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Effect of seed priming on germination and seedling growth of bitter gourd (*Momordica charantia*) under salinity stress

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Abstract: Efficient seed germination is prerequisite for subsequent plant growth and development. Seed priming are being used to increase the germination percentage of seed under both optimal and stress conditions such as salinity, temperature and drought stress. Salinity the most brutal environmental factors severely affects the seed germination and seedling growth stages of plants. Under salinity condition seed priming can improve emergence and stand establishment by stimulating the pre-germination metabolic processes of seed. The experiment was conducted to evaluate the effect of different priming methods on germination of bitter gourd. The effect of different level of NaCl priming agents on seed germination was also evaluated. Fresh seeds of bitter gourd were subjected to seed priming with various chemicals (20 mgl^{-1} gibberellic acid, 4% mannitol, 2 dS mol^{-1} NaCl) and 3 level of saline (0 dS mol^{-1} NaCl, 4 dS mol^{-1} NaCl, 6 dS mol^{-1} NaCl, 8 dS mol^{-1} NaCl) and the results were compared to identify the best priming treatments. Changes in physiological attributes like germination percentage, seedling length, fresh weight, dry weight and vigour index were measured. The best mean results for germination (85.11%), shoot length (9.73 cm), fresh weight of shoot (1.76 g), dry weight (0.13 g), and seedling vigor index (828.29) were observed in 20 mgl^{-1} GA3 solution. The priming of bitter gourd seeds in different level of NaCl solution reveals that seed priming with NaCl could be used as a method for improving seed performance in salinity condition. However, further investigations are needed to evaluate the priming effect on later growth and development stages of bitter gourd.

Kew words: Priming, salinity, germination, growth characteristics

Introduction

Soil salinity one of the severe problem limiting agricultural production worldwide, including Bangladesh, resulting in significant losses in crop yield. Soil salinity adversely affects almost every developmental stage of plants where germination and early seedling establishment are more sensitive to salt stress.Therefore, development of salt tolerant plant variety is essential to overcome the increased salinity problem. To develop salt tolerance against salt stress plants need to hardened in saline condition(Levitt, 1980).

Pre-conditioning seeds (priming) by NaCl could be used as technological alternative to induce plant tolerance against salt stress. Seed priming is a seed improvement process that allow pre-germinative metabolic activity to continue germination and seedling development of variable crop species (Hussein et al. 2007). Priming has practical implications in improving performance of many crop species under adverse physical conditions including drought, extreme temperature and salinity (Cayuela et al. 1996; Cuartero and Fernández-Muñoz, 1998; Nascimento, 2002). Besides germination and seedling emergence, seed priming has potential impact on crop development and stress tolerance under various biotic and abiotic stresses (Cayuela et al. 1996; Cuartero and Fernández-Muñoz, 1998; Nascimento, 2002). Different types of seed priming techniques (like osmopriming, halo-priming, solid matrix priming, biopriming) are widely used to improve germination, early seedling emergence and vigor of seedling (Kaya et al. 2006; Zhang et al. 2006; Patade et al. 2012). Among those hydropriming and osmopriming are the most commonly used techniques because of simplicity and satisfactory results (Ibrahim, 2016). In hydropriming seeds are soaked in water (Casenave and Toselli, 2007) and in osmopriming seeds are soaked in adverse osmotic solution (mannitol, polyethylene glycol) or salt solution (chlorides, sulphates, nitrates) to regulate water potential within the seed (Chen et al. 2010; Papastylianou and Karamanos, 2012). Seed priming with NaCl is important to increase salt tolerance of plant (Cayuela et al. 1996). Osmopriming of seeds with NaCl has already been reported in various plant species (Casenave and Toselli, 2007).

Bitter gourd (*Memordica charantia* L.) is one of the most popular vegetable in Bangladesh. Bitter gourd have many nutritional and medicinal value used as ayurvedic medicines for treating many diseases like diabetes andrheumtism etc. (Kumar and Bhowmik, 2010). Bitter gourd is normally sown in December to January and harvested in April to May. But at sub optimal temperature (temperature below18^{0}C) the bitter gourd seed germination is occasionally severely hindered which ultimate affect the yield. Moreover, production of bitter gourd is also hindered due to poor or slow seed germination, thick seed coat enclosing embryo etc. (Kanwar and Mehta, 2017). Additionally the thick seed coat restricts embryo growth by imposingmechanical restriction (Pandita and Nagarajan, 2004). As Bitter gourd has tremendous economic and dietic importance so the production of bitter gourd need to increase by adopting different advanced methods in seed technology. Generally bitter gourd is moderately tolerant to salinity and gives good percentage of yield up to EC 4-5 dS mol^{-1}(Raman and Lau, 1996). However, the high salinity and the thick seed coat hamper the field emergence of bitter gourd seeds. The present study was conducted to evaluate the physiological effect of osmopriming and NaCl priming on salt tolerance, germination and seedling characters of bitter gourd.

Materials and Methods

The experiment was conducted at Biotechnology laboratory (located at 22.47°N, 90.38°E) of Patuakhali Science and Technology University, Bangladesh. Fresh and graded bitter gourd seeds were primed with four levels of priming solution (control, GA3, Manitol, NaCl) for 24 hours. 20 mgl^{-1} gibberellic acid, 4% Manitol, 2 dS mol-^{1} NaCl and distilled water were added in the plastic petridish containing the seeds at 25±2 °C temperature. Three replications of 20 seeds for each treatment were placed in each petridish. After 24 hours the seeds were dried at the laboratory temperature. In the second step artificial salinity stress were created by adding 5 ml of salt solutions (4, 6 and 8 dS mol-^{1}) to10 cm plastic Petri dish and 20 seeds were placed in each petridish. The emergence of 2 mm or more of radicle was considered as germination and the germination was checked for every 24 hours. Data was recorded considering following parameters:

Germination percentage

Germination of bitter gourd was checked on every alternative day for upto sixth day of plantation Germination percentage was calculated following the Larsen and Andreasen (2004) equation:

$GP = \Sigma n/ N \times 100$; (n is the number of germinated seeds at each counting; N is total seeds in each treatment).

Number of days to emergence

Mean Germination Time (MGT) was calculated following Ellis and Roberts (1981) equation:

$MGT = \sum Dn \sum n$ (Where n is the number of seeds on number of days counted from the beginning of germination).

Seedling Vigor Index (SVI)

Seedling Vigor Index (SVI) was calculated by using Abdul-Baki and Anderson (1970) formula:
Vigor index (VI) = [seedling length (cm)× germination percentage].

Fresh weight of seedling (g): Ten seedlings from each sample were taken randomly.Seedling fresh weight were recorded using electronic balance in gram.

Seedling dry weight (g): 14 days oven dryed seedlings were used to measure the dry weight of seedling. The seedlings excluding the cotyledon were drying at 85ºC for 24 hrs. in oven. The lot exhibiting the maximum seedling dry weight is considered as vigorous. Shoots and roots Fresh weigh were measured using digital weighting balances.

Results and discussion

The present study investigated positive effect of priming on salinity tolerance and germination of bitter gourd seeds. The data indicated significant differences in per cent germination among four priming treatments and three salinity levels (Table 1, Table 2). Among the treatments 20 mgl^{-1} GA3 resulted in highest per cent germination (85.11%) followed by 4% Mannitol (81.94%) and 2 dS $m-^{1}$ NaCl (76.30%) compared to control (68.55%) (Table 1). Successful crop production in normal and stress conditions generally depends on germination and subsequent seedling establishment stages of plant life cycle. Priming is one of the important pre-sowing treatment of seeds that improves germination mechanisms and empower the seeds to combat with six stresses that occur during germination (Farooq et al. 2006). The primed seeds give better and more uniform germination and good seedling emergence and establishment (Bradford, 1986).The different priming solution improve the embryonic activity of seed during germination and enhance germination by overcoming the seed dormancy. Demir and Oztokat (2003), reported that when watermelon seeds pre-treated with KNO_3 gavebetter germination percentage with minimum time (Demir and Oztokat, 2003). Bradford (1986) mentioned more uniform and better germination and good seedling establishment of primed seed compared to unprimed one. Priming treatment enhance uniform and good germination of seeds under both normal and saline situations (Basra et al. 2002; Mavis et al. 2006; Souguir et al. 2013).

Besides germination, marked variation in shoot length, dry weight, root number and vigour index was noticed due to seed priming when compared to unprimed one (Table 1). The shoot length was affected by different priming treatment. Compared to the control all priming treatments (20 mg-l GA3 following 4% mannitol and 2 dS $mol-^{1}$ NaCl) increased the shoot length (Table 1). Between the three treatments priming with solutions of 20 mgl-1 GA3 following 4% mannitol exhibited the greatest shoot length, while priming with 2 dS $mol-^{1}$ NaCl resulted least shoot length (Table 1). Earlier seed germination may have positive effect on plumule length in seeds treated with different priming solutions (Table 1). Nascimento et al. (2004), reported the positive effect of priming solutions regarding the plumule length and faster germination of seeds (Nascimento and Aragão, 2004). Besides shoot length, increased fresh and dry weight of seedling was noticed due to seed priming when compared to unprimed seed. All priming treatments significantly raised seedling fresh and dry weight compared to control. The greatest seedling fresh and dry-weight was observed in 20 mg-l GA3 followed by 4% manitol compared to control (Table 1). The result is consistent with earlier work of Afzal et al. (2008), in which they reported that various seed priming treatments increased germination percent, germination index, seedling fresh and dry weight under cool condition (Afzal, Rauf et al. 2008). Hassanpouraghdam et al. (2009), reported that fresh weight primed seed derived seedling was higher than unprimed seedlings(Pardaz et al. 2009).

Although all of the three priming treatments influenced shoot traits compared to control, however, did not show any significant effect on root characteristics (root number and dry weight).These results are in accordance with Tzortzakis (2009), and Čanak et al. (2016) (Tzortzakis 2009; Mirosavljević et al. 2016). Marked increase in vigour index length was observed in primed seed compared to unprimed ones. Highest increase in vigour index length occurs in 20 mgl-1 GA3 (828.29) followed by 4% mannitol while lowest vigour index length (426.15) was observed with unprimed control treatment. The positive effect of seed priming on vigor index length was also reported by Venkatasubramanian and Umrani (2007) (Venkatasubramanian and Umarani, 2007). Increased seedling vigour and growth in primed seedling had also been found in tomato (Pandita and Shantha, 2000). During treatment priming often induces the physiological, biochemical and metabolic process of seeds and raised amylase production resulting in higher seed vigor (Suk-Soon, 2000).

Table 1 Effect of seed priming treatment on germination percentage, shoot length, seedling fresh and dry weight, vigour index and root number of bitter gourd

Treatment	FGP (%)	SL	FW	DW	RN	VI
Control	68.55d	6.13d	1.04d	0.1270d	5.33b	426.15d
2 dS m^{-1} NaCl	76.30c	7.37c	1.43c	0.1780b	7.17a	507.96c
20 mgl-1 GA3	85.11a	9.73a	1.76a	0.1880a	5.67b	828.29a
4% Manitol	81.94b	8.33b	1.58b	0.1487c	6.33b	682.64b
LSD	10.05	2.10	0.42	0.03	1.12	249.21

FGP = Final germination percentage; SL= shoot length; FW= Seedling fresh weight; DW=Seedling dry weight; RN=Root number; VI=Vigour index. Means with the same letters in each column are not significantly different at the 0.05 level of probability

Figure 1 Biter gourd seedling growth after different priming treatment

Effects of different level of NaCl priming on germination and seedling growth characteristics

Both the priming and salinity level have the significant effects on the germination of bitter gourd seeds. The seeds were primed at different level of NaCl (0, 4, 6 and 8 dS mol^{-1}) and observed their effect on different growth parameters of seedling (Table 2, Figure 2). Generally increased NaCl level decreased the germination percentage and germination rate of bitter gourd seeds (Table 2, Figure 2). Similarly, shoot length, seedling fresh and dry weight, vigour index and root number were also found to be decreased with increased NaCl level (Table 2). In increased salinity condition the poisonous effect of sodium and chlorine ions may prevent the water uptake of seeds and thereby hamper the germination process (Khajeh-Hosseini, Powell et al. 2003). Mohammadi, (2009), reported that increased NaCl level reduced the different growth attributes of Canola (Mohammadi, 2009).

Table 2 Effects of different level of NaCl on germination and seedling growth behavior of bitter gourd

Salinity level (dS m^{-1})	FGP (%)	SL	FW	DW	RN	VI
0	86.21a	9.13a	3.15a	1.01a	7.12a	754.34a
4	74.41b	8.37b	2.10b	0.21b	6.35b	624.6b
6	71.10c	7.25c	1.86c	0.17c	6.01c	512.8c
8	70.56c	7.10c	1.75bc	0.13d	5.56d	508.12c
LSD	10.10	1.33	1.09	0.58	0.91	160.87

FGP = Final germination percentage; SL = Shoot length; FW = Seedling fresh weight; DW = Seedling dry weight; RN = Root number; VI= Vigour Index. Means within each column followed by the same letter are not significantly different (P=0.05).

Priming with NaCl positively declined the negative effects of salinity on bitter gourd germination as compared to non-primed seeds (Figure 3). At all NaCl levels all the seedling growth parameters such as final germination percentage, shoot length, fresh and dry weight, vigour index, root number were also observed much enhanced in primed seeds compared to non- primed seeds (Figure 3). Similar observations were reported by Sivritepe et al. (2003), on melon seeds (Sivritepe et al. 2003). Priming by NaCl has positive effect in reducing the negative effect of salinity in wheat seed (Ashraf et al. 2006).

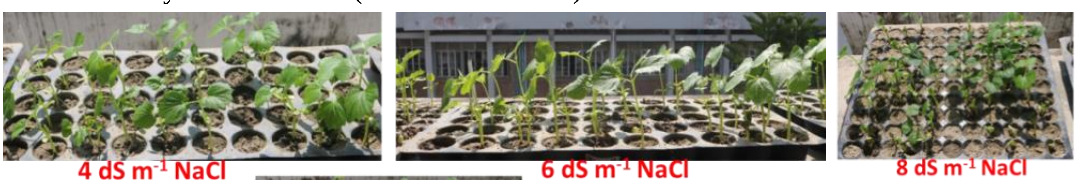

Figure 2 Bitter gourd seedling growth after NaCl treatment

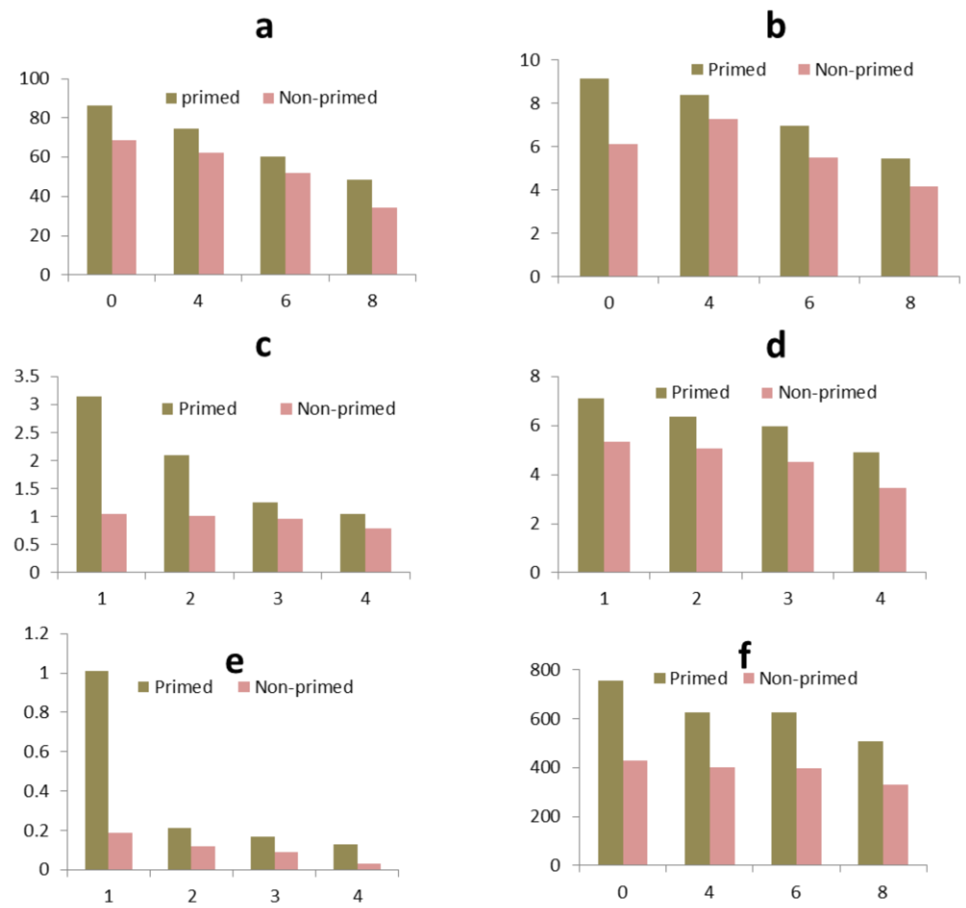

Figure 3 Effect of different levels of NaCl on a) Final germination percentage; b) Shoot length; c) Seedling fresh weight; d) Seedling dry weight; e) Root number; f) Vigour index

Conclusion

The results of the experimentrevealed that seed priming has remarkable beneficial influence on germination and seedling establishment of bitter gourd. Among the different priming materials 20 mg-l GA3 was the most promising priming agent for all seedling parameters. The study was also observed that seed priming with different level of NaCl potentially enhance the germination percentage and seedling growth characteristics. However, further more detail studies are needed to explore the various effects of different priming treatments on later growth and development of bitter gourd.

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Competing interests

The authors declare that they have no competing interests.

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