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Effect of drought stress on germination characteristics of *Tanacetum polycephalum* under different temperature regimes

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ABSTRACT: Drought stress is one of the most important environmental stresses which limit plants growth andproductions in different regions of the world. An experiment was performed to investigate the effects of drought stress and differenttemperatureregimeson germination and seedling growth of Tanacetumpolycephalum. Temperatures included 5, 10, 15, 20,25, 30 and 35 °C and for each temperature six level of osmotic potentials (0, -2, -4, -6, -8 and -10 bar) imposed by polyethylene glycol) simulating drought conditions. The results showed that, the effects of osmotic stress and temperature was significant on total germination percentage, normal germinationpercentage,mean germination time, rootlength,shoot length and vigor index. At all temperatures germination percentage and other parameters decreased by increasing stress level. At 5 and 10°C drought tolerance was not considerableandseedswere germinated in -2 bar of PEG only. The highest drought tolerance was observed at 25°C which reached to 100% in control (0 bar PEG) and 14.66% in -10 bar of PEG at this temperature. Germination percentage and rate decreased under temperature of 35°C, and it reached to 79.33% in control seeds. In conclusion,25°C was determined as the optimum temperature of germination for this plant. This isdue to exhibition of the highest germination parameters under different water potentials.Also, this herb can tolerate osmotic potential, up to -10bar. Therefore, it is a semi drought tolerant plant species.

Key words: Drought stress,temperature,germination,Tanacetumpolycephalum

INTRODUCTION

Medicinal plants are defined as a group of plant species that contains some special properties or virtues which make them suitable to be used asthetherapeutic agentsorother medicinal purposes.The genus Tanacetum(Compositae) is represented by 26species in the flora of Iran, 12 of them are endemic (Nezhadali et al., 2010).Tanacetumpolycephalumsubsp. Duderanumgrow up wild in North-East of Iran.These species have traditionally been used as a spicy additive for food.In cosmetics and asherbal remedies, due to existence of biologically active compounds, this species is known as a goodtreatment for infectious disease (Nezhadali et al.,2009).This genus isrich in essential oils, bitter substances and sesquiterpene lactones (Akpulat et al., 2005).

Drought is a severe limitation of plant growth, development and productivity, particularly in arid and semi-arid regions (Galle et al., 2007) Response, mechanismand characteristics of plants to face drought stress have become a crucial environmental research topic in drought-prone regions.Seed germination is a complex physiological process largely determined in non-dormant seeds by temperature and water potential of the seedbed. Each plant species has a specific range of environmental requirements necessary for germination (Baskin and Baskin, 1989).Under conditions of drought stress and low and irregular precipitation, achievement to desirable vegetative covers is considered as suitable features of crops. Under such conditions, seedling emergence potential is one of the most important traits related to seedling establishment (Saeidiet al., 2007). According to previous studies, it is obvious that germination percentage and promptness index decreases with increasing drought stress level induced by polyethylene glycol (De and Kar, 1995). It has also been shown that the inhibition of radicle emergence is mainly because of a decrease in water potential gradient between the external environment andtheseeds (EneasFilho et al., 1995). Decrease in germination on account of polyethylene glycol application might be due to decrease in water contact with seeds and low hydrolic conductivity of water around the seeds (Emmerich and Hardegree, 1991). It has been reported that germination promptness index is more sensitive to drought or osmotic stress than germination percentage (Abdul-baki and Anderson, 1970).

Till now, there is not comprehensive information about germination characteristicsofTanacetumpolycephalum. According to the importance of this plant in Iran medicinal industry and also high potential of this plant to be cultivated as an agronomic plant we placed the objective of this study in determination of the germination characteristics of Tanacetumpolycephalumseeds under different osmotic potentials in combination with different temperature regimes.

MATERIAL AND METHODS

Study site

The study was conducted in the Seed Lab, Department of Agronomy and Plant Breeding, University of Tehran, Iran in 2011.

PEG-osmopriming test

The seeds were sterilized in 5% (v/v) sodium hypochlorite solution for 10 min and washed three times with sterile distilled water. The seeds were placed in Petri dishes with 9 cm in diameter and incubated in the dark at range of temperatures(5, 10, 15, 20, 25, 30 and 35°C) and moisture potential.Forsimulation of drought stress,Poly ethylene glycol (PEG 6000) solutions with a water potential of 0, -2, -4, -6, , -8 and -10 bar were prepared (Michel and Kuffman, 1973). These solutions were mixed separately for each temperature regime to account for the thermal dependence of PEG-solution (Michel and Radcliffe, 1995). Each water potential treatment was used for alltemperatures.

Germination test

Seeds were germinated at constant temperatures (T) of 5, 10, 15, 20, 25, 30 and 35 ℃, maintained in a thermostatically controlled incubator. Samples of 150 seeds (three replicates of of 50 seeds each) were placed in 9-cm petri dishes, containing 5 mlofindividualPEG solutions. The seeds were kept in the dishes to assess germination. This was scored when the radicle reached approximately 2 mm length and data were collected daily until no new seeds germinated during 5 consecutive days. At the end of the experiments, the final germination percentage and the Mean Germination Time (MGT) were calculated.

Radicle and Shoot measurement

Radicle length measurements were also carried out for each experimental treatment during germination tests. To this purpose, 10 seeds were chosen randomly, within those germinated first per each petri dish. Radicle and Stemwere excised from seeds after end of germination and measured for length.

Seed germination parameters calculation

Total germination percentage (Gt) was calculated as Gt= (n/N*100), where n= total number of germinated seeds (normal and abnormal) at the end of the experiment and N= total number of seeds used for germination test. Normal and abnormal seedlings were determined based on ISTA,(1997). Normal germination percentage (Gn) was calculated the same as Gt. But in Gnonly normal seedlings were counted and calculated. Mean germination time (MGT) was calculated based on the following equation (Manjkhola et al. 2003):

$$MGT = \frac{\sum(ni \times di)}{N}$$

Where n_i is the number of seeds germinated after each period of incubation in days (DI) and N is the total number of seeds germinated normally at the end of the experiment. The seed vigor index was calculated as follows(Rahnama and Tavakkol-Afshari, 2007):

$$VI = \frac{Ls \times Pg}{100}$$

Where VI is vigor index, Ls is the mean of seedling length (mm) and Pg is germination percentage.

RESULTS AND DISCUSSION

Germination parameters such as total germination percentage, normal germination percentage, mean germination time, radical length, shoot length and vigor index,under different temperatures, were decreased significantly with increase in severity of drought stress and there was significant difference between the control and all of the levels of stress (Table 1-7).Results showed that, tolerance to drought stress increased with an increase in temperatures from 5 to 25℃ and germination parameters showed the best tolerance in all level of drought at 25℃ .At 5 and 10 ℃, tolerance to drought stress was not considerableandunder drought stress conditions, seed germination was observed up to -2 bar of PEG (10.66 and 18.88%) respectively and reached to 0% in other potentials.(Table 1, 2). There was significant difference between the control and all of the levels of stress at these temperatures.

Table 1.Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 5 ^{o}C.

PEG	Gt	Gn	MGT	RL	SL	VI
0	14^{a}	14^{a}	5.56^{a}	4.33^{a}	6.66^{a}	1.53^{a}
-2	10.66^{b}	9.33^{b}	5.84^{b}	2.6^{b}	4.33^{b}	0.73^{b}
-4	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
-6	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
-8	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
-10	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
Treatment						
M.S	655.53	100.72	21.60	8.97	22.53	2.07
Significant	**	**	**	**	**	**

**, significant in 1%

Table 2. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 10 ^{o}C

PEG	Gt	Gn	MGT	RL	SL	VI
0	$24/66^{a}$	24.66^{a}	$5/05^{a}$	5.33^{a}	8.66^{a}	3.46^{a}
-2	$18/88^{b}$	16^{b}	$5/35^{b}$	3.66^{b}	5.66^{b}	1.74^{b}
-4	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
-6	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
-8	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
-10	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
Treatment						
M.S	330.19	301.66	26.01	14.54	37.39	5.36
Significant	**	**	**	**	**	**

**, significant in 1%

At 15 ℃ germination percentage and drought tolerance was improved and germination was observed up to -8 bar (9.33%)while it reached to 0 in -10 bar of PEG. In addition,germination rate, radical length, stem length and vigor index was better than 5 and 10 ℃ (Table 3). The highest drought tolerance and germination parameters was observed at temperatures of 20 and 25 ℃that germination percentage reached to 100%℃in control seeds (0 bar). Results showed that drought tolerance and germination percentage was better in all potentials under 25 ℃ compare to 20℃. At 20 and 25 ℃ germination was observed in all water potentials and reached to 8.66 and 14.66% in -10 bar of PEG respectively (Table 4, 5).

All germination parametersexhibited the highestvalue of tolerance at 25℃ than other temperatures and also mean germination time was at lowest amount under this temperature. By increasing temperature to 30 and 35℃ germination percentage decreased again and reached to 0% in -10 bar of PEG (Table 6, 7).At all temperatures germination percentage decreased significantly with an increased level of drought stress.The most sensitive stages for successful establishment of many crops in response to environmental stresses are germination and early seedling growth (Jones, 1986). Two stages of germination process are enzymatic hydrolysis of stored material and building of new tissue by hydrolysis (Bahrami et al., 2012). Moisture deficit can affect enzymatic activity and consequently germination percentage decreases under more osmotic potential.

Results showed that, water stress impose considerable changes in the mean germination time of tanacetumpolycephalumand a significant delay in the initiation and completion of germination process under water stress treatments, at all temperatures. Under water stress conditions, delay in completion of germination is a common response, because seeds require more time to absorb sufficient amount of water, which is important for the act of initiation of germination (Tesche,1975). Metabolic disorders such as slower hydrolysis of storage compounds in endosperm and slower transportation of hydrolysed material to developing embryo axis could be the reasonsfor reduction of seed germination under water stress condition (Ayazet al., 2000). By decreasing osmotic potential, water absorption also decreases and as a result, turgidity of cell decrease and finally, radicle and stem growth and germination percentage are decrease (Zaefizadehet al., 2011).

Radical length reduced as drought level increased at all temperatures and had the highest length in all level of drought at 25℃ . Root length is one of the most important characters for drought stress because rootlengthisimportantto increase the area of water absorption. Therefore, root length has an important role to a plant's response to drought resistance (Mostafavi et al.,2011). Stem length reduced as drought level increased at all temperatures but was more resistant to drought stress than radical. The hypocotyls are the equivalent of stems of a germinating seedling, found below the cotyledons or seed leaves and above the root. Hypocotyls are responsible for the elongation of growth and development into the stem.

Shi and Ding (2000) stated the growth of hypocotyl and radicle at germination stagecan reflects the tolerance of the shoot to drought. From those studies that have been reviewed, it may be concluded that reductions in root and shoot lengths could be due to reductions in cell division and enlargement caused by water stress.Plants apply several mechanisms to cope with the challenges caused by abiotic stresses, for instance in osmotic stress defense mechanisms is to accumulateorganicosmolytes (such as proline, soluble sugars and other organic acids)in the cytoplasm to savewaterpotential during drought stress (Li et al., 2013). Temperature might affects, these mechanisms and as results, protective mechanisms of plants in facing water stress will disturb by increasing temperature. Many of proteins are sensitive to high temperatures.

Table 3. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 15 ^{o}C

PEG	Gt	Gn	MGT	RL	SL	VI
0	73/33[a]	72.66[a]	3/28[a]	6.33[a]	13.16[a]	14.31[a]
-2	65/33[b]	58[b]	3/73[b]	4.33[b]	10.66[b]	9.79[b]
-4	51/33[c]	39.33[c]	5/24[c]	2.83[c]	9[b]	6.07[c]
-6	22/66[d]	10.66[d]	5/36[d]	1.83[d]	5.66[c]	1.69[d]
-8	9/33[e]	2.66[e]	5/55[e]	1.4[d]	2.16[d]	0.33d[e]
-10	0[f]	0[f]	0[f]	0[e]	0[e]	0[e]
Treatment						
M.S	2920.82	2755.42	12.95	16.11	84.34	96.20
Significant	**	**	**	**	**	**

**, significant in 1%

Table 4. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 20 ^{o}C

PEG	Gt	Gn	MGT	RL	SL	VI
0	100[a]	100[a]	2/16[a]	10[a]	27.66[a]	37.66[a]
-2	89/33[b]	86[b]	2/80[b]	7[b]	20.66[b]	24.72[b]
-4	66[c]	60[c]	2/92[b]	4.66[c]	9.76[c]	9.43[c]
-6	50/66[d]	39.33[d]	3/86[c]	3.16[c]	10.33[c]	6.85[c]
-8	25/33[e]	11.33[e]	3/83[c]	3.10[c]	4.33[d]	2.15[d]
-10	8/66[f]	2.66[f]	4/08[d]	3[c]	3.10[d]	0.51[d]
Treatment						
M.S	4553.07	4939.30	1.74	29.54	305.77	612.76
Significant	**	**	**	**	**	**

**, significant in 1%

Table 5. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 25 ^{o}C

PEG	Gt	Gn	MGT	RL	SL	VI
0	100[a]	100[a]	1/6[a]	12.66[a]	23[a]	35.66[a]
-2	93/33[b]	88[b]	1/91[b]	8[b]	21[a]	27.06[b]
-4	80[c]	73.33[c]	2/15[b]	5.33[c]	15.33[b]	16.53[c]
-6	50[d]	38.66[d]	2/95[c]	3.66[d]	10[c]	6.84[d]
-8	28[e]	15.33[e]	3/01[cd]	2.5[e]	4.66[d]	2.02[e]
-10	14/66[f]	4.66[f]	3/18[d]	2.33[e]	2.26[d]	.053[e]
Treatment						
M.S	4736.82	5208.88	1.32	57.01	250.54	603.13
Significant	**	**	**	**	**	**

**, significant in 1%

Table 6. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 30 ^{o}C

PEG	Gt	Gn	MGT	RL	SL	VI
0	86/66^{a}	86.66^{a}	1/93^{a}	10.66^{a}	22.33^{a}	28.58^{a}
-2	48/66^{b}	45.33^{b}	2/90^{b}	6^{b}	20.66^{a}	13.01^{b}
-4	38^{c}	30.66^{c}	3/20^{c}	3.83^{c}	13.66^{b}	6.65^{c}
-6	32^{d}	16.66^{d}	4/1^{d}	2.5^{d}	7.66^{c}	3.12^{d}
-8	12^{e}	4.66^{e}	4/43^{e}	2^{d}	2.66^{d}	0.55^{e}
-10	0^{f}	0^{f}	0^{f}	0^{e}	0^{e}	0^{f}
Treatment M.S	2871.93	2984.82	7.95	43.22	268.63	327.83
Significant	**	**	**	**	**	**

**, significant in 1%

Table 7. Results of drought on total germination (Gt), normal germination (Gn), Mean germination time (MGT), Root length (RL) (mm), Stem length (SL) (mm), Vigor Index at 35 ^{o}C

PEG	Gmax	Gn	MGT	RL	SL	VI
0	79/33^{a}	78^{a}	1/98^{a}	10.33^{a}	19.33^{a}	23.54^{a}
-2	66/66^{b}	56^{b}	2/29^{b}	4.66^{b}	13^{b}	11.78^{b}
-4	42^{c}	38^{c}	2/93^{c}	3.66b^{c}	9.33^{c}	4.81^{c}
-6	16^{d}	9.33^{d}	3/90^{d}	2.66^{c}	4^{d}	0.81^{d}
-8	8/66^{e}	2.66^{e}	5/11^{e}	2.2^{c}	2.1^{e}	0.25^{d}
-10	0^{f}	0^{e}	0^{f}	0^{d}	0^{f}	0^{d}
Treatment M.S	3186.15	2960.82	9.40	41.39	162.17	237.25
Significant	**	**	**	**	**	**

**, significant in 1%

Table 8.Effect of water potential in PEG on final seed germination and mean germination time (MGT) at different temperatures (℃). Mean values with the same letter are not significantly different at 0.05 level.

Water	5℃		10℃		15℃		20℃	
potential (bar)	Gmax	MGT	Gmax	MGT	Gmax	MGT	Gmax	MGT
0	14no	5/56^{a}	24/66^{l}	5/05^{b}	73/33^{e}	3/28^{f}	100^{a}	2/16^{i}
-2	10/66^{pq}	5/84^{a}	18/88^{m}	5/35^{b}	65/33^{f}	3/73^{e}	89/33^{c}	2/80^{h}
-4	0^{r}	0^{k}	0^{r}	0^{k}	51/33^{g}	5/24^{b}	66^{f}	3/00^{gh}
-6	0^{r}	0^{k}	0^{r}	0^{k}	22/66^{l}	5/36^{b}	50/66^{g}	3/86^{ed}
-8	0^{r}	0^{k}	0^{r}	0^{k}	9/33^{pq}	5/55^{a}	25/33^{lk}	3/83^{ed}
-10	0^{r}	0^{k}	0^{r}	0^{k}	0^{r}	0^{k}	8/66^{q}	4/08^{ed}

Water potential (bar)	25 ℃		30 ℃		35 ℃	
	Gmax	MGT	Gmax	MGT	Gmax	MGT
0	100a	1/6j	86/66c	1/93i	79/33d	1/98i
-2	93/33b	2/15i	48/66g	2/90gh	66/66f	2/29i
-4	80d	2/10i	38i	3/20c	42h	2/93gh
-6	50g	2/95gh	32j	4/1d	16nm	3/90ed
-8	28k	3/01gfh	12po	4/43c	8/66q	5/11b
-10	14/66no	3/18gf	0r	0k	0r	0k

CONCLUSION

It can be concluded that in relation to various levels of induced water stress, the total germination percentage, mean germination time,vigor index, root and shoot length significantly changed ($P<0.05$). Distinction of significant differences in germination and seedling growth in induced water stress tested in this study leads to the conclusion that these parameters may be used as criteria in screening for tolerant against drought stress at germination and seedling stages. Results also showed that the maximum drought tolerance and minimum decrease in germination parameters was observed at 25^{o}C than other temperatures and it can be optimum temperature for Tanacetumpolycephalum(Table 8).Also the value of germination parameters, showing that, this plant is also semi-drought tolerant. Moreover, due to high germination parameters, in 35 ^{o}C for control treatment, we suggest that, this plant have a good and considerable tolerance to high temperatures so it could be a good choice for plantation in warm and hot climates.

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