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EFFECTS OF WATER STRESS ON GERMINATION OF YARROW POPULATIONS (*ACHILLEA* SPP.) FROM DIFFERENT BIOCLIMATIC ZONES IN IRAN

ABSTRACT

The effects of water potential on germination were studied in 18 wild populations of three yarrow species (*Achillea tenuifolia*, *A. vermicularis* and *A. filipendulina*) from different bioclimatic zones in Iran. Water potential between 0 and -0.6 MPa were obtained using polyethylene glycol 6000 (PEG-6000) solutions. The study of water stress on germination showed that, regardless of the species and the populations, the lowering of the water potential reduced the capacity for germination and early seeding growth. These results indicated a strong genetic potential for drought tolerance during germination within each species. These differences in germination ability of wild populations of each species might be attributed to intraspecific variations resulting from the effects of natural selection and genetic pool background.

Key words: germination; polyethylene glycol; water potential; Yarrow

INTRODUCTION

The genus *Achillea* (Yarrow) is one of the most important genera of the Asteraceae family and is presented by about 85 species widespread throughout the world (Chevalier 1996). Yarrow is a drought-tolerant herbaceous perennial plant that is best suited to cottage rather than a formal garden (Halevy 1999). It has medicinal and cosmetic uses (Rohloff *et al.* 2000), and extensively grown in droughtprone environments due to its numerous leaf and several stems developed from the horizontal radiclestock (Bartram 1995). Due to over collection, essentially in the

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flowering period, land conversion and also land degradation, the *Achillea* species are considered now at risk for local extinction, Which affect greatly their financial income and subsequently their livelihoods. Many healers recognized that recently the species become very scarce and that 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, which restricted in Iran by water deficiency and water scarcity. More than 82% of Iran's territory is located in arid and semi-arid zones and faces shortages of water and the challenges caused by drought in its various different regions (Khoshbakht 2011). The water constraint constitutes one of the main environmen-tal problems for development and crop productivity of plants. In front of this problem, the selection of drought tolerant species and varieties remains the best economic approach for exploitation and rehabilitation of arid and semiarid regions (Shannon 1985; Alonso *et al.* 1999; Ghoulam *et al.* 2001). The effectiveness of such approach depends on the availability of genetic variation in relation with drought toler-ance and its exploitation by screening and selection of the pow-erful plants under drought stress (Al-Khatib *et al.* 1992; Ali *et al.* 2007; Hussain *et al.* 2010).

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri *et al.* 2001). Crop establishment depends on an interaction between seedbed environment and seed quality (Brown *et al.* 1989; Khajeh-Hosseini *et al.* 2003). Factors adversely affecting seed germination may include sensitivity to drought stress. A large number of studies have been carried out on the effects of water stress on the germination of plant species, and most species and populations have shown a different sensitivity to water stress with regards to germination and subsequent radicle growth. (Bonner and Farmer 1966; Barnett 1969; Djavanshir and Reid 1975; Kaufmann and Eckard 1977; Calamassi *et al.* 1980; Falusi and Calamassi 1982; Falusi *et al.* 1983; Dunalp and Barnett 1984; Thanos and Skordillis 1987; Falleri 1994; Lopez *et al.* 2000). However, in none of the studies was the selection of populations at the beginning of the research based on a bioclimatic classification. Soil water supply is an important environmental factor controlling seed germination (Kramer and Kozlowski 1979). If the water potential is reduced, seed germination will be delayed or prevented, depending on the extent of its reduction (Hegarty 1978). One technique for studying the effect of water stress on germination is to simulate stress conditions using artificial solutions to provide variable water potentials (Larson and Shubert 1969; Sharma 1973; Falusi *et al.* 1983). In the present work, the effects of water stress were examined in 18 wild populations of three yarrow species (*Achillea tenuifolia*, *A. vermicularis* and *A. filipendulina*) using PEG solutions with water potentials ranging from 0 to -0.6 MPa. The purpose of this study was to evaluate the influence of water stress on germination and to determine whether there was a significant intraspecific variation in drought tolerance between wild populations of *A. tenuifolia*, *A. vermicularis* and *A. filipendulina* seeds from different bioclimatic zones.

MATERIAL AND METHODS

Seed material and experiment layout

Seed material of 18 wild populations of yarrow from four different species including *Achillea tenuifolia*, *A. vermicularis* and *A. filipendulina* were obtained from National Natural Resources Gene Bank, Iran (Table 1).

Water stress was applied through incubation in four different concentrations of PEG 6000 that provide solutions with water potentials ranging from 0 to *-*0.6 MPa (Michel and Kaufmann 1973). Polyethylene glycol 6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Hohl and Peter 1991; Lu and Neumann 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough not to be absorbed by plants (Carpita *et al.* 1979). Because PEG does not enter the apoplast, water is withdrawn from the cell and the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low M_r osmotica, which infiltrate the cell wall with solutes (Verslues *et al.* 1998). Four replicates of 25 seeds per wild populations were used to determine germination rates in the absence or presence of osmotic stress. Washed seeds of *Achillea* were surface sterilized with 70% ethyl alcohol for five minutes. Seeds were thoroughly rinsed with deionized water, 25 seeds were placed in 90 mm diameter glass Petri dishes on double Whatman papers (TP) and irrigated with 5 ml of distilled water or with PEG solution (0 *-* 0.6 MPa). The Petri dishes immediately transferred into a germinator at (20±4°C) with 1000 lux light for 15 days. The percent and rate of germination were recorded at 3, 6, 9, 12 and 15 days. The length of radicles and shoots of 10 randomly-selected seedlings from each replicate were measured in 15 days seedlings. The vigor index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time (Abdul-Baki and Anderson 1973). The experiment was designed as a completely randomized design with two factors. The first factor was the populations and the second the drought stress treatments. Differences in germination between populations and between treatments, as well as the interaction between these variables, were tested for an analysis of variance.

RESULTS

Analysis of variance showed highly significant differences among species, populations and water potentials (Table 2). The interaction between species, populations and water potentials was also significant (Table 2). Mean comparison at different stress levels indicated that lowering of water potential causes a decrease in seed germination per-centage, which was higher in distilled water than in any PEG concentration. In all populations, PEG concentrations delayed the beginning of germination and reduced the final germi-nation percentages (Figs 1-3).

Results from the ANOVA on Germination parameters

Table 2

**, Significant at 0.01 level

Fig. 1. Effect of PEG induced drought (MPa) on Germination rate (GR), vigor index (VI), Radicle length (RL) and Germination % (G %) of seeds from different populations of *A. tenuifolia*

Fig. 2. Effect of PEG induced drought (MPa) on Germination rate (GR), vigor index (VI), Radicle length (RL) and Germination % (G %) of seeds from different populations of *A. vermicularis*

Fig. 3. Effect of PEG induced drought (MPa) on Germination rate (GR), vigor index (VI), Radicle length (RL) and Germination % (G %) of seeds from different populations of *A. filipendula*

Achillea tenuifolia,

Analysis of variance showed highly significant differences among all populations and water potentials as well as their interaction (Table 3). Although lowering the water potential to -0.6 MPa reduced germination for most populations by more than 50%, the reaction to increased water stress differed among the nine populations: T-Dehgelan and T-Sanandaj1 seem to be the most tolerant, with a threshold between -0.4 and -0.6 MPa; and T-Golestanpark and T-Semnan1 the least tolerant, with a tolerance threshold between control and -0.2 MPa (Table 4, Fig. 1). A moderate tolerance was observed in T-Khalkhal, T-Semnan2, T-Sanandaj2, T-Divandareh1 and T-Divandareh1 with a threshold between -0.2 and -0.4 MPa (Table 4).

Table 3

Results from the ANOVA on Germination parameters of *A. tenuifolia***,** *A. vermicularis* **and** *A. filipendulina*

	DF	F									
Source		Germination				Radicle/shoot					
		$\frac{0}{0}$	rate	Vigour index	Radicle	Shoot	Seedling	length ratio			
				A. tenifolia							
(P) Population	8	57.07**	44.40**	84.39**	46.51**	$20.21**$	52.90**	15.02**			
(W) Water	3	146.60**	129.07**	105.75**	16.75**	45.26**	35.78**	$16.52**$			
$P \times W$	24	2.97**	$3.71**$	9.24**	$7.11**$	4.42**	6.95**	$5.21**$			
$P \times S$	$\overline{2}$	0.45	1.35	1.67	24.47	26.54	20.21	32.35			
				A. vermicularis							
(P) Population	$\overline{4}$	$61.02**$	28.37**	183.29**	44.27**	$13.1**$	38.79**	23.56**			
(W) Water	3	40.77**	103.19**	122.41**	19.65**	47.15**	30.52**	2.83**			
$P \times W$	12	$5.65**$	$5.80**$	23.53**	$5.36**$	5.99**	5.43**	$3.51**$			
$P \times S$		19.86	18.77	18.47	23.69	19.28	19.66	31.33			
				A. filipendula							
(P) Population	$\overline{3}$	1.63	$10.05**$	$21.30**$	51.85**	2.03	48.15**	17.89**			
(W) Water	3	28.74**	37.89**	20.87**	4.36**	$4.02**$	5.25**	0.14			
$P \times W$	9	$3.91**$	$2.63*$	$10.65**$	7.88**	$3.47**$	$8.06**$	$3.45**$			
$P \times S$		18.75	20.02	26.22	20.81	18.1	17.64	35.75			

**, Significant at 0.01 level

According to Duncan's test for germination percentage, T-Dehgelan and T-Sanandaj1 were combined in an independent group as the most drought resistant populations. T-Khalkhal, T-Sanandaj2 and T-Divandareh2 (all three in a group); and T-Semnan2 were included in two different groups as moderate tolerance populations. T-Divandareh1 and T-Semnan1 were in two different groups as least drought tolerant populations. T-Golestanpark was included in another group with Divandareh1 and T-Semnan1. On the other hand, there were significant differences between all stress levels according to the germination percentage of different populations (Table 4).

The mean germination rate decreased in response to water stress in all species and populations. However, the magnitude of this response varied among populations (Table 4). The *A. tenifolia* population T-Dehgelan germinated similar to T-Sanandaj1 and both germinated significantly $(p<0.05)$ more rapidly than any other accessions (Table 4).

	Germination [%]						Germination rate				
Population	$\boldsymbol{0}$	-0.2	-0.4	-0.6	Ave.	$\mathbf{0}$	-0.2	-0.4	-0.6	Ave.	
					A. tenifolia						
T-Dehgelan	100.0	96.0	66.7	52.2	78.7a ¹	27.2	22.2	17.5	10.7	19.4a	
T-Sanandaj1	100.0	76.0	85.3	60.3	80.4a	23.2	17.7	19.5	14.5	18.7a	
T-Khalkhal	89.3	92.0	57.3	28.0	66.7b	19.5	19.7	13.3	9.8	15.6b	
T-Semnan2	65.3	70.7	30.7	6.7	43.3c	20.1	20.6	11.8	4.1	14.2 _{bc}	
T-Sanandaj2	100.0	84.0	35.3	26.3	61.4b	22.3	17.3	9.2	3.8	13.2cd	
T-Divandareh2	93.3	81.3	48.0	36.0	64.7b	20.6	14.8	7.4	7.6	12.6cd	
T-Golestanpark	60.0	29.3	10.7	6.0	26.5ed	19.1	14.6	5.5	1.4	11.2d	
T-Divandareh1	49.3	48.0	21.3	12.0	32.5d	8.7	10.7	9.1	3.0	7.9e	
T-Semnan1	39.2	29.3	19.9	2.0	22.6e	6.8	6.6	3.1	0.9	4.4f	
Average ²	77.4a	67.4b	41.7c	25.5d		18.6a	16 _b	11.2c	6.2d		
					A. vermicularis						
V-Baneh	85.3	90.3	77.4	22.2	68.0a	19.3	17.2	8.8	4.0	12.3ab	
V-Mahabad	86.1	83.0	72.6	30.5	68.8a	16.8	16.1	16.9	3.8	13.4a	
V-Sardasht	73.3	65.3	44.0	44.0	57.7b	15.0	12.9	9.6	5.6	10.8bc	
V-Tehran	40.0	40.0	24.0	29.3	33.3c	8.4	7.7	3.2	3.5	6.0d	
V-Yazd	30.7	26.7	8.3	11.3	19.3d	12.9	15.3	9.3	0.6	9.5c	
Average ²	60.4a	61.1a	49.8b	26.4c		14.5a	13.8a	9.6a	3.4c		
					A. filipendula						
F-Meshkinshahr	89.7	69.3	48.0	32.0	59.5a	18.9	14.5	10.8	6.3	12.6ab	
F-Urmieh	93.3	76.6	55.5	32.2	64.4a	21.1	15.8	11.2	9.4	14.4a	
F-Khalkhal2	80.0	85.0	58.7	34.7	69.5a	9.5	10.7	9.5	6.6	9.1c	
F-Khalkhal1	78.4	72.0	60.0	34.7	61.3a	17.4	15.6	9.8	5.5	12.1 _b	
Average ²	79.8a	74.5b	62.2c	38.3d		16.7a	14.1b	10.3c	7.0 _d		

Effect of PEG induced drought (MPa) on Germination parameters of seeds from different populations of *A. tenuifolia***,** *A. vermicularis* **and** *A. filipendulina*

Table 4.

Continued

Table 4

1 Values in the column with the same initial(s) are not significantly different ($P < 0.05$). 2 Values in the row with the same capital initial are not significantly different $(P < 0.05)$

Vigor index and Radicle length differed in the nine populations and were markedly influenced by water potential (Table 4). Even a stress of -0.2 MPa caused a significant decrease in vigor index and Radicle length of most populations. Each further increment in water stress produced additional significant decrease in both characters. The vigor index and radicle length for T-Dehgelan, T-Sanandaj1 and T-Khalkhal were higher than those for other

populations at the lowest water potential (-0.6 MPa), and the lowest tolerance again occurred in T-Golestanpark, T-Divandareh1 and T-Semnan1.

Achillea vermicularis

Table 4 and Fig. 2 show that, for all populations, the lowering of the water potential resulted in a reduction of germination capacity. For V-Mahabad and V-Baneh, germination was not affected when the seeds were exposed to a water potential of *−*0.4 MPa. Germination only dropped dramatically once the potential fell under -0.4 MPa. The other three populations, namely, V-Sardasht, and V-Tehran, V-Yazd retained some germination capacity with water potential *−*0.4 MPa. The study of the influence of water stress on germination showed that, regardless of the populations, the lowering of the water potential reduced the capacity for germination. However, the final germination capacity for 0 MPa stress levels of V-Mahabad and V-Baneh as most tolerance populations, and V-Tehran, V-Yazd as least tolerance populations showed significance differences.

According to Duncan's test for germination percentage, V-Mahabad and V-Baneh were combined in an independent group as the most drought resistant populations. V-Sardasht and V-Tehran were included in two different groups as more sensitive populations. V-Yazd was in a different group as least drought tolerant populations. On the other hand, there were no significant differences between 0 and -0.2 MPa stress levels according to the germination percentage of different populations, while -0.4 and -0.6 MPa stress levels differed significantly (Table 4).

The mean germination rate decreased in response to water stress in populations. However, the magnitude of this response varied among populations (Table 4). The population V-Sardasht germinated significantly $(p<0.05)$ more rapidly than any other populations (Table 4).

Germination rate, vigor index and radicle length differed in the five populations were markedly influenced by water potential (Table 4). A stress level of -0.2 MPa caused a significant decrease in vigor index of most populations, whereas germination percentage and rate of different populations influenced markedly by -0.4 MPa. Population V-Sardash with highest amount of germination percentage and rate showed lowest vigor index and radicle length.

Achillea filipendulina,

According to the experimental data presented in Table 4 and Fig. 3, lowering the water potential to -0.6 MPa reduced germination for all populations by more than 60%. The final germination in this water potential showed only minor differences among the populations, and such differences were statistically not significant. However, at the lowest water potential (-

0.6 MPa), there were significant differences among the populations in terms of germination rate, vigor index and radicle length.

The mean germination rate decreased in response to water stress in all species and populations. However, the magnitude of this response varied among populations (Table 4). The population F-Urmieh germinated more rapidly than any other populations.

The higher vigor index and radicle length in F-Meshkinshahr at the lowest water potential (-0.6 MPa) may be related to its better adaptation to water deficit compared to other three populations.

DISCUSSION

The study of water stress on germination showed that, regardless of the species and the populations, the lowering of the water potential reduced the capacity for germination and early seeding growth.

Seed germination and early seeding growth are usually the most critical stages in seedling establishment, determining successful crop production (Almansouri *et al.* 2001). Crop establishment depends on an interaction between seedbed environment and seed quality (Brown *et al.* 1989; Khajeh-Hosseini *et al.* 2003). Factors adversely affecting seed germination may include sensitivity to drought stress. Results of *A. tenuifolia* allow distinguishing two main types of response to water stress. The first is represented by the T-Dehgelan and T-Sanandaj1 populations, which are tolerant to water stress during the germination phase, since they continued to germinate at *−*0.6 MPa by more than 50%. The second type of response characterized the populations of T-Golestanpark and T-Semnan1, which are the more sensitive. In this case, the germination capacity reduced at -0.2 MPa. Although some authors (Fady 1992; Abulfatih 1995) tented to demonstrate the existence of relationships between the germination properties of seeds (response to temperature and to water stress) and the ecology of the plant, these relationships are not confirmed in our study. Even if this response could be an acceptable indicator of their germination potentials under similar conditions of stress in the natural environment, it cannot be considered as an indicator of drought tolerance in the adult plants (Manohar *et al.* 1968), or as a criterion for the selection of varieties adapted to aridity (Saint-Clair, 1980). The establishment and development of the species depend on the ecological conditions prevailing at the time of emergence and during the later phases of development. They also depend on the genetic characteristics of each species. In order to refine the selection of species offering the best potential, it would be useful to continue investigations into the tolerance to water stress at the seedling stage, since the establishment of the species also depend on the ecological conditions prevailing at the time of seedling emergence. Our results confirm the findings of Khajeh-Hosseini

et al. (2003) in soybean and those of Murillo-Amador *et al.* (2002) in cowpea. However, our findings showed that PEG had greater inhibitory effects on germination because of the significant decrease in germination in the four populations. We can conclude that the higher germination percentage and value in T-Dehgelan and T-Sanandaj1 at the lowest water potential (*−*0.6 MPa) may be related to their better adaptation to water deficit compared to the other populations. Furthermore, differences under water stress also showed that populations are characterized by a significantly different tolerance to drought.

Our findings revealed that inhibition of germination by water potential resulted from osmotic effect. PEG affected the germination and seedling growth of *Achillea* spp. and had a greater inhibitory effect. These results agree with those given by Murillo-Amador *et al.* (2002), and Sadeghian and Yavari (2004), who stated that seedling growth was severely diminished by water stress in sugar beet. Moreover, distinct genetic differences were found among the populations with respect to germination and seedling growth subjected to PEG. This variability would be useful to exploit in a program of Yarrow rehabilitation and reintroducing.

PEG-induced osmotic stress also had an adverse effect on radicle growth. Comparing *A. tenuifolia* and *A. filipendula*, the final germination of different populations of *A. filipendula* at *−*0.6 MPa showed only minor and insignificant differences. However, at this water potential level (-0.6 MPa), there were significant differences among the populations in terms of germination rate, vigor index and radicle length. The higher germination rate, vigor index and radicle length in F-Meshkinshahr at the lowest water potential (-0.6 MPa) may be related to its better adaptation to water deficit compared to other three populations. Radicle length is one of the most important characters for drought stress because radicles are in direct contact with the soil and absorb water from the soil. For this reason, radicle length provides an important clue to a plant's response to drought stress (Mostafavi *et al.* 2011). The hypocotyl is the primary organ of extension of the young plant that develops into the stem, it emerges after emergence of the radicle and in typical cases of drought the radicle will develop faster than the hypocotyl in order to compensate for water stress (Zhu *et al.* 2005). Therefore, the growth of hypocotyl and radicle at germination and seedling stages should reflect the tolerance of the shoot to drought (Shi and Ding 2000). From those studies that have been reviewed, it may be concluded that reductions in radicle and shoot lengths could be due to reductions in cell division and enlargement caused by water stress.

In contrast to *A. tenuifolia* and *A. filipendula* that revealed positive and significant correlation between germination percentage and rate and vigor index and radicle length, results of *A. vermicularis* showed population V-

Sardash with highest amount of germination percentage and rate had lowest vigor index and radicle length.

The mean germination rate decreased in response to water potential in all species and populations. In *A. tenifolia* and *A. filipendula* the ranking order of populations under water potential was similar to that under non-stress (control) treatment. Thus in these two specie rapid germination under water potential represents an intrinsic capability of the seed to tolerate drought. However, in *A.vermicularis*, order of populations under water potential was different from that under non-stress (control) treatment. The *A.vermicularis* population V-Baneh ranked first in the absence of water potential, whereas it ranked 2nd in the presence of water potential. In contrast, population V-Sardasht ranked first to attain final germination capacity in the presence of water potential, whereas it ranked 3rd in the absence of water potential. Interestingly, the V-Sardash with highest amount of germination percentage and germination rate at the lowest water potential (-0.6 MPa), showed lowest vigor index and radicle length. The results indicated that the ability to germinate rapidly under water potential is not merely an overall reflection of the ability for rapid germination under non-stress conditions.

It is noteworthy that many populations that germinated rapidly under water potential (e.g., T-Dehgelan in Table 4) also germinated rapidly under non-stress condition. Thus observations are consistent with the suggestion that some common factors contribute to rapid germination under stress and non-stress conditions (Bradford, 1995; Foolad, 1996). However, several populations (e.g., V-Yazd and T-Sanandaj2 in Table 3) germinated rapidly under control conditions but germinated poorly in the presence of water potential. Consequently, in these populations, the physiological processes required for germination were sensitive to drought exposure. Thus, these populations might be deficient in genetic elements required for coping with drought.

Evaluation of selected populations at the three water potential levels, 0.2, 0.4 and 0.6 MPa, demonstrated that populations that germinated rapidly at lower water potential also germinated rapidly at higher water potential (Table 4). Germination processes under these water potential levels possibly were controlled by similar genetic and physiological mechanisms. This hypothesis is consistent with the previous finding that similar QTLs (quantitative trait loci) contributed to rapid seed germination under differing water potential levels.

Distinction of significant differences in germination and seedling growth in induced water stress between the *Achillea* spp. populations tested in this study leads to the conclusion that these parameters may be used as criteria in screening for tolerant populations against drought stress at germination and seedling stages. In addition, the relatively wide variation between the populations suggested that these stages of growth may be used effectively

to select drought tolerant populations. This study demonstrated that potential sources of drought tolerance during germination exist within *Achillea tenuifolia*, *A. vermicularis* and *A. filipendulina.*

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