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Seed pre-treatment effect on seedling emergence, chlorophyll content and plant weight of dill under salt stress

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Abstract

A factorial experiment with three replications on the bases of randomized complete block design was conducted in 2015 at the Greenhouse of the university of Tabriz, to investigate the effects of different seed pre-treatments (Control, 1 mM GA_3 and SA and polymer seed coating) on seedling emergence, chlorophyll content and plant weight of dill (*Anethum graveolens* L.) under different levels of NaCl salinity (0, 4, 8 and 12 dS/m). Hormonal treatments of seeds significantly improved emergence percentage of dill seedlings under moderate (8 dS/m) and severe (12 dS/m) salinities. Seedling emergence rate of GA_3 treated seeds was superior under 0-4 dS/m, but SA treated seeds showed the highest emergence rate under 8-12 dS/m salinities. Seed coating reduced emergence percentage and rate of dill seedlings under all salinity levels. Seed treatment by SA and GA_3 improved chlorophylls a and b contents. Whereas, the lowest chlorophyll content was observed in plants from polymer coated seeds. Plants from GA_3 treated seeds had the highest plant weight.

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Introduction

Dill (*Anethum graveolens* L.) is an aromatic herb native to the Mediterranean area. While it is sometimes grown as a biennial, it is most commonly grown as an annual. Dill is commonly used in the countries of the temperate and even of the cooler climatic zone. The plants have an attractive appearance, pleasant fragrance and a rich chemical composition. It is used as a general aromatic herb, a vegetative and an inhibitor of sprouting in stored potatoes and an antispasmodic (Hassan, 2012). Fruits of dill contain 1 - 4 % essential oil comprising of major compounds: carvone (30 - 60 %), limonene (33 %), phellandrene (20.6 %), pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiole. Although dill somewhat tolerate salt stress, the growth and essential oil production of this plant could be affected by severe salinity (Shannon and Grieve, 1998).

Abiotic stresses greatly influence the growth and development of crop plants. Among these stresses, salt stress is affecting over 6% of land area in the world (Meloni *et al.,* 2004). Most of the salt stresses in nature are due to Na^+ salts, particularly NaCl (medhat, 2002). Salinity causes a reduction in hydraulic conductivity in plants (Peyrano *et al.,* 1997). The stress forced by excess salts is an important limitation for the productive use of lands (Sanderson *et al.,* 1997) as it reduces plant growth at a soil conductivity above 4.5 dS/m (Muscolo *et al.,* 2003). High salt content, especially chloride and sodium sulphates, affects plant growth by modifying their morphological, anatomical (Kalaji and Pietkiewicz, 1993; Huang and Redmann, 1995) and physiological traits (Muscolo *et al.,* 2003; Ghassemi-Golezani and Hosseinzadeh-Mahootchi, 2015). The general effect of salinity is to reduce the growth rate resulting in smaller leaves, shorter stature, and fewer leaves and flowers (Ghassemi-Golezani *et al.,* 2012).

Seed germination, seedling emergence and plant establishment are important aspects of agricultural and horticultural production, and are important components of seed and seedling vigor. These factors are related to early growth of the crop, and may be related to resistance to early-season stresses and final yield (Medhat, 2002). A method to improve the rate and uniformity of germination is the priming or physiological advancement of the seed lot (Finch-Savage, 2004; Halmer, 2004; Ghassemi-Golezani *et al.,* 2013). Seed pre-treatment by water or plant growth regulators is one of the simple ways to improve plant performance under salt stress.

Seed priming reduces leakage of metabolites, permits DNA replication, increases RNA and protein synthesis and enhances seedling growth and establishment (McDonald, 2000). One of the effective priming methods is the pre-treatment with plant growth regulators. Plant growth regulators such as GAs and SA are involved in the induction of plant stress responses (Pedranzani *et al.,* 2003). Gibberllic acid is one of the important growth regulators that is used for promoting cell elongation and cell division in plants (Golenberg and West, 2013), that act at all stages in the plant life cycle by promoting germination, stem elongation, flowering and seed development. The advantageous effects of GA_3 has been shown to be through increasing the water content of the seedlings and improving protein and RNA synthesis (Banyal and Rai, 1983). Salicylic acid (SA), is considered as a hormone like endogenous regulator, and its role in the defence mechanisms against biotic and abiotic stresses has been well characterized (Ghassemi-Golezani *et al.,* 2015). Seed coating with polyacrylamide may also be effective in improving water absorbance and seedling growth (Taylor and Harman, 1990). Thus, the aim of this research is to evaluate the effects of seed pre-treatments on seed germination and essential oil production of dill under salt stress.

Materials and methods

Experimental design and treatments

A Factorial experiment based on randomized complete block design with three replications was carried out to investigate the effects of seed polymer coating and pre-treatments on germination, chlorophyll content and essential oil production of dill (*Anethum graveolens* L.) under a non-saline and three saline (4, 8 and 12 dS/m NaCl).

Seeds of dill were divided into four sub-samples, one of which was kept as control (unprimed). A sample was primed with 1 mM GA_3 (GA_3) and another with 1 mM SA (SA) for 4 hours at 25 ± 2°C under dark conditions and then dried back to initial moisture content at a room temperature of 20-22°C. The other seed sample was coated with water-absorbent polymer (Sc). The hydrophilic polymer used for seed coating was polyacrylamide. Ten grams of seeds were soaked in 0.5% of Arabic gum solution and uniformly were coated with polymer. All seeds were sown 1.5 cm deep in pots, each filled with 900 g perlite, using 48 pots in general. Pots were then placed in the glass greenhouse under natural light and temperature around 25 ± 2˚C. Tap water (EC = 0.59 dSm^{-1}) and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC (field capacity). After emergence, seedlings were thinned to keep 10 plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC =1.3 dSm^{-1} and pH= 6.5-7). Perlites within the pots were washed every 30 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC) due to adding the Hoagland solution.

Seedling emergence

Seedling emergence was recorded in daily intervals until final establishment in each pot. The emergence percentage was determined after 21 days. Rate of seedling emergence ($\bar{R}$) was calculated as:

$$\bar{R}=\frac{\sum n}{\sum Dn}$$

Where n is the number of seedlings emerged on day D, D is the number of days from sowing and $\bar{R}$ is the mean emergence rate.

Chlorophylls a and b

Chlorophylls a and b were determined by the method introduced by Sukran *et al.* (1998). A leaf sample of 0.1 g from each pot was ground and solved in 5 mL of 100% acetone. After 24 h incubation in darkness within a refrigerator at 3-5 °C, the absorbance of the solution was measured at 662 nm and 645 nm, using a spectrophotometer (Analytikjena Spekol 1500). Then chlorophylls a and b were estimated as:

Chlorophyll a (µg/g) = $(11.75\,A_{662} - 2.350\,A_{645}) \times 1000$

Chlorophyll b (µg/g) = $(18.61\,A_{645} - 3.960\,A_{662}) \times 1000$

Plant weight

At maturity, 5 plants were harvested from each pot and then were dried in an oven at 80°C for 48 h. Subsequently, the dry plants of each pot were weighed and mean plant weight was calculated.

Statistical analysis

Analysis of variance of the data appropriate to the experimental design and comparison of means (Duncan multiple range test) at $p \leq 0.05$ were carried out, using SPSS16 software.

Results and discussion

Seedling emergence

Effects of salinity and seed pre-treatment and also the Interaction of these factors were significant for seedling emergence percentage and rate ($p \leq 0.01$). Emergence percentage of seedlings from seeds treated by GA_3 and SA under non-saline condition and low salinity (4 dS/m) did not show significant difference with those from untreated seeds. However, hormonal treatments of seeds significantly improved emergence percentage of dill seedlings under moderate (8 dS/m) and severe (12 dS/m) salinities. The highest percentage of seedling emergence under moderate and severe salinities was recorded for SA treated seeds, followed by GA_3 treated seeds. Seed coating (SC) reduced emergence percentage of dill seedlings under all salinity levels. Seedling emergence rate of GA_3 treated seeds was superior under 0 – 4 dS/m, but SA treated seeds showed the highest emergence rate under 8-12 dS/m salinities. Emergence percentage and rate of seedlings from untreated and pre-treated seeds was generally reduced under moderate and high NaCl salinities (8-12 dS/m) (Table 1).

Table 1. Means of germination percentage, germination rate, chlorophyll a and b contents in dill plants from non-treated and pre-treated seeds under non-saline and saline conditions.

Salinity (dS/m)	Seed treatment	Seedling emergence (%)	Seedling emergence rate	Chlorophyll a (µg/g FW)	Chlorophyll b (µg/g FW)
0	Control	78.00 a	0.072 c	21.80 abc	14.32 bc
	GA_3	79.15 a	0.080 a	23.26 a	15.46 a
	SA	78.00 a	0.075 bc	22.98 a	15.42 ab
	SC	68.00 c	0.053 f	21.02 bc	13.74 c
4	Control	78.50 a	0.071 c	21.77 abc	14.27 bc
	GA_3	79.70 a	0.078 ab	23.26 a	15.45 a
	SA	79.20 a	0.075 bc	22.98 a	15.42 ab
	SC	64.2 d	0.051 fg	20.70 c	13.51 cd
8	Control	55.00 e	0.058 e	20.36 c	11.09 e
	GA_3	68.00 c	0.061 e	22.26 ab	12.61 d
	SA	73.00 b	0.066 d	22.60 a	13.71 c
	SC	42.36 g	0.042 i	16.53 d	8.05 gh
12	Control	41.8 0g	0.044 hi	14.81 e	7.13 h
	GA_3	51.00 f	0.048 gh	15.11 e	8.79 g
	SA	56.00 e	0.058 de	15.98 de	9.77 f
	SC	30.06 h	0.030 j	10.40 f	4.15 i

Different letters in each column indicate significant difference at $p \leq 0.05$.

Reduction in seedling emergence percentage and rate of dill seeds under salinity (Table 1) can be attributed to Na^+ toxicity and decreasing water potential (Medhet, 2002; Munns *et al.,* 2006). Na^+ accumulation in seeds also reduced alpha amylase activity under severe salinity (Ashraf *et al.*, 2002), leading to decrease seedling emergence percentage and rate of dill. Improving emergence rate and percentage of dill seedlings with priming by GA_3 may be related to increasing alpha amylase activity and gene expression (Paleg, 1960; Gubler *et al.*, 1995). SA alleviates ion toxicity and regulates sugar metabolism in growing seedlings (He *et al.,* 2010; Dong *et al.,* 2011), thereby improving emergence percentage and rate of seedlings.

Chlorophylls a and b

Salinity and seed pre-treatment significantly influenced chlorophyll a and b contents. The Interaction of these factors on chlorophyll content was also significant ($p \leq 0.01$). Changes in leaf chlorophyll a content of dill plants from untreated and GA_3 and SA treated seeds under non-saline condition, low (4 dS/m) and moderate salinities (8 dS/m) were statistically similar, but it was considerably reduced under severe salinity (12 dS/m).

The highest chlorophyll a content under severe salinity was recorded for plants from SA treated seeds. Chlorophyll b content did not change up to 4 dS/m salinity, but thereafter reduced in all treated and untreated seeds. Differences in chlorophyll b content of plants from seeds treated by GA_3 under non-salinity and low salinity were not significant, but under 8 and 12 dS/m, plants from SA treated seeds had the highest chlorophyll b content, compared with other treatments (Table 1). Plants from polymer coated seeds showed the lowest chlorophylls a and b contents under all salinity levels (Table 1).

Chlorophyll is the main pigment of photosynthesis in plants. To some extent, the chlorophyll content can reflect the photosynthesis rate of plants. Reduction in chlorophyll content under moderate and severe salinities (Table 1) may be associated with a salt-induced weakening of protein-pigment-lipid complex (Strogonove *et al.,* 1970) or increased chlorophyllase enzyme activity (Noreen and Ashraf, 2009).

Increasing chlorophyll content with GA_3 and SA treatments (Table 1) may be related to increasing nitrogen absorption and therefore, enhancing nitrate reductase activity (Singh and Usha 2003; Afroz *et al.,* 2006). Leslie and Romani (1988) have showed that seed priming with salicylic acid stimulated photosynthetic machinery and increased chlorophyll content. El-Tayeb (2005) has also found that barley seeds presoaked with 1mM salicylic acid under salinity increased the photosynthetic pigment of shoots compared with seedlings treated with NaCl alone.

Plant weight

Plant weight was significantly affected by salinity and seed pretreatment ($p \leq 0.01$). The interaction of salinity × seed pretreatment on this trait was not significant ($p > 0.05$). Dill plant weight was not significantly changed in salinities up to 8 dS/m, but it was significantly reduced with further increase in salinity (Fig. 1 A). The highest and the lowest plant weight were recorded for plants from GA_3 treated and polymer coated seeds, respectively. The dry weight of plants from untreated seeds and SA treated seeds was statistically similar (Fig. 1 B).

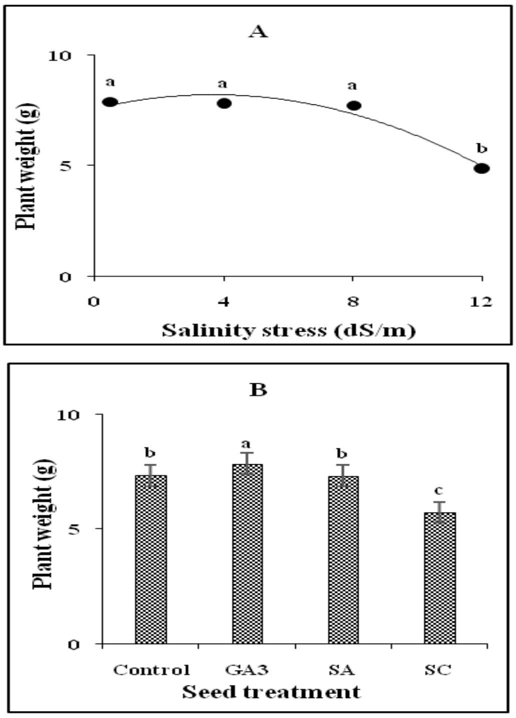

Fig. 1. Dill plant weight for different levels of salinity (A) and seed pretreatments (B).

Changes in plant weight due to salinity and seed pre-treatments (Fig. 1) were largely associated with variation in seedling emergence rate and leaf chlorophyll content under these treatments (Table 1). Seed priming with GA_3 plays an important role in the induction of tolerance to salinity and overcoming the limitations created by the environmental stress such as osmotic effects, ion toxicity and nutritional imbalance (Jamil and Rha, 2007). This was also related to gibberellic acid physiological roles on promoting cell elongation, cell division and growth stimulating in many plant species (Bose *et al*., 2013). Decreasing plant weight due to seed polymer coating (Fig. 1. B) could be attributed to holding more Na^+ ions around the roots, leading to higher absorption of this ion into roots and shoots.

Conclusion

Hormonal treatments of seeds significantly improved emergence percentage of dill seedlings under moderate (8 dS/m) and severe (12 dS/m) salinities. Seedling emergence rate of GA_3 treated seeds was superior under 0 – 4 dS/m, but SA treated seeds showed the highest emergence rate under 8-12 dS/m salinities. Seed coating reduced emergence percentage and rate of dill seedlings under all salinity levels. Seed treatment by SA and GA_3 improved chlorophylls a and b contents. Whereas, the lowest chlorophyll content was observed in plants from polymer coated seeds. Plants from GA_3 treated seeds had the highest plant weight.

High salinities can reduce seedling emergence percentage and rate, chlorophyll content and plant weight of dill. These deleterious effects of salinity could be largely overcome by treatment of seeds with plant hormones such as GA_3 and SA before sowing. However, seed polymer coating is not useful under saline conditions.

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