J. Crop Sci. Biotech. 2015 (March) 18 (1) : 21 ~ 26
DOI No. 10.1007/s12892-014-0078-1

RESEARCH ARTICLE

Seed Halopriming Improves the Germination Performance of Black Seed (*Nigella sativa*) under Salinity Stress Conditions

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Received: July 16, 2014 / Revised: December 2, 2014 / Accepted: December 9, 2014

Abstract

Soil salinity is one of the most serious agricultural problems. There are many biological strategies by which salinity tolerance of potential crops can be increased. The ameliorative efficiency of salt priming on emergence and seedling growth was examined in black seed (*Nigella sativa*), a valuable medicinal herb. The seeds were soaked for 24 h at 25ºC in the dark in distilled water (control) or 1 or 2% of KNO_3, $CaCl_2$, NaCl, $ZnSO_4$, or $CuSO_4$. After the all treatments, the seeds were washed with distilled water and sown. Although all priming agents were effective in alleviating adverse effects of salt stress on black seed at germination stage, NaCl proved to be the most effective since the seeds primed with this salt had significantly higher germination performance than those treated with other salts or distilled water. Next to NaCl, KNO_3, $CaCl_2$, and $ZnSO_4$ were also effective in promoting seed germination and early growth under saline conditions.

Key words : medicinal crops, NaCl, priming, salinity tolerance, salt

Introduction

High salinity is the most widespread soil problem limiting plant distribution and productivity (Qin et al. 2010). Coping with salinity stress in order to sustain food production is a major issue in many arid and semiarid regions (Kaya et al. 2013). The biological approach to this problem focuses on the management, exploitation, or development of plants able to thrive on salt-affected soils (Ashraf et al. 2008). Seed priming is one of the biological strategies by which salinity tolerance of potential crops can be increased. In priming, seeds are exposed to restricted water availability under controlled conditions which allows some of the physiological processes of germination to occur, before germination is completed (Sharma et al. 2014). In plant defense, priming is defined as a physiological process which have a plant prepare to respond to imminent abiotic stress more quickly or forcefully (Jisha et al. 2013). Many studies have shown that salt tolerance of plants can be improved by treating seed with water or solutions of inorganic or organic salts before sowing (Jafar et al. 2012; Kaya et al. 2006; Patade et al. 2009; Srivastava et al. 2010; Yadav et al. 2011). Seed priming has become as a promising strategy in modern stress management (Van Hulten et al. 2006). There are various priming approaches including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices), and biopriming (hydration using biological compounds) (Ashraf and Foolad 2005; Tzortzakis 2009).

A variety of chemicals are used to create low-water-potential solutions, including KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, NaCl, etc. (Jisha et al. 2013). Pretreatment of the mungbean seeds with NaCl ameliorated the injurious effects of NaCl stress (Saha et al. 2010). It has been shown that priming of sunflower seeds with KNO_3 (500 ppm) alleviates NaCl-induced stress during germination (Kaya et al.

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2006). In the case of wheat, Jafar et al. (2012) pointed out that priming with $CaCl_2$ (50 mg L^{-1}) for 12 h had positive effects on stand establishment and harvest index of wheat when salt treatments were applied with seed sowing.

Black seed (*Nigella sativa*) is an important medicinal herb. In many Arabian, Asian and African countries, black seed oil is used as a natural remedy for a wide range of diseases, including various allergies (Kalus et al. 2003). It has long been used in traditional medicine for treating various conditions related to the respiratory and gastrointestinal systems as well as different types of cancers (Majdalawieh et al. 2010). Also, high antifungal activity of *N. sativa* makes it a promising candidate for engineering pathogen-resistant plants (Rogozhin et al. 2011). One of the main problems that prevent sustainable use of medicinal plants, native to the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions or when cultivation is attempted (Nadjafi et al. 2006). Different osmotica can be used in seed priming and these have got different characteristics and levels of efficacy (Foti et al. 2008). On the other hand, different seed lots responded differently to priming, even within the same cultivar (Bassel et al. 2008).

The aim of this research was to determine treatment(s) which are able to stimulate and enhance germination of *N. sativa* seeds. Furthermore, the study examined the possibilities to overcome salinity stress by using seed treatments with different types of salts.

Material and Methods

Plant material

Native seed lots of black seed (*N. sativa*) were obtained from a local seed market in Isfahan, Iran which collected the seed from Mobarakeh in the same year that the study was undertaken. This native self-pollinated seed lot was widely cultivated in central regions of Iran.

Preliminary salt stress experiment

To determine the threshold level of salt concentration at which black seed germination decreased, solutions of NaCl were prepared at concentrations of 0, 10, 20, 30, and 40 mM. Fifty seeds of black seed were sown in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 10 mL distilled water (control) or test solution and four replicates of each treatment were used. All Petri dishes were placed under constant temperature (25ºC) and darkness conditions (Kaya et al. 2006). Every 2 days, the solution in each Petri dish was removed, a further 10 mL test solution added, and removed again as completely as possible before 5 mL of the test solution was added. Germinated seeds were recorded daily. Seeds were considered to have germinated when the emerging radicle was at least 2 mm.

Seed priming treatment

The inorganic salts $CaCl_2$, NaCl, KNO_3, $ZnSO_4$, or $CuSO_4$ were used as priming agents and 1 and 2% (w/v) solutions of each salt was used for seed priming. Priming of seeds with distilled water served as the control (dry seeds had low germination percentage (data not shown)). Solutions volumes were three times the seed weights and priming was performed at 25ºC for 24 h under dark conditions as described by Yuan-Yuan et al. (2010). During priming, containers were kept well aerated with an aquarium water pump, because sufficient oxygen is a requirement for seed respiration in osmotic seed priming. After priming, the seeds were rinsed with distilled water two times and dried at 25ºC to the original moisture content as the unprimed seeds and immediately used for germination tests. In Petri dishes, the primed seeds were treated with 10 mL of NaCl in the concentration of 0 or 15 mM which was the threshold concentration in the preliminary salt stress experiment (above). For the germination test, a total of 200 seeds in four replicates (50 seeds/replicate) were sown evenly on double-layered Wathman No. 1 filter paper and the Petri dishes were maintained in the dark at room temperature (25ºC) (Kaya et al. 2006; Mahmoudi et al. 2012). The number of germinated seeds was counted at 24-h intervals from the onset of germination.

Germination tests

Germination was considered to have occurred when the radicles were 1 mm long. Final germination percentage (FGP) was recorded every 24 h for 25 days. Germination rate (T_{50}) was defined as days needed to reach 50% of FGP. Mean germination time (MGT) was calculated as follows (based on Zhu et al. 2006):

$$MGT = \sum Dn / \sum n$$

where MGT is the mean germination time (day), *D* is the time in days from the starting / sowing day and *n* is the number of germinated seeds on a given day.

Germination index (GI) was calculated as:

$$GI = \sum_{i=1}^{t} Gi/t$$

where *Gi* is the number of seeds germinated in the *i*th day, *t* is the number of days.

Those seeds that germinated but were failed to emerge were determined. The vigor index (VI) was calculated as the product of seedling dry weight by germination percentage (ISTA 1996). Root and shoot length were measured after the 25th day. Finally, those seeds that germinated but failed to emerge were determined.

Data analysis

The experimental design was a factorial with two factors (11 x 2) arranged in a completely randomized design; with four replications and 50 seeds per replicate. The first factor was seed treatments (control, 1 or 2% KNO_3, $CaCl_2$, NaCl, $ZnSO_4$, or $CuSO_4$) and the second was germination solution (distilled water or 15 mM NaCl). Percentage data were arcsin-transformed before analysis. All data were subjected to an analysis of variance. Mean comparisons were performed by Duncan's multiple range test if *F*-test was significant at $P = 0.05$.

Results

Percentage of germination and emergence are shown in Figs. 1 and 2, respectively. In general, considering all the treatment together and on the basis of FGP, significantly positive effects of halopriming with NaCl, $CaCl_2$, or $ZnSO_4$ were recorded. Notably, halopriming with 1% $ZnSO_4$ increased the FGP up to 82% in comparison to non-primed seeds (68%) (Fig. 1). Salinity stress showed a substantial reduction of FGP in unprimed seeds (40%). Primed seeds of black seed performed significant higher FGP in salinity stress compared to unprimed seeds. Halopriming with 2% KNO_3 and 1% NaCl were equally effective to increase the FGP up to 87 and 88%, respectively (Fig. 1). The percentage of seeds that germinated but failed to emerge was lower in primed seeds than in control (unprimed seeds). For many treatments, irrigating seeds with saline water increased the percentage of emerged seeds (Fig. 2).

Except for 1% $CuSO_4$, salinity treatment did not influence significantly the MGT of black seed. Priming with 2% NaCl or 2% $CaCl_2$ shortened the time to seed germination under both saline and non-saline conditions compared with unprimed seeds. Priming of seeds with 2% NaCl for 24 h resulted in MGT of about 5.8 days compared to control (about 7.5 days) (Fig. 3). Except for $CuSO_4$ treatments, germination rate was not significantly influenced by salinity. The highest germination rate (the lowest T_{50}) (2.7 days) was recorded in the seeds haloprimed with NaCl and irrigated by distilled water (Fig. 4).

After treatment with NaCl (15 mM), GI was decreased significantly. However, osmopriming with KNO_3, NaCl, $CaCl_2$, or $ZnSO_4$ resulted in no significant decreases, even increases of GI. Maximum GI was recorded in the osmoprimed seeds with NaCl for 24 h (Fig. 5). All seed priming treatments gave better VI than control (untreated) under salt stress or without it, with clear effectiveness of halopriming with KNO_3 or NaCl (Fig. 6).

Root and shoot lengths of seedlings are shown in Figs. 7 and 8, respectively. Of the five salts for seed priming tested, all the salts increased root length even up to 2.8 cm (osmopriming with 2% KNO_3) under Petri dish conditions. Except for KNO_3 treatments, there was no significant difference

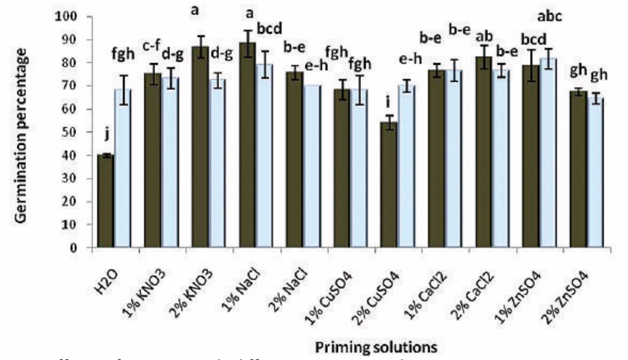

Fig. 1. Effects of priming with different inorganic salts on germination percentages of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

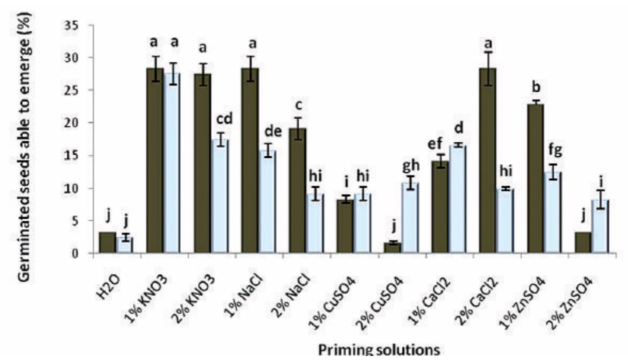

Fig. 2. Effects of priming with different inorganic salts on percent of germinated seeds of black seed able to emerge at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

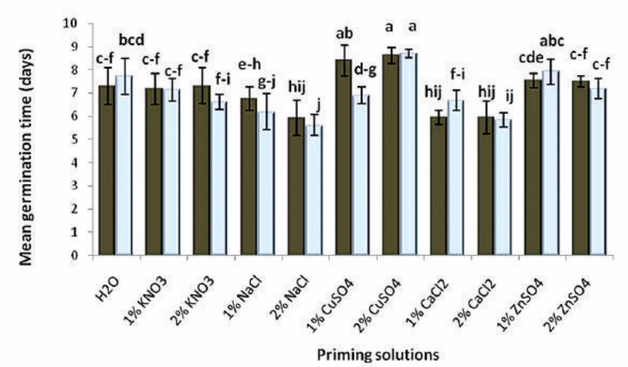

Fig. 3. Effects of priming with different inorganic salts on mean germination time of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

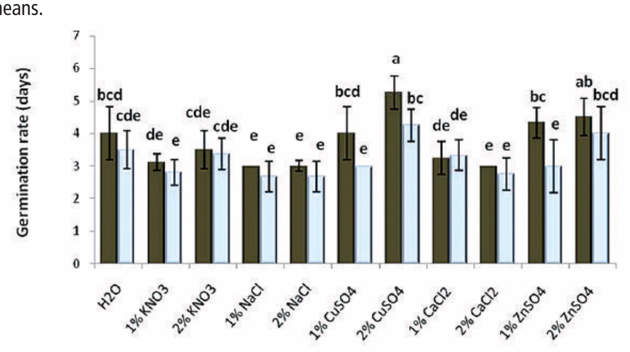

Fig. 4. Effects of priming with different inorganic salts on germination rate of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

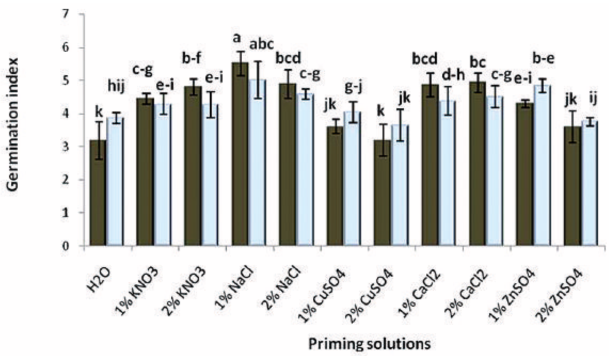

Fig. 5. Effects of priming with different inorganic salts on germination index of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

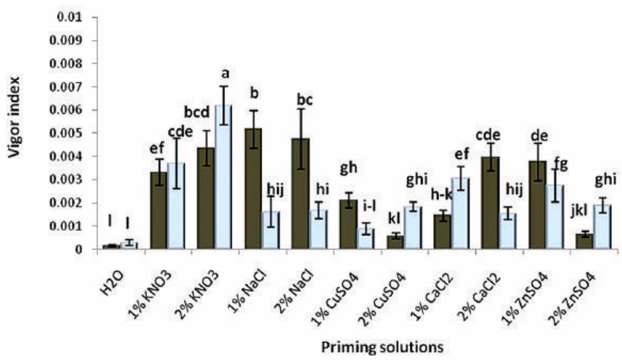

Fig. 6. Effects of priming with different inorganic salts on vigor index of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

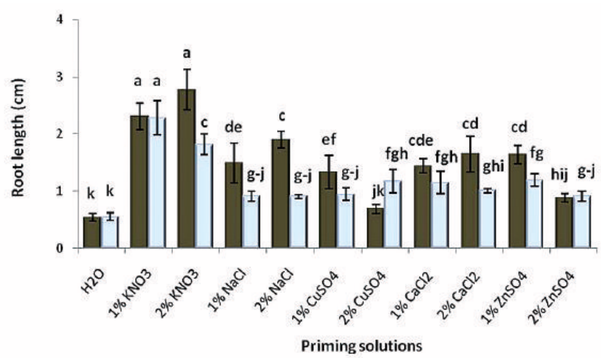

Fig. 7. Effects of priming with different inorganic salts on root length of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

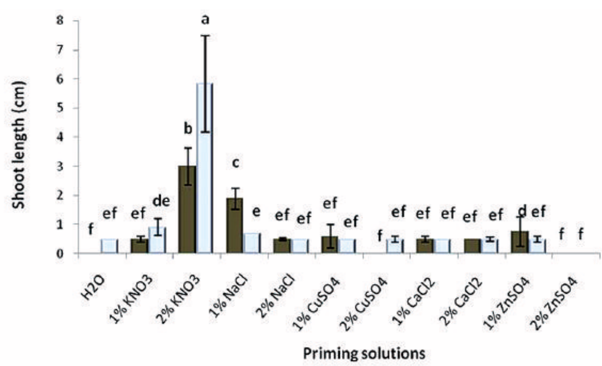

Fig. 8. Effects of priming with different inorganic salts on shoot length of black seed at 15 mM NaCl (black) or distilled water (grey). Nonprimed seeds (control) were imbibed and germinated in H2O. Vertical bars are standard deviation (SD) of means.

among different priming agents in their effects on root length of distilled water irrigated seeds (Fig. 7). Seedlings derived from seeds treated with 2% KNO_3 showed a maximum shoot length. There was no significant difference among control and priming agent of $CuSO_4$, $CaCl_2$, or $ZnSO_4$ in terms of their effect on shoot length of black seed (Fig. 8).

Discussion

In the present study, following halopriming treatments, seeds always germinated better in NaCl than distilled water. Under salt stress, Na^+ and Cl^- may be taken up by theseeds. The better germination performance in NaCl than distilled water may be due to this uptake, maintaining a water potential gradient allowing water uptake during seed germination (Kaya et al. 2006).

Osmopriming is the soaking of seeds in aerated, low-water-potential solutions. Exposing seeds to a low external water potential restricts the rate and extent of imbibitions and progress various pre-germination metabolic activities (Jisha et al. 2013). It resulted in the faster imbibition process in primed seeds as a result of the steeper osmotic potential gradients between distilled water in the germination papers and osmotic potential on the seed surfaces (Foti et al. 2008). It has been shown that different priming methods could be used as an adaptation method to improve salt tolerance of seeds (Mahmoudi et al. 2012).

Of the different priming agents used in this experiment, NaCl was found to be very effective in encouraging seed germination under both saline and non-saline conditions. Saha et al. (2010) working with mungbean have concluded that pretreatment of seeds with a sub-lethal dose of NaCl could be an interesting strategy for ameliorating the injurious effects of salt stress in plants by increasing antioxidant enzymes activities and accumulation of osmolytes for osmotic adjustments. In melon, NaCl pretreatments enhanced K and Ca concentrations of leaves and stems, and prevented toxic effects of salinity because less Na accumulated in stems (Sivritepe et al. 2005).

The second most effective priming agent in our study was KNO_3. Relative to control and other treatments, priming with KNO_3 promoted root and shoot length and VI. The results are in line with Anosheh et al. (2011) in maize who reported that KNO_3 alleviated effects of salt stress and led to increased germination and seedling growth as well as the root length. Root elongation in other reported plants (medicinal) was positively influenced by KNO_3 (Kattimani et al. 1999). The beneficial effects of KNO_3 might be due to overcoming some seed dormancy or improving embryonic development (Demir and Mavi 2004).

The effects of $CaCl_2$ and $ZnSO_4$ as priming agents in this experiment were also prominent on the germination performance of black seed. The beneficial effects of $CaCl_2$ may have resulted from the role of calcium as a second messenger in plant cells, from the protective role of it against adverse environmental stress or the effects of calcium on hormonal balance (Iqbal et al. 2006). Germination performance improvement after treatment with moderate $CaCl_2$ concentration was in agreement with the finding of Basra et al. (2006) in wheat. According to Cakmak (2008), sufficient Zn concentration of seed is essential for seedling vigor and plant resistance to environmental stress factors. Prom-u-thai et al. (2012) reported that seed priming with $ZnSO_4$ increased Zn concentrations of rice seedlings grown on Zn-deficient soil. Zink is essential in protein synthesis and gene expression. Since production of reactive oxygen species is a common phenomenon during seed germination, Zn can play a critical role in the detoxification of reactive oxygen species in cells (Cakmak 2000). It must be noted, however, that high concentrations of Zn priming may cause toxicity in plant cells and inhibit seedling development (Prom-u-thai et al. 2012).

The low effect of $CuSO_4$ on seed germination performance is not in agreement with the study of Foti et al. (2008) in which FGP of the maize seeds increased by $CuSO_4$. The finding that germination performance did not improve with $CuSO_4$ halopriming suggests that osmotic potential of this salt not being low enough to partially hydrate the seed (Foti et al. 2008).

Conclusions

In conclusion, of the different priming agents used in the present study, NaCl was the most effective in alleviating the adverse effects of salt stress. However next to NaCl, KNO_3, $CaCl_2$, and $ZnSO_4$ were also effective in promoting germination and early growth of black seed under saline conditions. Priming with NaCl was found to be simple, cheap, and effective practice for overcoming salinity-induced negative effects in black seed and therefore was found suitable to be recommended to farmers, so they can get better crop stand and synchrony of emergence in medicinal plants under the environmental stresses.

Acknowledgment

This research was supported by the Isfahan University of Technology.

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