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EFFECT OF DIFFERENT PRIMING METHODS ON GERMINATION AND SEEDLING ESTABLISHMENT OF TWO MEDICINAL PLANTS UNDER SALT STRESS CONDITIONS

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ABSTRACT. Priming is one of the seed enhancement methods that might be resulted in increasing seed performance (germination and emergence) under stress conditions, such as salinity. Salinity is a major environmental stress which adversely affects germination and seedling establishment in a wide variety of crops. The experiment was arranged as a factorial in completely randomized design (CRD) at Seed Research Laboratory of College of Agriculture, University of Tehran, Iran. The objective of this research was to evaluate the effect of different priming methods on seed germination of two medicinal plants including lemon balm (*Melissa officinalis* L.) and cumin (*Cuminum cyminum* L.) under salinity stress. Treatments were combinations of two levels of salinity stress (0 and 10 dsm^{-1}) and five levels of priming (control = non-priming), GA_3, manitol, NaCl and distilled water) with three replications. Seeds of lemon balm and cumin were primed for 24 h at 25°C. Results revealed that different growth traits (including germination percentage, germination rate, seedling dry weight, plumule and radical length) significantly (p=0.05) decreased with applying salinity. However, priming of seeds with different materials particularly GA_3 was useful for alleviating salt stress effects and improving germination and seedling establishment under salt stress. Under salinity condition, primed seeds possessed more germination and emergence than control. The result of this experiment is consistent with the hypothesis that under salinity stress, priming can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment.

Key words: Cumin; Germination; Lemon balm; Priming; Salinity.

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is a perennial herb in the mint family *Lamiaceae*, native to center-southern Europe and the

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Mediterranean region. It grows to 70-150 cm tall. The leaves have a gentle lemon scent, related to mint. During summer, small white flowers full of nectar appear.

Cumin (*Cuminum cyminum* L.) is a flowering plant in the family *Apiaceae*, native from the east Mediterranean to India. Its seeds (each one contained within a fruit, which is dried) are used in the cuisines of many different cultures, in both whole and ground form. The cumin plant grows to 30-50 cm tall and is harvested by hand. It is an annual herbaceous plant, with a slender, branched stem 20-30 cm tall.

Seed germination, one of the most critical phases of plant growth and development, is adversely affected under saline conditions (Ghavami and Ramin, 2007). Germination of seeds, one of the most critical phases of plant life (El-Keblawy and Al-Rawai, 2005), is greatly influenced by salinity (Misra and Dwivedi, 2004). Salinity slows the germination rate and higher levels of salinity reduce the germination percentage. Low concentrations of salinity affect the germination rate, but not the total percentage of seeds germinated. Thus, reported data are dependent upon the time of observation as well as on the germination conditions (Patanea *et al.,* 2009). In general, salinity affects almost every aspect of the physiology and biochemistry of plants (Lee *et al.,* 2008). The need to develop crops with higher salt tolerance has increased greatly within the last decade due to increased salinity problems throughout the world (Sivritepe *et al.,* 2003).

Seed priming is a technique of seed enhancements that improves germination or seedling growth. Seed priming enhances seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination in different crops (Ashraf and Foolad, 2005). There is several priming techniques used for seeds. Effects of priming or pre- treatment of seeds persist under suboptimal field conditions, such as salinity (Foti *et al.,* 2008), and low soil moisture availability (Du *et al.,* 2002). Different seed priming methods have been successfully integrated (Basra *et al.,* 2004; Farooq *et al.,* 2006).

The purpose of this research was to evaluate the efficiency of employing priming with different materials to mitigate salt stress effects on improve germination and seedling establishment of two medicinal plants, lemon balm and cumin, at two levels of salinity.

MATERIAL AND METHODS

The experiment was conducted in the seed laboratory of College of Agriculture, Tehran University, Iran. The experiment was arranged as a factorial in completely randomized design (CRD). There were three replications for each treatment. Seeds of lemon balm and cumin were used in this experiment. Seeds of the both species were sterilized (2% sodiumhypochlorite, containing 4 ml^{-1} Tween 20) for 7-10 min, then washed several times with distilled water (Bhuvaneswari *et al.,* 1980). The seeds were subjected to seed priming with different materials (control, GA_3, manitol, NaCl and distilled water) at various levels of salinity for 24 h. First, 25 seeds were placed in the plastic vessel. Priming solutions including 20 mg l^{-1} gibberellic acid (432k6943, Sigma-Aldrich), 25% manitol (k91614582, Merck), 3% NaCl (k29042800, Merck) and distilled water were added, after 24 hours, the seeds were dried at the laboratory temperature. Then, seeds were placed in 10 cm Petri dishes with double-layer filter paper and 5 ml of salt solutions (0= control and 10 dsm^{-1}) were added to every Petri dish. 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, germination rate, mean of germination time, plumule and radicle dry and fresh weight, plumule and radicle length.

- Germination percentage was evaluated by counting the numbers of normal seedlings at the end of standard germination test.
- Germination rate (GR, seed number day) was calculated by using the data related to germinated seeds through germination course and the following formula: $\sum \frac{ni}{di}$, where *ni* is the number of germinated seeds in every counting and *di* is the day of counting (Ellis and Roberts, 1981).
- Mean of germination time (MGT, day) that is the reverse of germination rate: $\sum \frac{di}{ni}$.
- Seedling dry and fresh weight (plumule and radicle).
- Plumule and radicle length.

Results were analysed statistically by analysis of variance (ANOVA), using SAS software (SAS Institute Inc., 1988). When analysis of variance showed significant treatment efects, the least significant diferences test (LSD) was applied to make comparisons among the means at the 0.05 level of significance.

RESULTS

Analysis of variance results showed that effect of salinity on all studied traits and also the effect of seed priming on germination percentage, plumule and radical length, plumule dry weight were significant. Interaction between salinity and priming on GP, GR, MGT and plumule and radical length in both lemon balm and cumin was significant.

GP decreased by applying salinity (*Table 1*), but by comparing lemon balm and cumin, this shows the tolerance of cumin at the primary stages of germination to salinity stress. The highest GP in lemon balm and cumin obtained under control salinity level and priming with GA_3 and the lowest amount at 10 dsm^{-1} salinity level and priming with manitol and non – primed seed (*Table 2*). The maximum GP in cumin obtained in priming with GA_3 that had no significant difference by priming with NaCl (*Table 2*).

Table 1 - The effect of salinity and priming on germination traits in two medicinal plants

Salinity	GP	GR	PL	RL	MGT	PDW	RDW
Control	93.2^{a}	0.54^{a}	0.21^{a}	0.16^{a}	0.240^{a}	0.04^{a}	0.0020^{a}
10 dsm^{-1}	89.0^{b}	0.43^{b}	0.13^{b}	0.09^{b}	0.353^{b}	0.03^{b}	0.0011^{b}
Species							
Lemon balm	89.0^{b}	0.47^{b}	0.17^{a}	0.12^{a}	0.290^{b}	0.04^{a}	0.0015^{a}
Cumin	95.2^{a}	0.50^{a}	0.18^{a}	0.13^{a}	0.303^{a}	0.04^{a}	0.0016^{a}
Priming							
Control	89.7^{c}	0.41^{b}	0.11^{c}	0.08^{c}	0.389^{d}	0.04^{b}	0.0008^{d}
GA_3	96.5^{a}	0.60^{a}	0.24^{a}	0.16^{a}	0.245^{a}	0.05^{a}	0.0021^{a}
NaCl	93.5^{b}	0.54^{a}	0.21^{a}	0.13^{b}	0.266^{b}	0.04^{b}	0.0017^{b}
Manitol	86.1^{d}	0.44^{b}	0.17^{b}	0.12^{b}	0.310^{c}	0.04^{b}	0.0018^{b}
Distilled water	94.8^{ab}	0.43^{b}	0.14^{bc}	0.12^{b}	0.273^{b}	0.04^{b}	0.0015^{c}

Means of three replicates. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

Table 2 - Mean comparison for salinity and priming interaction on germination traits in two medicinal plants

Salinity (dsm^{-1})		GP	GR	PL	RL	MGT	PDW	RDW
Lemon balm								
Control	Control	87c	0.45c	0.15c	0.11c	0.325d	0.04a	0.0012c
	GA3	95a	0.63a	0.27a	0.19a	0.185a	0.05a	0.0021a
	NaCl	90b	0.57b	0.24a	0.17ab	0.205b	0.045a	0.002a
	Manitol	83d	0.48c	0.2ab	0.15b	0.248c	0.045a	0.0024a
	Distilled water	92ab	0.47c	0.18b	0.15b	0.212b	0.04a	0.0018b
10	Control	85cd	0.34d	0.07e	0.05e	0.435f	0.03b	0.0004e
	GA3	93ab	0.52b	0.19b	0.12c	0.295c	0.04a	0.0013c
	NaCl	88c	0.46c	0.16c	0.09d	0.315d	0.035b	0.0012c
	Manitol	81d	0.37d	0.12d	0.08d	0.358e	0.035b	0.0016b
	Distilled water	90b	0.36d	0.1de	0.08d	0.322d	0.03b	0.001d
Cumin								
Control	Control	95.7b	0.5d	0.16b	0.12c	0.34e	0.04b	0.0013de
	GA3	100a	0.68a	0.29a	0.21a	0.193a	0.05a	0.0024a
	NaCl	99a	0.62b	0.26a	0.18ab	0.214b	0.05a	0.0022ab
	Manitol	91.3c	0.52d	0.21ab	0.16b	0.259c	0.05a	0.0026a
	Distilled water	96b	0.51d	0.19b	0.16b	0.222b	0.04b	0.0019b
10	Control	91c	0.37f	0.07d	0.05e	0.455g	0.03c	0.0004d
	GA3	98ab	0.56c	0.20b	0.13c	0.308d	0.04b	0.0014d
	NaCl	96.8b	0.50d	0.17b	0.10cd	0.329e	0.04b	0.0013de
	Manitol	89.1c	0.40e	0.13c	0.09d	0.374f	0.04b	0.0017c
	Distilled water	95b	0.39e	0.11c	0.09d	0.336e	0.03c	0.0011e

Means of three replicates. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

In both species, MGT increased by applying salinity (*Table 1*). By definition, MGT is associated to the time length (day) that radicle exits. The low MGT was at control level and the high was at 10 dsm^{-1} salinity level. Also, in both species the highest MGT achieved at priming with manitol and non primed seed, but the lowest MGT was obtained priming with GA_3 (*Table 2*). Probably, this is the result of the effect of gibberellic acid under salinity conditions that can decrease the negative effects of sodium ions through germination.

In both species, the longest plumule observed at control salinity. Salinity stress probably decreases cell division and its expansion in aerial parts of plant. Also, the longest plumule obtained in the interaction of control salinity and priming with GA_3. The shortest plumule obtained at 10 dsm^{-1} salinity level and priming with manitol and non- primed seed (*Table 2*).

The effect of priming on radicle and plumule length was significant. The maximum radicle and plumule length in both studied species was seen in priming with GA_3 that had no significant difference with NaCl priming (*Table 2*). In lemon balm was no significant difference between priming and non- primed seed at control salinity, but in cumin hormonal priming had significant effect on dry weight of plumule as compared to non-primed seeds. In both species, there were no significant differences in dry weight of radicle activity between both GA_3 and NaCl primed seeds grown under NaCl and non-NaCl conditions.

DISCUSSION

According to our results, by increasing salinity levels, germination decreased in both medicinal plants. Germination of seeds, one of the most critical phases of plant life (Davies, 2004), is greatly influenced by salinity (Dezfuli *et al.,* 2008). Salinity can affect germination and seedling growth either by creating an osmotic pressure that prevents water uptake or by toxic effects of sodium and chloride ions (Khan *et al.,* 2009). Decreasing germination percent by the effect of increasing salt concentration is in consistence with the results of Sannazzaro *et al.* (2006).

In some plants, such as halophytes or salt resistant plants, the existence of sodium ions even at low amounts, could have positive effect on seed germination, and even could increase germination than control (Sabahat and Khan, 2004). Distilled water has zero osmosis potential and some seeds show lower germination at this potential.

The most researchers believe that the decrease of germination rate is the result of decreasing water potential and seed accessibility to water (Mohammadi, 2009). In this experiment and in the both species, the highest germination rate obtained at control salinity level and priming with GA_3.

By applying salinity, dry weight of plumule and radicle decreased. It is probably due to decreasing in remobilization of reservoirs from cotyledons to embryo axis. The factors that affect the growth rate of embryo axis, also are affecting the mobility of reservoirs and its remobilization from cotyledons to embryo axis (Munns, 2008). Mer *et al.* (2000) observed that by increasing salinity, plumule length in wheat, barley, pea and cabbage seeds decreased. They pointed out that decreasing the growth of young seedlings, by increasing salinity, was because of the most decreasing of water absorption by radicle, and subsequently by accumulation of soluble salts in cells, water potential of root cells decreases and biological processes occur in roots, even in low water potentials.

In this research, the low seed germination rate obtained with manitol priming. It looks that manitol may increase osmosis potential and force seeds under the moisture deficit that, in these conditions, the activity of enzymes will decrease and the rest of metabolic activities will confront with problems. In other words, at higher osmosis potential, the moisture will be accessible for seed and its germination will decrease. It is reported that in sunflower, watermelon and melon seeds, priming with salt (NaCl) causes an increase in germination rate and rapid establishment of seedlings from treated seeds (Demir Kaya *et al.,* 2006; Foti *et al.,* 2008). Ruan *et al.* (2002) reported that osmo-priming of rice seeds by salt solution in compare with polyethilenglicole, increases the germination index and decreases germination time significantly. Priming with GA_3 will accelerate metabolic reactions before germination process and make possible seed germination under salinity stress conditions with low moisture (Iqbal and Ashraf, 2010).

GA_3 increases the synthesis of hydrolytic enzymes at aleuron layer and by the activity of these enzymes, storage compounds convert to transferable ones (sucrose and glucose) and transfer to embryo (Sedghi *et al.,* 2008). The main factor in transferring of reservoirs is their solubility in water that with decreasing moisture because of salinity stress, their remobilization to embryo will not possible (Khan *et al.,* 2009).

Pre-treatment with NaCl and GA_3 accelerates some metabolic processes even in low potential. This causes improvement at metabolic activities in germination, especially under salinity stress conditions and, subsequently, the weight of radicle and plumule increases in less time. Since priming is simple and cheap, we can propose this method to farmers, so they can increase percent and homogeneity of emergence of medicinal plants under environmental stresses.

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