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Effects of Hydro and Hormonal Seed Priming on Seed Germination of Milk Thistle under Saline Stress Condition

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Abstract

Salinity is an abiotic stress which has harmful effects on germination of many plants. Therefore, high germination rate and vigorous early growth under salty soils is preferred. Seed priming is a way to increase salt tolerance of plants. An experiment was conducted to investigate the effect of seed priming on germination of milk thistle under salinity condition. The treatments were 4 levels of seed priming (no priming, distilled water as hydro priming and 0.5 and 1.0 mM salicylic acid) and 5 levels of salinity (0, 40 and 80 mM NaCl and 40 and 80 mM $CaCl_2$). The experiment arranged as a factorial in a completely randomized design (CRD) with three replications. Results showed that salinity decreased germination percentage and germination rate to about 16 and 32% in 80 mM $CaCl_2$ level compared to control, respectively. The highest mean germination time (5.7 day) were belonged to 80 mM $CaCl_2$. Radicle and plumule length significantly decreased by 80 mM NaCl and 40 and 80 mM $CaCl_2$. The lowest seedling weight and seed stamina observed in 80 mM $CaCl_2$. 0.5 mM salicylic acid improved all traits except mean germination time as compared to control. Salicylic acid (0.5 mM) improved radicle length under 0, 40 and 80 mM NaCl salinity levels as well as increased plumule length at the 0 and 40 mM NaCl salinity conditions.

Keywords: germination, salicylic acid, salt stress, seed priming, *Silybum marianum*

Introduction

Milk thistle (*Silybum marianum* L.) is an annual or biannual plant of the Asteraceae family. This plant is known commercially as a medicinal plant in Europe, Egypt, China, Argentina and Iran but it has been reported as a deleterious weed in some countries (Khan *et al*., 2009; Shokrpour *et al*., 2007; Zehtab-Salmasi and Moghbeli, 2008). Milk thistle is used for treatment of some disorders such as liver and gastrointestinal disease and poisoning caused by several funguses (Dvorak *et al*., 2003; Ram, *et al*., 2000). Seeds of this plant are commonly used as a medicinal drug. Its therapeutic properties are due to the presence of silymarin. The seeds contain the highest amount of silymarin, but the whole organs of plant are used medicinally (Geneva *et al*., 2008; Veres and Tyr, 2012).

Soil salinity as one of the main factors limit agricultural production and have been affected almost 80 million hectares of arable lands in arid, semi-arid and seaside regions of the world (Abo-Kassem, 2007; Allakhverdiev *et al*., 2000; FAO, 2008; Liopa-Tsakalidi *et al*., 2011; Mohammadi, 2009; Yamaguchi and Blumvald, 2005). Salinity is defined as the presence of high levels of minerals (Cations such as K, Mg, Ca, Na and anions such as NO, HCO, SO, Cl) in water and soil (Tanji, 1995). Seed germination and seedling growth are the most sensitive stages in the biological life cycle of the plants. These stages affected by environmental factors such as temperature, soil moisture and salinity (Abo-Kassem, 2007; Cheng and Bradford, 1999; Khan and Gulzar, 2003). Salt and osmotic stresses are serious factors limit seed germination and are responsible for delayed seed germination and seedling establishment (Mohammadi, 2009; Thiam *et al*., 2013). Salinity affects the germination rate and percentage, seedling growth and many germination parameters such as germination index, radicle and plumule length, fresh and dry weight etc. in different methods depending on the plant species (El-Keblawy and Al-Rawai, 2005; Demir and Mavi, 2008; Sayar *et al*., 2010; Thiam *et al*., 2013; Zahedi *et al*., 2011).

Seed priming as a pre-sowing technique can improve radicle emergence, germination rate, germination vigor, seedling establishment and yield by making changes in metabolic activities in the seeds of many crops (Bodsworth and Bewley, 1981; Dell Aquila and Tritto, 1991; Taylor and Harman, 1990). Different priming techniques such as hydropriming, osmopriming, halopriming and hormone priming are used to improve seed germination and seedling growth (Basra *et al*., 2002; Carvalho *et al*., 2011; Eisvand *et al*., 2011). Priming is a procedure that partially hydrates seed in a specific environment, followed by drying of seed, so that germination processes begin, but radicle emergence does not occur (Dell Aquila and Tritto, 1991; Giri and Schillinger, 2003).

Salicylic acid (SA) as an endogenous regulator of plant metabolism plays an important role in plant defense mechanisms against adverse environmental conditions. It is well documented that SA is involved in biotic and abiotic stress (Dat *et al*., 1998; Shakirova *et al*., 2003; Yalpani *et al*., 1994). SA may affect directly on specific enzymes function or may activate the genes responsible for protective mechanisms (Horvath *et al*., 2007). It is found that SA can enhance activity of antioxidant enzymes like catalase and peroxidase to cope with oxidative stress. Application of exogenous SA enhanced the drought and salt stress resistance of plants (Senaratna *et al*., 2000; Tari *et al*., 2002). Application of SA for seed priming of different crops to improve seed germination and reduce the effects of soil salinity has been reported in many studies (Entesari *et al*., 2012; Erdal *et al*., 2011; Khan *et al*., 2009; Misra and Misra, 2012).

Farahmandfar *et al*. (2013) reported that seed priming of fenugreek by salicylic acid improved the dry weight and length of plumule and radicle under salt stress. Farhbakhsh (2012) also reported that application of 0.25 and 0.5 mM of salicylic acid for seed priming of fennel were effective on germination percentage and rate, seed stamina index, hypocotyl and radicle length, fresh and dry weight under different water potentials (0, -2, -4 and -6 bar). Increase the tolerance to salinity by seed priming with salicylic acid reported in faba bean genotypes (Azooz, 2009). Dry weight of seedlings of wheat raised from seeds primed with SA improved as compared to the seeds non-treatment of SA under non salinity and salinity conditions (Bahrani and Pourreza, 2012).

Since, salinity is one of the most important factors that limit seed germination and production of many crops, and seed priming is a technique for decrease effects of salinity, so, this research was carried out to evaluate the effect of seed priming with water and different concentrations salysilic acide on germination and seedling growth behaviors of milk thistle under salinity stress conditions.

Materials and methods

This study was conducted in a seed laboratory of Agronomy and Breeding Plants Department, University of Maragheh, Maragheh, Iran in 2013. The experiment with seeds of milk thistle was conducted as a factorial in a completely randomized design (CRD) with three replications. Treatments included 4 levels of seed priming (no priming as control), hydro priming (HP) and 0.5 & 1.0 mM salicylic acid (SA)) and 5 levels of salinity (0, 40 and 80 mM sodium chloride (NaCl) and 40 and 80 mM calcium chloride ($CaCl_2$)). Before seed planting, salicylic acid solutions in three concentrations (0, 0.5 and 1.0 mM) were prepared. Similar seed size and weight were surface sterilized in 5% (v/v) sodium hypochlorite for 5 min to eliminate microorganisms and thoroughly washed with sterile tap water several times (Khan, *et al*., 2009; Korkmaz, 2005). Then 50 grams of sterilized seeds were immersed in 250 mL of the SA solutions and distilled water (with the ratio 1:5 gmL^{-1}) for 6 hours at room temperature. Thereafter, the seeds from each solution were washed in a sieve and rinsed under running tap water for one minute and left to surface dried on filter paper under room conditions until their moisture content reached to the original weight (Carvalho *et al*., 2011).

Before placing the seeds in the seed bed, the petri dishes and filter papers were sterilized into Autoclave at 121 °C for 20 min. The 25 seeds from each SA solution and without priming collection were transferred on Whatman filter paper No. 1 in sterilized Petri dishes (9 cm diameter) separately and 5 ml of each saline solution were added to related treatment. All petri dishes containing seeds were sealed to prevent the loss of moisture and avoid contamination. Finally, the petri dishes containing seeds were transferred to a growth chamber at a day/night cycle of 16h/8h at 25 °C. Seeds were watered with desired concentration of salinity treatments that are already prepared as needed. A seed germination scored when root length reached 2 mm (AOSA, 1990; Bewley and Black, 1994). Germinated seeds counted daily until complete germination and no further germination occurred.

Data were recorded daily on germination for 14 days and finally on various aspects of seed dry weight of seedling, radicle length, plumule length. Radicle, plumule and seedlings Dry weights were determined by drying the plant material at 70 °C for 24 h in an oven prior to weighting. Germination percentage (GP), germination rate (GR), mean germination time (MGT) and seed stamina index (SSI) were calculated using the following formula:

$GP = (n/N)*100$ (ISTA, 2013)

$GR = \sum (ni/Di)$ (Maguire, 1962)

$MGT = \sum Dn / \sum n$ (Ahmad *et al*., 2012; Ellis and Roberts, 1981)

$SSI = [GP*(PL+RL)]/100$ (Abdul-Baki and Anderson, 1970)

n: number of seeds that were germinated (on the day D), N: total number of seed in each petri dishes, ni: number of germinated seeds in each numeration, D: number of days counted from the beginning of germination. $\sum n$: total number of germinated seed, and PL: average of plumules length, RL: average of Radicles length.

Data obtained from experiment were subjected to analysis variance using MSTATC software and mean separation was performed by Fisher's least significant difference (LSD) test if F test was significant at $p<0.05$. And the Excel software was used for drawing diagrams.

Results and discussion

Germination

The ANOVA for germination percentage (GP) and Germination Rate (GR) of milk thistle (Tab. 1) show that the effect of salinity and priming on these characteristics were significant ($p<0.05$ for GP and $p<0.01$ for GR). Data presented in Tab. 2 show that GP was significantly reduced by 80 mM $CaCl_2$ salinity which was 16.3 % less than that of control. There was no significant difference among the control, 40 and 80 mM NaCl and 40 mM $CaCl_2$ in germination percentage value. The GR was the highest at control treatment (Distilled water) compared to other salinity treatments (Tab. 2). The lowest values of GR belonged to the 80 mM $CaCl_2$, 40 mM $CaCl_2$ and 80 mM $CaCl_2$ by 32.5, 15.6 and 11.9% decreases compared to control, respectively.

According to results on Tab. 1, GP and GR were affected by seed priming treatments. So that, the maximum values for GP and GR belonged to 0.5 mM SA by 11.4 and 17.3% increment compared to control, respectively. There were no significant differences between 0.5 and 1.0 mM SA treatments and hydro priming (HP) with control in this regards. Although, the 1.0 mM SA also increased GP compared to the control and HP but these increases were not significant.

Mean germination time (MGT)

Results showed that the effect of salinity on MGT was significant ($p<0.001$). The highest (5.71 day) and the lowest (5.02 day) MGT were recorded for 80 Mm $CaCl_2$ and control, respectively. Differences between other treatments (40 and 80 NaCl and 40 mM $CaCl_2$) were not significant but GMT for them significantly increased compared to control (Tab. 2). MGT response to priming and priming × salinity were not significant (Tab. 1).

Radicle and plumule length

Analysis of variance results showed that radicle length (RL) and plumule length (PL) of seedlings were significantly affected by salinity, seed priming and interaction between these two factors (Tab. 1). The response of radicle length to salinity and seed priming was the same with plumule length. The length of radicle and plumule of milk thistle decreased significantly ($p<0.001$) with increasing in salinity concentration from 40 mM to 80 mM at both NaCl and $CaCl_2$ salinity solutions (Tab. 2). The highest RL and PL obtained for the control treatment that had no significant differences with 40 mM NaCl treatment. On other hand the lowest values of these parameters observed in 80 mM $CaCl_2$ treatment with 60.2 and 59.8 % decrement than those of control for RL and PL respectively.

Seed priming had a significant effect on radicle and plumule length (Tab. 3). Seed priming with SA at 0.5 mM

Tab. 1. Analysis of variance for seed priming and salinity effects on milk thistle seed germination

Source of variation	df	Germination percentage	Germination rate	Mean Germination Time	Radicle length	Plumule Length	Seedling dry weight	Seed Stamina Index
Salinity	4	250.62^{*}	2.24^{**}	0.79^{***}	3909.5^{***}	214.9^{***}	2.93^{*}	42.3^{***}
Priming	3	232.99^{*}	0.96^{**}	0.08^{ns}	279^{***}	46.7^{***}	2.49^{*}	6.3^{***}
Salinity×Priming	12	8.4^{ns}	0.19^{ns}	0.05^{ns}	70.3^{*}	15.6^{*}	1.18^{ns}	0.5^{ns}
Error	40	67.03	0.15	0.03	28	6.13	0.86	0.39
CV (%)		13.17	13.6	3.75	11	20.64	8.2	13.38

ns: Non-significant, *, ** and ***: Significant at α=0.05, α=0.01 and α=0.001, respectively. CV: Coefficient of variation

Tab. 2. Mean comparisons of effect of salinity on milk thistle seed germination behaviors

Treatment	Germination percentage	Germination rate (seed/day)	Mean Germination Time (day)	Radicle length (mm)	Plumule length (mm)	Seedling dry weight (mg)	Seed Stamina Index
Control	66.2^{a}	3.26^{a}	5.02^{c}	63.6^{a}	16.7^{a}	11.71^{a}	6.56^{a}
40mM NaCl	66.6^{a}	3.23^{a}	5.19^{b}	60.6^{ab}	15.9^{a}	11.52^{a}	6.22^{a}
80 mM NaCl	61.5^{ab}	2.87^{b}	5.22^{b}	31.8^{c}	10.6^{b}	11.15^{ab}	3.27^{c}
40mM $CaCl_2$	61^{ab}	2.75^{b}	5.25^{b}	59.1^{b}	9.9^{b}	11.61^{a}	5.23^{b}
80 mM $CaCl_2$	55.4^{b}	2.2^{c}	5.71^{a}	25.3^{d}	6.7^{c}	10.5^{b}	2.26^{d}
LSD (0.05%)	6.75	0.32	0.16	4.36	2.04	0.76	0.50

Means followed by the same letter in each column are not significantly different according to LSD Test at 5 % level

Tab. 3. Mean comparisons of effect of salicylic acid on milk thistle seed germination behaviors

Treatment	Germination percentage	Germination rate (day)	Radicle length (mm)	Plumule length (mm)	Seedling dry weight (mg)	Seed Stamina Index
Control	60.2^{b}	2.66^{b}	45.2^{b}	9.6^{c}	10.81^{b}	4.17^{b}
HP	58.0^{b}	2.63^{b}	48^{b}	11.8^{b}	11.29^{ab}	4.4^{b}
SA (0.5 mM)	67.07^{a}	3.12^{a}	54.2^{a}	13.7^{a}	11.81^{a}	5.63^{a}
SA (1.0 mM)	63.33^{ab}	3.03^{a}	45^{b}	12.9^{ab}	11.29^{ab}	4.62^{b}
LSD (0.05%)	6.04	0.28	3.9	1.83	0.68	0.45

HP: hydro priming, SA: Salicylic acid.
Means followed by the same letter in each column are not significantly different according to LSD Test at 5 % level

concentration significantly increased RL compared to other treatments. There was no significant affect among control, hydro priming and SA (1mM) treatments. The difference in PL was statistically significant between control and all seed priming treatments. The concentration of 0.5 mM SA caused an increment of 29.9 % in PL compared to control.

Mean comparisons showed that treatment of milk thistle seeds with SA (0.5 mM) could prevent the decrease in radicle length caused by salinity stress at 40 and 80 mM NaCl. But seed priming with 0.5 mM SA at other salinity levels did not show such results (Fig. 1). Regarding to PL 0.5

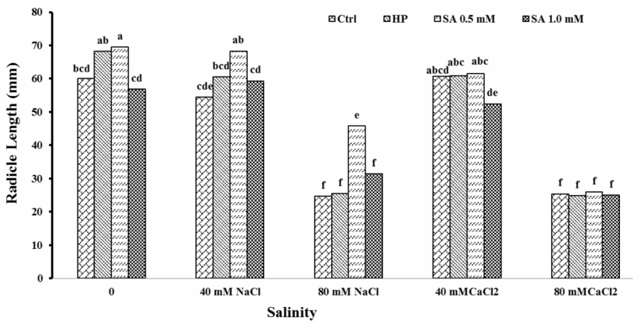

Fig. 1. Mean comparisons of the interaction of priming and salinity on radicle length of milk thistle. Ctrl: control (no priming), HP (hydro priming), SA (salicylic acid). Different letters on columns refer to significant differences between treatments at 5 % level according to LSD Test

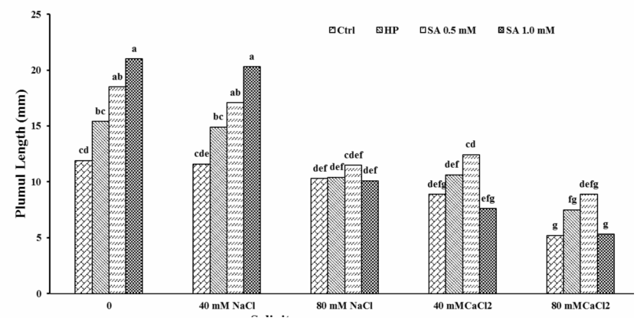

Fig. 2. Mean comparisons of the interaction of priming and salinity on plumule length). Ctrl: control (no priming), HP (hydro priming), SA (salicylic acid). Different letters on columns refer to significant differences between treatments at 5 % level according to LSD Test

and 1 mM SA seed priming treatments only at level of 40 mM NaCl salinity showed such behavior (Fig. 2). Moreover, seedling pretreatment with 0.5 mM SA had the highest value of these traits under normal and stress condition (Fig. 1 and 2).

Seedling dry weight

As shown in Tab. 1 the effects of salinity and seed priming on seedling dry weight were significant (p<0. 05). The salinity of 80 mM $CaCl_2$ caused remarkably decreases in mentioned trait compered to control (Tab. 2). The greatest increase in seed dry weight was observed in seed priming with SA at concentration of 0.5 as 9.3 % compared to non-primed seedlings (Tab. 3). The integration of salinity and seed priming on this trait was not significant.

Seed stamina index

According to data in Tab. 1 Seed stamina index (SSI) was significantly affected by salinity and seed priming. This trait decreased significantly under salinity of 80 mM NaCl, 40 and 80 mM $CaCl_2$ by 50.1, 20.3 and 65.5 % when compared to the control respectively (Tab. 2). On the other hand, SSI increased with priming of seeds by 0.5 mM SA significantly compared to other treatments. Treatments of Hydro priming and 1 mM SA did not significantly increase the SSI compared to the control (Tab. 3). The interaction of salinity and seed priming on SSI did not significant in conditions of our study.

The results of this study showed that salt stress caused significant decreases in germination and seedling growth of milk thistle. So that, the greatest decrease in the germination percentage and germination rate was observed in 80 mM $CaCl_2$ treatment. GP in concentrations of 40 and 80 mM NaCl and 40 mM $CaCl_2$ salinity and GR in 40 mM NaCl were not significantly reduced compared to control. From these results, it seems that those traits are partly salt-tolerant at those concentrations of salinity. A negative effect of salinity on germination of seed has been reported for many plants (Bahrani and Pourreza, 2012; Jamil *et al.*, 2006; Sivritepe *et al.*, 2003). Such a responses of seeds to the excess salinity might be related to the inhibitory effect of the solution low osmotic potential and/or ionic toxicity (Zhu, 2002). So, reduced water uptake by seeds under salt stress decreased metabolic and physiological processes in seeds. Results showed that seed pretreatment with SA significantly increased GP and GR compared to control. As the greatest values of them were obtained from 0.5 mM SA. These results are in agreement with those obtained by other researchers such as Farahbakhsh (2012) on fenel, Bahrani and Pourreza, (2012) on wheat, Dallali *et al.* (2012) on Sulla (*Hedysarum carnosum* and *Hedysarum coronarium*). The mechanism that salicylic acid enhances seed germination is not yet clearly understood (Jamshidi-Jam *et al.*, 2012), but according to Nun *et al.* (2003), salicylic acid can inhibit the activity of catalase. Reduction of catalase activity lead to increased hydrogen proxide that it can improve some seeds germination. It is possible that salicylic acid stimulates the seed germination via bio-synthesis of GA and acts as a thermogenesis inducers (Shah, 2003). Moreover increased germination rate due to seed priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds (Farooq *et al.*, 2005; Khan *et al*, 2009).

The different concentrations of both saline solutions led to increase the mean germination time of milk thistly seeds, as the highest average of MGT recorded for seeds under 80 mM calcium chloride condition. In general, salinity causes an increase in mean germination time, this may be due to effects of Na^+ and Cl^- in the decrease of seed germination rate that was previously discussed. Increasing MGT of seed in salinity condition reported for safflower (Jamshidi-Jam *et al.*, 2012), wheat (Afzal *et al.*, 2006) and mustard genotypes (Sharma *et al.*, 2013). MGT was unaffected by all priming treatments and this result is consistent with the findings of Afzal *et al.* (2005) on wheat.

In the present study, salt stress at 80 mM NaCl and 40 and 80 mM $CaCl_2$ causes a significant decrease in radicle and plumule length and seed stamina index, but seedlings dry weight significantly decreased compared to control only at 80 mM $CaCl_2$ salinity solution. Such a finding demonstrated that seedling weight of milk thistle can be partly tolerant to salinity. Decreasing of radicle and plumule length, seedling dry weight and seed stamina index as a result of salinity stress has been informed in several plants (Bahrani and Pourreza, 2012; Cicek and Cakirlar, 2002; Elouaer and Hannachi, 2012; Khan *et al.*, 2009; Pasandipour *et al.*, 2012). Reduced growth of these traits may be due to toxic effects of ions (Na^+ and Cl^-) that harmfully affect on plant metabolism as well as unbalanced nutrient uptake by the seedling. High salinity may inhibit radicle and plumule elongation due to slowing down the water uptake by plant (Grieve and Fujiyama, 1987; Werner and Finkelstein, 1995).

Results also showed that seed priming with 0.5 mM SA significantly increased radicle length (RL), seedling weight (SW) and seed stamina index (SSI) compared to control. But in the case of plumule length (PL) both hydro priming and salicylic acid (0.5 and 1 mM) were led to a significant increase in this trait (Tab. 3). Increase of those traits may be due to early emergence induced by priming treatment as compared to un-primed seeds (Khan *et al.*, 2009). These results are also in confirmation with Farahbakhsh (2012) and Ghoohestani *et al.* (2012) showed that seeds primed with salicylic acid resulted to improve of plant growth characters. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Sivritepe *et al.*, 2003).

Our results also showed that interactions of salinity and priming on RL and PL were significant. So that, seed priming with 0.5 mM SA at the salinity levels of 0, 40 and 80 mM NaCl led to an increase in RL compared to control. Regarding to PL, 0.5 and 1 mM SA seed priming treatments only at 0 and 40 mM NaCl salinity levels showed such a behavior (Fig. 2). According to these results treatment of milk thistle seeds with SA could prevent the decrease in plumul and radicle length under low salinity stress conditions. Khan *et al.* (2009) presented the same results by observing that priming of the pepper seeds with SA significantly improved radicle and plumul length under salinity and non-salinity stress conditions. These results are also in agreement with those obtained by other authors that showed saline tolerance was increased in seeds were primed with SA as indicated by shoot and root length (Afzal *et al.*, 2005; Pasandipour *et al.*, 2012; Tari *et al.*, 2002). Salinity tolerance in seeds primed with 0.5 or 1 mM salicylic acid might be due to increased activities of alpha amylase and proteinase in endosperm and the contents of soluble sugar, protein and free amino acids under stress conditions (Afzal *et al.*, 2005; Zhang *et al.*, 1999).

Conclusions

It is well established that seed priming is a new method for improvement of seed germination under stress and non-stress condition. Our finding also showed that seed priming especially hormonal seed priming by SA improved seed germination parameters except MGT that were investigated in this study. On other hand, saline tolerance of seeds primed with SA especially at 0.5 mM concentration increased as indicated by radicle and plumule length. Moreover, from results of this study, we conclude that germination of seeds of milk thistly under saline stress with calcium chloride showed less resistance than sodium chloride.

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