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N. Ghavami [a] & A. A. Ramin [b]

[a] Department of Horticulture, Shahid Chamran University, Ahwaz, Iran

[b] Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

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Salinity and Temperature Effects on Seed Germination of Milk Thistle

N. Ghavami
Department of Horticulture, Shahid Chamran University, Ahwaz, Iran

A. A. Ramin
Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Abstract: Milk thistle [*Silybum marianum* (L.) Gaertn] is an annual plant belonging to the *Asteraceae* family whose ripe seeds contain flavonoid substances, which are important in the modern pharmaceutical industry. Seed germination is a major factor limiting the establishment of plants under saline conditions. The effect of salinity and temperatures on germination and seedling establishment was studied in two genotypes of milk thistle, an Iranian wild type and German (Royston) type in the laboratory and in the field. Experiments were done with seven salt concentrations [0.1 (control), 1, 3, 6, 9, 12, and 15 dS/m] and three temperatures (15, 25, and 35°C). There were three replications for each treatment, and the experiment was run three times. The results showed that the percentage of germination and the number of normal seedlings at different salt treatment at 15°C were higher than at 25 or 35°C. The mean time to 50% germination was least at this temperature for both genotypes. Results suggested all germination indices and seedling emergence (50%) were achieved at levels up to 9 dS/m salinity at 15°C. Also, seeds at a salinity of 9–15 dS/m will germinate and up to 25% of the control nonstress treatment could emerge at the low temperature of 15°C.

Keywords: Germination, milk thistle, salt stress, seedling emergence, temperatures

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Address correspondence to A. A. Ramin, Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan 84156, Iran. E-mail: aa-ramin@cc.iut.ac.ir

INTRODUCTION

Milk thistle [*Silybum marianum* (L.) Gaertn] is an annual or biennial; it is annual in its native range. It grows well under less fertile soil and may be also found along roadsides, in fields, and in wastelands. Milk thistle has long been used in Europe as a food. Despined leaves were used in salads, whereas stalks, roots, and flowers, were cooked. Seeds were used as a coffee substitute. It has been used as a medicine for more than 2000 years as a milk stimulant and for liver, kidney, and spleen problems; jaundice; gallstones; and menstrual pain (Varma, Talwar, and Gray 1980). The active ingredients in milk thistle seeds are a group of related flavonolignan compounds collectively referred to silymarin (Hammouda 1991).

Milk thistle is very adaptable to many different growing conditions. Conditions such as rainfall, average temperature, and salinity also affect growth, development, and silymarin production. However, germinating seed and standing plants may be influenced highly by salinity and temperatures. Salinity is a determining factor for seed germination. Germination is one of the most critical periods in the life of the plants, when plants are more sensitive to mortality caused by direct action of the high salinity of areas such as saline deserts and salt marshes (Ungar 1987). Many studies have suggested that all growth stages may be affected by saline stress. The start of germination is delayed, germination is reduced, and many seeds remained dormant because of low water potentials under high salinity levels (Chapman 1974; Tobe et al. 2001). However, plant species differ in their sensitivity or tolerance to salts (Brady and Weil 1996). There are many different types of salts and an almost equally diverse set of mechanisms of avoidance or tolerance. In addition, organs, tissues, and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns 1993; Ashraf 1994).

The southwest region of Khuzestan in Iran can be divided into two zones:

i. a northern state without a salt problem in the soil and
ii. a coastal area along the shore of the Persian Gulf with increased salinity in the soil.

The average annual rainfall is about 350 mm in both zones. Milk thistle grows naturally by seed germination in both the coastal area and in the nonsaline and marginal central area. However, the potential of this medical plant to grow and survive in the saline conditions is not fully known. The present investigation was carried out to determine the response of milk thistle to salinity and temperatures.

MATERIALS AND METHODS

Seeds of two types of milk thistle, an Iranian wild type and German cultivated line (Royston), were used for this study. Capitula of the Iranian wild type were collected from populations in Khuzestan along the Persian Gulf coast. Seeds were separated from capitula, cleaned, and dry stored at room temperature. Seeds of the German type (Royston) were obtained from the Research Institute of Forests and Rangelands, Tehran, Iran. Seeds of both genotypes were produced by growing parental plants under the same field conditions and self-pollinating these plants. Specific care was taken to produce seed of similar physiological age and quality.

After surface sterilization with 0.1% sodium hypochlorite for 1 min, germination was carried out by placing 50 seeds on Whatman filter paper in 9-thinsp;cm Petri® dishes containing 15 mL of distilled water (control) or solutions of various salinity levels. A mixture of sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and calcium chloride ($CaCl_2$) in the ratio 2:1:1 was then thoroughly mixed to give electrical conductivities of 1, 3, 6, 9, 12, and 15 dS/m by diluting in water and adjustment by an electrical conductometer (Model K620, Consort, Belgium). (Electrical conductivity of saturated soil of area ranged from 1 to 20 dS/m). The concentration of each ion for the given EC is shown in Table 1. The effect of temperature on germination was determined in the darkness in incubators set at constant temperatures of 15, 25, and 35°C. The Petri® dishes were opened every 12 h to count germinated seeds, which were then discarded, and to add water or salt solution, if necessary. An additional sample from each treatment was provided to measure abnormal seedlings after 25 days from the beginning of the experiment by visual selection, according to ISTA (1999) rules. Germination was considered to have occurred where a radical length of approximately 2 mm had been reached. Final germination was determined at day 16 of incubation when for 2 consecutive days no further seeds germinated. Final germination and rate of germination (1/t) for 50% was calculated according to Ellis, Simon, and Covell (1987). The experiment was carried out using a completely randomized design. There were 2 genotypes $\times 7$ salinity levels $\times 3$ temperatures and 3 replicates. Data were analyzed using Minitab and Excel computing systems. Germination data was transformed (arcsine) before statistical analysis. The analysis of variance procedure was

Table 1. Concentration of each ion in final salt solution for different salinity levels

	Ion concentration (mM)			
EC (dS/m)	Na	Cl	Ca	SO_4
1	6.4	7	2.3	4.4
3	22.6	24	3	5.37
6	27.82	50	3.2	9.5
9	50	97.5	8.5	13.75
12	91.3	100	9	14
15	157.6	152.5	10.75	27.92

used, least-square means were computed, and differences in mean were tested using least square difference (LSD) at the $P < 0.05$ level.

A second experiment was carried out to examine the effect of soil salinity on germination and seedling emergence under ambient air temperature. The same batches of seeds were used for this experiment. Surface soil was collected from a nearby field and passed through a 2-mm mesh screen. This soil is a noncalcareous sandy loam containing 68% sand, 14% silt, and 18% clay. The total organic carbon content was 1%, and pH of soil was 7.5. The electrical conductivity (EC) of the soil ranged from 0.2 to 0.4 dS/m. A mixture of NaCl, Na_2SO_4, and $CaCl_2$ (in the ratio of 2:1:1) was thoroughly mixed with the soil to give electrical conductivities of 1, 3, 6, 9, 12, and 15 dS/m in seven lots of soil, respectively. There was no addition of salt to one lot of soil, and it was maintained as control. The electrical conductivity of the control soil was 1 dS/m. Polyethylene pipes 20 cm in diameter and 30 cm high for each level of soil salinity were each filled with the appropriate salinity. Tap water was added to the soil to achieve field capacity, and soils were then allowed to dry. The soil was then raked, and 100 seeds were sown in each pipe at a depth of about 20 mm. The mean air temperature ranged from 18°C at the time of seed sowing to 13°C at the end of the experiment. Immediately after sowing, soils were watered. Thereafter, watering was carried out on alternate days. Emergence of seedlings was recorded every day. The experimental design was completely randomized with 7 salinity $\times 2$ genotypes and 4 replications for each treatment.

RESULTS

Germination percentages of milk thistle were significantly different between treatments and were highly negatively correlated with salinity levels (Figures 1 and 2). Milk thistle showed a very high germination percentage, close to 95% at 15°C treatments for the two lowest salinities (0.1 and 1 dS/m). At levels greater than 3 dS/m salinity, a slight decrease in germination took place. At 15°C and salinity of 15 dS/m, there were about 70% germinated seeds in both genotypes (Figures 1 and 2). However, with increasing temperatures (25°C) and salinity greater than 3 dS/m, a significant decrease in germination appeared. At 35°C, there were few seeds that germinated even under very low salinity treatments (Figures 1 and 2).

The most critical phase of a germination test is the classification of seedlings into normal and abnormal categories. Seedlings of milk thistle were classified as normal when they had a well-balanced symmetrical growth pattern for all their essential parts. At 15°C, increasing the salinity concentration from 0.1 to 15 dS/m caused a gradual but steady decrease in the percentage of normal seedlings (Figs 3 and 4). But, there was no significant difference in percentage of normal seedlings up to 6 dS/m for both

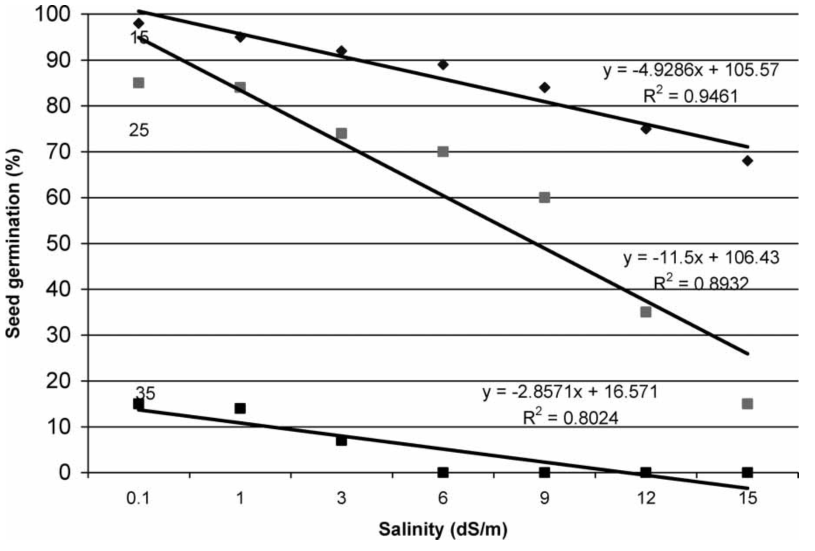

Figure 1. Effects of salinity and temperatures on seed germination of milk thistle (wild type).

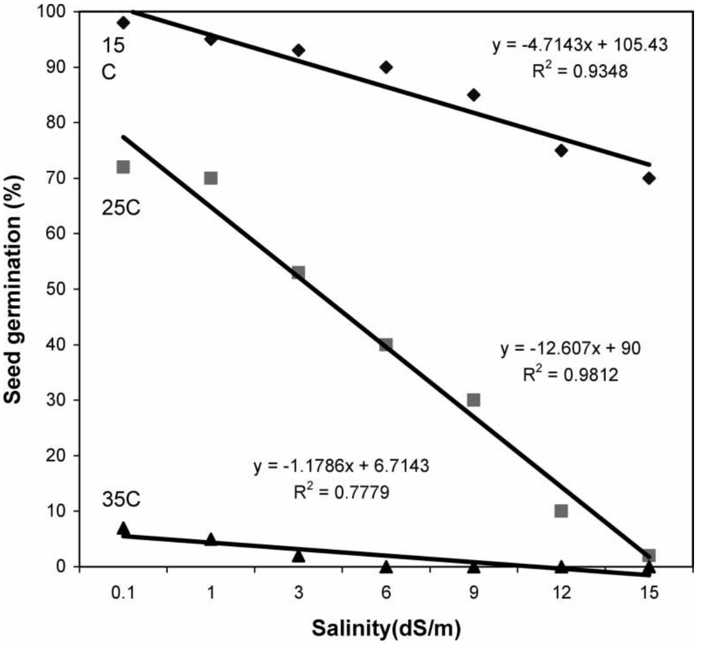

Figure 2. Effects of salinity and temperatures on seed germination of milk thistle (German type).

genotypes. However, both genotypes produced 60% normal seedlings at the salinity of 9 dS/m at the 15°C treatment. Both genotypes at temperatures of 25°C under 6 dS/m or greater produced fewer normal seedlings compared to nonstressed controls. There were a few normal seedlings for plants germinated at 35°C even at very low salinity levels (Figures 3 and 4).

Changes in the rate of germination (1/t) for 50% germination due to the interaction effects of temperature and salt are shown in Figures 5 and 6. There were highly significant relationships between salt concentration and rate of germination for all treatments. The decrease in the rate of germination depended, mostly, on the sensitivity of the temperature stress and on salt concentration. In both genotypes and at 15°C, there was no significant difference in the rate of germination (50%) between control nonstressed seeds up to those in the 6 dS/m salinity treatment. Seeds of milk thistle germinated significantly more rapidly in all salinity concentrations at 15°C. However, at either 25 or 35°C treatment, germination was delayed with increasing salt concentration (Figures 5 and 6).

There were highly significant relationships ($P < 0.01$) between salinity levels and seedling emergence in both genotypes (Figure 7). Generally, increasing soil salinity caused reduction in seedling emergence in two genotypes. In the control soil, 82% of seeds emerged over a period of 9 days. Low soil salinity of 6 dS/m conductivity did not cause any significant effects on seedling emergence in the wild type, but seedling emergence from the soil surface was reduced by about 15% at this salinity level in the

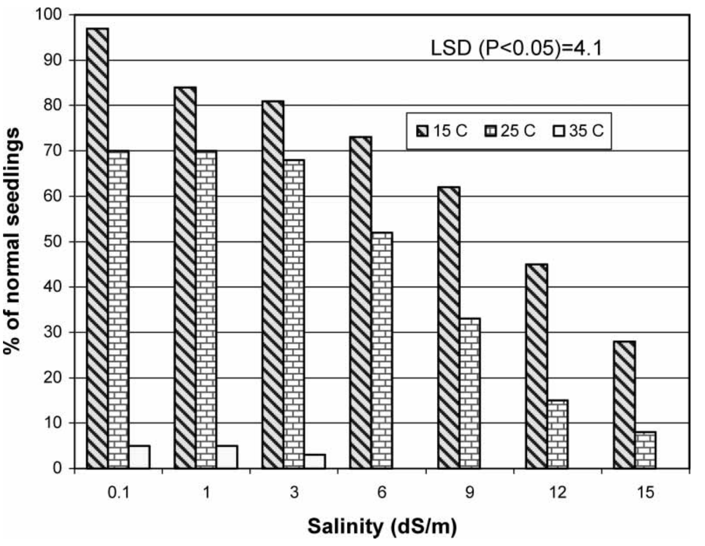

Figure 3. Effects of salinity and temperatures on normal seedlings in milk thistle (wild type).

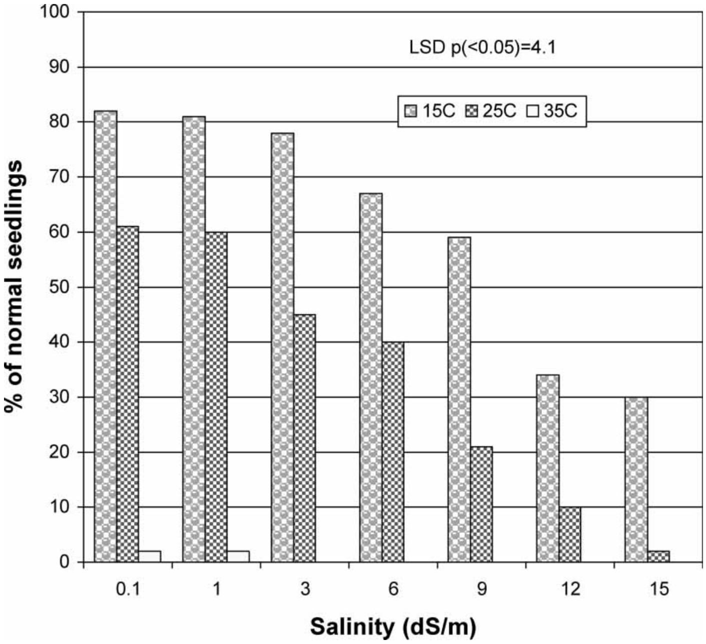

Figure 4. Effects of salinity and temperatures on normal seedlings in milk thistle (German type).

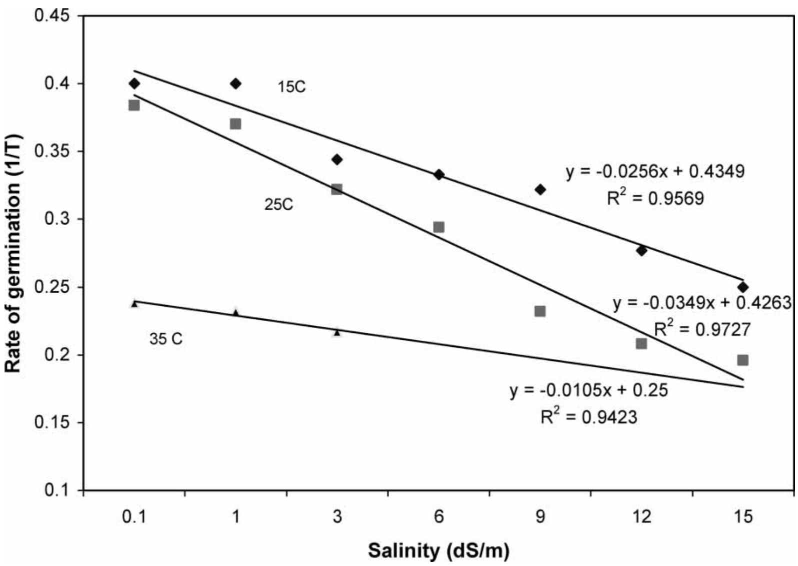

Figure 5. Relationship between salinity and rate of germination (50%) in milk thistle (wild type).

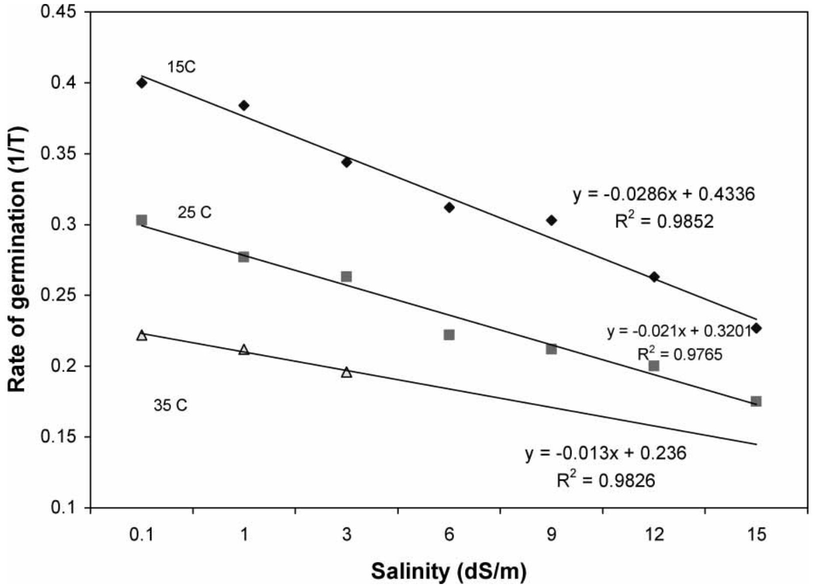

Figure 6. Relationships between salinity and rate of germination (50%) in milk thistle (German type).

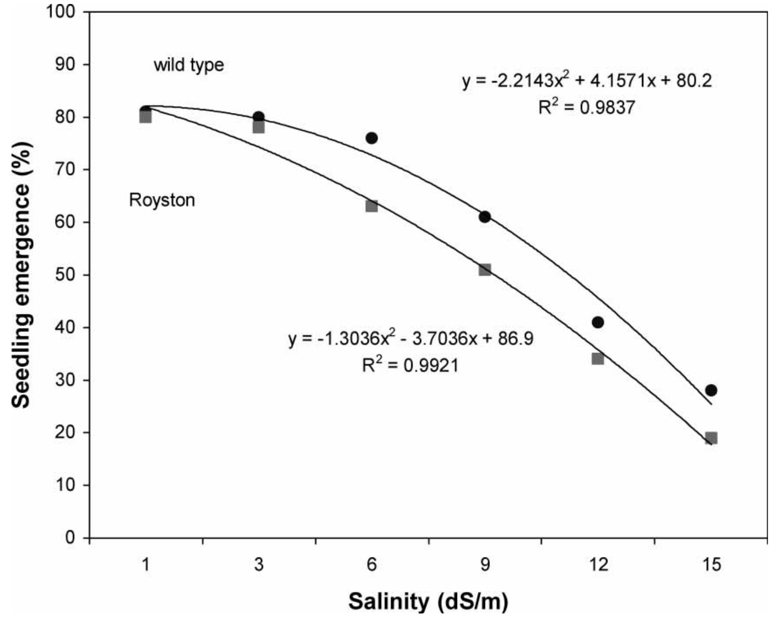

Figure 7. Relationship between salinity and seeding emergence in milk thistle.

German genotype (Figure 7). From the regression equations in Fig. 7, 40% of the seeds of the wild type emerged at a salinity of 13 dS/m compared to 80% of control nonstress seedlings, whereas for German genotype, 40% emerged at about 11 dS/m.

DISCUSSION

The results indicated that all germination characteristics and field establishment of milk thistle under temperature stress and salt stress were reduced, especially under unfavorable temperatures. In general, a higher concentration of salts was detrimental to seed germination, but the critical concentration differed among the temperature treatments. Seeds of both genotypes germinated satisfactorily up to a conductivity of 6 dS/m under 15°C treatment, but at temperature regimes of 25 and 35°C, seeds did so only up to a conductivity of 3 dS/m. This finding is in agreement with those for other plants; Powell, Thornton, and Mitchell (1991) found this in brassica, Zekri (1993) in citrus, Wheeler and Ellis (1994) in onions, and Al-Karaki (2001) in barely.

Salt tolerance during seed germination is a measure of the plant's ability to withstand the effects of high concentrations of salts in the medium. Excessive levels of salt depress the external water potential, making water less available to the seed. Slower seed germination under salt-stressed compared with nonstressed conditions, however, could be due to osmotic and/or ionic effects of the saline germination medium. Physiological investigations to distinguish between these two types of effect have been very limited. However, accumulating evidence suggests that low water potential of the external medium rather than ion toxicity effects is the major limiting factor to germination under salt stress in different crop species (Bliss, Platt-Aloia, and Thomson 1986; Haigh and Barlow 1987; Bradford 1995). Furthermore, in a recent investigation using different ionic and nonionic germination media with identical osmotic potential, it was demonstrated that tomato seed germination was mainly affected by osmotic rather than ionic effects of the medium (Foolad, Hyman, and Lin 1999). Although the effects of high salt content on metabolic process are yet to be fully elucidated, it is thought that the high concentration of salt keeps cell water potential low and reduces protein hydration and enzyme activity (Kramer 1983; Noguchi and Macias 2005). Temperature stress is known to also affect the water status of the cells and could conceivably delay seed germination by causing water stress (Liptay and Schopfer 1983). In the context of this discussion, the term *salt tolerance* during germination is used only to imply situations where the seed has the ability to germinate rapidly under salt-stress conditions. High levels of salinity depressed and delayed seedling emergence of the two genotypes studied, but the degree of reduction and delay varied among temperatures. Similar results were obtained by Mer et al. (2000) in *Cicer arrietinum*, Foolad, Hyman, and Lin (1999) in tomato, Alam, Stuchbury, and

Naylor (2002) in rice, and Rubio-Casal et al. (2003) in Mediterranean salt pans.

Based on results from this study, it may be concluded that milk thistle at the seed germination stage and at optimum temperature may be classified as a facultative halophyte, showing a slight growth at low salinity levels. These conditions are matched naturally and come with late autumn rain in Khozestan during early December each year. These conditions are suitable for dry-land cultivation of milk thistle in poor land area. However, for other growth stages, more work is needed to clarify this point.

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