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Effect of seed priming on germination and seedling traits of Marigold (*Calendula officinalis*) at saline condition

Azam Mirlotfi*, Saeed Bakhtiari** and Amir Behzad Bazrgar***
**Graduate Student Islamic Azad University, Neyshabur branch. Neyshabur, IRAN.*
***Department of Agronomy, Neyshabur Branch, Islamic Azad University, Neyshabur, IRAN.*
****Department of Agronomy, Neyshabur Branch, Islamic Azad University, Neyshabur, IRAN.*

(Corresponding author: Saeed Bakhtiari)
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ABSTRACT: An experiment was conducted in laboratory and greenhouse to investigate the effect of salinity and priming on seed germination and other plant traits of calendula at 2013. Two separate factorial experiment based on randomized complete block design with three replications was conducted. Treatments were four priming levels (distilled water, 0.1% solution of manganese sulfate, 0.5% solution of calcium sulfate, 0.6% solution of potassium phosphate, control) and four salinity levels (S1: 0, S: 25, S3: 50 and S4:75 mmol/lit solutions of NaCl). Germination rate promoted by priming treatments which result in better establishment. Stem length and root length reduced by salinity. Root length enhanced by applying distilled water as priming treatment. Root dry weight enhanced by salinity. Root dry weight at 75 mmol/lit NaCl level, enhanced by applying manganese sulfate as priming treatment. Chlorophyll and carotenoid content did not affect by salinity levels and priming treatments.

Keywords: Germination rate, root length, shoot dry weight, chlorophyll content

INTRODUCTION

Pot marigold (*Calendula officinalis*), is an herbaceous plant in *Calendula* genus of Asteraceae family. It is an aromatic perennial and medical plant. The cosmetic and therapeutic applicability of Calendula is well established especially when concerned with skin-related disorders (Mishra *et al.*, 2011).

The flowers of *Calendula* contain flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins, and asesquiterpene glucoside (Ukiya, 2006). Salinity is one of the major constraints limiting plant growth in some of the most productive agricultural regions of the world (Boyer, 1982). Increased salinity leads to a reduction or delay in germination of plant seeds (Ungar, 1982). A high concentration of salt causes ion imbalance, hyperosmotic stress, and oxidative damage (Zhu, 2002). Priming improves germination and emergence of several plant species (Singh, 1995, Bailly *et al.*, 2000). This approach has proven its effectiveness to improve crop establishment on saline soil (Ashraf *et al.*, 2001, Basra *et al.*, 2005).

Seed priming stimulates many of the metabolic processes involved with the early phases of germination and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously and perform better in adverse conditions (Neto *et al.*, 2000). Plant growth, chlorophyll content, flower number per plant and flower diameter of calendula (*Calendula officinalis* L.) decreased by salinity reported that *Calendula officinalis* seeds germination percent decreased by higher salt levels. Maximum germination percent, radicle and stem length observed for control group. declared that hydro-priming results in better water absorption by seeds and high germination rate. They also reported that plant growth of primed seeds accelerated compare with control seeds. The present experiment conducted to investigate the effect of seed priming on germination and plant traits of Calendula at saline condition (Bayat *et al.*, 2012).

MATERIALS AND METHODS

An experiment was conducted in laboratory and greenhouse to investigate the effect of salinity and priming on seed germination and other plant traits of calendula at 2013. Two separate factorial experiment based on randomized complete block design with three replications was conducted. Treatments were four priming levels (distilled water, 0.1% solution of manganese sulfate, 0.5% solution of calcium sulfate, 0.6% solution of potassium phosphate, control) and four salinity levels (S1: 0, S: 25, S3: 50 and S4:75 mmol/lit solutions of NaCl). 700 seeds of Par-par variety of *Calendula* kept in rolled filter papers and placed in 1000 cc beakers of different priming solutions. Beakers placed in germinators with 25 and 18°C temperature for day and night respectively.

Primed seeds divide in two sets and used for laboratory and greenhouse experiments. 50 seed of each priming treatment placed in different NaCl solutions in order to estimate germination rate and percentage. Five seeds of different priming levels planted in 15 × 30 cm pots which filled with sand. Pots irrigated with proper salt solutions. Hoagland nutrient solution applied to all treatments.

According to seed standard test directions, germinated seed counted every day until the 14th day after planting. Germination percentage and rate calculated using the following equitation:

Germination (%) = (number of germinated seed)/(total seeds number) × 100

$$GR = \sum \frac{ni}{di}$$

Where n_i is the number of germinated seeds per day and d_i is the day of counting (Ellis *et al.*, 1981).

In the greenhouse, root and stem length of 5 samples measured by a ruler. Then samples placed in an oven for 48 hours at 70°C. Dry weight measured by a digital scale. Chlorophyll content measured based on (Lichtenthaler, 1987). Calendula leaves area measured using a LEAF AREA METER. The data of experiment analyzed by ANOVA using the SAS statistical package and significance of differences between means was conducted using Duncan's multiple range test at 5% probability level.

RESULTS AND DISCUSSIONS

Results showed that both germination rate and percentage affect by different priming levels. There was significant difference between salinity levels in respect of germination rate (Table 1).

Table 1: Analysis of variance of laboratory traits.

Source of variation	Degree of freedom	Germination percentage	Germination rate
Prim	4	12.95*	0.07**
Salinity	3	10.26 ns	0.03**
Prim*salinity	12	2.89 ns	0.008 ns
Error	60	4.1	0.003
%CV		2.09	15.95

*, **: significant at 5 and 1 percent probability levels and ns: not significant

Patade *et al.*, (2009) suggest that salt priming is an effective pre-germination practice for overcoming salinity and drought induced negative effects in sugar-cane. The benefits of seed priming methods on seedling emergence reported in some medicinal plants, such as cumin and marigold (Tabrizian and Osareh 2007). Germination rate reduced by higher NaCl concentrations.

Decrease in germination rate of seeds is due to decrease in osmosis potential of solution or increase in toxic ions and changing in the remobilization balance of seed reservoirs (Demir Kaya *et al.*, 2006).

Root and stem length affect by priming levels (Table 2).

Table 2: Analysis of variance of root length and stem length.

Source of variation	Degree of freedom	Root length	Stem length
Prim	4	14.15 **	73.36**
Salinity	3	7.47 ns	55.41**
Prim*salinity	12	8.14 ns	63.91 **
Error	40	3.71	9.77
%cv		14.49	11.78

*, **: significant at 5 and 1 percent probability levels and ns: not significant

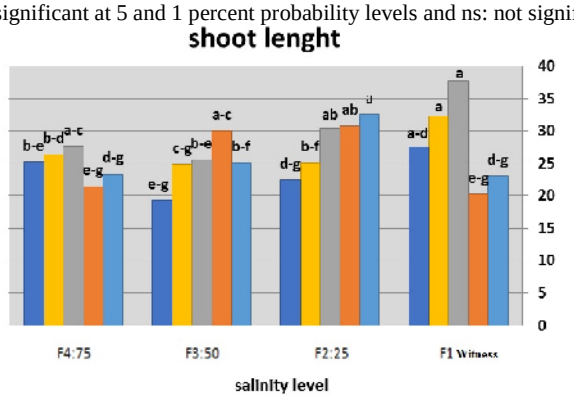

Fig. 1. Effect of salinity level and priming treatment on stem length.

Stem length affected by salinity levels and interaction between salinity and priming too (Table 2). The highest root length produced by applying distilled water as priming treatment. There was no significant difference between other treatments in respect of root length. Stem length affected by salinity levels, priming treatments and interaction between them ($p<0.01$) (Table 2). Stem length reduced by salinity. The highest stem length produced by applying potassium phosphate at control treatment. At S2, the highest stem length produced by not primed seeds. At S3 the highest stem length produced by applying calcium sulfate (Fig. 1). In response to salt stress the production of endogenous gibberellic acid (GA) decreases (Xu *et al.*, 2011). GA promotes hypocotyl length during seed germination, and promotes early seedling development (Gallardo *et al.*, 2002). Thus plants which exposed to salinity showed lower stem length. Root and shoot dry weight affected by salinity levels, priming and interaction between them ($p<0.01$) (Table 3). Root and shoot dry weight increased by salinity (Fig. 2 and 3). Results showed that the highest root and shoot weight produced by interaction between 75% salinity level and applying manganese sulfate (Fig. 2 and 3). Gallardo *et al.*, 2002) reported that root and shoot dry weight of marigold decreased by high salinity levels but dry weight did not affect by low salinity levels. Flower and leaf dry weight affected by salinity level, priming and interaction between them ($p<0.01$) (Table 4).

Table 3: Analysis of variance of root and shoot dry weight.

Source of variation	Degree of freedom	Root dry weight	Shoot dry weight
prim	4	0.014**	0.041**
Salinity	3	0.012**	0.13**
Prim*salinity	12	0.02**	0.10**
Error	40	0.001	0.004
%cv		14.67	14.95

*, **: significant at 5 and 1 percent probability levels and ns: not significant

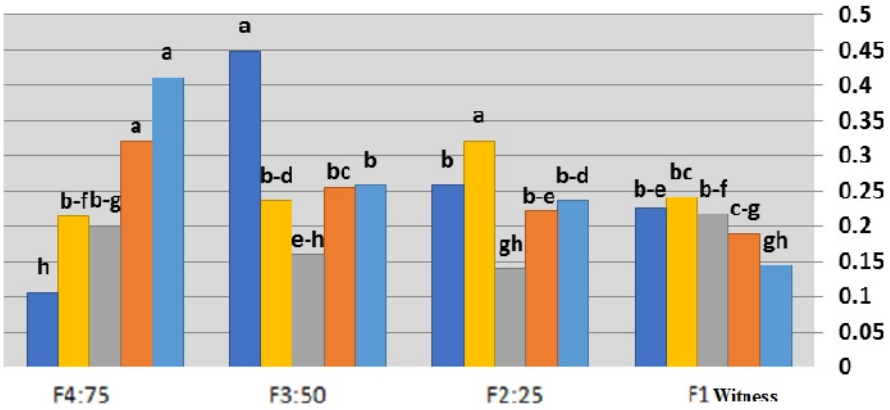

Fig. 2. Effect of salinity level and priming treatment on root dry weight.

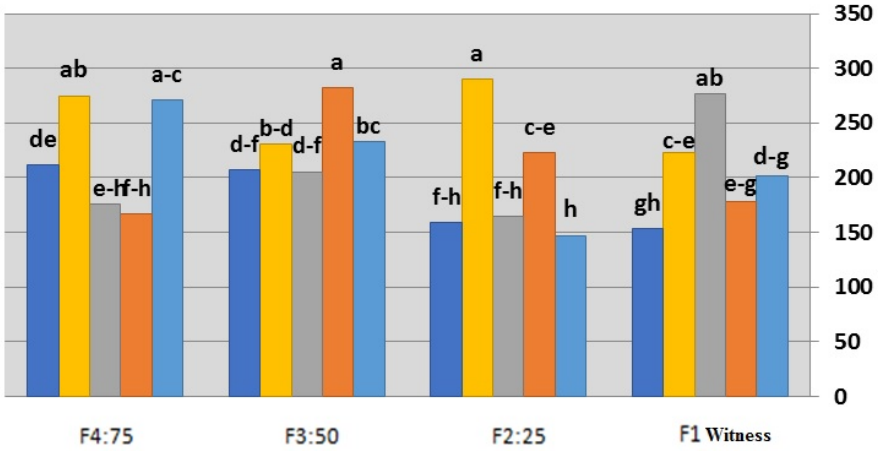

Fig. 3. Effect of salinity level and priming treatment on shoot dry weight.

Table 4: Analysis of variance of leaves and flower dry weight.

Source of variation	Degree of freedom	Leaf dry weight	Flower dry weigh
Prim	4	0.35**	0.0004**
Salinity	3	0.086**	0.0039**
Salinity*prim	12	0.224**	0.037**
Error	40	0.011	0.0001
%cv		12.37	10.54

*, **: significant at 5 and 1 percent probability levels and ns: not significant

The highest leaf and flower weight observed for hydroprimed seeds at S4 salinity level (Fig. 4 and 6). Gallardo *et al.,* (2002) stated that pot marigold is somehow tolerant to low salt concentrations. Leaf area affected by salinity, priming and interaction between them ($p<0.01$) (Table 5), but chlorophyll and carotenoid content did not affect by applied treatments (Table 5). The highest leaf area observed for S2, S3 and S4 by applying distilled water and calcium sulfate as priming treatment (Fig. 6). The toxic effect of sodium at high salt levels and physical damage to roots decreased their ability to absorb water and nutrient which caused strong reduction in photosynthesis, enzymatic process and protein synthesis. This resulted in limited growth and poor leaf area development (Akram *et al.,* 2010).

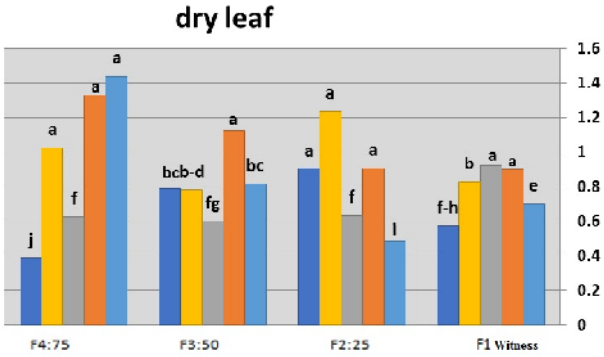

Fig. 4. Effect of salinity level and priming treatment on flower dry weight

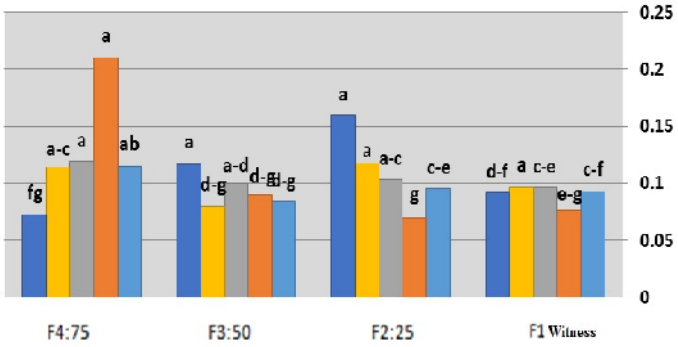

Fig. 5. Effect of salinity level and priming treatment on leaf dry weight.

Table 5: Analysis of variance of leaf area, chlorophyll and carotenoid content.

sov	Degree of freedom	Leaf area	Chlorophyll content	Carotenoid content
Prim	4	8181**	0.019 ns	0.0012ns
Salinity	3	3488 **	0.061 ns	0.006 ns
Prim*salinity	12	6661 **	0.041 ns	0.001 ns
Fault	40	707	0.071	0.002
%cv		**12.45**	**24.45**	**20.21**

*, **: significant at 5 and 1 percent probability levels and ns: not significant

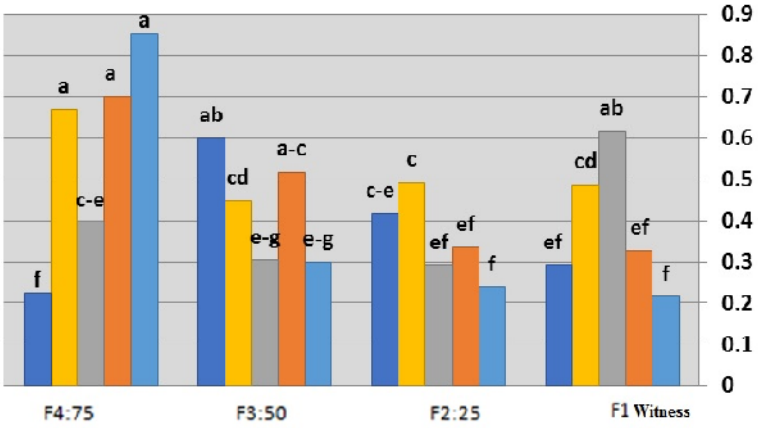

Fig. 6. Effect of salinity level and priming treatment on leaf area.

CONCLUSION

Results showed that salinity inhibits early seedling growth of marigold seeds. The inhibitory effects of salinity decreased by priming treatments. Germination rate promoted by priming treatments which result in better establishment and higher shoot and root dry material production in marigold. Plant tolerance to environmental stresses such as salinity will improve in better established plants. The best results observed by applying distilled water and calcium sulfate as priming treatments. The lowest germination percentage gained by applying manganese sulfate as priming treatment.

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