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Influence of Different Priming Materials on Germination and Seedling Establishment of Milk Thistle (*Silybum marianum*) under Salinity Stress

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Abstract: Soil salinity is a major environmental stress which adversely affects germination and seedling establishment in a wide variety of crops. However, priming of seeds with different materials may helpful to alleviate the deleterious effects of salinity and enhance germination in salt stress environment. In present experiment we investigated the effects of various priming materials (gibberellic acid, manitol, NaCl and distilled water) on germination and seedling establishment of milk thistle grown at different salinity levels (0, 2.5, 5.0, 7.5 and 10.0 dS m^{-1}). Results revealed that different growth traits (like germination percentage, plumule and radicle length, plumule fresh and dry weight) significantly (p = 0.05) decreased with increasing salinity but maximum reduction was found at 10.0 dS m^{-1} EC. However, priming of seeds with different materials particularly GA_3 and NaCl and amplification of α-amylase activity were useful for alleviating salt stress effects and improving germination and seedling establishment under salt stress. These results warranted further verification for confirmation.

Abbreviations Used: FUSR (fraction of utilized seed reservoir), GA_3 (gibberellic acid), GR (germination rate), MGT (mean of germination time), SDW (seed dry weight), SFDW (seedling fresh and dry weight), SOD (super oxide dismutase), SRUR (seed reservoirs using rate).

Key words: α- amylase · Germination traits · Gibberellic acid · Manitol · Milk thistle

INTRODUCTION

Milk thistle (*Silybum marianum* L.) is an annual plant belonging to the Asteraceae family, grows natively in the Mediterranean and is widespread in other regions in the world including Iran [1]. Since the 4th century B.C. milk thistle extracts were used as medicine and became a favored remedy for *hepatobiliary* diseases in the 16th century [2]. The silymarin flavonolignans protect liver cells against oxidative stress and tissue damage [3,4].

Seed germination, one of the most critical phases of plant growth and development, is adversely affected under saline conditions [1]. Germination of seeds, one of the most critical phases of plant life [5], is greatly influenced by salinity [6]. Salinity is reported not only to delays, but also decreases germination of most crops. Lower levels of salinity delay germination, whereas higher levels reduce the final percentage of seed germination [7]. In addition, salinity imposes other stresses such as ion toxicity on plants, as a result of ion entry in excess of appropriate concentrations and nutrient imbalances, osmotic stress and oxidative damage [8]. Improved seed priming techniques are used to reduce emergence time, accomplish uniform emergence, better allometric (changes in growth of plant parts over time) attributes and requisite stand in many horticultural and field crops [9,10]. These techniques include hydropriming, osmoconditioning, osmohardening, hardening and hormonal priming or soaking prior to sowing [9,11]. Effects of priming or pre-treatment of seeds persist under suboptimal field conditions, such as salinity [12,13], low or high temperature [14] and low soil moisture availability [15]. Different seed priming methods have been successfully integrated [16,17].

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The purpose of this research was to evaluate the efficiency of different priming materials to mitigate salt stress effects and improve germination and seedling establishment of milk thistle at various levels of salinity.

MATERIAL AND METODS

A laboratory experiment was conducted at the University of Mohaghegh Ardabili (38°15"N; 48°15"E; altitude of 1,350 m, northwest of Iran) on priming of milk thistle with different materials under salinity stress. Treatments included seed priming with different materials (control, GA_3, manitol, NaCl and distilled water) at various levels of salinity (0, 2.5, 5.0, 7.5 and 10.0 dS m^{-1}) which arranged on a completely randomized design with three replications. In the first step, 20 g of medicinal plant seeds was weighted and placed in a plastic vessel. The priming solutions were added to seeds. After 24 h, the seeds were dried at the laboratory temperature at 25°C. In the second step, 50 seeds were placed in 10 cm petridishes and then 5 mL of salt solutions (0, 2.5, 5, 7.5 and 10 dS m^{-1}) were added to every dish. The purpose of using salt solutions was creating artificial salinity stress. The counting of germinated seeds was done regularly after every 24 h and the appearance of 2 mm or more of radicle was considered as germination. Germination test was ended after 14 days when the number of germinated seeds was equal in two sequential counting. Following traits were observed:

- Germination percentage was evaluated by counting the numbers of normal seedlings at the end of standard germination test.
- The germination rate (GR, seed number day^{-1}) was calculated by using the data related to germinated seeds through germination course and the formula $\sum \frac{ni}{di}$ where ni is the number of germinated seeds and di is the day of counting [18].
- Mean of germination time (MGT, day) that is the reverse of germination rate $\sum \frac{di}{ni}$ [18].
- Seedlings fresh and dry (SFDW) and fresh weight (plumule and radicle).
- Plumule and radicle length.
- Residual seed dry weight (RSDW, without plumule and radical).
- Seed reservoirs using rate (SRUR, mg $seed^{-1}$), seed reservoirs using efficiency (SRUE, mg mg^{-1}) and fraction of utilized seed reservoirs (FUSR, mg mg^{-1}) were calculated according to Soltani *et al.* [19]:

$$SRUR = SDW - RSDW$$
$$SRUE = SFDW/SRUR$$
$$FUSR = SRUR/SDW$$

Where, SDW is the seed dry weight.

Alpha-amylase (EC 3.2.1.1) activity was determined after 5 days of germination according to Doman *et al.* [20]. Seeds were homogenized in 60 mM phosphate buffer (pH 6.8), filtered and centrifuged at 12000 g for 15 min. Enzyme activity was determined in a reaction medium containing 60 mM phosphate buffer (pH 6.8), 400 µg mL^{-1} calcium chloride and 500 µg mL^{-1} starch. Enzyme extract (1 mL) was added to the reaction medium following incubation in a water bath for 20 min. Alpha-amylase activity was assayed at 620 nm using starch and demonstrated as µg of starch min^{-1} g fresh matter.

The normality test and analysis of variance of data were conducted by the MSTATC and SPSS softwares and comparison of means was performed by Duncan's multiple range test [21].

RESULTS AND DISCUSSION

Effect of salinity levels on all studied traits and also the effect of seed priming on germination percentage, plumule and radicle length, plumule fresh and dry weight were significant. Also, interaction between seed priming and salinity on germination rate, germination percentage and plumule length was significant. The germination rate reduced by increasing salinity, such that the maximum germination rate was observed in control that was in the same group with 2.5 dS m^{-1} salinity level (Table 1). These results were consistent with Ghoulam and Fares [7] and Soltani *et al.* [22]. Many researchers had reported that reduction in germination rate under salinity stress was due to the reduced water potential [23]. The maximum germination rate was observed at zero salinity level and priming with GA_3 and the lowest was observed at 10 dS m^{-1} salinity level and priming with manitol. Seed priming with GA_3 might cause the acceleration in metabolic reactions before germination and make germination of cultivated seeds possible under salinity stress [24]. Since manitol is made up of just one ionic particle, so, according to Van't Hoff's law $\pi = icRT$ (where π= osmotic pressure, ic= solution molarity, R=constant value for gases and T=absolute temperature in°K) it could not have great effect on osmotic pressure and also its behavior was like the conditions of control.

Table 1: Comparison of means for salinity and priming on milk thistle germination traits

	GR (seed day^{-1})	MGT (days seed^{-1})	GP (%)	PL (cm)	RL (cm)	PFW (g)	RFW (g)	PDW (g)	RDW (g)	PDW RDW	PFW RFW
Salinity (dS m^{-1})											
Control	6.16a	0.20b	75.18a	4.00a	7.68a	0.24a	0.037a	0.17a	0.026a	5.03b	5.21b
2.5	6.05a	0.17b	74.98a	3.93a	7.20a	0.18b	0.025b	0.12b	0.018b	9.58b	9.91b
5	5.33ab	0.19b	67.86b	2.20b	4.25b	0.13c	0.018c	0.09c	0.013c	7.69b	7.95b
7.5	4.51b	0.23b	62.78c	1.59c	1.97c	0.14c	0.008d	0.10c	0.005d	19.01a	19.67a
10	3.06c	0.47a	49.72d	1.03d	1.22c	0.09d	0.004d	0.06d	0.003d	23.50a	24.31a
Priming											
Control	-	-	65.80b	2.00c	3.37c	0.14b	-	0.099b	-	-	-
GA_3	-	-	69.57a	3.20a	5.52a	0.18a	-	0.127a	-	-	-
Manitol	-	-	60.28c	2.43b	4.44b	0.14b	-	0.099b	-	-	-
NaCl	-	-	70.42a	2.88a	4.97ab	0.19a	-	0.135a	-	-	-
Distilled water	-	-	67.46ab	2.26c	4.29b	0.13b	-	0.095b	-	-	-

Different letters at each column indicate significant differences at 5% probability level. GR: germination rate. MGT: mean of germination time. GP: germination percent. PL: plumule length. RL: radicle length. PFW: plumule fresh weight. RFW: radicle fresh weight. PDW: plumule dry weight. RDW: radicle dry weight.

By increasing salinity levels, MGT increased (Table 1). As MGT is related to the time (day) in which radicle appeared, the lower the MGT, the rapid the germination. Germination percentage decreased by increasing salinity level (Table 1). The decrease of seed germination percentage by increasing salt concentration may be due to the decreasing osmotic potential of solution, increasing toxic ions and changing in the equilibrium of the remobilization of seed reserves. Decreasing germination percentage under the effect of increasing salt concentration was inconsistent with Xiao-Fang *et al.* [25]. In some halophytes, the existence of sodium ions in less quantity might have positive effect on germination of seeds [26]. The observation regarding the effect of priming materials on germination percentage indicated that NaCl treatment resulted in maximum germination which was not significantly (p = 0.05) different from GA_3 priming (Table 1). It had been reported that priming with NaCl increased the activity of superoxide dismutase (SOD) and peroxidase and by increasing the respiration rate, improved the germination percentage and rate [27]. GA_3 might increase synthesis of hydrolytic enzymes in alurun layer and by the activity of these enzymes the seeds convert the transferable metabolites (sucrose and glucose) and transferred them to the embryo [28]. The main factor involved in transfer of soluble compounds, was their solubility in water which was decreased under salinity stress due to reduced water availability [29].

Salinity stress higher than 2.5 dS m^{-1} probably decreased the cell division and expansion and consequently reduced plant growth and development. Mer *et al.* [30], observed that by increasing salinity, radicle length decreased in wheat, barley, pea and cabbage. They noted that the decrease in growth of young seedling by increasing salinity was mostly because of decrease in water absorption by radicle and by accumulation of soluble salts in the cell. The maximum length of plumule and radicle was observed in case of priming with GA_3 that had no significant (p = 0.05) difference from NaCl treated seedlings (Table 1). The longest plumule was recorded in control and priming with GA_3 and the lowest value obtained at the 10 dS m^{-1} salinity level and control priming. At highest level of salinity (10 dS m^{-1}) there was no significant difference (p = 0.05) among various priming materials (Table 2).

By increasing salinity, fresh and dry weight of plumule decreased, which might be attributed to decrease in remobilization of the seed reserves from cotyledons to the embryonic axis. The factors that affected the growth rate of embryonic axis also had affect on the reserves remobilization and transfer from cotyledons to the embryonic axis [31]. The maximum fresh and dry weight of plumule obtained at the priming with NaCl, which had not significant difference (p = 0.05) from GA_3 priming. Priming with NaCl and GA_3 might accelerate some metabolic processes even at low water potential. This improved metabolic activities at germination stage particularly under salinity stress.

Table 2: Mean comparison for salinity and priming interaction on germination traits and plumule length

Salinity(dS m^{-1})	Priming	GR (seed day^{-1})	GP	PL (cm)
Control	Control	6.70abc	77.93ab	2.97cdefg
	GA_3	7.65a	83.66a	5.80a
	Manitol	6.07abcd	60.90bcdefg	4.34abc
	NaCl	3.20abcd	73.86abc	3.97bcd
	distilled water	7.17ab	79.53ab	3.59bcde
2.5	Control	5.52abcd	72.90abc	3.28bcdef
	GA_3	6.61abc	77.36ab	4.82ab
	Manitol	5.72abcd	69.53abcde	3.34bcdef
	NaCl	6.42abc	80.63ab	4.06bcd
	distilled water	6.01abcd	74.50abc	3.54bcde
5	Control	5.17abcd	66.36abcde	1.86fghij
	GA_3	4.95abcd	66.83abcde	2.69defgh
	Manitol	4.34abcd	60.73bcdefg	1.81fghij
	NaCl	6.85abc	76.33abc	2.65defghi
	distilled water	5.34abcd	69.06abcde	2.01efghij
7.5	Control	4.13abcd	62.26bcdef	1.07ij
	GA_3	4.30abcd	60.43bcdefg	1.77fghij
	Manitol	3.51abcd	56.36cdefg	1.74fghij
	NaCl	5.75abcd	70.83abcd	2.20efghij
	distilled water	4.85abcd	64.00abcdef	1.19hij
10	Control	2.80bcd	49.53efg	0.82j
	GA_3	2.43cd	44.56fg	0.92j
	Manitol	1.70d	40.90g	0.93j
	NaCl	4.74abcd	63.40abcdef	1.54ghij
	distilled water	3.61abcd	50.23defg	0.95j

Different letters at each column indicate significant differences at 5% probability level. GR: germination rate. GP: germination percentage. PL: plumule length.

Table 3: Mean comparison of salinity and priming on milk thistle germination traits and α-amylase activity

	SRUR (mg $seed^{-1}$)	SRUE (mg mg^{-1})	FUSR (mg mg^{-1})	α-amylase activity (µg min^{-1} g fresh matter)
Salinity(ds m^{-1})				
Control	0.68a	0.59d	0.65a	2.2a
2.5	0.59b	0.68c	0.51b	1.9b
5	0.43c	0.77b	0.45c	1.4c
7.5	0.27d	0.92a	0.35d	0.98d
10	0.25d	0.98a	0.31d	0.85d
Priming				
Control	0.60b	0.77b	0.53b	2.04b
GA_3	0.71a	0.57c	0.69a	2.6a
Manitol	0.31c	0.88a	0.35c	1.8c
NaCl	0.69a	0.55c	0.68a	1.9c
Distilled water	0.58b	0.79b	0.55b	1.5d

Different letters at each column indicate significant differences at 5% probability level. SRUR: Seed reservoirs using rate. SRUE: seed reservoirs using efficiency. FUSR: fraction of utilized seed reservoirs.

Salinity decreased the fresh and dry weight of radicle. The effect of salinity stress on the ratio of fresh and dry weight of plumule to dry and fresh weight of radicle was significant. The highest ratio of fresh and dry weight of plumule to fresh and dry weight of radicle was observed at 7.5 and 10 dS m^{-1} salinity treatments (Table 1). The reason of this increase was the sensitivity of root to salinity stress at high concentrations. The increase in the ratio of above-ground portion to root in response to increased salinity was also reported in wheat and barley [30,32].

The highest value for SRUR was seen at control salinity level and priming with GA_3 and NaCl, but with increasing salinity, SRUE increased in a constant manner (Table 3). Soltani *et al.* (2008) concluded that the decrease in SRUR and FUSR probably happened due to decrease in GA_3 level and hydrolytic enzymes during germination.

Salinity had pronounced effect on α-amylase activity (Table 3). The results are in accordance with Ashraf *et al.* [33]. Priming with GA_3 increased enzyme activity over the control and the rest priming materials had the lower enzyme activity than the control (Table 3). Priming with NaCl also had the benefit in enzyme stimulation.

Consequently priming with NaCl and GA_3 improved germination and seedling establishment in studied plants. Although priming with NaCl and GA_3 caused an increase in germination percentage of milk thistle at all salinity levels, but this increase was greater at lower levels. It seemed that priming with NaCl increased the activity of SOD and peroxidase and by increasing the respiration rate improved the germination rate and percentage. The germination of primed seeds started earlier than control seeds and under salinity stress seedling emergence was accelerated. Seed priming with NaCl and GA_3 had more germination rate than control and produced more dry matter under the salinity stress. As priming, specially with NaCl is simple and cheap, therefore we can offer this method to the farmers, so they can get better crop stand and synchrony of emergence in medicinal plants under the environmental stresses. However, these results warranted further studies to test and verify the profitability and effectiveness of this method.

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