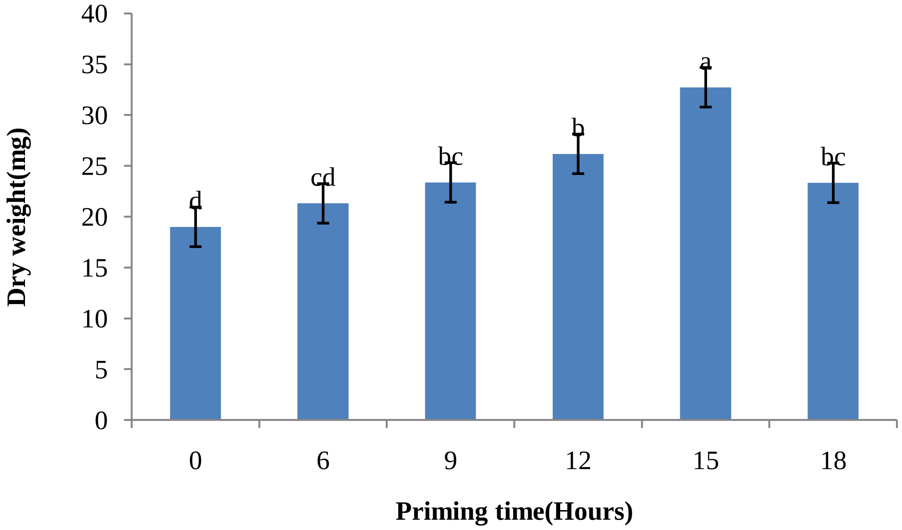


Figure 15. Effect of different priming time on vigour index of rice seeds (primed with water) (LSD $_{0.05}$= 3.004)

4.2.4 Shoot length

Priming time significantly affect on the shoot length. The highest shoot length (48.07 mm) was observed from priming time with 15hr. The lowest seedling length (32.17mm) was observed in priming time with 6hr (Fig. 16). It has been reported that the greatest shoot length was obtained with seeds primed with 10% for 24 hr (Yari *et al*., 2012) Priming time was also significantly affect on shoot length. The highest shoot length (45.03 mm) was observed from priming time 15hr. The lowest shoot length (21.45mm) was observed in priming time with 0hr (Fig. 17).

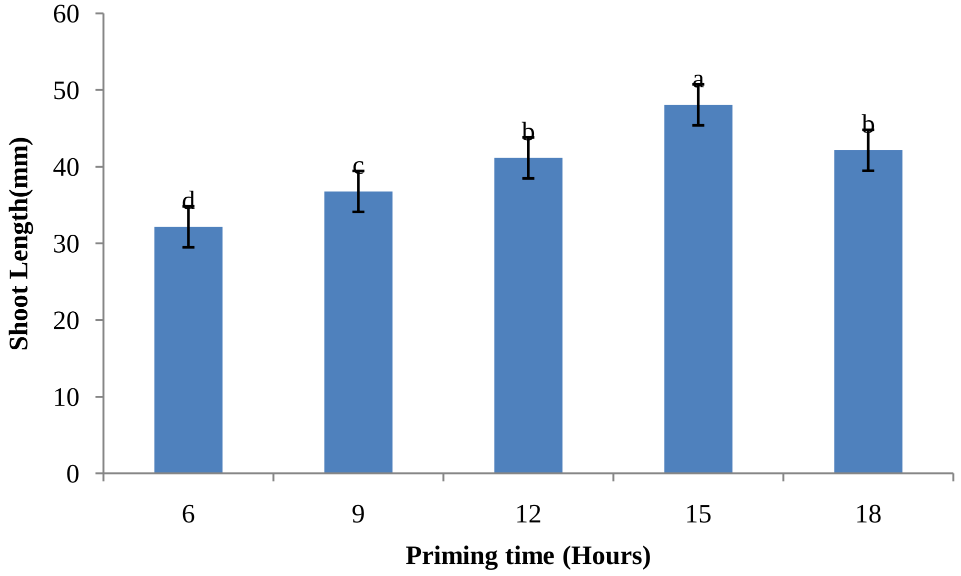


Figure 16. Efect of different priming time on shoot length of rice seeds (primed with 150 Mm NaCl) (LSD $_{0.05}$= 4.097)

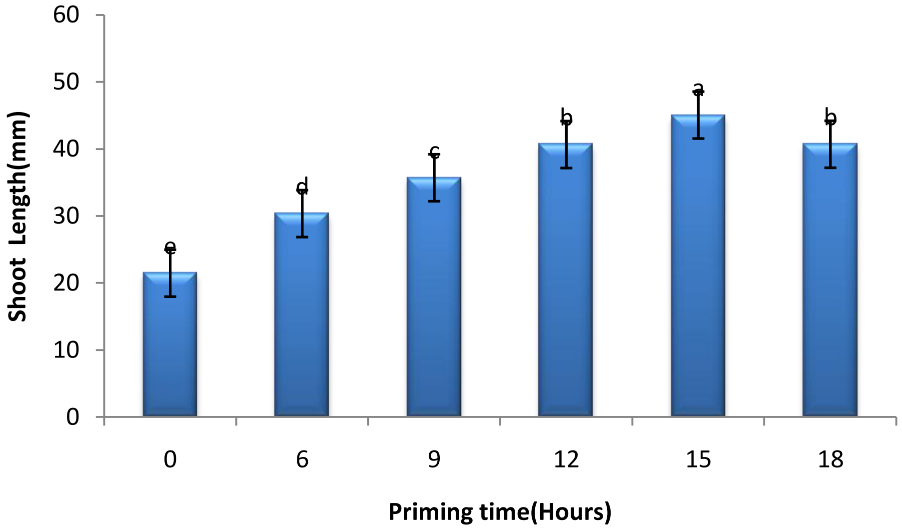


Figure17. Effect of different priming time on shoot length of rice seeds (primed with water) (LSD $_{0.05}$= 2.822)

4.2.3 Root length

Priming time of seed primed with 150 mM NaCl solution was significantly affect on the root length. The highest root length (58.81 mm) was observed from priming time with 15hr. The lowest root length (44.33 mm) was observed in priming time with 6hr (Fig. 18).

Priming time of seed primed with water significantly affect on root length. The highest root length (52.99 mm) was observed from priming time with 15hr. The lowest root length (20.10 mm) was observed in priming time with 0hr (Fig.19). Afzal *et al.* (2007a) observed the maximum shoot length (18mm) and root length (28mm) of wheat by hydropriming followed by 24 h of chilling.

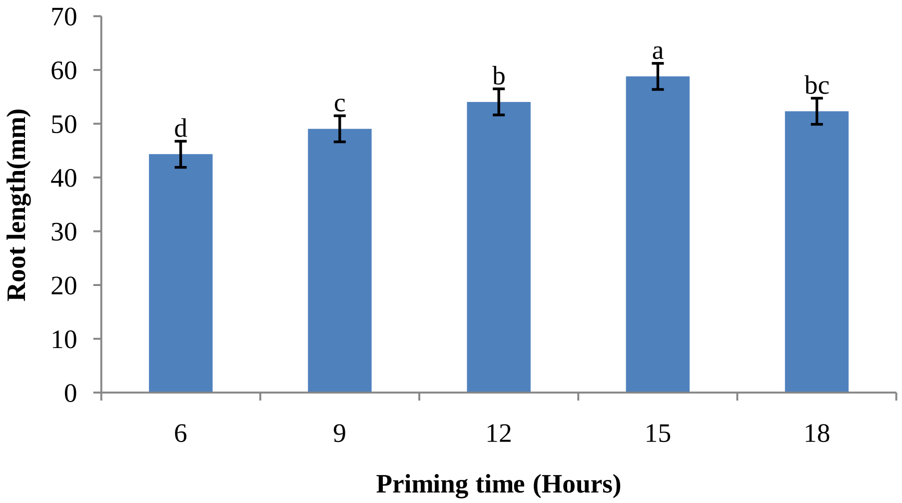


Figure 18. Effect of different priming timeon root length of rice seeds (primed with 150mM NaCl) (LSD $_{0.05}$= 3.287)

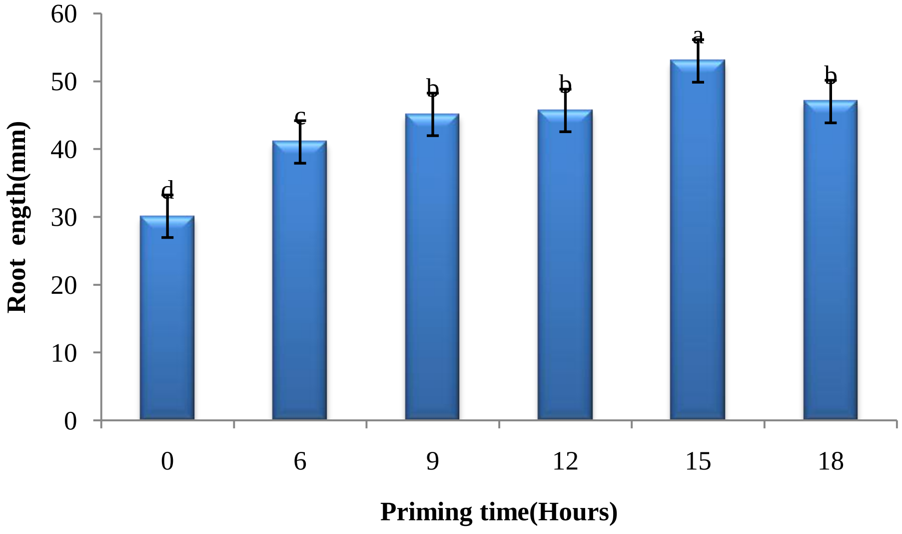


Figure 19. Effect of different priming time on root length of rice seeds (primed with water) (LSD $_{0.05}$= 4.428)

4.3 Third experiment

This experiment was conducted under laboratory condition. BRRI dhan28 and BRRI dhan47 were primed in distilled water, 100 and 150 mM NaCl solution for 15 hrs. Dry seed used as control and was exposed to 0, 50, 100, 150 and 200 mM NaCl induced salt stress conditions in Petri dishes. The results are presented separately under different headings .

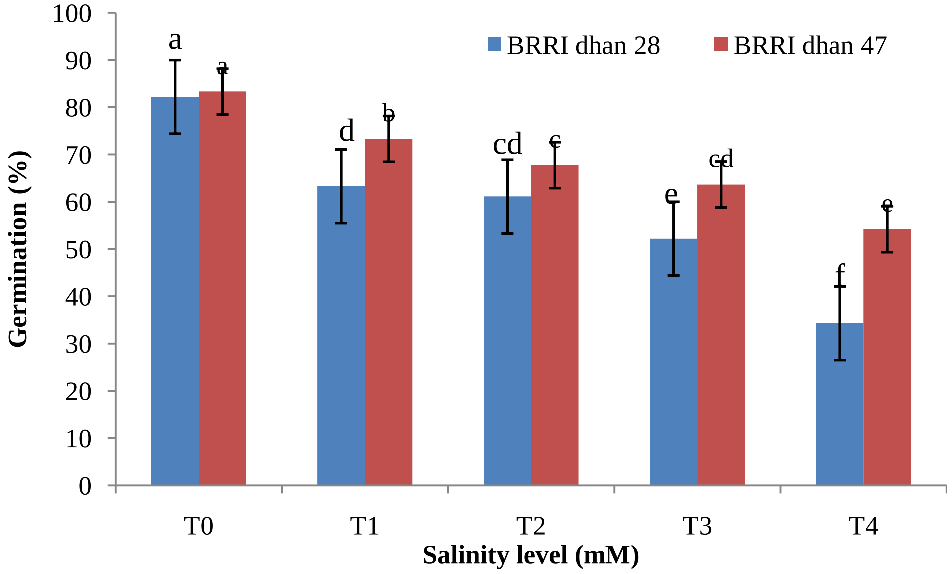
4.3.1 Germination Percentage

Seed germination percentage was significantly influenced by NaCl concentration. When seed was primed in distilled water, the highest seed germination percentage (85.55 %) observed from BRRI dhan47 in T_0 treatment. Then germination % was decreased gradually. The lowest seed germination percentage (25.33 %) was observed in BRRI dhan28 in T_5 treatment (Fig. 20).

Seed germination percentage was significantly influenced by NaCl concentration. The highest seed germination percentage (83.33 %) was observed from BRRI dhan47 in T_0 treatment. The lowest seed germination percentage (34.32%) was observed from BRRI dhan28 in T_5 treatment (Fig. 21).

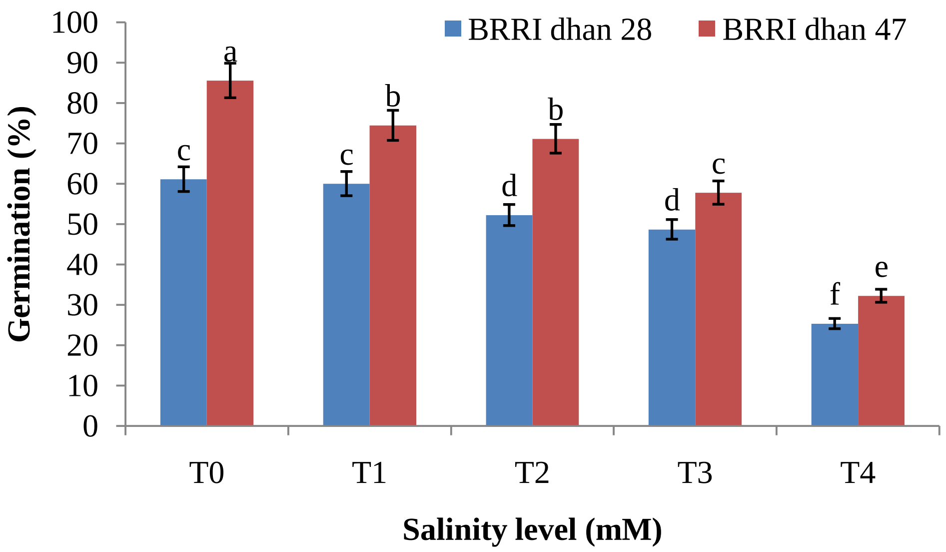
BRRI dhan47 and BRRI dhan28 seeds were used as dry seed. The highest seed germination percentage (61.11 %) observed from BRRI dhan47 in T_0 treatment. The lowest seed germination percentage (17.77 %) was observed in BRRI dhan28 in T_5 treatment (Fig. 22). NaCl primed seed gave the best result than non primed and hydro primed (water)seed.

This study demonstrated that germination recorded from primed seeds were significantly different from nonprimed seeds when exposed to different salinity levels. Similar findings were reported by Kaya *et al.*, (2006). They observed that increasing salinity causes a decrease in safflower germination; this may be due to the toxic effects on Na+ and Cl- in the process of germination (Khajeh-Hossain *et al.*, 2003)



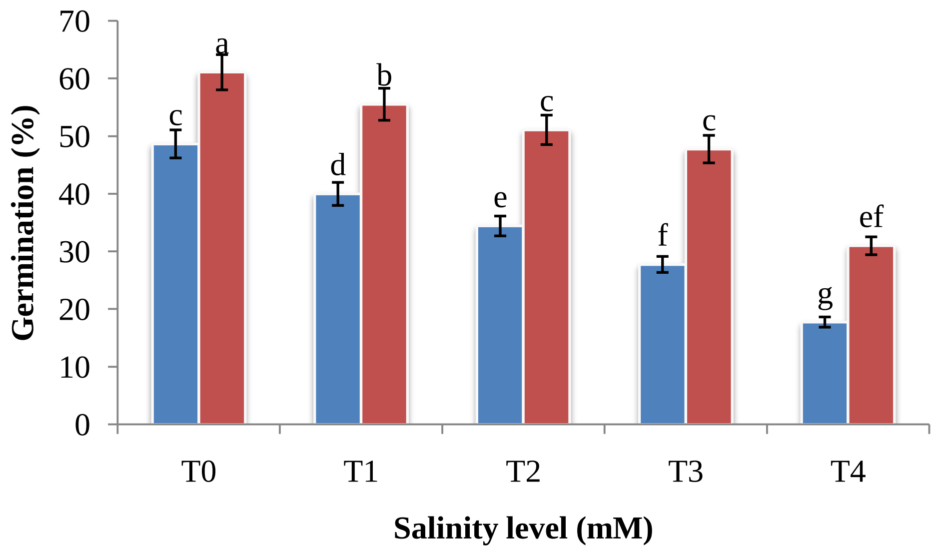
T0= Seeds placed without salt (control), T1= Seeds placed with 50 mM salt, T2= Seeds placed with 100 mM salt, T3= Seeds placed with 150 mM salt , T4 =Seeds placed with 200 mM salt

Figure 20. Effect of different salinity levels on germination percentage of rice seed (seeds primed with water) (LSD $_{0.05}$= 4.822)



T0= Seeds placed without salt (control), T1= Seeds placed with 50 mM salt, T2= Seeds placed with 100 mM salt, T3= Seeds placed with 150 mM salt , T4 =Seeds placed with 200 mM salt

Figure 21. Effect of different salinity levels on germination percentage of rice seed (seeds primed with NaCl) (LSD $_{0.05}$= 4.50)



T0= Seeds placed without salt (control), T1= Seeds placed with 50 mM salt, T2= Seeds placed with 100 mM salt, T3= Seeds placed with 150 mM salt , T4 =Seeds placed with 200 mM salt

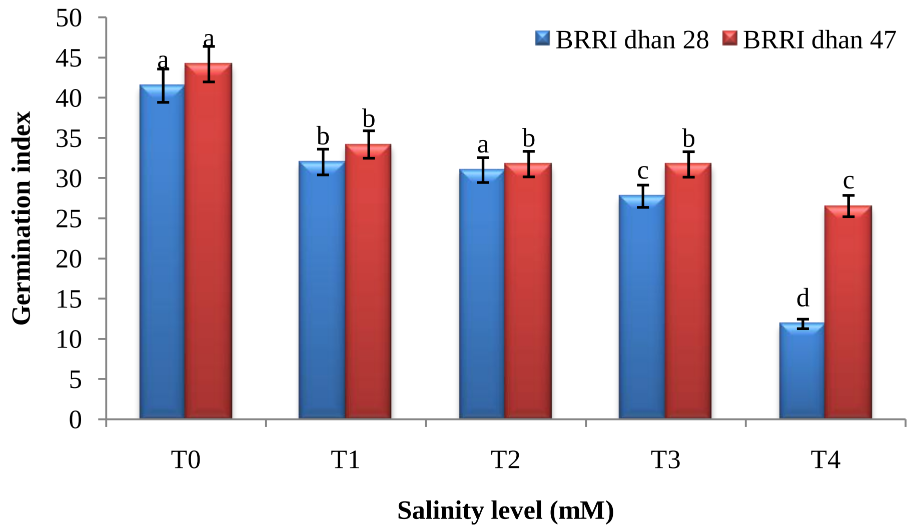
Figure 22. Effect of different salinity levels on germination percentage of rice seed (non pried seed) (LSD $_{0.05}$= 4.048)

4.3.2 Germination index

Seed germination index significantly influenced by NaCl concentration. The highest seed germination index (44.18) observed from BRRI dhan47 in T_0 treatment. The lowest seed germination index (11.18) was observed in BRRI dhan28 in T_5 treatment (Fig. 23).

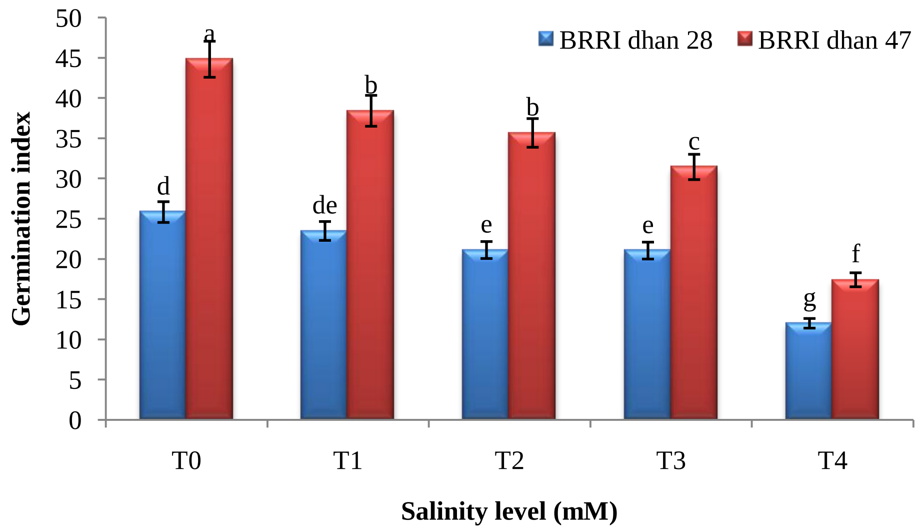
Seed germination index was also significantly influenced by NaCl concentration. The highest seed germination index (44.83) was observed from BRRI dhan47 in T_0 treatment. The lowest seed germination index (12) was observed from BRRI dhan28 in T_5 treatment (Fig. 24).
Incase of non primed seed, seed germination index was significantly influenced by NaCl . The highest seed germination index (31.48) was observed from BRRI dhan47 in T_0 treatment. The lowest seed germination index (7.9) was observed from BRRI dhan28 in T_5 treatment (Fig. 25).

Ruan *et. al*. (2002a) demonstrated that priming the rice seed with KCl and $CaCl_2$ had improved germination index. Greater efficiency of seed priming with KCl is possibly related to the osmotic advantage that K has in improving cell water saturation and that they act as co-factor in the activities of numerous enzymes Taiz and Zeiger., 2000)



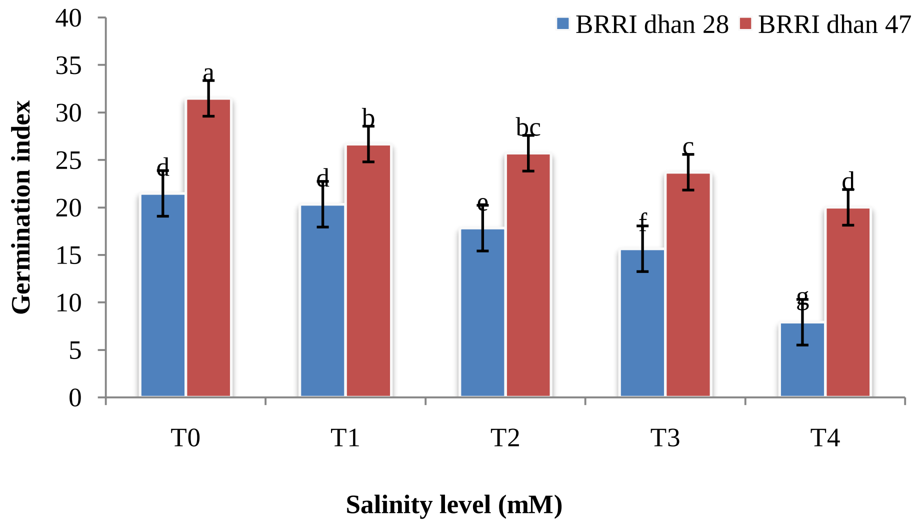
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 23. Effect of different salinity levels on germination index (primed with water) (LSD $_{0.05}$= 3.22)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 24. Effect of different salinity levels on germination index (primed with water) (LSD $_{0.05}$= 2.97)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 25. Effect of different salinity level on germination index of rice seeds (nonprime seeds) (LSD $_{0.05}$= 2.099)

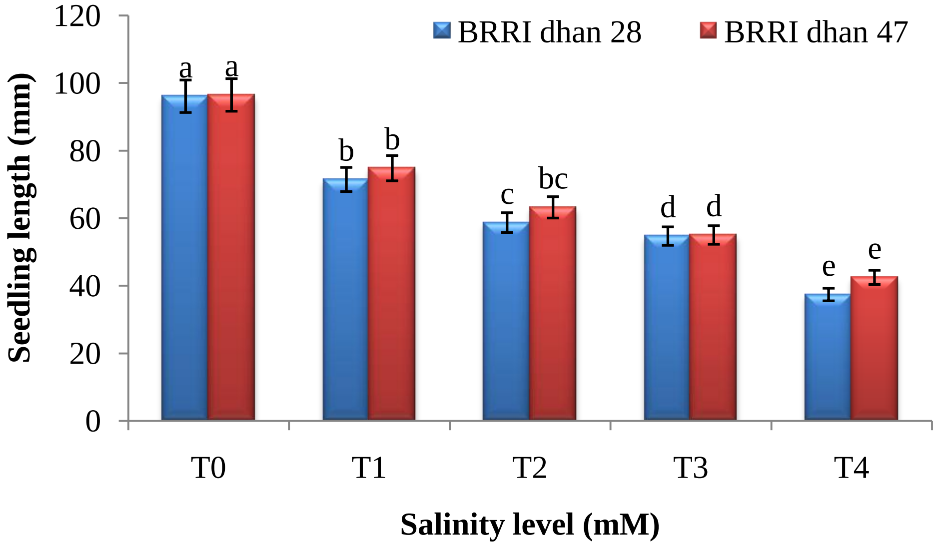
4.3.3 Seedling length

Seedling length was significantly influenced by NaCl concentration. In case of hydroprimed seed the highest seedling length (96.47mm) was observed from BRRI dhan47 in T_0 treatment. The lowest seed germination percentage (37.37) was observed from BRRI dhan28 in T_5 treatment (Fig. 26).

In case of NaCl primed seed, seedling length significantly influenced by NaCl concentration. The highest seedling length (123.9mm) was observed from BRRI dhan47 in T_0 treatment. The lowest Seedling length (27.63mm) was observed from BRRI dhan28 in T_0 treatment (Fig. 27).

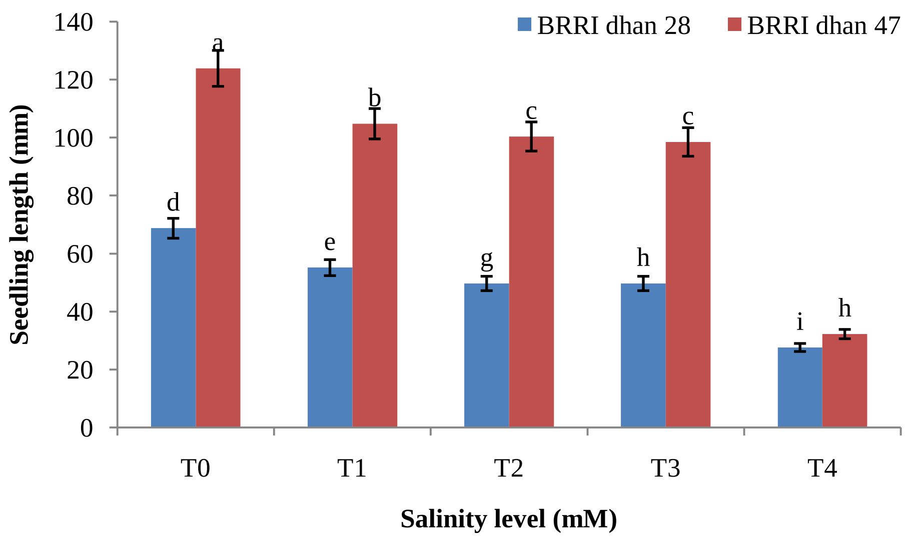
Seedling length was also significantly influenced by NaCl concentration. In case of non primed seed, the highest Seedling length (59.53mm) was observed from BRRI dhan47 in T_0 treatment. The lowest Seedling length (32.28mm) was observed from BRRI dhan28 in T_5 treatment (Fig. 28).

In the present study salt stress causes a significant reduction in seedling lenth. Reduction in seedling growth as a result of salt stress has been reported in several others species. Because salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita., 2000)



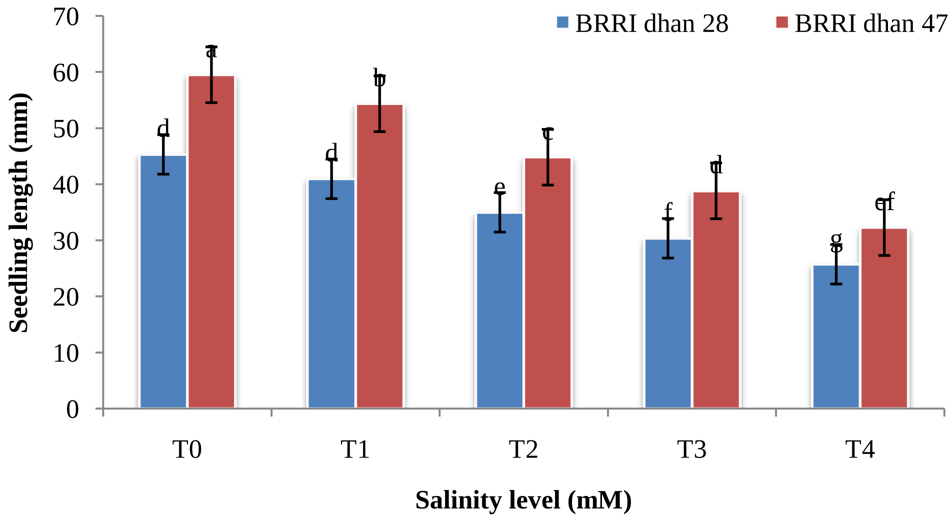
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 26. Effect of different salinity levels on seedling length of rice seeds (primed with water) (LSD $_{0.05}$= 5.207)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 27. Effect of different priming levels on seedling length of rice seed (primed with NaCl) (LSD $_{0.05}$= 4.191)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 28. Effect of different salinity levels on seedling length (non primed seed) (LSD $_{0.05}$= 3.28)

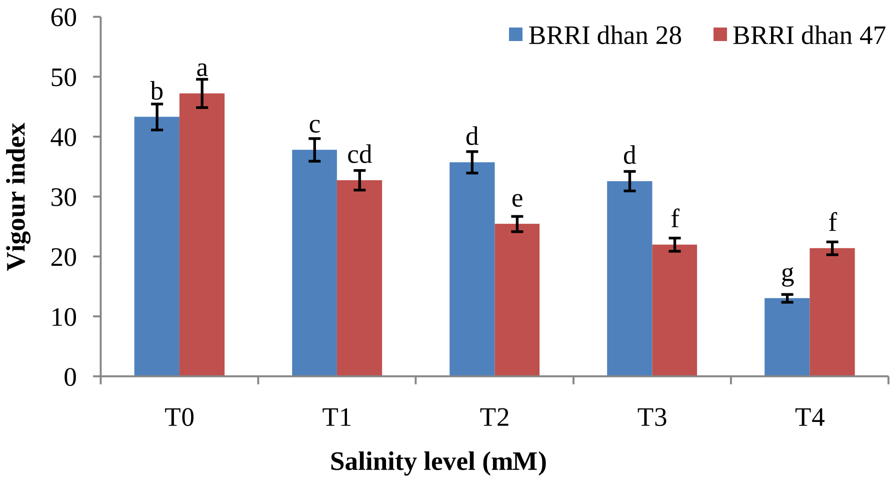
4.3.4 Vigour index

Vigour index was significantly influenced by NaCl concentration. In case of hydro primed seed . the highest seed vigour index (47.23) was observed from BRRI dhan47 in T_0 treatment. The lowest Vigour index (13.04) was observed from BRRI dhan28 in T_5 treatment (Fig.29).

Seed germination percentage was significantly influenced by NaCl. The highest vigour index (51.00) was observed from BRRI dhan47 in T_0 treatment. The lowest vigour index (11.74) was observed from BRRI dhan28 in T_5 treatment (Fig. 30).

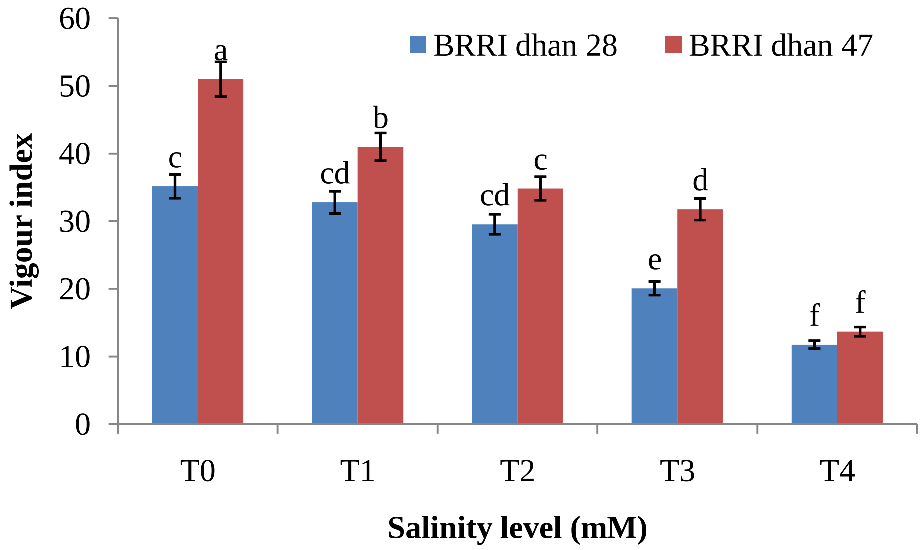
Vigour index was significantly influenced by NaCl concentration. In case of non primed seed, the highest Vigour index (33.33) was observed from BRRI dhan47 in T_0 treatment. The lowest Vigour index (13.01) was observed from BRRI dhan28 in T_5 treatment (Fig. 31).

Seed priming improved vigour index under saline conditions. Ruan *et al.* (2002b) reported that primed rice seeds showed higher vigour index than non-primed rice. Halopriming was found to be more efficient than hydropriming in enhancing the seedling vigor, overall growth, and stress tolerance potential of rice varieties.



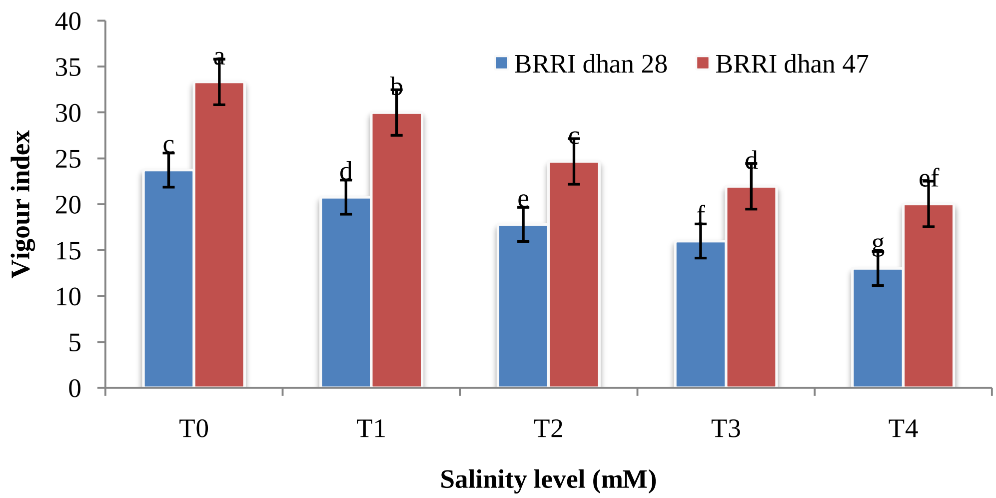
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 29. Effect of different salinity levels on vigour index (primed with water) (LSD $_{0.05}$= 3.59)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 30. Effect of different salinity levels on vigour index (primed with NaCl) (LSD $_{0.05}$= 4.177)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 31. Effect of different salinity levels on vigour index (nonprimed seeds) (LSD $_{0.05}$= 2.27)

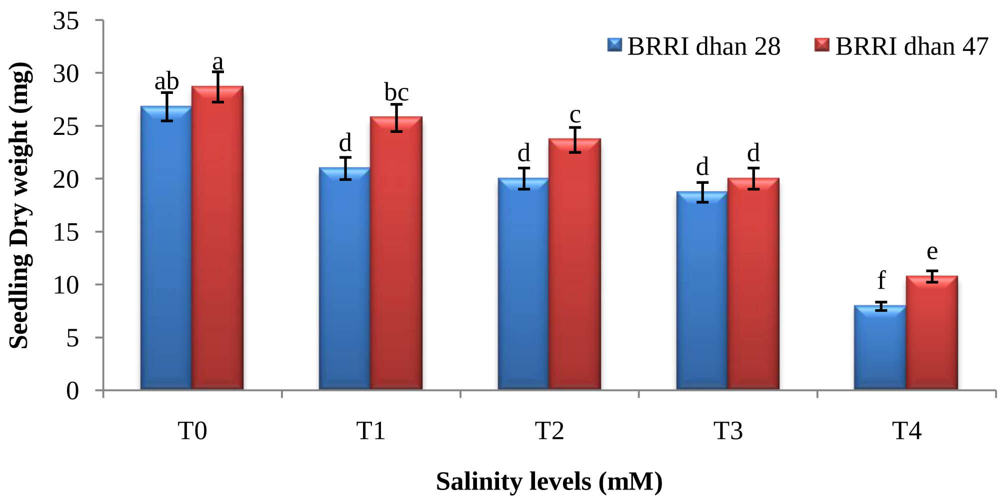
4.3.5 Dry weight of seedling

Dry weight of seedling was significantly influenced by NaCl concentration. In case of hydroprimed seed, the highest dry weight of seedling (28.67mg) was observed from BRRI dhan47. in T_0 treatment . The lowest dry weight of seedling (7.923mg) was observed in BRRI dhan28 from T_5 treatment (Fig. 32).

Dry weight of seedling was significantly influenced by NaCl concentration. The highest dry weight of seedling (29.37mg) was observed from BRRI dhan47 in T_0 treatment. The lowest dry weight of seedling (6.627mg) was observed from BRRI dha 28 in T_5 treatment (Fig. 33).

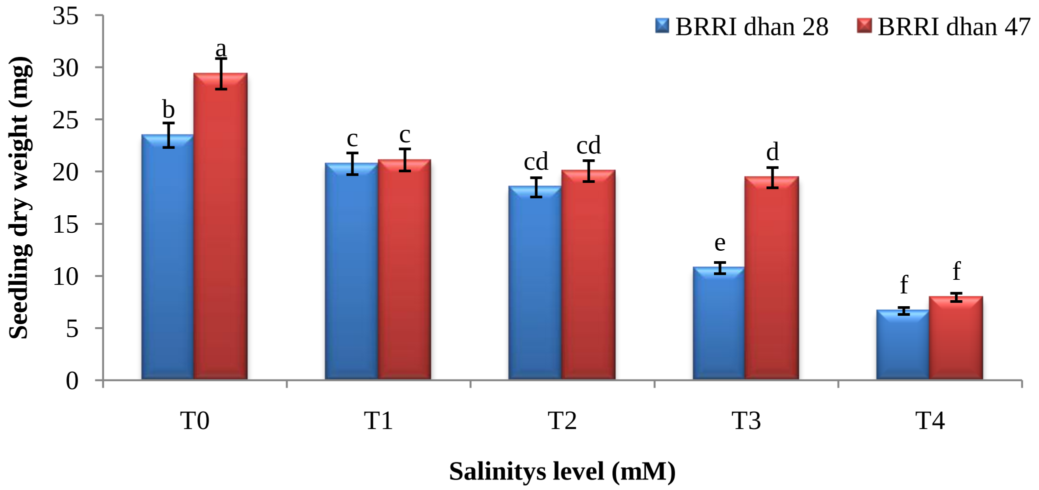
In case of non primed seed, the highest dry weight of seedling (20.37 mg) observed from BRRI dhan47 in T_0 treatment. The lowest dry weight of seedling (6.443mg) was observed in BRRI dhan28 in T_5 treatment (Fig. 34).

In this study, seedling dry weights decreased with increasing salinity. The same results were also obtained by other researchers (Monsour *et al.*, 2005). Increased seedling fresh and dry weight in primed seeds over the nonprimed seeds were also observed by Sivritepe *et al*. (2003).



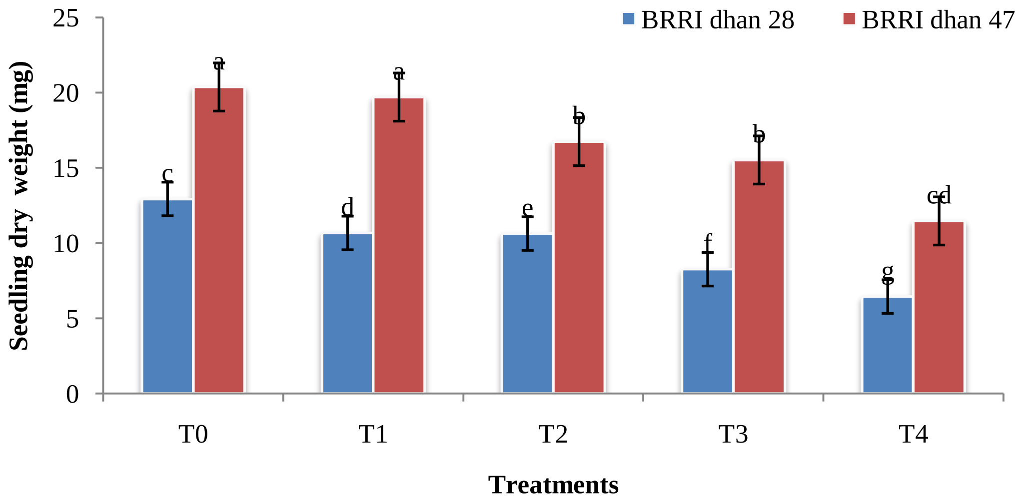
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 32. Effect of different salinity levels on seedling dry weight (primed with water) (LSD $_{0.05}$= 2.613)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 33. Effect of different salinity level on seedling dry weight (primed with NaCl) (LSD $_{0.05}$= 1.67)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 34. Effect of different salinity levels on seedling dry weight(non primed seeds) (LSD $_{0.05}$= 1.741)

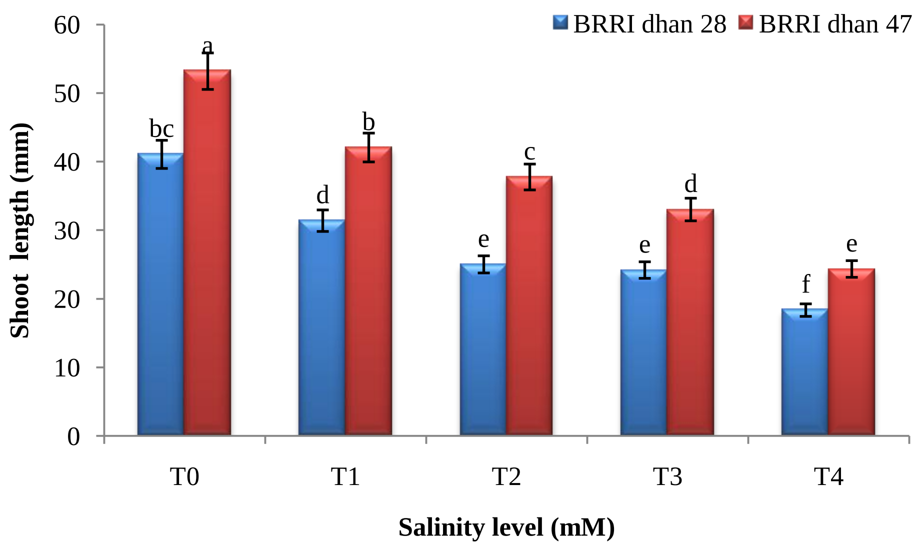
4.3.6 Shoot length

Shoot length was significantly influenced by NaCl concentration. In water primed seed, the highest shoot length (53.17mm) observed from BRRI dhan47 in T_0 treatment. The lowest lengt shoot (18.33mm) was observed in BRRI dhan28 in T_5 treatment (Fig. 35).

Shoot length was significantly influenced by NaCl concentration. The highest shoot length (56.00mm) was observed from BRRI dhan47 in T_0 treatment . The lowest shoot length (10.63mm) was observed in BRRI dhan28 with in T_0 treatment (Fig. 36).

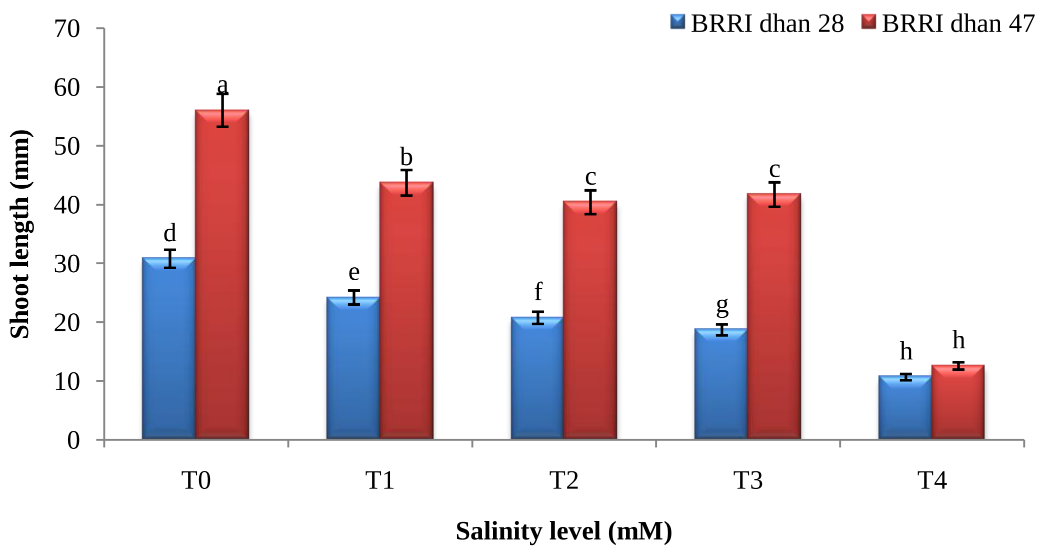
Shoot length was significantly influenced by NaCl concentration. In case of non primed seed, the highest shoot length (25.17mm) was observed from BRRI dhan47 in T_0 treatment. The lowest shoot length (11.07mm) was observed in BRRI dhan28 in T_5 treatment (Fig. 37).

In the present study, salt stress causes a significant reduction in shoot length. Our results confirm the findings of Stofella *et al.* (1992), who reported that priming of pepper seeds significantly improved shoot length.



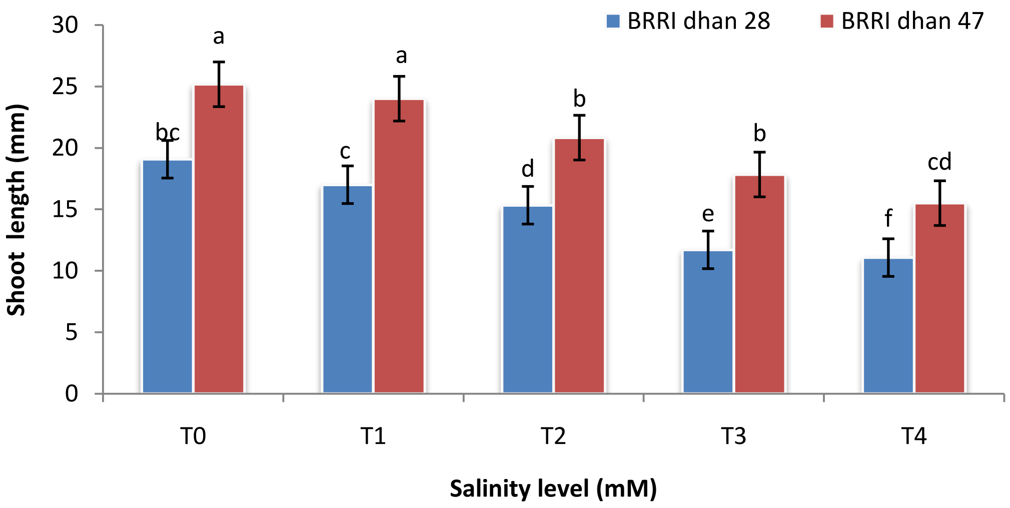
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 35. Effect of different salinity levels on shoot length (primed with water)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 36. Effect of different salinity levels on shoot length (primed with NaCl) (LSD $_{0.05}$= 1.99)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 37. Effect of different salinity levels on shoot length(nonprimed seeds) (LSD $_{0.05}$= 1.83)

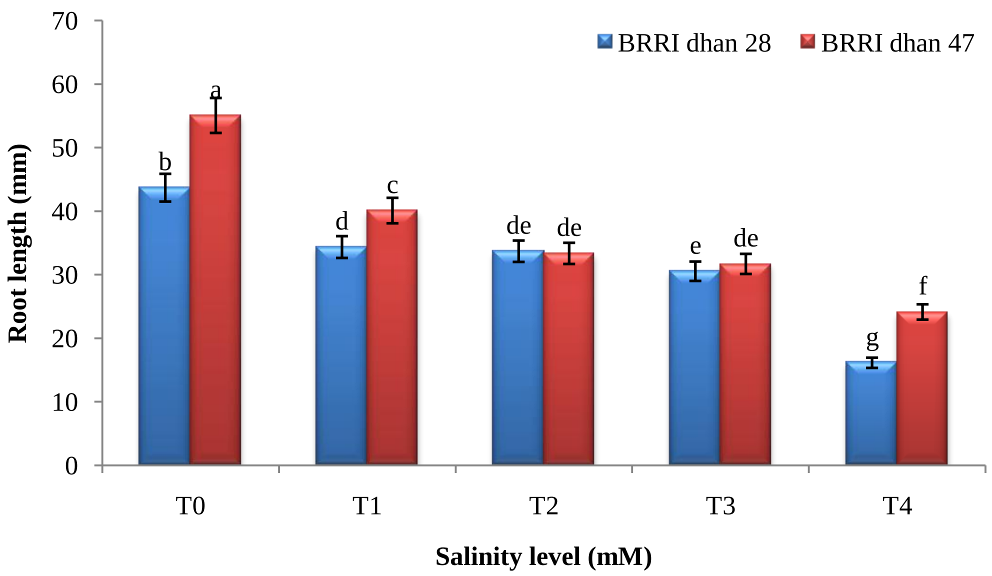
4.3.6 Root length

Root length was significantly influenced by NaCl concentration. In case of hydro primed seed, the highest root length (55.04mm) was observed from BRRI dhan47 in T_0 treatment . The lowest **Root** length (16.11mm) was observed from BRRI dhan28 in T_5 treatment (Fig. 38).

Root length was significantly influenced by NaCl concentration. The highest root length (67.9mm) was observed from BRRI dhan47 with in T_0 treatment. The lowest root length (17mm) was observed in BRRI dhan28 in T_5 treatment (Fig. 39).

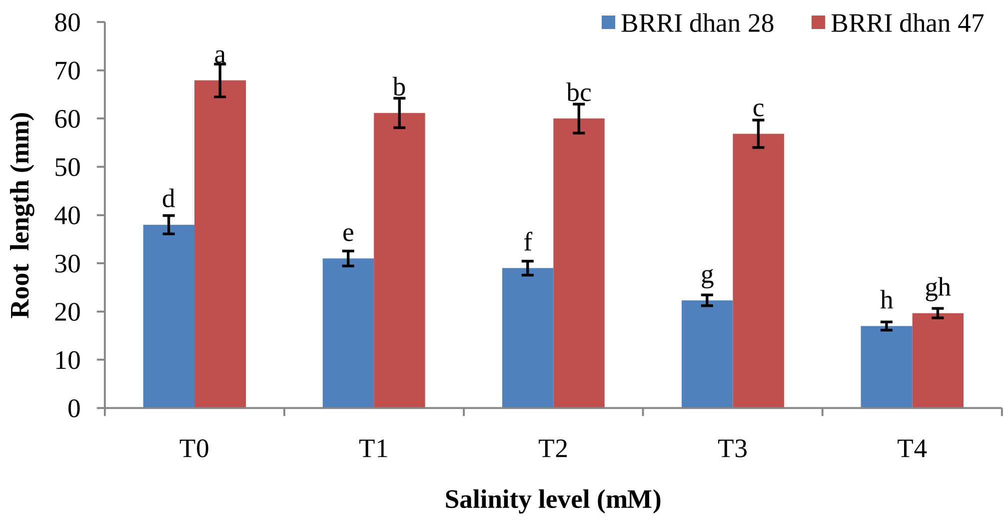
In non primed seed, the highest root length (34.22mm) observed from BRRI dhan47 in T_0 treatment. The lowest root length (13.33mm) was observed from BRRI dhan28 in T_5 treatment (Fig. 40).

In the present study, salt stress causes a significant reduction in root length. Our results confirm the findings of Stofella *et al*. (1992), who reported that priming of pepper seeds significantly improved root length.



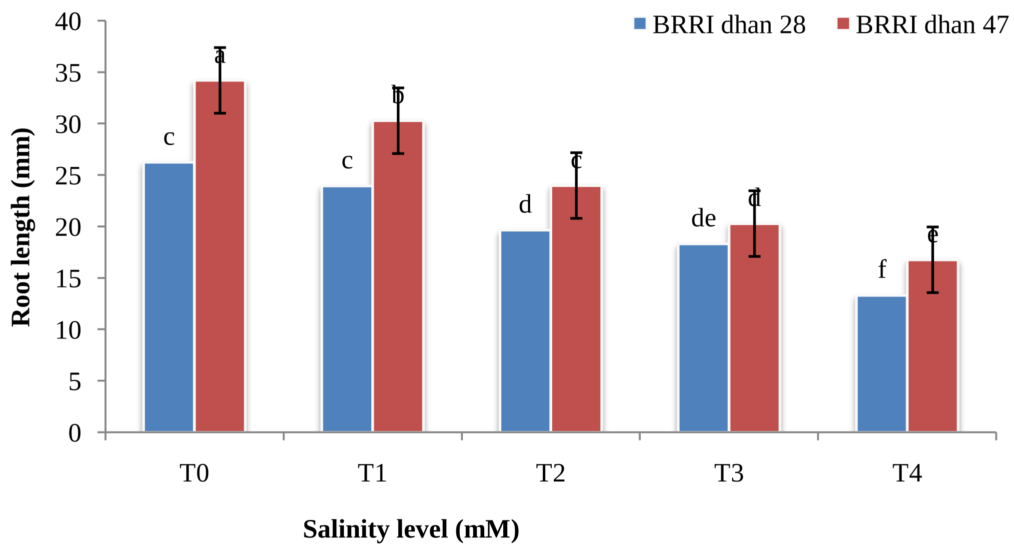
T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 38. Effect of different salinity levels on root length (primed with water) (LSD $_{0.05}$= 3.195)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 39. Effect of different salinity levels on root length (primed with NaCl) (LSD $_{0.05}$= 3.19)



T_0= Seeds placed without salt (control), T_1= Seeds placed with 50 mM salt, T_2= Seeds placed with 100 mM salt, T_3= Seeds placed with 150 mM salt , T_4 =Seeds placed with 200 mM salt

Figure 40. Effect of different salinity levels on root length(non primed seeds) (LSD $_{0.05}$= 2.33)

Chapter 5

Summary and conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

An experiment was conducted at the Agronomy lab of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from December 2012 to July 2013 to improvement of germination and seedling growth of rice through seed priming under salt stress. Two sets of treatments included in the experiment were two varieties viz. BRRI dhan28 , BRRI dhan47 and six Priming treatment (NaCL concentration) viz. 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl. The experiment was laid in a completely randomize design with five replications. This experiment was completed in three steps. In the 1st step the best Priming concentration, 2nd step the best Priming time and 3rd step best result was explored.

The results of the experiment revealed that all characters of rice seed were significant due to seed primig under salinity. The highest seed germination percentage (97.75 %) , highest seed germination index (40.95) , highest seedling length (149.9 mm) ,highest seed vigour index (144.90) and highest dry weight (57.73 mg) observed from BRRI dhan47 with 150 mM NaCl concentration and the highest seed germination percentage (85%) , highest seed germination index (37) , highest seedling length (139.4 mm) , highest seed vigour index (135) highest dry weight (57.73 mg) observed from BRRI dhan28 with 100 mM NaCl concentration.

In second step, Seed germination percentage was significantly influenced by different priming time of primed with 150 mM NaCl concentration. The highest seed germination percentage (85.55%) , highest seed germination index (49.22), highest seedling length (94. 48mm) , highest vigour index (82.44) , highest dry weight (39.22 mg) , the highest shoot length (48.07 mm) and the highest seedling length (58.81 mm) observed from priming time 15 hr when seed primed with 150 mM NaCl concentration. Seed germination percentage was significantly influenced by different priming time of primed with water. The highest seed germination percentage (82.22%), highest seed germination index (43.81), highest seedling length (100.00 mm), highest dry weight (32.73

mg), highest shoot length (45.03 mm) and highest root length (52.99mm) observed from priming time 15 hr when seed primed with distilled water.

All the characters were significantly influenced by NaCl concentration. Seed was primed in distilled water. The highest seed germination percentage (85.55 %), seed germination index (44.18), seedling length (96.47mm), vigour index (47.23), dry weight of seedling (28.67 mg), shoot length (53.17 mm) and root length (55.04 mm) were observed from BRRI dhan47 with 0 mM NaCl concentration .

All the characters was significantly influenced by salinity. When seed primed with water the highest seed germination percentage (83.33 %), seed germination index (44.83), seedling length (123.9mm), vigour index (51.00), dry weight of seedling (29.37mg), shoot length (56.00mm) and root length (67.9mm) were observed from BRRI dhan47 with 0 mM NaCl concentration.

All the characters were significantly influenced by salinity. The highest seed germination percentage (61.11 %), seed germination index (31.48), seedling length (59.53mm), vigour index (33.33), dry weight of seedling (6.443mg) and root length (34.22mm) were observed from BRRI dhan47 with 0 mM NaCl concentration.

The results showed that priming improves germination and growth of rice. In this present study, rice seed showed clear response to priming treatments. Reduction in germination parameters and seedling growth was more pronounced in control seeds than primed seeds. Seed priming with NaCl increase germination of rice seeds. Thus priming may be an effective method to meet the demands of framers 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. Pictorial view of the effect of different priming agent on the seedling of BRRI dhan28

Plate 1. Seedling from non primed Seeds

Plate 2. Seedling from seeds primed With Water

Plate 3. Seedling from seeds primed with 50 mM NaCl concentration

Plate 4. Seedling from seeds primed with 100mM NaCl concentration

Plate 5. Seedling from seeds primed with 150 mM NaCl concentration

Plate 6. Seedling from seeds primed with 200 mM NaCl concentration

Appendix II. Pictorial view of the effect of different priming agent on the seedling of BRRI dhan47

Plate 1. Seedling from non primed

Plate 2. Seedling seed primed with 50mM NaCl concentration

Plate 3. Seedling from seeds primed with 100mM NaCl concentration

Plate 4. Seedling from seeds primed with 150mM NaCl concentration

Plate 5. Seedling from seeds primed with 200mM NaCl concentration

Plate 6. Seedling from seeds primed with water

Appendix III. Pictorial view of the effect of different priming time on the seedling of BRRI dhan47

Plate 1. Seedling from seeds primed with 150mM NaCl concentration for 6 hrs

Plate 2. Seedling from seeds primed with 150mM NaCl concentration for 9 hrs

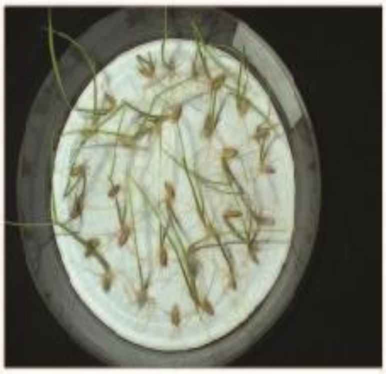

Plate 3. Seedling from seeds primed with 150 mM NaCl concentration for 12 hrs

Plate 4. Seedling from seeds primed with 150mM NaCl concentration for 15 hrs

Plate 5. Seedling from seeds primed with 150mM NaCl concentration for 18 hrs

Appendix IV. Pictorial view of the effect of different priming time on the seedling of BRRI dhan47

Plate 1. Seedling from non primed seeds with water for 0 hrs

Plate 2. Seedling from seeds primed with water for 6 hrs

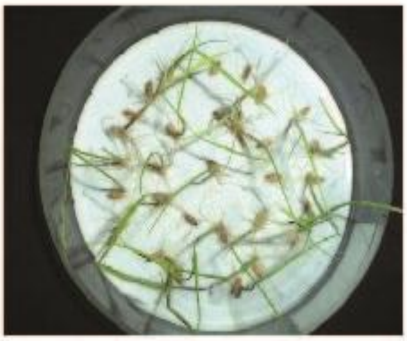

Plate 3. Seedling from seeds primed with water for 9 hrs

Plate 4. Seedling from seeds primed with water for 12 hrs

Plate 5. Seedling from seeds primed with water for 15 hrs

Plate 6. Seedling from seeds primed with water for 18 hr

Appendix V. Pictorial view of the effect of different salinity level on the seedling of BRRI dhan28

Plate 1. Seedling from seeds primed with 100 mM NaCl concentration when salinity level was control

Plate 2. Seedling from non primed seeds when salinity level was control

Plate 3. Seedling from seeds primed with water when salinity level was control

Appendix VI. Pictorial view of the effect of different salinity level on the seedling of BRRI dhan47

Plate 1. Seedling from non primed seeds when salinity level was control

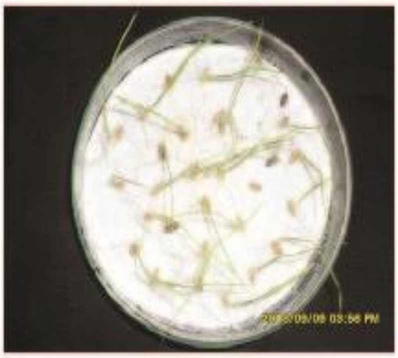

Plate 2. Seedling from seeds primed with 150 mM NaCl concentration when salinity level was control

Plate 3. Seedling from seeds primed with water when salinity level was control