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Seed germination and seedling physiology of ajowan (*Trachyspermum ammi* L.) under chloride salinity

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Abstract

Seed germination and seedling physiology of ajowan (*Trachyspermum ammi*) was investigated under salinity induced by NaCl. Five different salinity levels (0, 25, 50, 100 and 150 mM) were used. The results revealed that seed germination and seedling growth were significantly retarded due to salinity. Significant accumulation of various osmolytes like reducing sugars and total sugars, proline, glycine betaine and total free amino acids was recorded with increasing salinity. The reduction in protein and starch content of seedlings under stress condition was concentration dependent. The studies suggested that ajowan is moderately tolerant to salt stress at seed germination stage.

Keywords: osmolytes, salinity, seedling growth, *Trachyspermum ammi*

Salinity is one of the most important abiotic stresses limiting seed germination, seedling growth and crop productivity. Osmotic adjustment plays an important role in sustaining growth under salt stress. The response of plants differ significantly at various levels, depending on the intensity and duration of stress as well as plant species and stage of development (Chaves et al. 2003). Ajowan (*Trachyspermum ammi* L.) is mainly grown as a medicinal spice crop for its fruits, having commercial value. The fruits are antispasmodic, antiflatulent, antirehumatic, diuretic, antibacterial and antimicrobial, anthelmintic, antihypertensive and gastroprotective (Malhotra & Vijay 2004). In India, it is widely cultivated in the states like Rajasthan, Gujarat, Uttar Pradesh, Punjab, Maharashtra, Karnataka and Andhra Pradesh, occupying an area of about 20,628 ha and producing about 8,950 tonnes in 2009–10. Although extensive research has been carried out on the effect of salinity stress on germination of most crops, very little studies have focused on the effect of environmental stresses like salinity on seed germination and seedling physiology of seed spices. Therefore, the objective of the current study was to examine the responses of the seeds of ajowan to different salinity levels at germination stage and to predict the possibility of its cultivation in salt affected areas.

The seeds of *T. ammi* L. var. NRCSS AA-2 were procured from National Research Centre on Seed Spices, Tabiji Farm, Ajmer (Rajasthan). Salinity stress was induced using NaCl.

Different concentrations of NaCl *viz*., 0 (distilled water), 25, 50, 100, and 150 mM were used for seed treatment in completely randomized block design with three replicates. Seed germination bioassay was carried out in petri plates (9 cm diameter) lined with germination paper in seed germination chamber. The seeds were surface sterilized using 0.1% $HgCl_2$ for five minutes, followed by thorough rinsing with distilled water. About 20 healthy and uniform seeds were used for germination and 5 mL solution of respective concentration of NaCl was added to each petri plate. The petri plates were then kept in 12/12 hrs in light and dark conditions with no special light treatment. The criterion for the germination was emergence of 1mm radicle.

Germination percentage was recorded on 12 DAS and 10 seedlings were randomly selected from each treatment and their average length (shoot and root) and fresh weight were measured. Then, they were oven-dried at 65°C for 24 h to acquire their mean dry weight. Seedling vigour index was determined by the formula given by Abdul Baki & Anderson (1973); Seedling vigor index = Germination percent × (Shoot length + Root length). The data was recorded from 10 randomly selected seedlings of each treatment and control. The seedlings (20 DAS) were washed with distilled water and 100 mg was used for the estimation of protein, reducing sugars, total sugars, starch, proline and total free amino acids by the methods of Sadasivam & Manickam (1996). Glycine betaine content was determined by the method of Ishitani *et al*. (1993). The data was statistically analyzed by one way ANOVA using SPSS (version 20.0). The significance of differences was determined according to Duncan's Multiple Range Test (DMRT).

Increasing salinity caused significant reduction in seed germination, shoot and root length, fresh and dry weight, seedling vigor index as compared to control (Table 1). As salt concentration increased to 100 and 150 mM, there was 50%-80% reduction in seed germination over control (67.14%). The decrease in shoot and root length was highly significant at higher salinity. The root length was comparatively more affected than the shoot length. There was about 50% and 85% decrease in shoot and root length, respectively at 150 mM NaCl concentration. Significant decrease in seedling vigor index was observed at all the concentrations. About 70% and 84% reduction in seedling fresh and dry weights was recorded at 150 mM NaCl concentration. Our results are in agreement with Ozturk (2008), Belaqziz *et al*. (2009) and Ghanavati & Sengul (2010). The salt treated seeds might have developed osmotically enforced inhibition by salinity stress. It has been claimed that decrease in the water potential gradient between seeds and their surrounding media may adversely affect the germination and subsequent metabolic events of seedling growth and development (Afzali *et al*. 2006). The activity of enzymes like lipase, amylase, protease might be hampered by

Table 1. Effect of different concentrations of NaCl on seed germination and seedling growth of ajowan

NaCl (mM)	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)	Seedling vigor index
0	67.14±1.87a	3.2±0.2a	1.39±0.09a	62±2.77a	13±0.35a	308.85±19.8a
25	60.67±2.31b	3.09±0.09a	1.29±0.09a	56±1.85ab	12±0.23b	266.05±16.38b
50	42.81±2.44c	2.25±0.28b	0.93±0.05b	50±5.3bc	11±0.46c	136.55±20.63c
100	31.46±1.28d	2.01±0.12b	0.5±0.01c	46±5.38c	10±0.69d	79.21±5.8d
150	12.03±1.76e	1.42±0.39c	0.21±0.17d	18±5.41d	2±0.06e	20.11±7.89e
SEm±	5.34	0.19	0.12	4.15	1.03	29.48
Significance	**	**	**	**	**	**

Data are the pooled means of three estimates ± SD; The values followed by different letters differ significantly by DMRT at P<0.05

salt stress, resulting in non-availability of soluble food material for the developing embryo, which might inhibit seed germination and seedling growth (Taiz & Zeiger 2002).

NaCl induced osmotic stress caused reduction in proteins of treated seedlings as compared to the control (Table 2). The decrease in protein content under salt stress might be due to proteolysis and degradation or inhibition of protein synthesis. The stress mediated decrease in protein may be due to the damage of the protein synthesis apparatus and blocks transcription of RNA from DNA with subsequent depressed translation into protein through binding of DNA and/or RNA with certain inhibitors as claimed by Datta *et al*. (1997). There was accumulation of reducing sugars and total sugars in the seedlings subjected to NaCl stress. The accumulation was highly significant at higher concentrations (150 mM) as compared to control. However, a reverse trend was found for starch content. The enhancement of soluble sugar fraction was accompanied with concomitant decrease in starch as the water potential dropped under stress conditions. The results of the present investigation are in confirmity with Patakas & Noitsakis (2001) and Mohammadkhani & Heidari (2008).

Increasing concentrations of NaCl showed significant increase in content of proline, glycine betaine and total free amino acids (TFAA) as compared to control (Table 3). Under stress conditions, protein degrades and consequently osmolytes like proline, glycine betaine and free amino acids increase (Roy *et al*. 2009). Proline is considered a compatible solute, an osmoprotectant and might be conferring protective effect by inducing the synthesis of stress-protective proteins (Khedr *et al*. 2003). Similar results were reported for plants under salinity by Mohammadkhani & Heidary (2008), Monirifar & Barghi (2009) and Farissi *et al*. (2011).

Table 3. Effect of different concentrations of NaCl on osmolyte content of ajowan seedlings

NaCl (mM)	Proline	Glycine betaine	TFAA*
	mg g^{-1}		
0	0.2±0.01e	0.11±0.01e	4.8±0.72c
25	0.31±0.02d	0.15±0.02d	5.81±0.73c
50	0.42±0.04c	0.18±0.04c	6.89±0.84b
100	0.57±0.06b	0.21±0.02b	7.67±0.58b
150	0.7±0.01a	0.25±0.03a	8.89±0.84a
SEm±	0.05	0.12	0.63
Significance	**	**	**

Data are the pooled means of three estimates ± SD; The values followed by different letters differ significantly by DMRT at P<0.05; * TFAA=Total free amino acids

The present investigation indicated that ajowan behaves as a glycophyte and can tolerate moderate salt concentration upto 100 mM, atleast during seed germination and seedling growth stage. The levels of accumulation of proline, reducing sugars, glycine betaine and

Table 2. Effect of different concentrations of NaCl on organic constituents of ajowan seedlings

NaCl (mM)	Total soluble proteins	Total sugars	Reducing sugars	Starch
	mg g^{-1}			
0	8.63±2.38a	16.18±0.32e	5.08±0.14d	9.25±0.44a
25	8.25±0.44b	19.15±0.25d	7.14±0.25c	7.11±0.18b
50	7.06±0.1c	21.13±0.23c	8.65±0.56b	6.19±0.32c
100	6.08±0.13d	22.07±0.13b	9.61±0.53b	5.59±0.53cd
150	5.04±0.07e	23.15±0.26a	11.08±0.89a	5.22±0.39d
SEm±	1.38	0.66	0.57	0.39
Significance	**	**	**	**

Data are the pooled means of three estimates ± SD; The values followed by different letters differ significantly by DMRT at P<0.05

free amino acids may serve as indices for salinity tolerance in ajowan.

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