Physiological and Biochemical Evaluation of *Achillea tenuifolia* Lam. and *A. vermicularis* Trin. wild populations under drought stress

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ABSTRACT

Five wild populations of *Achillea tenuifolia* Lam. (three populations from hot/dry and cold/dry climate regions) and *A. vermicularis* Trin. (two populations from cold/wet climate region), were subjected to drought stress in order to assess the levels of drought tolerance through the analysis of relative water content, leaf moisture percentage, osmotic solutes (proline and soluble sugars), antioxidant enzymes (peroxidase and polyphenol oxidase), soluble proteins content, and pigments content. Variation among populations under different levels of drought shows that populations are characterized by a different tolerance to drought. In *A. tenuifolia*, the hot/dry climate populations (T-Semnan and T-Sabzevar) had higher capacity of osmotic adjustment and antioxidant protection than the cold/dry climate population (T-Khalkhal). In *A. vermicularis* populations, from the same cold/wet climate region, V-Baneh2 vs. V-Baneh1 seems to be the most tolerant. These results showed that drought tolerance may be closely related with efficient photoprotective system, accumulation of osmoprotectants, and the increased capacity of the antioxidative system to scavenge reactive oxygen species.

Keywords: Peroxidase, proline, soluble sugars, water stress, Yarrow.

INTRODUCTION

Water is one of the most important environmental factors regulating plant growth and development. Drought is therefore a major threat affecting the life of plants and limiting crop yields globally (Boyer, 1982). There are many studies which demonstrate that drought stress induces numerous metabolic, biochemical and physiological changes in plants (Levitt, 1980). These include water status, growth, membrane integrity, pigment content, osmotic adjustment and photosynthetic activity (Dhanda *et al.*, 2004, Serraj *et al.,* 2004, Benjamin and Nielsen, 2006, Khanna-Chopra and Selote, 2007, Praba *et al.*, 2009).

Osmotic adjustment in terms of accumulating compatible solutes has been considered as an important physiological adaptation for plant to resist drought (Morgan, 1984), which facilitate extracting water from dry soils and maintaining cell turgor, gas exchange and growth in very dry environments (White *et al.*, 2000, Chaves *et al.*, 2003). Soluble sugars and proline are two kinds of most important compatible solutes in plants (Chaves *et al.*, 2003, Ben Ahmed *et al.*, 2009, Hessini *et al.*, 2009). Besides their roles in osmotic adjustment, they may protect plant cell membranes from damages and stabilize the structures and activities of proteins and enzymes (Iyer and Caplan, 1998, Samuel *et al.*, 2000, Villadsen *et al.*, 2005, Lee *et al.*, 2008, Ben Ahmed *et al.*, 2009, Hessini *et al.*, 2009). Drought stress usually

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leads to oxidative stress due to stomatal closure (Lei *et al.*, 2006, Ozkur *et al.*, 2009), which causes the over-reduction of photosynthetic electron chain (Bacelar *et al.*, 2007, Ben Ahmed *et al.*, 2009) and high formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Asada, 1999, Fu and Huang, 2001). ROS could disrupt normal metabolisms of plants through oxidative damages to lipids, proteins, nucleic acids, and photosynthetic pigments and enzymes (Smirnoff, 1993, Fu and Huang, 2001, Ozkur *et al.*, 2009).

In order to overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993). Peroxidase (POD) is one of the antioxidant enzymes which scavenge accumulation of hydrogen peroxide (H2O2) in tissue (Reddy *et al.*, 2004). Besides, non-enzymatic antioxidative carotenoids (Car) such as B-carotene and xanthophylls can also quench ROS and stabilize photosynthetic complexes (Adams III *et al.*, 1999, Bassi and Caffarri, 2000, Munné-Bosch and Peñuelas, 2003).

Plants also contain a number of natural secondary products with antioxidant properties, including different phenolics (Rice-Evans *et al.*, 1997). There is no universal pattern for phenolic compound activity in the drought stress of different plants and organs (Bagniewska-Zadworna *et al.*, 2007). Enzymes, such as Peroxidase (POD) and Polyphenol oxidase (PPO), may oxidize phenolics and thus take part in the regulation of the phenolic concentration in plants. Recent studies have also indicated that phenol oxidizing enzymes may participate in the response to various abiotic stresses including drought (Sofo *et al.*, 2005, Veljović-Jovanović *et al.*, 2006, 2008).

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 drought-prone environments due to its numerous leaf and several stems developed from the horizontal radiclestock (Bartram, 1995). Due to over collection, essentially in the 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 in order to ensure the sustainable utilization to meet the growing demand of these wild species, it has become necessary, to develop rapid methods of their commercial cultivation. Seeds culture is an alternative and easy method of commercial propagation in Iran. More than 82% of Iran’s territory is located in arid and semi-arid zones and faces shortages of water and drought in its various different regions (Amiri and Eslami, 2010). The water constraint constitutes one of the main environmental 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 tolerance and its exploitation by screening and selection of the powerful plants under drought stress (Al-Khatib *et al.*, 1992, Ali *et al.*, 2007).

Investigations of intra-specific variation to drought provide an opportunity to better understand species specific adaptations, including the relative importance and variation of physiological adaptations within species. Therefore, achieving a greater understanding of intra-specific variation to drought is of value both from a scientific perspective, which includes the identification of traits associated with greater stress resistance, as well as from an applied perspective, which includes the identification of superior planting stock for specific restoration needs and breeding programs. In order to provide more detailed knowledge for the selection of plant populations of *Achillea tenuifolia* Lam. and *A. vermicularis* Trin. and contribute to the success of breeding and re-vegetation programs, we compared osmotic adjustment, antioxidant enzymes and pigments content of different populations of *A. tenuifolia* and *A. vermicularis* under experimental drought conditions. The specific objectives of this study were to evaluate short-term physiological/biochemical responses to drought stress and to determine interaspecific variation in drought tolerance among Iranian populations of *Achillea tenuifolia* and *A. vermicularis*.

MATERIALS AND METHODS

Seed material of five populations of *Achillea tenuifolia* Lam. (three populations from hot/dry and cold/dry climate regions) and *A. vermicularis* Trin. (two populations from cold/wet climate region), provided from the Iranian Natural Resources Gene Bank at Research Institute of Forests and Rangelands (RIFR), was evaluated in the present study. The research was performed during the summer 2012 (Table 1). Plants were grown in plastic pots (16 cm height and 18 cm diameter) containing peat moss and sand (5:1) in a greenhouse (temperature: 25 °C ± 2 and relative humidity: 60% ± 5). Prior to drought stress treatments, all plants were well-watered. After 60 days, when the plants well established, drought stress was applied in 35% field capacity (FC), 55% FC, 75% FC, and well-watered (100% FC). During the experiment, which lasted for 30 days, the soil water potentials and corresponding soil water contents used in the study were calculated from soil water retention curves. The pots were kept at the designated drought stress levels by weighting. The study was carried out in a greenhouse at the RIFR in Tehran, Iran. During the experiment, the minimum and maximum temperatures inside the greenhouse were 16.2 ◦C and 33.5 ◦C, respectively.

To determine relative water content (RWC%), 3 leaves from each plant were weighed immediately (FW) after harvesting the plant. Leaves were then placed in distilled water for 4 hr and then turgid weight (TW) was measured. Then the leaves were dried in oven at 80˚C for 24hr to obtain their dry weight (DW). RWC% was calculated by using the equation: RWC% = FW-DW/TW-DW ×100 (Weatherley, 1950 and 1951). Leaf moisture% was calculated by using the equation: (FW-DW/FW ×100). Proline was determined by the ninhydrin method (Bates *et al.*, 1973). Soluble sugars were determined by the Anthrone method (Yemm and Willis, 1954). Activities of peroxidase (POD) and polyphenol oxidase (PPO) were determined using the methods of Fu and Huang (2001). For POD, the oxidation of guaiacol was measured by the increase in absorbance at 470nm for 1min. For PPO, the decomposition of H2O2 was measured by the decline in absorbance at 240nm for 1min. One unit of POD and PPO activity was defined as an absorbance change of 0.01 units per min. Total content of foliar protein was measured according to Bradford (1976), using bovine serum albumin as a standard. The activity of each enzyme was expressed on protein basis. Chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophylls (Chla+b), and carotenoids (Car) were determined spectrophotometrically using 80% acetone as a solvent (Lichtenthaler, 1987).

In all analysis three replicates per species and treatment were obtained from the youngest fully expanded leaves of different individuals during midday after 30 days of treatments.

Table 1. Ecogeographical data of the wild populations of *A. tenuifolia* and *A. vermicularis*.

Populations	Latitude (N)	Longitude (E)	Elevation from sea level (m)	Annual average precipitation (mm)	Annual average maximum temperature (ºC)	Annual average minimum temperature (ºC)
A. tenuifolia						
T-Semnan[1]	35˚ 35'	53˚ 33'	1130	142	24	13
T-Sabzevar[1]	36˚ 20'	57˚ 30'	977	188	24	10
T-Khalkhal[2]	37˚ 38'	48˚ 31'	1796	363	15	2.5
A. vermicularis						
V-Baneh1, 2[3]	36˚ 00'	45˚ 54'	1600	707	16	5

[1], [2] and [3]: Hot/dry, cold/dry and cold/wet climate population, respectively

Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA) to determine differences among populations and treatments for each variable. The significant differences between means were determined using Duncan's test at $P < 0.01$ level. Data were tested for normality by Kolmogorov–Smirnov test. When necessary, data were transformed to meet the assumptions of ANOVA. Linear regression coefficients between contents of osmotic solutes and activities of antioxidant enzymes were calculated. Statistical tests were performed with MiniTab 16 (SPSS, Chicago, USA).

RESULTS

Analysis of variance showed highly significant differences among drought treatments (Table 2). However species were significant in antioxidant enzyme activities (POD and PPO). Populations were also significant in antioxidant enzyme activities as well as leaf moisture%. The interaction between population and drought treatment was significant in RWC%, leaf moisture%, proline content, soluble sugars, antioxidant enzyme activities (POD and PPO) and the ratio of Car/Chla+b. The interaction between population and species was significant in POD activity, Chla, Chlb and Chla+b (Table 2).

Table 2. Results from the ANOVA on different studied characteristics.

Source of variance	df	F value												
		Leaf moisture%	**RWC%**	**Proline content**	**Soluble sugars**	**PPL**	**POD**	**Soluble proteins**	**Chla**	**Chl_b**	**Car**	**Chla/ Chlb**	**Chl_{a+b}**	**Car/ Chla+b**
Species (S)	1	1.17	1.38	0.46	0.18	20.34**	36.21**	0.41	1.15	1.12	0.12	0.15	1.18	0.66
Population (P)	4	3.20*	0.65	0.68	1.29	8.43**	31.27**	0.85	2.09	2.29	0.71	0.75	2.23	0.37
Treat (T)	3	15.87**	13.09**	100.67**	19.52**	19.42**	263.93**	3.82**	4.55**	6.20**	9.54**	7.46**	5.19**	17.65**
P × T	12	3.78**	2.65**	16.00**	4.47**	3.67**	25.89**	1.3	1.41	1.62	1.52	1.24	1.52	2.39**
S × P	4	2.89	0.5	0.48	1.46	2.48	10.25**	0.95	3.33*	3.15*	0.39	0.42	3.11*	0.23
CV		9.605	27.338	36.522	33.669	14.945	20.550	65.755	20.268	28.173	17.250	14.517	22.134	30.160

*, **, significant at 0.05 and 0.01 level, respectively.

Mean comparisons at different stress levels indicated that lowering of water potential causes a decrease in RWC% and leaf moisture% of leaves in all populations of both species, which were higher under well-watered condition (Fig. 1a, b). Species and the interaction of species and population had no significant effect on RWC% and leaf moisture% of leaves. Among the five populations, the RWC% of leaves exhibited relatively similar responses to drought intensities (Fig. 2b), exhibiting lowest values under severe stress. As can be seen from figure 1a, similar variations in leaf moisture% of leaves were obtained in both populations of *A. vermicularis* (V-Baneh1 and V-Baneh2, from the same cold/wet climate region). However drought stress affected the leaf moisture% of all *A. tenuifolia* populations with different responses to drought (Fig. 1a). The largest decrease was recorded in population T-Khalkhal (cold/dry climate population), while the populations T-Semnan and T-Sabzevar (hot/dry climate populations) showed higher value of leaf moisture% under severe stress.

Drought treatments significantly increased proline content in populations V-Baneh2 and T-Sabzevar under mild stress. Proline content was increased in all populations under moderate and/or severe stress (Fig. 2a). The largest increase in proline content was recorded in the populations V-Baneh2 and T-Sabzevar. Under severe stress the populations V-Baneh2 and T-Sabzevar seem to be the most tolerant, and the populations V-Baneh1 and T-Khalkhal the moderate tolerant. The accumulation of soluble sugars was also affected by drought stress. Among the three populations of *A. tenuifolia*, the accumulation of soluble sugars exhibited similar responses to drought intensities (Fig. 2b), exhibiting highest values under severe stress. However, drought treatments significantly increased soluble sugars in both populations of *A. vermicularis* under mild and/or moderate stress, but decreased that in the population V-Baneh1 under severe stress (Fig. 2b).

Drought stress affected antioxidant enzyme activities of all populations with different responses to drought. The activities of POD, PPO, and soluble proteins content are presented in Figures 3a-c. Drought stress significantly increased POD activity in the all five populations under moderate and/or sever stress (Fig. 3a). The largest increases in POD activity of each species were recorded in the populations T-Sabzevar and V-Baneh2. Drought stress increased PPO activity in the all five populations except population T-Khalkhal and V-Baneh1 under mild and moderate stress, but increased that in the all populations under severe stress (Fig. 3b). Under well-watered condition, the soluble proteins were significantly lower in the all five populations of both species. As drought stress intensified, soluble proteins content increased in the all populations (Fig. 3c). The accumulation of soluble proteins content exhibited similar responses to drought intensities in the all populations (Fig. 3c).

Pigment contents were affected by drought stress. The Chla and Chlb contents in the all the five populations of both species gradually decreased as drought stress intensified. The values of Chla and Chlb exhibited similar responses to drought intensities (Fig. 4a, b), exhibiting highest values under well-watered condition. Among the three populations of *A. tenuifolia*, the Car content exhibited similar responses to drought intensities (Fig. 4c), exhibiting highest values under severe stress. As drought stress intensified, the ratio of Car/Chla+b increased in the all populations under moderate and/or severe drought stress (fig. 4d).

Proline content was linearly and positively correlated with soluble sugars in the all populations (Fig. 5a). Proline content was also correlated with POD and PPO activity in the all populations (fig. 5b and c). The soluble sugars content was positively correlated with POD activity in the all five populations (Fig. d) and was positively correlated with PPO activity in the all populations except population V-Baneh1 (Fig. 5e). The soluble proteins content, POD and PPO activities correlated positively each others in the all populations (Fig. 5f-h).

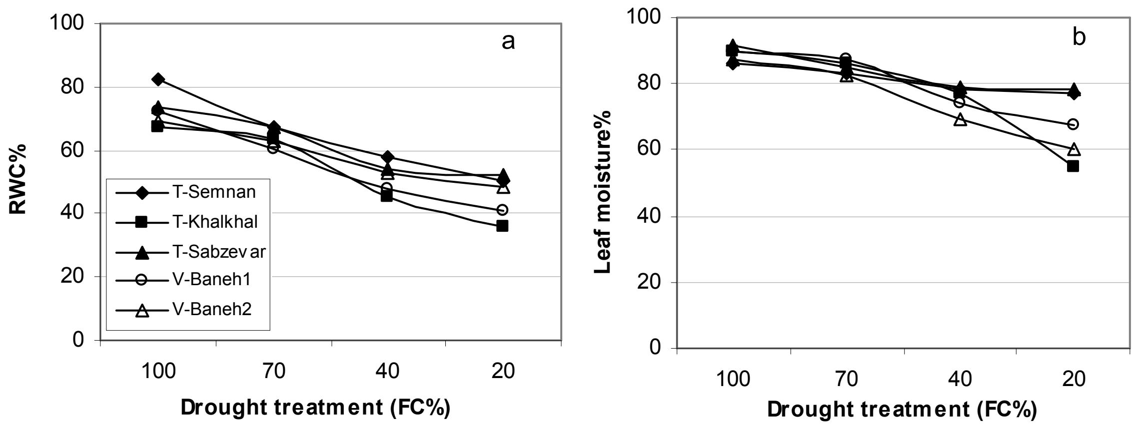

Fig. 1. The percentages of relative water content (RWC%) and leaf moisture% of five populations of *A. tenuifolia* (T-Semnan, T-Khalkhal and T-Sabzevar) and *A. vermicularis* (V-Baneh1 and V-Baneh2) of four drought treatments (n = 3).

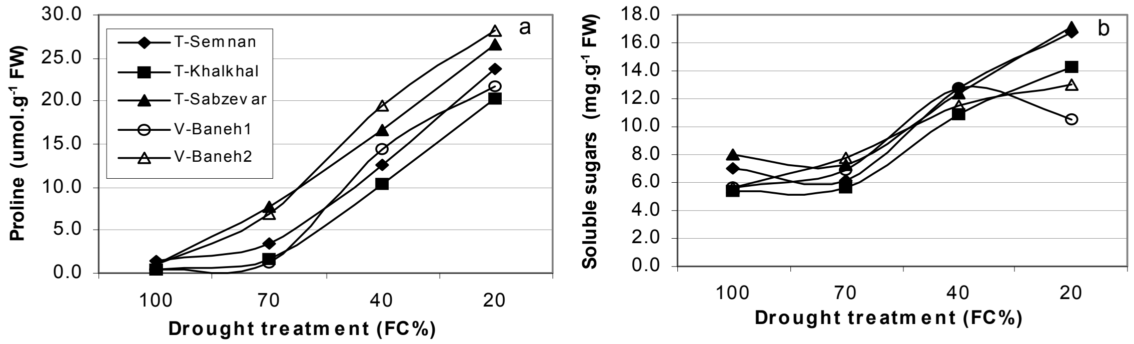

Fig. 2. The contents of proline and soluble sugars of five populations of *A. tenuifolia* (T-Semnan, T-Khalkhal and T-Sabzevar) and *A. vermicularis* (V-Baneh1 and V-Baneh2) of four drought treatments (n = 3).

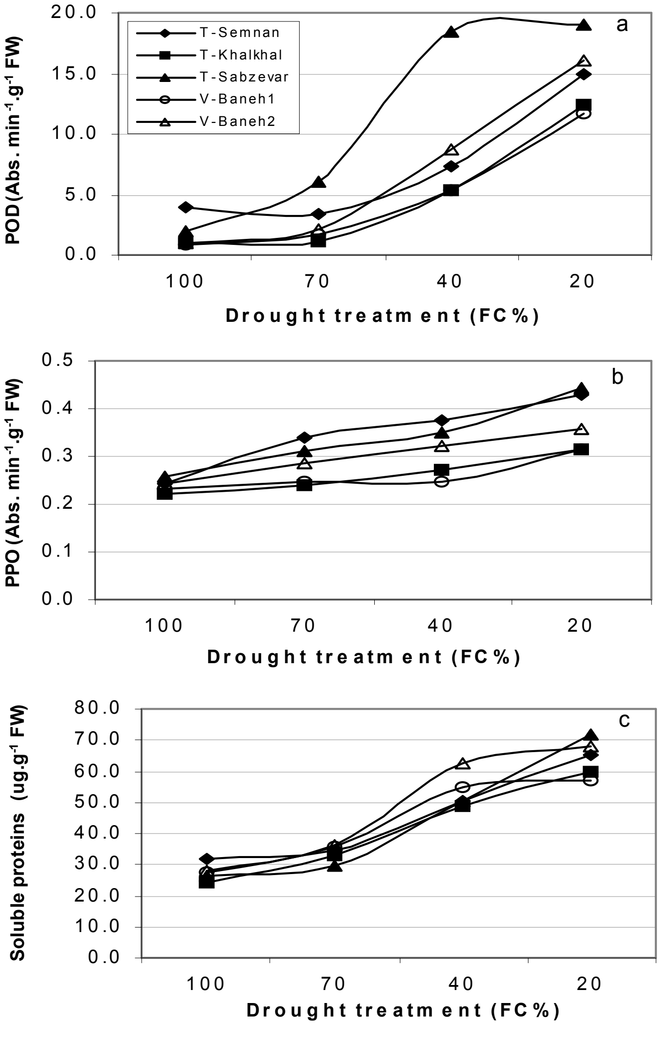

Fig. 3. Activities of POD and PPO, and soluble proteins content five populations of *A. tenuifolia* (T-Semnan, T-Khalkhal and T-Sabzevar) and *A. vermicularis* (V-Baneh1 and V-Baneh2) of four drought treatments (n = 3).

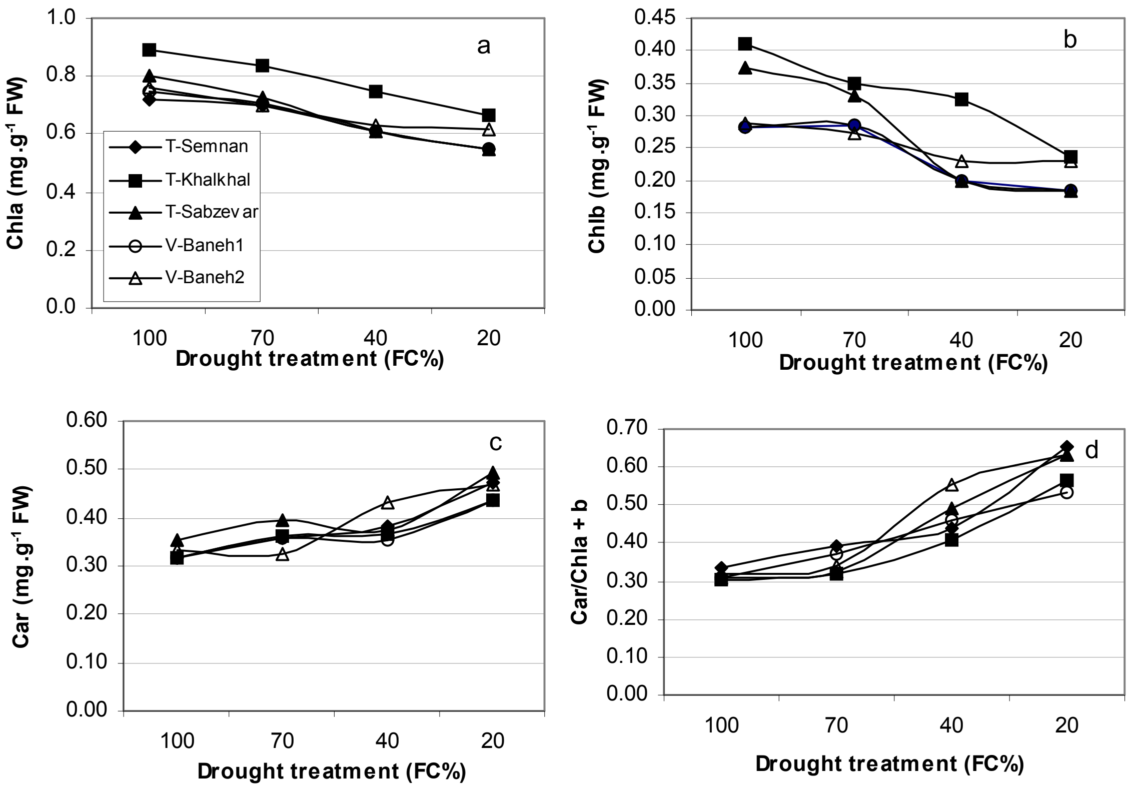

Fig. 4. Chlorophrll a (Chla), Chlorophrll b (Chlb), Cartenoids (Car) and the ratio of Cartenoids to total chlorophylls (Car/Chla+b) of five populations of *A. tenuifolia* (T-Semnan, T-Khalkhal and T-Sabzevar) and *A. vermicularis* (V-Baneh1 and V-Baneh2) of four drought treatments (n = 3).

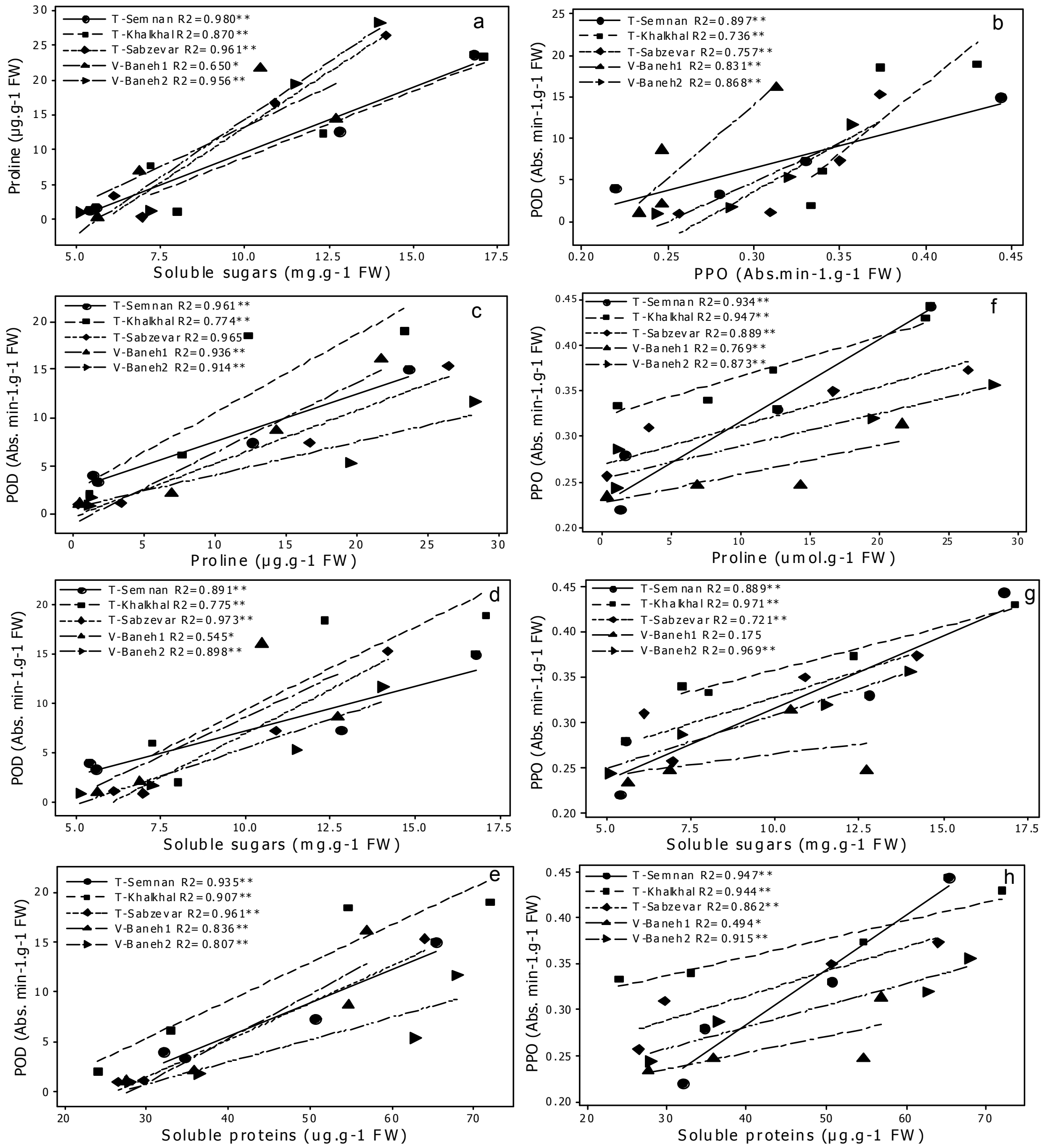

Fig. 5. Correlations between proline content and soluble sugars content (a), between POD activity and PPO activity (b), Proline content (c), soluble sugars content (d) and soluble proteins (e), between PPO activity and proline content (f), soluble sugars content (g) and soluble proteins (h). Population T-Semnan (•), T-Khalkhal (■), T-Sabzevar (◆), V-Baneh1 (▲) and V-Baneh2 (▶). Values are means of three replicates per population and treatment. The solid lines represent the best-fit linear regressions for each species: *P < 0.05, **P < 0.01.

DISCUSSION

The RWC% and leaf moisture% of leaves were affected by drought stress. The RWC% and leaf moisture% of leaves in all populations gradually decreased as drought stress intensified. The values of RWC% and leaf moisture% of leaves exhibited relatively similar responses to drought intensities, exhibiting highest values under well-watered condition. However drought conditions induced a slightly larger decrease in RWC% and leaf moisture% of leaves in the populations of T-Khalkhal vs. T-Semnan and T-Sabzevar (*A. tenuifolia*), and V-Baneh1 vs. V-Baneh2 (*A. vermicularis*). Experimental data on the other species indicated that drought resistance cultivars or genotypes had higher RWC% (Abraham *et al.*, 2004, Shahrokhi *et al.* 2011) and leaf moisture% (Batlang, 2006, Shahrokhi *et al.* 2011). The high RWC% of resistant genotypes was probably the result of their better ability for water uptake at low soil water potential (Volaire *et al.*, 1998). It is well documented that a critical component of the dehydration tolerance for grasses is cell membrane stability (Crowe *et al.*, 1987, Volaire and Lelievre, 2001).

Under drought conditions, the accumulations of proline and soluble sugars seemed to be associated with drought tolerance in many plant species. The rate of proline accumulation was significantly higher in drought-tolerant cultivars than drought-sensitive cultivars of wheat (Nayyar and Walia, 2003), mulberry (Reddy *et al.*, 2004), and olive (Ben Ahmed *et al.*, 2009). Soluble sugars also contributed to improving drought tolerance of peas (Sánchez *et al.*, 1998), sugar beets (Choluj *et al.*, 2008) and black poplars (Regier *et al.*, 2009). In two mango cultivars, a cultivar, which exhibited more active accumulations of soluble sugars and proline, also revealed higher resistance to drought than the other one (Elsheery and Cao, 2008). In our study, proline content appeared to increase sharply in all five populations under mild and moderate drought stress. However, severe drought stress caused serous metabolic damages and largely decreased proline accumulation in the population V-Baneh1. On the contrary, severe drought stress did not reduce proline content in others and even significantly increased that in populations T-Semnan and T-Sabsevar. The responses of soluble sugars to drought intensity showed the similar trends in the five populations. These results suggested that among *A. tenuifolia* populations the T-Semnan and T-Sabzevar vs. T-Khalkhal, and among the *A. vermicularis* populations the V-Baneh2 vs. V-Baneh1 had higher capacity of osmotic adjustment in terms of accumulating proline, especially under severe drought, which could maintain water absorption under such harsh conditions (White *et al.*, 2000, Chaves *et al.*, 2003).

Specific activity of antioxidant enzymes in different species (Baisak *et al.* 1994) and even in different cultivars of the same species (Bartoli *et al.* 1999) is highly variable in response to drought stress. Antioxidative mechanisms consist of many enzymes including POX and PPO. In the current study, severe drought stress largely increased the activities of POD in all populations, indicating that the scavenging function of antioxidant enzymes was not impaired by severe stress (Fu and Huang, 2001). Under drought conditions, the activity of POD increased to a greater extent, resulting in lower levels of lipid peroxidation and electrolyte leakage, in a drought-tolerant clone than in a drought-sensitive one of *Coffea canephora* (Lima *et al.*, 2002). The drought-resistant *Phaseolus acutifolius* also revealed higher activities of POD than the drought-susceptible *P. vulgaris* (Türkan *et al.*, 2005). Khanna-Chopra and Selote (2007) attributed lower membrane injury to the higher activity of POD in a drought-tolerant wheat cultivar than in a drought sensitive cultivar under severe drought stress. High activities of antioxidant enzymes also improved drought tolerance of cultivars of mulberry (Reddy *et al.*, 2004), tea (Upadhyaya *et al.*, 2008) and olive (Ben Ahmed *et al.*, 2009). It seemed to be that higher activity of POD provided higher protection against oxidative stress in the populations T-Sabzevar and V-Baneh2 under severe drought stress, as judged from higher increases of proline and soluble proteins content in these populations.

In response to severe drought, PPO activity was also increased in the all populations, moreover, this value in the populations of *A. tenuifolia* was significantly higher than the populations of *A. vermicularis*. There are several reports regarding the role of PPO in response to drought stress and even osmotic stress in plants. The activity of this enzyme was dependent on the species and variety (Takeuchi *et al.* 1992). In a study of *Aegiceras corniculatum* increasing PPO content in the plant tissues was observed under stress and was proved by other studies (Parida *et al.*, 2004). In another study on seedlings under osmotic stress, PPO activity increased in root, shoots and leaves (Demir & Kocacaliskan, 2001). Thus, higher levels of PPO in *A. tenuifolia* compared to *A. vermicularis* may indicate that this species more tolerant to drought stress.

In current study, soluble proteins content appeared to increase significantly in all populations under sever drought stress. The responses of soluble proteins content to drought intensity showed similar trends in the populations of both species. Some proteins are up-regulated under water stress while others are degraded or down regulated. According to Ashraf *et al.* (2003) accumulation of proteins in leaves under water stress conditions might be an adaptive mechanism. Contrasting reports regarding the changes in protein contents are available in literature. Increasing water stress was found to cause a significant reduction in soluble protein content in moth, beans (Garg *et al.*, 2001) and *Vigna radiata* L. (Farooq and Bano, 2006). The increase in total soluble proteins under drought stress in different populations of *A. tenuifolia* and *A. vermicularis* is consistent with other findings (Bensen *et al.*, 1988, Riccardi *et al.*, 1998, Ti-da *et al.* 2006, Iqbal *et al.*, 2010).

The observed positive correlations among activities of POD and PPO in the most studied populations (Fig. 5) suggested that these enzymes might be involved in the elimination of the reactive oxygen species (ROS) within the peroxide/ phenols/ ascorbate system in drought stress (Takahama & Oniki, 1997, Sgherri *et al.*, 2003, 2004). The intimate relationships between enhanced or constitutive antioxidant enzyme activities in response to drought stress were also observed in many other species (Türkan *et al.*, 2005, Chen and Cao, 2008, Zhu *et al.*, 2009). The positive relationships between contents of osmotic solutes (proline and soluble sugars) and antioxidant enzyme activities (POD and PPO) were also observed in the current study. It was reported that proline accumulation could activate the antioxidant defense mechanisms (Türkan *et al.*, 2005, Ben Ahmed *et al.*, 2009). Since proline and soluble sugars could stabilize the structures and activities of enzymes (Chaves *et al.*, 2003), the high accumulations of proline and soluble sugars in the populations T-Sabzevar and V-Baneh2 under severe drought stress may largely permit their high activities of antioxidant enzymes.

The limitation of water supply induced chlorophyll degradation in present experiment. Reduction of pigments content, as a result of either slow synthesis or fast breakdown, has been considered as a typical symptom of oxidative stress (Smirnoff, 1993). Authors explained this phenomenon as a photo protection mechanism through reducing light absorbance by decreasing pigments content (Munné-Bosch and Alegre, 2000, Galmés *et al.*, 2007, Elsheery and Cao, 2008). The significant increase recorded in content of cartenoids in the different population under drought condition. Cartenoids content and Car/Chla+b ratio are correlated with the capacity of light protecting mechanisms (Boardman 1977). Cartenoids have essential functions in photosynthesis and photoprotection. Besides their structural roles, they are well known for their antioxidant activity by quenching ^{3}Chl and ^{1}O2, inhibiting lipid peroxidation, and stabilizing membranes (Knox *et al.* 1985, Demmig-Adams and Adams III 1992, Frank and Cogdell 1996, Niyogi 1999). They also play a critical role in the assembly of the light-harvesting complex and in the radiationless dissipation of excess energy (Streb *et al.* 1998, Munné-Bosch and Alegre 2000). According to the mentioned carotenoids functions, their comparative levels in a genotype will determine its relative tolerance. However significant differences in carotenoid contents were not recorded among all studied populations. Since carotenoids played an important role in photoprotection (Demmig-Adams III and Adams III, 1996*,* Adams *et al.*, 1999*,* Munné Bosch and Peñuelas, 2003), the increased ratio of Car/Chla+b in some populations under drought conditions (Fig. 4) indicated a higher need of photo protection by carotenoids (Baquedano and Castillo, 2006*,* Elsheery and Cao, 2008).

CONCLUSIONS

As drought stress intensified, all populations of both species exhibited lower leaf moisture% and RWC% of leaves. Severe drought stress significantly increased accumulations of proline, soluble sugars, soluble proteins contents and activities of POD and PPO enzyme almost in all five populations. The positive relationships were observed among activities of antioxidant enzymes, and between contents of osmotic solutes and activities of antioxidant enzymes. Drought stress decreased chlorophyll content but increased cartenoids content and the ratio of Car/Chla+b in the studied populations. Variation among populations under different levels of drought shows that populations are characterized by a different tolerance to drought. In *A. tenuifolia*, compared with the cold/dry climate population (T-Khalkhal), the hot/dry climate populations (T-Semnan and T-Sabzevar) had higher capacity of osmotic adjustment and antioxidant protection and hence a higher potential to adapt to arid and semi-arid conditions which makes it a candidate of choice in breeding programs. In *A. vermicularis* populations, from the same cold/wet climate region, V-Baneh2 vs. V-Baneh1 seems to be the most tolerant, especially under severe drought condition. These results showed that drought tolerance may be closely related with efficient photoprotective system, accumulation of osmoprotectants, and the increased capacity of the antioxidative system to scavenge reactive oxygen species.

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التقييم الفسيولوجي والبيوكيميائي لصنفي النباتات البرية *Achillea tenuifolia* Lam. و *A. vermicularis* Trin . تحت ظروف إجهاد الجفاف

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ملخــص

خمسة أصناف برية من *Achillea tenuifolia*. (ثلاثة أصناف من مناطق المناخ الحار الجاف و/ الجافة والباردة) و نبات *A. vermicularis Trin* . (اثنين من أصناف المناخ البارد الرطب) ، تم تعريضهم لإجهاد الجفاف من أجل تقييم مستويات تحمل الجفاف من خلال تحليل المحتوى المائي النسبي، نسبة الرطوبة للورقة، والمواد الاسموزية (البرولين والسكريات القابلة للذوبان) ، والأنزيمات المضادة للأكسدة (بيروكسيديز وبولي فينول و اكسيديز)، والبروتينات القابلة للذوبان، والأصباغ التي يحتويها النبات. الاختلاف بين الأصناف تحت مستويات مختلفة من مستويات الجفاف أظهرت بأن الأصناف تتميز بمستويات تحمل مختلفة للجفاف . في نبات A. tenuifolia، وكان الصنف الذي يعيش في المناخ الحار / الجاف وهو (T-Semnan و T-Sabzevar) له قدرة أعلى على التكيف لاحتوائه على مستويات أعلى من التحمل و الضبط الاسموزي و الحماية المكونة من مضادات الأكسدة أكثر من الأصناف النباتية التي تعيش في المناخ البارد / الجافة وهو الصنف (T-Khalkhal) . في نبات *A. vermicularis*، الذي يعد من أصناف المناطق ذات المناخ البارد/ الرطب، كان الصنفين _ V- Baneh2 و V- Baneh1) الأكثر تحملا . وأظهرت هذه النتائج أن تحمل الجفاف قد تكون ذات صلة بشكل وثيق مع نظام الحماية الضوئية photoprotective ، وتراكم المواد المنظمة و الحامية للاسموزيةosmoprotectants ، و زيادة قدرة منظومة مضادات الأكسدة لمختلف أنواع الاكسجين التفاعلية .

الكلمات الدالة: البيروكسيداز ، البرولين ، السكريات قابل للذوبان ، والإجهاد المائي ، يارو.

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