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EFFECT OF SALINITY AND DROUGHT STRESS ON GERMINATION INDICES OF TWO THYMUS SPECIES

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ABSTRACT. The environmental stress such as, salinity (soil or water) and drought are serious obstacles for medical plants and field crops in further areas of the world, especially arid and semiarid regions. In order to investigate salinity and drought stress on *T. daenensis* and T. *kotschyanus* germination indices, an experiment was carried out. To create salinity stress, sodium chloride (NaCl) at the levels of 0 (as control), -3, -6 and -9 bar, and for drought stress, polyetylenglycol 6000 (PEG 6000) in osmotic levels at 0 (as control), -3, -6 and -9 bar were used. Effect of drought and salt levels on investigated traits significant at 0.01. Result showed significance different between evaluated indices. Increasing of stress levels lead to reduction of germination and root and shoot length. Salt stress only in high levels reduced germination rate. Therefore, *T. daenensis* and *T. kotschyanus* have relative resistance to salinity in germination stage. Decrease in osmotic potential progressively inhibited seed germination of the two thyme species. In all salt treatments, maximum germination percentage in *T. kotschyanus* related to West Azerbaijan population and maximum germination rate related to Gazvin population. But in T. daenensis the highest values (both rate and percentage germination) were observed in Arak population. The germination rate of T. *daenensis* to the drought stress was more than the *T. kotschyanus* germination rate like the germination percentage. Comparing the two stresses, it appears that drought has more effect than salt in germination indices.

Key words: Salinity; Drought; Germination; Thymus species.

INTRODUCTION

Medicinal plants play an important role in the provision of health care in many developing countries (Timmermans, 2003). The genus Thymus comprising of around 350 species of perennial, aromatic herbs and sub shrubs is predominantly found in Mediterranean region, Asia,

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Southern Europe and North Africa (Maksimovic *et al.,* 2008).

Thymus is a polymorphic genus and belongs to the family *Lamiaceae*. Thyme species (*Lamiaceae*) are amongst medicinal plants that are largely used in the Mediterranean basin (Ismaili *et al.,* 2004). Some of Thymus species are used in folk medicine. For example, *Thymus vulgaris* L. and *Thymus zygis* L. extracts have been used orally to treat dyspepsia and other gastrointestinal disturbances; coughs due to colds, bronchitis and pertussis; and laryngitis and tonsillitis (as a gargle). Theantiviral and analgesic activities have been reported for this species (Elhabazi *et al.,* 2008). *Thymus daenensis* and *Thymus kotschyanus* are aromatic medicinal plants endemic to Iran. *Thymus kotschyanus* and *Thymus daenensis* known as members of thyme species is used as traditional medicine among people of Iran. Nickavar *et al.* (2005) characterized the essential oils of *Thymus daenensis* and *Thymus kotschyanus* by using GC and GC-MS. Both the essential oils were found to be rich in thymol and carvacrol.

It's evident that many of these species which are slow-growing and slow reproducing are especially vulnerable to this situation. Consequently, many medicinal species are threatened and are in danger of extinction (Zschocke *et al.,* 2000). Due to this over collection, essentially in the flowering period, land conversion and also land degradation, the species is considered now at risk for local extinction. Many healers recognized that recently the species become very scarce and that affect greatly their financial income and subsequently their livelihoods. In order to ensure the sustainable utilization and to meet the growing demand of these wild species, it has become necessary, therefore, to develop rapid methods of their commercial cultivation. Seeds culture is an alternative and easy method of commercial propagation and is being used widely for the commercial propagation of a large number of plant species, including manymedicinal plants. The environmental stress such as, salinity (soil or water) and drought are serious obstacles for medical plants and field crops in further areas of the world, especially arid and semiarid regions.

Salt stress leads to suppression of plant growth and development at all growth stages, however, depending upon plant species, certain stages such as germination, seedling or flowering stage could be the most critical stages for salts stress. Seed germination is first critical and the most sensitive stage in the life cycles of plants (Ahmad *et al.,* 2009) and the seeds exposed to unfavorable environment conditions like salts and drought stresses may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003).

The aim of this study was to evaluate the effects of drought and salinity stresses on *Thymus daenensis* and *Thymus kotschyanus* germination rate, germination percentage, root length, shoot length and ratio of root length to shoot length (R/S).

MATERIALS AND METHODS

Seeds of Thymus kotschyanuswere collected from West Azerbaijan, Gazvin, Semnan (provinces of Iran) but seeds of *Thymus daenensis* were collected from West Azerbaijan, Arak and Guilan (provinces of Iran) during the June 2010. Factorial experiment was carried out in completely randomized design with four replications on *Thymus kotschyanus* and *Thymus daenensis* in the summer of 2010 in the research laboratory of Tehran University Iran. Sodium chloride (NaCl) was used as a salt stimulator and four osmotic potential levels (salt stress accompanied with drought stress; PEG6000) of zero (control), -3, -6, and -9 bar were used. Twenty-five seeds were sown in Petri dishes (8 cm diameter) lined with two layers of filter paper. The seeds of *T. kotschyanus* and *T. daenensis* were surface sterilized in 3% Sodium hypochlorite for 2 min, rinsed five times with distilled water and imbibed in deionized water for 24 h at 25°C.

Five mL of designated treatment solution was added to each Petri dish and distilled water (5 mL) was used as control. All petri dishes were covered with lids and placed in growth chamber 8/16 h light, darkness at 25° for 14 days. Added periodically to maintain the filter wet during the course of the experiment. Only seeds with 1 mm emerged radicle were considered germinated. The numbers of germinated seeds were recorded daily. Based on daily germinated seeds counting, the number of seeds that germinated during the experiment period was recorded. Then, some parameters including germination percentage, germination rate, shoot and root lengths were evaluated. Germination rate was calculated using the following equation (Maguire, 1962):

$$\text{Germination rate} = \sum \frac{\text{number of germinated seeds}}{\text{day of count}}$$

Statistical analysis was carried out through MSTATC software. The graphs were designed by using Microsoft Office Excel 2007 software.

RESULTS AND DISCUSSION

Analysis of variance results showed that the effect of salinity was significant ($P<0.01$) on the whole studied traits among two plants (*Tables 1, 2*).

Seeds of *T. daenensis* and *T.kotschyanus* germinated best in the absence of salt. The response of germination rate, germination percentage, root length, shoot length and ratio of root length to shoot length (R/S) to salt stress induced by sodium chloride was different depending on concentrations. The salt stress has not remarkable effect on germination rate and germination percentage at low concentration of NACL (-3 bar) but at the value of - 6 bar osmotic potential or higher levels of salt stress, both rate and percentage germination was significantly decreased compared to the control (*Figs. 1, 2*).

Table 1 - ANOVA effects due to salt on measured variables in *T. kotschyanus*

	df	Germination (%)	Germination rate	Root length	Shoot length	R / S
Salinity	3	0.8**	82.41**	1930**	48.72**	130.51**
Population	2	0.38**	98.71**	79.57**	1.97**	5.06ns
Salinity * Population	6	0.025ns	10.25*	36.6*	1.1**	5.06*
Error	36	0.012	3.4	13.96	0.31	1.63
Coefficient of Variation (%)		19.04	36.35	28.53	18.32	33.82

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

Table 2 - ANOVA effects due to salt on measured variables in *T. daenensis*

	Df	Germination (%)	Germination rate	Root length	Shoot length	R / S
Salinity	3	0.82**	193.86**	1730**	64.24**	33.98**
Population	2	0.083**	3.71*	26.46ns	0.75ns	.66ns
Salinity * Population	6	0.022*	2.46*	9.62ns	1.5*	.84ns
Error	36	0.008	0.91	20.11	0.56	0.51
Coefficient of Variation (%)		13.42	15.8	36.73	18.73	29.4

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

In all salt treatments, maximum germination percentage in *T. kotschyanus* related to West Azerbaijan population and maximum germination rate related to Gazvin population. But in *T. daenensis* the highest values (both rate and percentage germination) were observed in Arak population (*Figs. 1, 2*). Also the root length and shoot length were significantly decreased at high concentrations of salt. In all salt treatments, maximum shoot length in *T. daenensis* related to West Azerbaijan population (*Figs. 1, 2*).

Sodium chloride application at low concentration (-3 bar) significantly increased the ratio of root length to shoot length (R/S). The increase root length with NACL application at the low concentration would have the potential to enhance root's absorptive capacity and nutrient uptake in natural and agricultural ecosystems.

However, the ratio of root length to shoot length (R/S) was significantly decreased by the application of a higher levels of NaCl, and this decrease was greater at the higher concentration. This suggesting that the effect of NaCl stress on ratio of root to shoot biomass of *T. daenensis* and *T. kotschyanus* in a concentration-dependent manner.

According to analysis of variance (*Tables 3, 4*) all drought stress treatments (-3,-6,-9 bar) in two plants showed a significant reduction ($P<0.01$) in germination rate, germination percentage, root length, and shoot length, compared with control treatment but the ratio of root length to shoot length (R/S) was increased with increasing of levels of drought except at -9 bar osmotic level.

Table 3 - Analysis of variance for effects of drought stress on germination indices in *T. kotschyanus*

	Df	Germination (%)	Germination rate	Root length	Shoot length	R / S
Drought	3	1.28**	144.02**	2354**	109.5**	219.59**
Population	2	0.19**	29.96**	958.99**	8.75**	84.66**
Drought * Population	6	0.035**	5.68**	51ns	1.4*	43.1**
Error	36	0.008	0.55	42	0.5	11.43
Coefficient of Variation (%)		25.51	21.59	33.07	25.26	55.01

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

Table 4 - Analysis of variance for effects of drought stress on germination indices in *T. daenensis*

	Df	Germination (%)	Germination rate	Root length	Shoot length	R / S
Drought	3	1.72**	231.22**	1877.15**	149.7**	160.83**
Population	2	0.004ns	0.37ns	9.8ns	0.4ns	7.098ns
Drought * Population	6	0.05**	5.47**	34.27*	1.34*	1.99ns
Error	36	0.005	0.8	14.41	0.52	5.94
Coefficient of Variation (%)		15.87	18.28	12.98	22.83	47.25

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

In all drought treatments, maximum germination rate, root length, shoot length traits in *T. kotschyanus* related to Gazvin population. But in *T. daenensis* the highest values for R/S and root length traits were observed in West Azerbaujan population (*Figs.1, 2*).

The germination rate of *T. daenensis* to the drought stress was more than the *T. kotschyanus* germination rate like the germination percentage. Our results indicated that *T. daenensis* was more resistance to salt and drought stress than *T. kotschyanus* in view of germination indices.

Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford, 1999). Seed germination and early seedling growth are affected by both salt and drought stresses.

The response of germination rate, germination percentage, root length, shoot length and root length/shoot length (R/S) to salt stress induced by sodium chloride was different depending on concentrations.

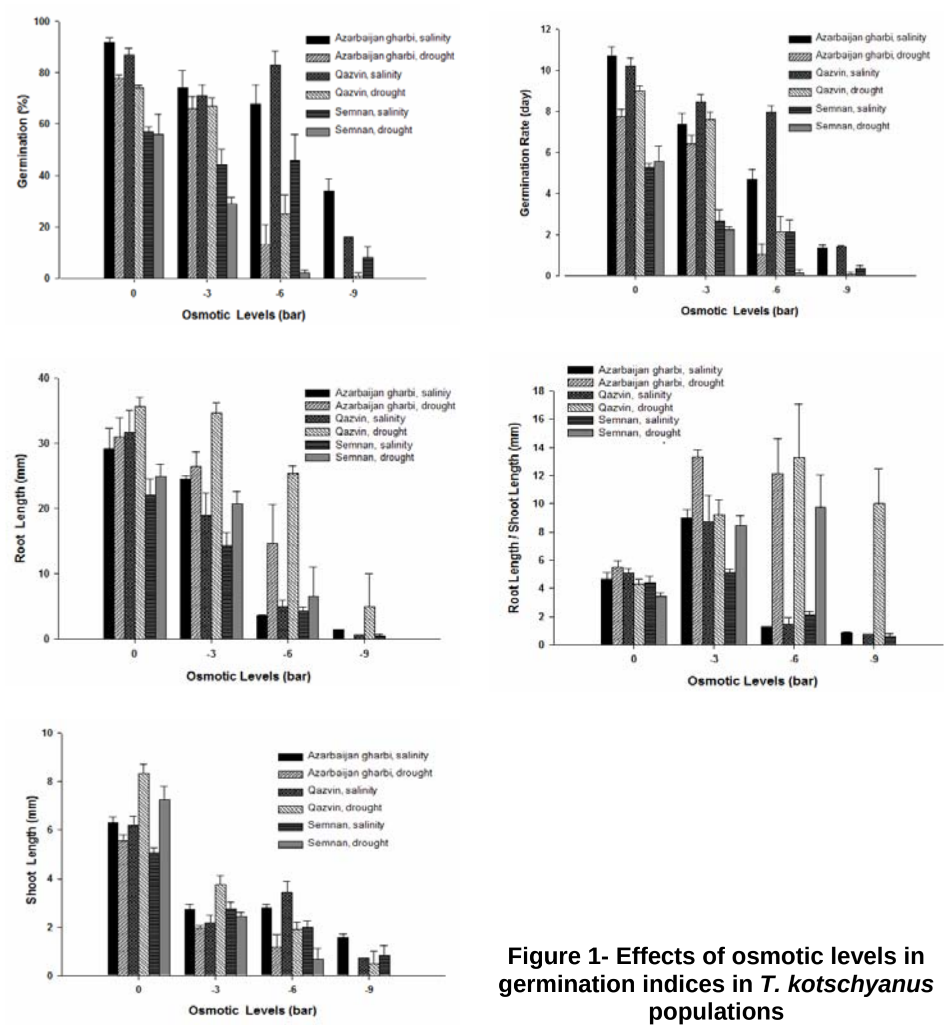
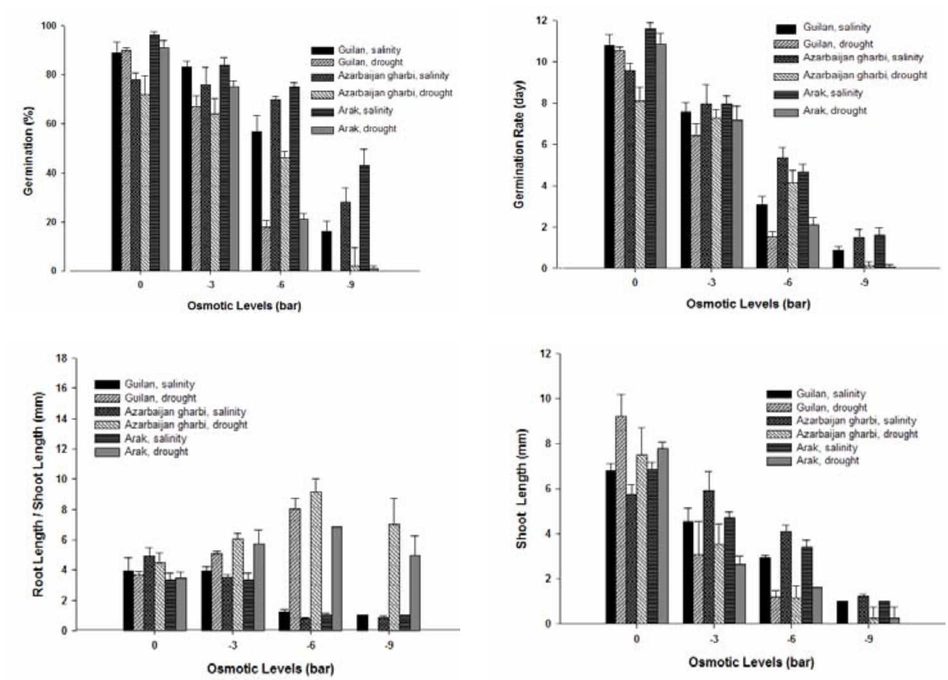


Figure 1- Effects of osmotic levels in germination indices in *T. kotschyanus* populations



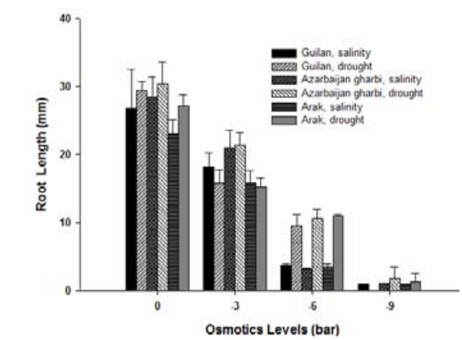


Figure 2 - Effects of osmotic levels in germination indices in *T. daenensis* populations

Salinity imposes other stresses such as ion toxicity on plants, as a result of ion entry in excess of appropriate concentrations and nutrient imbalances, as commonly seen in the displacement of potassium by sodium. Salinity damage is mainly due to altered water relation caused by high salt accumulation in the intercellular spaces (Zhang *et al.*, 2006). Increasing salinity levels caused delay in seedling emergence as a result of reducing cell division and plant growth metabolism (Maas and Nieman, 1978).

In this study drought stress caused the germination percentage decrease in all of the populations.

PEG causes the seed reserves materials hydrolysis decrease and finally the germination percentage decrease (Munns and Weir, 1981). Our results were fortified by those of Nayar and Gupta, 2006. They reported that drought stress decreased the root length in various plant species wheat and maize. The basal level of root length was also high in tolerant and moderately tolerant genotypes as compared to sensitive genotypes implying that root length is important for a plant to exploit the available water.

CONCLUSION

From this study, it can be concluded that low water potential and salt stress (in high levels) inhibit germination of the two thyme species. Salt stress only in high levels reduced germination rate. Therefore, *T. daenensis* and *T. kotschyanus* have relative resistance to salinity in germination stage. The reduction of the availability of water reduces the germination and the limits for seeds germination of the two thyme species occur between -3 and -9 bar. Comparing the two species, it appears that *T. daenensis* is more tolerant to the water stress than *T. kotschyanus.*

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