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Effect of drought stress on germination in 18 population of thyme (*Thymus kotschyanus*)

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Abstract

Thyme is an important medicinal plant of Iran that have Thymol and Carvacrol and used for antimicrobial and antibacterial agent. The present study was carried out to determine the effect of drought stress in 18 populations of this species. The environmental stress such as, drought are serious obstacles for medicinal plants and field crops in further areas of the world, especially arid and semiarid regions. To create drought stress, polyetylenglycol (PEG) 6000 in osmotic levels at 0 (as control), -0.2, -0.4, -0.6 and -0.8 MPa were used. Effect of drought 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. Comparison of traits in the populations and different levels PEG application showed the highest germination in the population of Zanjan 2, Unknown 2 and Zanjan 4, respectively. For this trait in normal conditions and severe stress, there was no major difference between *T. kotschyanus* population. In terms of the radicle length, population of Zanjan 2 and Ghazvin 3 were higher than others. There was a lot variety in seed vigor. Population of Zanjan 2, Ghazvin 3 and Zarand were higher than others. Accessions Zanjan 2, Az. Gharbi 1, Qazvin 3, Zanjan 4 and Qazvin 2 in the majority of traits related to germination, your positive characteristics somewhat maintain in different levels of PEG stress . Accession combination of the above can use as a mass suitable for cultivation in rainfed and low yields rangelands.

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Introduction

Medicinal plants play an important role in the provision of health care in many developing countries (Timmermans, 2003). The Thymus genus comprising of around 350 species of perennial, aromatic herbs and sub shrubs is predominantly found in Mediterranean region, Asia, Southern Europe and North Africa (Maksimovic, 2008). Thymus is a polymorphic genus and belongs to the family *Lamiaceae*. Thyme species 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 (Elhabazi *et al.*, 2008). Thyme Phenolic essential oil is one of the 10 important essences which had antibacterial, antifungal, antioxidant, preservative food and delay the aging mammals (Seidler *et al.*, 2008).

Thymus kotschyanus is medicinal plant aromatic endemic Iran. This specie known as members of thyme species is used as traditional medicine among people of Iran. Both the essential oils were found to be rich in thymol and carvacrol. It's evident that this specie which is 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 many medicinal plants. The environmental stress such as drought is serious obstacles for medical plants and field crops in further areas of the world, especially arid and semiarid regions. Certain stages such as germination, seedling or flowering stage could be the most critical stages for drought 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 drought stress may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003). The aim of this study was to evaluate the effects of drought stress on *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 18 *Thymus kotschyanus* populations were collected from several provinces of Iran. During the April 2015, factorial experiment was carried out in Completely Randomized Design with three replications in the research laboratory of Qom Agriculture Research Center. Five drought stress; (PEG6000) of zero (control), -0.2, -0.4, -0.6 and -0.8 MPa were used. Thirty seeds were sown in Petri dishes (8 cm diameter) lined with two layers of filter paper. Seeds were sterilized with fungicides Vitawax and a concentration of 2: 1000 and rinsed with distilled water. Five mL of designated treatment solution was added to each Petri dish and distilled water was used as control. All petri dishes were placed in growth chamber 8/16 h light, darkness at 25 °C for 14 days.

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, speed of germination, shoot and root lengths, R/S, root and shoot dry weight and seed vigor were evaluated. Germination speed was calculated using the Maguire, 1962 formula. Seed vigor was calculated using the Anderson and Abdulbaki,1973 formula. Statistical analysis was carried out through MSTAT-C software.

Results and discussion

Analysis of variance showed that the population effect was significant (P<0.01) on the whole studied traits. As well as between different levels of PEG for all traits (except speed of germination) significant difference was observed at 1% level, which shows the effect of different levels of drought traits in accessions. Population×PEG levels interaction was significant only in the germination percentage and seed vigor traits (table 1).

Table 1. Analysis of variance on the accession of the *T. kotschyanus* and levels of PEG.

traits	Mean Square				cv%
	Population df=17	level PEG df=4	Population*PEG level df=68	Error df=30	
germination percentage	0.084^{**}	5.56^{**}	0.024^{**}	0.009	18.4
germination speed	1.57^{**}	0.3^{ns}	0.6^{ns}	0.58	22.7
root length	126.7^{**}	3071^{**}	11.01^{ns}	14.52	16.6
shoot length	4.55^{**}	140.5^{**}	0.45^{ns}	0.77	27.1
R/S	34.5^{**}	272.8^{**}	8^{ns}	7.25	29.2
root and shoot dry weight	0.038^{**}	1.61^{**}	0.005^{ns}	0.012	21.4
seed vigor	3.76^{**}	185.3^{**}	0.84^{**}	0.47	32.9

*, ** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

Comparison of different levels PEG with Duncan's method showed, by increasing the use of PEG, germination, length and weight of root and shoot, decreased (Table 2). So the traits in each application of PEG, was in a separate group. As well as all levels of application PEG In the germination speed were in one group. In other words, by increasing the PEG, the germination speed did not change. Seed vigor decreased. Although there was no significant difference between -0.6 and -0.8 MPa.

Table 2. The results of traits mean comparison in different PEG levels.

Treatment	germination percentage	Ger. speed	root length	shoot length	R/S	R. & S. dry weight.	seed vigor
control	0.88a	3.17a	32.6a	5.25a	6.5d	0.72a	4.65a
-0.2 MPa	0.72b	3.17a	25.6b	3.98b	6.9d	0.61b	2.88b
-0.4 MPa	0.43c	3.12a	20.4c	2.64c	8.7c	0.48c	1.18c
-0.6 MPa	0.18d	3.14a	15.4d	1.56d	10.7b	0.36d	0.30d
-0.8 MPa	0.10e	3.31a	12.7e	1.09e	12.5a	0.23e	0.11d

In each column, the numbers have similar letters, together no significant difference in the 5% level.

Comparison of traits in the populations and different levels PEG application showed the highest germination in the population of Zanjan 2, Unknown 2 and Zanjan 4, respectively. As germination percentage in this populations at all levels of PEG was in group A and higher than other populations. In other words, the germination percentage of this population in rainfed conditions partly was preserved. The variation between populations was low in the control and treatment of -0.8 MPa. In other words, in normal conditions and severe stress, there was no major difference between T. *kotschyanus* populations. The lowest germination percentage in the population of Sanandaj 1, Lorestan and Divandare, respectively (table 3).

Table 3. The results of traits mean comparison in populations of *T. kotschyanus* and different PEG levels.

Population	germination percentage						germination speed					
	control	-0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa	mean	control	-0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa	mean
Ghazvin 1	0.96ab	0.88 a	0.23gh	0.09ef	0.02b	0.50bcd	3.3abc	3.33ab	3.6abc	3.2b	5a	3.48ab
Zanjan 1	0.89ab	0.88 a	0.66abc	0.19cde	0.06b	0.53a-d	3.37abc	2.93 ab	3.43a-d	2.23b	2.83ab	3a-d
Zanjan 2	0.92ab	0.82 a	0.78a	0.38a	0.2a	0.62a	2.3bc	2.53 ab	2.87 a-d	2.97b	3.3 ab	2.8cd
Az. Gharbi1	0.87ab	0.7abc	0.16h	0.08f	0.08b	0.38ef	2.83abc	3.5 ab	2.9 a-d	4.8a	3.4 ab	3.48ab
Zanjan 3	0.89ab	0.6bcd	0.34f-i	0.2cde	0.05b	0.46cde	4.13a	3.43 ab	3.93a	3b	3.7 ab	3.63a
Sanandaj1	0.82b	0.51cd	0.4c-h	0.07f		0.45de	3.4 abc	3.93a	2.53bcd	3.37b		3.3ab
Ghazvin 2	0.98a	0.88 a	0.71ab	0.15def	0.07b	0.56abc	3.87ab	3.33 ab	3.73ab	3b	3 ab	3.4ab
Divandare	0.63c	0.41d	0.16h	0.06f	0.07b	0.30f	2.03c	2.3b	2.5bcd	3.17b	3.7 ab	2.59d
Unknown1	0.83b	0.6bcd	0.16h	0.15def	0.04b	0.40ef	2.8 abc	3.13 ab	3.1 a-d	3.37b	3.7 ab	3.1a-d
Unknown 2	0.94ab	0.86 a	0.64abc	0.26abc	0.14ab	0.53a-d	3 abc	3.2 ab	3.5 a-d	3.67ab	4.1 ab	3.49ab
Lorestan	0.93ab	0.6bcd	0.25gh	0.11ef		0.47cde	3.5 abc	3.43 ab	3.53 a-d	3.27b		3.43ab
Zanjan 4	0.93ab	0.82a	0.46c-f	0.37ab	0.22a	0.56abc	3.2 abc	3.37 ab	3.07 a-d	2.73b	2.43b	2.9a-d
Tehran	0.82b	0.7abc	0.54b-e	0.19cde	0.05b	0.52bcd	3 abc	3 ab	2.73 a-d	3.13b	2.9 ab	2.9a-d
Nagade	0.87ab	0.74ab	0.37d-h	0.17c-f	0.05b	0.47cde	2c	2.63 ab	2.53bcd	2.63b	3 ab	2.53d
Zarand	0.88ab	0.76ab	0.61a-d	0.25bc	0.07b	0.59ab	3.4 abc	3.17 ab	3.33 a-d	3.47ab	2.2b	3.26ab
Sanandaj 2	0.9ab	0.73ab	0.52b-f	0.18c-f	0.05b	0.48cde	3.4 abc	2.9 ab	2.37d	2.67b	3.5 ab	3a-d
Oromiea 1	0.88ab	0.76ab	0.42c-g	0.19cde	0.09b	0.49b-e	4.43a	3.87 ab	3.53 a-d	2.8b	3.5 ab	3.64a
Oromiea 2	0.91ab	0.83a	0.57a-e	0.22cd	0.07b	0.52bcd	3.1 abc	3.07 ab	2.97 a-d	2.97b	3.5 ab	3.1a-d

In each column, the means with similar letters no significant difference in the %5level.

Table 3. The results of traits mean comparison in populations and different PEG levels (Continue).

Population	root length						seed vigor					
	control	-0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa	mean	control	-0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa	mean
Ghazvin 1	37.7ab	26.3a-d	17c	12.3cde	2d	22.2c-e	6.3ab	4.1ab	0.73bcd	0.15cd	0.01d	2.60ab
Zanjan 1	35abc	23bcd	20.7abc	16 a-e	14.7ab	21.9c-e	5.3abc	4.12ab	1.29bc	0.28cd	0.06cd	2.22a-d
Zanjan 2	36ab	32.3a	27.3a	21.3a	19.3a	27.3a	5a-d	3.59bcd	2.5a	0.81a	0.3a	2.4abc
Az. Gharbi1	38.3a	32.7a	27.3a	19.7ab	14.7ab	26.5a	4.3b-e	2.07def	0.31d	0.1cd	0.1bc	1.38fg
Zanjan 3	26cde	26.3a-d	19.3abc	15.3a-e	1.5d	21.2c-f	4.4b-e	2.1def	0.88bcd	0.3bcd	0.06cd	1.77def
Sanandaj 1	30.3b-e	25.3a-d	20abc	14.3a-e		22.5b-e	3.8cde	1.81ef	0.76bcd	0.07d		1.62def
Ghazvin 2	36.3ab	30ab	26.3ab	18.7abc	12.3abc	24.7abc	6.92a	5.12a	2.68a	0.38bcd	0.1bc	3.04a
Divandare	26.7cde	21cd	16.7c	13.7 a-e	1.5d	19.2e-h	2.94e	1.32f	0.38cd	0.08d	0.06cd	1.09g
Unknown 1	30.7b-e	26a-d	19.7abc	15.7 a-e	1d	22.4b-e	4.1cde	2.22def	0.47cd	0.36bcd	0.19b	1.65def
Unknown 2	35.3abc	27.3abc	20abc	18.3a-d	13.7ab	22.9b-e	4.2b-e	2.65b-f	1.03bcd	0.43bcd	0.13bc	1.69def
Lorestan	34a-d	27.3abc	21.7abc	18a-d		25.3ab	4.9a-d	2.5c-f	0.69bcd	0.17cd		2.1bcd
Zanjan 4	34.3a-d	27.7abc	19.7abc	13b-e	11.3abc	21.2c-f	3.5de	2.62b-f	0.81bcd	0.49abc	0.2b	1.52efg
Tehran	35.3abc	22.7bcd	20.3abc	16.3a-e	7bcd	22.7b-e	3.6de	2.47c-f	1.48b	0.27cd	0.06cd	1.80c-f
Nagade	27.7cde	23bcd	18.3c	12.3cde	8bcd	18.6fgh	4cde	2.52c-f	0.85bcd	0.23cd	0.06cd	1.64def
Zarand	34a-d	27abc	22.3abc	16.7a-e	9bcd	24abc	5.4abc	3.98abc	2.53a	0.66ab	0.29a	2.92ab
Sanandaj 2	33b-e	23.7bcd	19bc	14.7a-e	9.5bcd	20.5d-g	5.7abc	2.84b-f	1.64b	0.3bcd	0.05cd	2.1bcd
Oromiea 1	30b-e	20.3cd	16c	10.3e	4.5cd	17.6gh	4.5b-e	2.62b-f	0.91bcd	0.16cd	0.04cd	1.77def
Oromiea 2	26e	19.3d	16c	11.3de	8.3bcd	16.2h	4.7 b-e	3.22b-e	1.34bc	0.25cd	0.06cd	1.92cde

In each column, the means with similar letters no significant difference in the %5level.

There was not a lot variety between populations in germination speed. As most of the populations in the different levels of PEG were in common groups. As germination speed in the Az. Gharbi1 and Unknown 2 populations at all levels of PEG was in group A and higher than other populations. In different levels of PEG, in terms of the radicle lenght, population of Zanjan 2, Az. Gharbi 1 and Ghazvin 3 were higher than others. There was a lot variety between populations in seed vigor. As most of the populations in the different levels of PEG were in different groups. In this character populations of Zanjan 2, Ghazvin 3 and Zarand were higher than others at all levels of PEG.

Conclusion

The results indicated that *T. kotschyanus* was semi-resistance to drought stress. 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 drought stress. The response of germination percentage, root length, shoot length and root length/shoot length (R/S) to drought stress induced by PEG was different depending on concentrations.

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 wheat and maize species. 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. Our results are similar with Khoshsokhan *et al.,* (2012) report. They reported the Effect of drought 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.

Reduction in germination characteristics due to the water stress can be related to low water absorption by seed is adversely affected or slow absorption of water occurs, metabolic activity within the seed germination will take place slowly. Bagheri *et al.,* (2011) reported that *T. kotschyanus* was more resistant to water stress than *T. Daenensis* in view of germination speed and vigor index. Accessions Zanjan 2, Az. Gharbi 1, Qazvin 3, Zanjan 4 and Qazvin 2 in the majority of traits related to germination, your positive characteristics somewhat maintain in different levels of PEG stress . Accession combination of the above can use as a mass suitable for cultivation in rainfed and low yields rangelands.

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