Effect of Pre-soaking by distilled water on seeds germination of black seeds (*Nigella sativa* L.) under salinity stress

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Abstract

A laboratory experiment was conducted to study the effects of salinity and presoaking treatments on final germination percentage, days to 50% germination, and recovery percentage of *Nigella sativa* seeds. Seeds were divided into two groups and for 24h one group was presoaked in different NaCl concentrations (25, 50, 75, and 100 m*M*), while the other was presoaked in distilled water. They were then sown in Petri dishes and watered with salinity treatments. Results showed high significant difference between the two presoaking treatments. Final germination percentage of seeds of the first group was negatively affected where it decreased as salinity concentration increased. Only seeds at 25 m*M* reached to 50% of germination after ten days, while those at other concentrations failed. Results also showed that seeds of the second group were germinated well after being exposed to salinity stress. They also achieved more than 50% germination during the first five days of experiment. The recovery percentage of the un-germinated seeds of the two groups was low indicating toxic ionic effect on seed germination and viability. It was concluded that salinity effect on germination traits increases with the increase in concentration and presoaking treatment in distilled water helps seeds to reduce salinity stress.

Keywords: final germination, presoaking, distilled water, *Nigellasativa*, salinity.

Introduction

Land areas seriously suffer from salinization which increases day by day in arid, semiarid, and Mediterranean regions (30; 10). Currently, salinity transforms about one third of the world's land and half of the land in semi-arid and coastal regions into barren or unusable lands (13; 51; 44). That mostly happens because of low rain rate which is not enough for salt leaching from root medium to the ground water level; rapid and high rate of water evaporation and the use of salty ground water in irrigation which leads to more salt accumulation in soil to a harmful level to many plant species at different growth stages (13, 43).

As an important abiotic stress, salinity has been demonstrated to negatively affect a wide range of physiological processes in plants starting from germination initiation to productivity. Germination of seeds is critical in the survival; establishment and growth of plant populations under stress condition (33; 9;26; 42). Salt stress exerts osmotic effect by reducing water absorption and /or toxic effect through accumulation of Na^+ and Cl^-, which disturb nutrient uptake balance and finally leads to germination delay, inhibition or may prevent germination completely (11; 1; 36). Germination traits of several plant species, such as *Matricaria chamomilla* (1); *Lensculinaris* (19); many cotton cultivars (22); *Hordeum vulgare* (40) and number of salt marsh plants (41), were reported to be negatively reduced because of salinity.

Different methods have been employed to achieve better seed germination and healthy seedling vigor under stressful conditions. Of those methods, seeds presoaking treatment, with different types of materials, has been widely applied. A number of plant growth regulators, such as gibberellic acid, salicylic acid and kinetin (6; 27; 32; 35); organic composts (39) and inorganic solutes like cobalt and calcium (16; 52), proved their ability to induce plants to overcome several kinds of stresses conditions. Those materials were demonstrated to possess a compensatory mechanism for solute accumulation regulation, osmotic adjustment, and protection against injury to various growth vegetative and reproductive parameters (24; 25; 49; 54).

Black seed (*Nigellasativa* L.) is one of the important medical plants belongs to the Ranunculaceae family. The importance of this plant is due to its seeds which have wide applications and uses as a spice, food preservative, and curative remedy for numerous disorders. Healing with black seeds was previously recommended by the Prophet Mohammad (peace and pleasant be up on him) who described them as a remedy for every disease except death. Recent researches, in this context, have indicated that black seeds are effective for using as anti-microbial, anti-diabetic, anti-histaminic, anti-hypertensive, anti-inflammatory, and anti-tumor materials (2; 3; 4; 14; 55).

Several studies were carried out on *N. sativa* response to salinity or water stress at different growth stages (15; 18; 23). Some others evaluate the ameliorating effect of a number of growth regulators whether under stress or normal growth conditions (26; 27; 45; 46; 47; 48; 49). To our knowledge, to date, no study was performed to test the ameliorating effect of presoaking treatment in distilled or salinity solutions on germination of black seeds in Yemen. Hence, the sole objectives of this study were to compare the effects of presoaking treatment on the response of *N. sativa* in terms of germination traits and throw light in finding an applicable and simple method to help this important medical plant to withstand salinity stress at the more sensitive stage in its life history, i.e., seed germination.

Materials and methods

Seeds of *N.sativa* were purchased from a local market in the old Sana'a city. Species was then identified by the taxonomist Dr. Hassan Ibrahim, Biology Department, Science Faculty, Sana'a University, Yemen. Seeds were sterilized by soaking in 5 % (v/v) of sodium hypochlorite for 20 minutes (53) to eliminate surface contamination with microorganisms. Then, they were rinsed three times with distilled water.

Seeds were divided into two groups. The first group was then subdivided into small patches then each one was soaked in each salinity concentration (25, 50, 75, and 100 m*M*). The second group of seeds was soaked in distilled water. All seeds were left in soaking treatments for 24h in the darkness. After that, they were rinsed once with distilled water and dried with filter paper.

To study the effect of salinity on seeds germination, thirty seeds from each group were grown in 9 cm diameter Petri dishes (30 seed per dish)on filter paper moistened with each salinity concentrations and distilled water was used as control. Dishes were distributed with completely randomized design (CRD) with three replications for each treatment and then incubated in the darkness under laboratory conditions.Germination was recorded at 5 days interval through 15 days and addition amount of each treatment was added if necessary. The germination signal was the detected radicles grown to 2 mm at least. The un-germinated seeds were washed once with distilled water then they were re-germinated in Petri dishes lined with filter paper wetted with distilled water. They were incubated for another 15 days as recovery treatment to evaluate whether salinity treatments exerted osmotic, toxic or both effects (11; 31).

At the end of the experiment, germination percentage was counted with this equation (8):

$$G\,\% = \sum \frac{ni}{N} \times 100$$

Where: n is the number of germinated seeds till i^{th} day and N is the total number of seeds. Germination velocity was found through counting the number of days required to achieve 50% of germination. Inhibition percentage of germination over control was calculated using the equation recommended by (21):

$$\text{Inhibition } \% = \left[1 - \frac{\text{Germination } \% \text{ in sample}}{\text{Germination } \% \text{ in control}} \right] \times 100$$

Recovery percentage was detected through calculating with this equation (11):

$$\text{Recovery } \% = \frac{a - b}{c - b} \times 100$$

Where: a = number of seeds germinated after being transferred to distilled water, b = number of seeds germinated in saline solution, c = total number of seeds.

For statistical analysis, data were analyzed with two-way ANOVA to detect the significance difference between the two factors (presoaking treatments and salinity concentrations). Statistical analyses were performed using MINITAB for Windows and all graphs were plotted using Microsoft Office Excel.

Results

The response of seeds germination of the medical plant *N. sativa* to salinity stress and the ameliorating effect of presoaking treatment were carried out in this study.

Germination percentage and velocity:

At the end of the experiment period, 15 days, germination percentage of *N. sativa* seeds of the two groups were counted. Statistical analysis of the germination percentage revealed high significant difference between salinity concentrations (P=0.000) and between the applied presoaking treatments (P=0.000).For the first group of seeds, presoaked in salinity concentrations, germination percentage significantly (P= 0.000) decreased as salinity concentration increased and no germination was recorded for seeds at the highest salinity concentration (100 m*M*) (Figure 1).In contrast, for seeds of the second group, no significant difference was detected between salinity concentrations (P=0.183). In this respect, high percentages of germination were recorded and were arranged between 93.3% and 85.5% at control and 100mM, respectively (Figure 1). In respect to germination velocity of the first group of seeds, only those at 25 m*M* achieved 50% of germination after 10 days, while the rest at the other salinity concentration failed till the end of experiment (Figure 2). On the contrary, seeds of the second group, at all salinity concentrations, reached to more than 50% of germination during the first five days of the experiment period (Figure 2).

Inhibition percentage of germination:

Results of the percentage of germination inhibition for the seeds in the first group showed gradual increase with the increase in salinity concentrations. Low percentage was recorded for seeds at 25 m*M* (27.27%), while complete inhibition (100%) was recorded for seeds at the highest salinity concentration (100 m*M*) (Figure 3). By contrast, the percentage of germination inhibition was very low for seeds in the second group and the highest value (10.49%) was recorded at 100 m*M* (Figure 3).

Recovery percentage:

Percentage of recovery treatment of the un-germinated seeds of group 1 showed low percentage at 25 and 50 m*M* (8% and 10%, respectively). Then, the recovery percentage increased at 75 m*M* (45.65%) and reduced again at 100 m*M* (37.33%). The un-germinated seeds of the second group showed no response at all where all remaining seeds failed to germinate.

Discussion

Seeds of the medical plant *N. sativa* were germinated under salinity (NaCl) stress with two presoaking treatments to assess seeds response to salinity and any possible ameliorating effect of the presoaking treatment. Germination process is known to be generally divided into two phases where water physically moves into the free space (the apoplast) in the first phase, while it moves across cell membranes into the cells of the seeds in the second phase and that movement affects by the difference in the osmotic potential of the seed and that of the medium (7; 28). Physical process of water uptake accelerates some metabolic processes and any increase in salinity concentration retards water uptake which inhibits germination process (1; 38). In the present study, that inhibitory effect of salinity was found only for seeds presoaked in different salinity concentrations (group 1). Germination of seeds of this group drastically and significantly decreased with the increase in salinity concentrations and no germination was recorded at high NaCl concentration (100 m*M*). This result agrees well with the finding of Ghamarnia*et al.* (18) and Hussain*et al.* (23) on *Nigellasativa* who reported that NaClcause a consistent decrease in germination percentage and delay germination velocity of seeds. It also agreed with the results of many other authors on several plant species such as *Pennisetumglaucum* and *Sorghumbicolor* (5); *Chenopodiumglaucum* (11); *Prosopisjuliflora* (12); *Atriplextriangularis* (29); *Loliumperenne* (34) and *Ocimumbasilicum* (37).Results of the other group of seeds, presoaked in distilled water, showed that none of the germination traits significantly affected by salinity stress. It seems that presoaking treatment helpes seeds to overcome harmful effect of salinity where the first step in germination process started before exposing to salinity; i.e., in a medium-free of salinity stress. This result partly agrees with Çiçek, and Çakirlar (10) who applied a laboratory experiment to study salinity effect on two *Zeamays*cultivars where they found no significant inhibitory effect of salinity on seeds germination. In fact, they soaked seeds of their case study plant in nutrient solution ($^1/_2$ strength Hoagland solution) containing different concentrations of salinity, to induce germination which may be the cause of weak response to salinity stress. In other words, seeds may overcome harmful effect of salinity on ions homeostasis by compensating from other ions of the culture solution.

Velocity of germination of seeds of the first group also showed the negative effect of salinity where seeds reached to the end of experiment period before achieving 50 % of germination, except those at 25 m*M* which required 10 days for 50% germination. Seeds of the second group, at all salinity concentrations, showed no negative effect of salinity where only 5 days was required to achieve more than 50% of germination.The percentage of the germination inhibition was high for seeds of the first group, which already exhibited low percentage of germination under salinity stress, and the opposite was true for seeds of the second group.

At the end of the experiment, recovery treatment was applied to determine if salinity stress had osmotic, toxic or both effects on germination traits of *N. sativa*.Our findings revealed that, for seeds at group 1, low and moderate concentrations of NaCl, i.e., 25 and 50 m*M*, showed low recovery percentages (8% and 10%, respectively).That result contradicts with other results pointed in several studies where low concentrations was reported to mostly exhibit high recovery percentages, while the reverse was true for high salinity concentration. Such contrasting findings could indicate the interaction of many factors on plant response to stress such as plant species, organ, age, salt type, and time of exposure (17; 20; 50).

Other group of seeds, presoaked in distilled water, revealed no response. It seems important to consider that germination percentage was high under salinity treatments and only few seeds remained un-germinated where embryos may be affected by ion accumulation. It is commonly known that high recovery percentage indicates osmotic effect, while low one indicates ion toxicity (11). In the present study, it seems to be that the un-germinated seeds may suffer from lethal effect which could not be overcome by recovery treatment. The embryos may be killed by a toxic effect which may be resulted from high accumulation of ions in the cells. Khan *et al.* (31) pointed out that seeds of *Sarcobatus vermiculatus* which were previously exposed to high salinity showed little or no recovery, while those treated with low salinity concentration showed about 50% as recovery percentage. Lethal effect of salinity may be due to one or more than one of the following causes: (a) specific ion toxicity, which associates with excessive intake of chloride, sodium or other ion and causes nutritional imbalance, (b) elevation of osmotic pressure or (c) increases in alkalinity, which finally limit water availability, affect cellular physiology and metabolic pathway (38).

Conclusion

In the present study, the effect of NaCl, the most common salt in soil, on some germination traits of the important medical plant *Nigellasativa* and the ameliorating effect of presoaking treatment were studied. Our results showed that salinity treatments have a significant negative effect on all germination traits, only when seeds presoaked in salinity concentrations. Soaking of seeds in distilled water, before growing under salinity stress, seems to be a good step to grow plants against salinity stress where the first steps of germination and all necessary metabolic processes could be started safely. To have a clear evaluation of presoaking treatment, measurements of the other important growth stage and early seedling growth should be studied in a complement study.

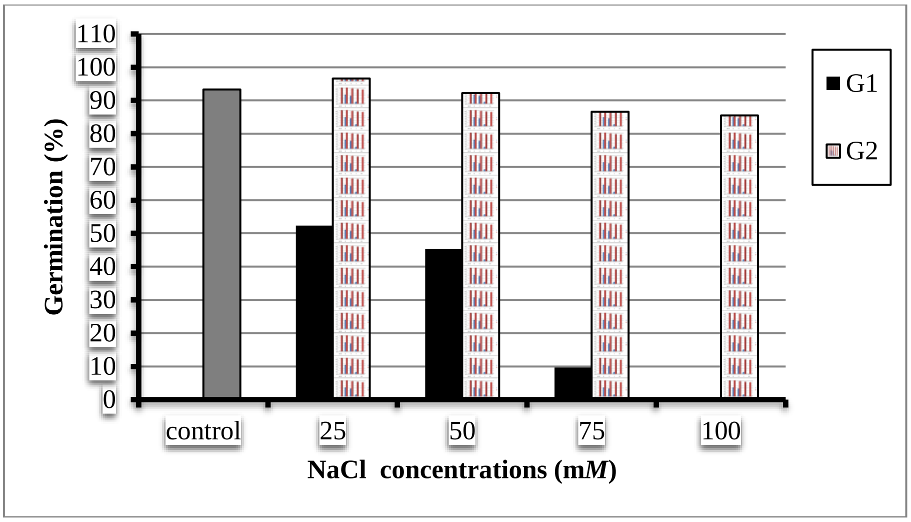

Figure 1: Effect of different concentrations of NaCl on germination percentage of *N. sativa* seeds presoaked in different salinity concentrations (G1) or distilled water (G2)

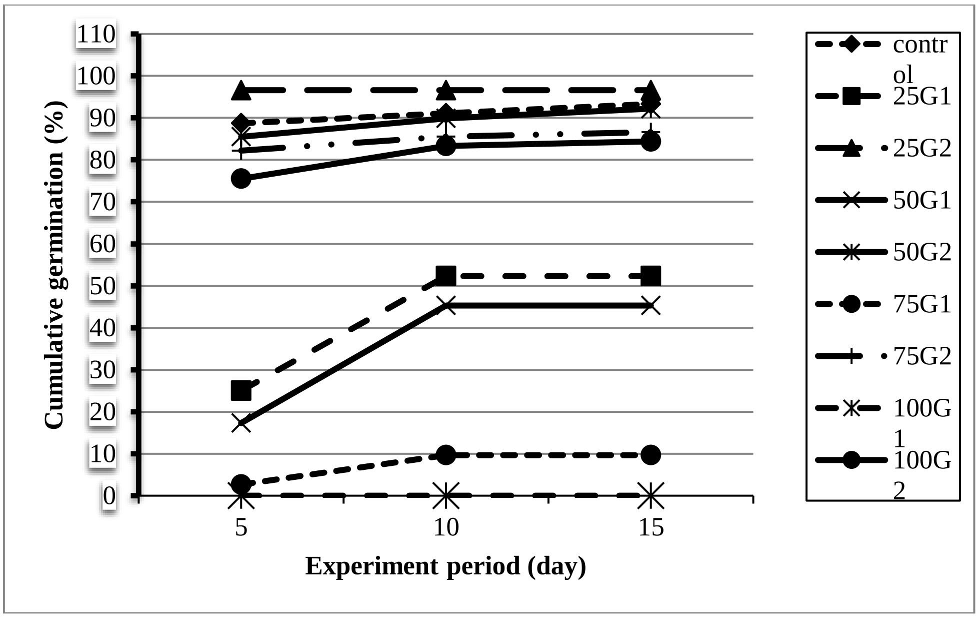

Figure 2: Cumulative germination percentage and days to 50% of germination of *N. sativa* seeds presoaked in different salinity concentrations (G1) or in distilled water (G2) through the period of the experiment

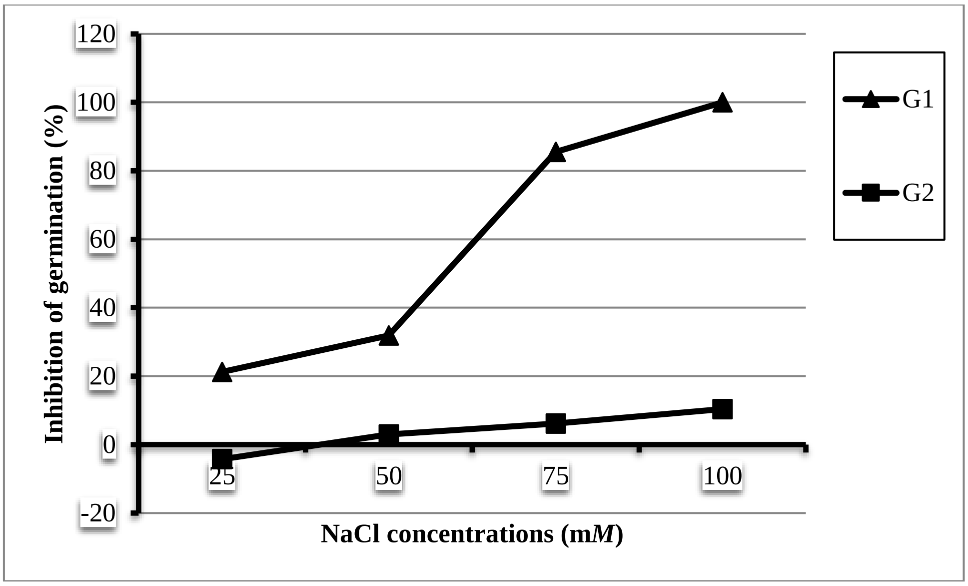

Figure 3: Percentage of germination inhibition of *N. sativa* seeds presoaked in different salinity concentrations (G1) or distilled water (G2)

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تأثير النقع المسبق بالماء المقطر على انبات بذور الحبة السوداء (*Nigella sativa* L.) تحت إجهاد الملوحة

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الملخص

نُفذِت التجربة تحت ظروف المختبر لدراسة تأثير إجهاد الملوحة و النقع المسبق للبذور على نسبة الإنبات النهائية، عدد الأيام حتى 50% انبات و النسبة المئوية للشفاء لبذور الحبة السوداء (*Nigellasativa*). قبل تعريض البذور لإجهاد الملوحة تم تقسيمها إلى مجموعتين و لمدة 24 ساعة نقعت المجموعة الأولى في تراكيز مختلفة من كلوريد الصوديوم (25، 50، 75 و 100 مللي مول) و نقعت المجموعة الثانية في الماء المقطر .أظهرت النتائج فرقاً عالي المعنوية في تأثير معاملات النقع المختلفة. تأثر إنبات المجموعة الأولى سلباً بالمعاملات الملحية حيث ازداد الانخفاض في الإنبات معنوياً بزيادة التركيز. البذور التي عوملت بالتركيز 25 مللي مول في هذه المجموعة وصلت إلى 50% إنبات في اليوم العاشر ب في حين فشلت البذور في بقية المعاملات الملحية حتى نهاية مدة التجربة. بذور المجموعة الثانية اعطت نسبة إنبات عالية في جميع المعاملات الملحية وحققت بذور هذه المجموعة أكثر من 50% إنبات خلال الأيام الخمس الأولى من فترة التجربة. النسبة المئوية لمعاملة الشفاء للبذور غير المنبتة كانت منخفضة جداً في كلا المجموعتين مما يعطي مؤشراً على التأثير السمَي للملوحة على إنبات و حيوية البذور. من نتائج هذه التجربة تم استنتاج انَّ إنبات بذور الحبة السوداء بالملوحة يزداد بزيادة التركيز وأنّ النقع المسبق للبذور في الماء المقطر يحسن من التأثيرات السلبية لإجهاد الملوحة.

الكلمات المفتاحية: الإنبات النهائي، النقع المسبق، ماء مقطر، الحبة السوداء، الملوحة.