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Original Article

Evaluating Domestic *Achillea millefolium* L. as a Suitable Plant to Use in the Urban Landscaping of Dry and Semi-dry Regions

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ABSTRACT

In this project, domestication of one of the wild flowering plants, *Achillea millefolium* L. (yarrow plant) was done to find out its resistance to drought stress condition. Plant samples were collected from the Isfahan region of Iran and were further multiplied by divisions of plants. The study was conducted on the improvement of seeds germination using GA_3, morphological and phenological study and to estimate drought tolerance of yarrow plants. The seeds of selected plants were treated with GA_3 hormone (0, 250 and 500 ppm) to break the dormancy and improve the germination percentage. GA_3 treatment improved the seed quality parameters and the best results were obtained with GA_3 @ 500 ppm. The plants propagated through division were cultivated in the field for the domestication of plants and to estimate their potential for landscape purposes. Also, the Phenological cycle of plants was monitored. Attractive flowers, Long duration of flowering and applying green cover during the year was positive points of yarrow for using in the landscape. Irrigation was applied at 25%, 50%, 75% and 100% levels of available water from April to September. Morphological and physiological parameters showed that *A. millefolium* could significantly tolerate drought treatments until 50% of available water and even at 75%, plants could survive and produce new stems.

INTRODUCTION

Yarrow (*A. millefolium*), belonging to the Asteraceae family is a wild flowering plant, which is raised in temperate regions of Asia and Europe [1, 2]. Mostly it is considered as an herbaceous weed. It is a perennial plant with an erect appearance, having several stems and rhizomes. Leaves are distributed on the stem in the way that leaves near the bottom of the stem are larger than leaves placed on the tips of stems. The leaves have different degrees of hairiness. The length of leaves is 5–25 cm, and arranged in helix form on the stems. The inflorescence with the white ray and disk flowers is placed in a flat-topped capitulum cluster and is visited by insects for pollination. The plant scent is sweet and strong [3].

Growth of yarrow happens in a wide range from sea level to 3500 meters in height. Commonly flowering occurs from May until July. Its active growth is in the spring, in the soil of open forests and grasslands and a yield of 43000 plants per acre could be achieved [3].

Severe droughts and climate change are associated with regional mortality in the world [4, 5, 6]. However, due to a poor understanding of the physiological mechanisms of drought survival and mortality, prediction in this case is difficult [7]. In nature, plants are subjected to several abiotic and biotic stresses continuously. Among them, drought is the most harmful parameter for plant growth and their productivity. Therefore, drought is considered as an intense threat to produce different crops in these conditions [8, 9].

Yarrow is a medicinal plant with a wide climatic range, drought-tolerance properties and fast-

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spreading characteristics. However, the rate of reproductive seeds of yarrow is low [3].

MATRIAL AND METHODS

A collection of germplasm: yarrow plants collected from the environment of the Isfahan region in Iran (Fig. 1), with the following properties: 33′17″ to 33′24″ latitude and 51′39″ to 51′49″ longitude with 147.22 mm annual rainfall and 15.41 ºC annual temperatures. The area is dry with seven months of drought conditions.

Experimental site: The experiment was performed in Mahallat (33.54 ºN, 50.28 ºE), located in Iran and 1750 m above sea level.

The collected plants were propagated by stem cuttings in the pots containing loam soil in April. After getting a high number of plants, the seedlings were divided into two parts. The first part was for domesticating experiment and the second part was for the drought-tolerance experiment.

Domestication

Seeds of *A. millefolium* have a low germination rate due to its dormancy. Therefore, GA_3 treatment in the levels of 0, 250 and 500 ppm was applied and seeds germination parameters were recorded. The experiment was carried out as a completely randomized design with three replications. 50 seeds were placed on filter paper in each petri-dish. GA_3 treatments were applied and placed in a germinator with 25 ±2 ºC, 1000 lux light (12 hrs light, 12 hrs dark) and 60% relative humidity for 15 days. The percentage and rate of germination [10], seedling vigor [11], shoot and rootlet length were recorded.

After preparing the land, fertilizer was added to the semi-loamy soil (EC = 0.78, pH = 7.9) and mixed well with the tillage. Seedlings, which were grown in the pots were transferred to the field and cultivated in the lands in April. The distance of plants between rows was 60 cm and the distance of plants on each row was 25-30 cm. First irrigation was done immediately, to prevent any shock after transplanting the seedlings. Afterward, during the plant growth, irrigation was done once a week in the warm season. No irrigation was done during autumn and winter. Different phenological and morphological parameters were evaluated as follows:

Phenological parameters: Different phenological parameters were recorded that include primary growth of the plant, the appearance of flowering stem, start of flowers flourishing, complete flowers flourishing, natural flowering period, a flowering period in the field, the start of seed production, seed maturity and senescence life cycle.

Morphological parameters: Different morphological parameters were recorded that include plant height, plant diameter, number of stems, leaves and flowers per plant, leaf area, fresh and dry weights of flowers and shrubs, flower diameter and flower color.

Fig. 1 Domestic *A. millefolium* L. in the time of vegetative growth (left) and in the time of flowering (right)

Drought-tolerance

After propagating the mother plants and getting high numbers of seedlings, the seedlings were allowed to grow until one year. Thereafter, the drought stress treatments were started with the start of the warm season, in June. Irrigation treatments contained 25%, 50% and 75% of the available water in the pots which contained the same size and age of yarrow plants. Different morphological and biochemical parameters were evaluated as follows:

Morphological parameters: Different morphological parameters were recorded that include plant height,

plant diameter, number of stems, leaves and flowers per plant, leaf area, fresh and dry weights of flowers and shrubs, flower diameter and flower color.

Biochemical parameters: Different biochemical parameters were estimated as; electrical conductivity, relative water content, proline content, chlorophyll content and the activities of the catalase and peroxidase enzymes.

Electrical Conductivity

After inducing water stress, electrical conductivity was measured to indicate membrane permeability, using an electrical conductivity meter (Control Dynamics, India) following the standard procedure according to Blum and Ebercon [9]. Four plants were selected randomly in each replicate and 10 discs from each treatment were separated. The vials were washed three times with distilled water to remove contaminations. Then leaf samples were placed in vials containing 25 ml distilled water. All the vials were covered with aluminum foil and then it was incubated on the shaker (100 rpm), at room temperature (25 ºC) for 24 hrs. After incubation, the electrical conductivity of the bathing solution was determined as EC1. Then, the samples were placed in an autoclave at 120 ºC for 20 min. After cooling the bathing solutions at room temperature, again the electrical conductivity was measured as EC2.

Relative water content

A leaf sample was taken from the plants and its fresh weight was recorded (FW). Then samples were immersed in the water and placed in darkness for 24 hrs. The extra water on samples was removed and turgescent weight (TW) was recorded. After that, samples were placed in the oven at 70ºC for 48 hrs and its dry weight (DW) was recorded. Finally, relative water content was calculated based on the Smart and Bingham [13] method.

Proline Content

The plant tissue (0.5 g) was taken and 10 ml of 3% aqueous sulpho salicylic acid was added. Then they were homogenized using a pestle and mortar and filtered through filter paper (Whatman No. 2). The glacial acetic acid (2 ml) (Merck, Germany) and ninhydrin (2 ml) (Merck, Germany) were added to 2 ml of the filtrated solution and were mixed. The mixture was kept in the water bath for 1hr and finally placed in the ice bath. At this stage, 4 ml of toluene (Merck, Germany) was added and was completely mixed. The toluene layer was separated and the absorbance of the red color solution was measured at 520 nm with a spectrophotometer (Jenway, spec gene, UK). The proline content was calculated based on the following formula as the Mm proline per g of tissue [14].

Chlorophyll

For calculating Chlorophyll, the leaf sample (1 g) was placed in a pestle and mortar. The acetone (80%, 20 ml) was added to the tissue and grounded well and then it was centrifuged at 5000 rpm for 5 min. The supernatant was transferred to a volumetric flask. Again, the residue was grounded and the procedure was repeated until achieving a colorless residue. The final volume was made up to 100 ml using acetone (80%). Then the absorbance of the solution was recorded at 645, 663 and 652 nm with a spectrophotometer (Jenway, spec gene, UK) [15].

Catalase Activity

3ml reaction mixture contained 50 mM phosphate buffer (pH 6.8), 75 µl of enzyme extract and 22.5 µl hydrogen peroxide (30%). The decrease in hydrogen peroxide was recorded with a spectrophotometer (Jenway Specgene 6015, UK) at 240 nm for 3 min [16].

Peroxidase activity

1gr of plant sample was homogenized with 2.5 ml of 0.5 M Tris –HCl buffer (pH 7) (Merck, Germany). Then, it was centrifuged (Sigma 1-14K, Germany) at 10000 rpm for 20 mins. The supernatant was used as a source of crude enzyme. 2.8 ml of the buffered o-dianisidine (16 ml o– dianisidine (Merck, Germany) + 48 ml sodium acetate buffer (pH 5.4) (Merck, Germany) + 43.6 ml distilled water), H_2O_2 (1%, 0.1 ml) and 0.1 ml diluted enzyme were added to a cuvette and completely mixed. Increasing absorbance values were recorded with a spectrophotometer (Spectronic 20 Geneses, 4001/4, USA) at 470 nm for 3 min [16].

RESULTS

Drought is one of the most important abiotic stresses, which reduces flowers growth and yield. Large areas of lands in the world are dry and semi-dry, due to water shortage. In this condition, introducing dry tolerant plants is a basic step for water saving in managing urban landscape. Therefore, the capability

of *A. millefolium*as as an ornamental plant in the landscape was studied.

Domestication of Plants

Fig. 2 Domestic *A. millefolium* L. at the time of flowering

Seeds of *A. millefolium* were exposed to different levels of GA_3 treatments. Results showed that GA_3 treatments increased all the seed quality parameters as germination percentage, speed of germination, seedling vigor, shoot and root length. With increasing GA_3 concentration, the germination percentage was increased. The highest rate of germination was achieved in the seeds, which were treated with 500 ppm GA_3 (86%). Which, had a significant difference compared to the control (32%) and 250 ppm treatment (58%) (Table 1). The highest rate of speed germination (13.96) and seedling vigour (29.60) were also achieved in the 500 ppm, which had a significant difference with speed germination (3.38) and seedling vigour (3.39) in control. Moreover, the highest seedling shootlet number (19.60) and rootlet length (14.80 mm) were obtained in 500 ppm treatment (Table 1).

The phenological cycle of *Achillea millefolium*in was studied in the research field of Ornamental Plants Research Center in Mahallat. The data was recorded from plants that started to grow on 20th March, with 10.5 ^{o}C mean daily temperature and 5.7 GDD (Growing Degree Days). After 20 days on 8th April, with 11.5 ^{o}C mean daily temperature and 134.0 GDD flowering stems occurred. After 15 days on 24th April, with 12.4 ^{o}C mean daily temperature and 273.8 GDD first flowers were observed and flowering was continued for 40 days. Therefore, on 4th June, with 17.3 ^{o}C mean daily temperature and 968.3 GDD complete flowering was seen in the field. Afterward for 150 days till 5th Nov, with 20^{o}C mean daily temperature and 4046.9 GDD flowering was continued. Then after 25 days at 25th Nov, with 9.5^{o}C mean daily temperature and 4131.1 GDD seed production was started and finally after 25 days on 20th December, with 6.3 ^{o}C mean daily temperature and 4146.3 GDD seeds were matured completely and seed collection was done (Table 2).

Fig. 3 Effect of different levels of drought stress (25%, 50%, 75% and 100% of field capacity) on the aerial part (left) and roots (right) of *A. millefolium* L

Morphological parameters of domestic *A. millefolium* (Fig. 2) in the research field of the Mahallat region is presented in Table (3). Also, no pests and diseases were seen on the plants. The flowering duration in the first year was from May to November and in the second year from May to September. In the winter, there was no flower on the plants, but their green cover was available and even in the chilling times, a

very attractive red color of the plants was covering the land.

Effect of Drought Stress

Drought stress causes a decrease in the morphological characteristics of the plants. If this decrease doesn't show a significance difference with control, the plant can be introduced as a suitable case for using in urban landscaping of dry and semi-dry areas. Therefore, domestic *A. millefolium* it was subjected to drought stress treatments for five months (May, June, July, August and September) (Fig. 3). Recorded data for morphological characteristics showed that drought stress in the 25% and 50% of field capacity did not have a negative effect on plants growth. The highest plant height (23.67 cm), plant coverage (44.67 cm), No. of stems (4.33), No. of leaves (190), leaf area (8.53 cm^2), No. of flowers (5.67), the diameter of flowers (6.70 cm), dry weight of flowers (10.50 g), wet (202.82 g) and dry weight of plants (66.53 g) was observed in the 50% drought stress (Table 4). In the 75% and 100% water stress, morphological parameters decreased and this reduction continued during five months of drought stress. Lowest plant height (9 cm), plant coverage (16.67 cm), No. of stems (1.67), No. of leaves (61.33), leaf area (3.07 cm^2), No. of flowers (0.67), the diameter of flowers (1.13 cm), dry weight of flowers (1.50 g), wet weight of plants (39.39 g) and dry weight of plants (9.63 g) was observed in the 100% drought stress (Table 4).

Table 1 Seed quality parameters under GA_3 treatment

GA_3 Tr.	G %	S.G.	S.V.	Sh.L. (mm)	R.L. (mm)
0 ppm	32.00 c	3.38 c	3.39 c	5.96 c	4.60 c
250 ppm	58.66 b	8.81 b	12.47 b	11.03 b	10.20 b
500 ppm	86.00 a	13.96 a	29.60 a	19.60 a	14.80 a

*Similar letters show a non-significant difference at the 5% level of significance on the basis of the Duncan test.
G %: Germination percentage, S.G.: Speed of Germination, S.V.: Seedling Vigour,
Sh.L.: Shootlet Length, R.L.: Rootlet Length.

Table 2 The phenological cycle of *A. millefolium*

Phenology of *A. millefolium*	Primary growth	Emergence of flowering stem	Emergence of first flower	Complete flowering	Flowering duration	Start of seed production	complete seed production
2016-2017	20 th March	8 th April	24 th April	4 th June	5 th Nov	25 th Nov	20 th Dec
GDD	5.7	134.0	273.8	968.3	4046.9	4131.1	4146.3
Mean daily temperature (°C)	10.5	11.5	12.4	17.3	20.0	9.5	6.3

Table 3 Morphological parameters of domestic *A. millefolium*

Morphological parameters/ Month	Plant height (cm)	Plant diameter (cm)	Shoot No.	Leaf No.	Leaf area (cm^2)	Flower No.	Flower diameter(cm)	Flower dry weight (g)	Plant wet weight (g)	Plant dry weight (g)
April	10	10	2	50	4	0	0	0	100	8.5
May	50	30	30	200	5	0	0	0	120	10
June	74.5	45	48	420	10	38	7	2.95	522.24	129
July	75	50	81	500	13.0	81	8.1	3.01	1015	130
August	77	60	85	550	15.0	85	8.5	3.2	1200	143
September	78	60	89	580	15.2	88	9	3.5	1100	143.75
October	80	62	90	580	17.8	90	9.4	3.8	10000	145.25
November	82	64	100	600	20	90	10	4.3	10000	150
December	75	55	85	400	10	50	7	2.67	900	120
January	68	50	70	300	5	20	5	2.01	835	90
February	50	40	50	200	4	0	0	0	500	50
March	40	40	45	200	4	0	0	0	200	20

Table 4 Morphological parameters under drought stress condition in *A. millefolium* L.

Time	Drought stress	P.H. (cm)	P.D. (cm)	Sh.N.	L.N.	L.A. (cm^2)	F.N.	F.D. (cm)	$D.W_f$ (g)	$F.W_p$ (g)	$D.W_p$ (g)
June	25%	14.33 d-g	30.33 e-i	3.33 ab	137.33 e-g	4.29 g-i	4.33 a-d	2.23 gh	5.90 hi	110.37 f-h	20.03 e-g
	50%	16.00 c-f	31.00 e-h	3.33 ab	150.00 c-f	4.37 g-i	5.00 ab	2.73 g	6.17 h	123.74 d-f	23.00 ef
	75%	14.00 d-h	28.67 f-i	2.67 bc	135.00 e-g	4.26 g-i	4.00 a-d	2.47 gh	5.50 h-j	105. 0 g-i	18.40 fg
	100%	12.00 f-i	25.67 g-j	2.00 c	103.33h-j	3.79 hi	3.00 c-e	2.07 h	4.57 kl	80.40 jk	16.47 f-h
July	25%	15.67 c-f	32.33 d-g	3.33 ab	143.33 d-f	4.86 f-h	4.67 a-c	3.43 f	6.97 g	119.15 e-g	27.03 e
	50%	17.00 b-e	34.33 c-f	3.33 ab	161.67 b-e	5.27 e-g	5.33 ab	3.77 ef	7.40 fg	137.03 cd	37.90 cd
	75%	13.33 d-i	27.33 g-i	2.67 bc	131.67 fg	4.03 g-i	3.67 b-e	2.37 gh	5.17 i-k	102.10 hi	18.20 fg
	100%	10.67 g-i	23.67 i-k	2.00 c	91.67 i-k	3.43 hi	2.67 de	2.03 h	4.03 lm	74.97 kl	15.47 f-h
Aug	25%	17.67 b-d	36.33 b-e	3.33 ab	151.67 c-f	5.92 d-f	5.00 ab	4.30 de	7.80 ef	130.97 c-e	35.80 d
	50%	19.33 a-c	38.33 a-d	3.33 ab	173.33 a-c	6.55 c-e	5.67 a	4.73 d	8.40 de	141.17 c	44.50 c
	75%	13.00 e-i	26.67 g-i	2.67 bc	127.67 f-h	3.72 hi	3.67 b-e	2.37 gh	4.77 j-l	99.10 hi	16.77 f-h
	100%	10.00 g-i	19.67 j-l	2.00 c	78.67j-i	3.39 hi	2.67 de	2.03 h	3.60 mn	62.33 lm	14.43 gh
Sept	25%	19.67 a-c	38.00 a-d	3.33 ab	167. 67 a-d	7.00 b-d	5.00 ab	5.37 c	8.97 cd	171.33 b	58.53 b
	50%	20.67 ab	41.00 a-c	3.33 ab	180.67 ab	7.39 a-c	5.67 a	5.77 bc	9.60 bc	183.35 b	62.03 ab
	75%	12.67 e-i	25.33 g-j	2.33 bc	115.67 g-i	3.56 hi	3.00 c-e	2.20 gh	4.20 lm	90.80 ij	18.13 fg
	100%	9.67 hi	17.67 k-l	1.67 c	69.33 kl	3.28 i	2.00 ef	2.00 h	2.93 n	53.77 mn	12.77 gh
Oct	25%	23.33 a	42.67 ab	4.33 a	173.67 a-c	8.19 ab	5.00 ab	6.23 ab	9.87 ab	183.63 b	61.60 ab
	50%	23.67 a	44.67 a	4.33 a	190.00 a	8.53 a	5.67 a	6.70 a	10.50 a	202.82 a	66.53 a
	75%	12.00 f-i	24.00 h-k	2.33 bc	103.33 h-j	3.39 hi	2.67 de	2.10 gh	3.67 mn	81.84 jk	16.23 f-h
	100%	9.00 i	16.67 l	1.67 c	61.33 l	3.07 i	0.67 f	1.13 i	1.50 o	39.39 n	9.63 h

*Similar letters show non-significant difference at the 5% level of significance on the basis of Duncan test.
P.H.: Plant Height, P.D.: Plant Diameter, Sh.N.: Shoot Number, L.N.: Leaf Number, L.A.: Leaf Area, F.N.: Flower Number, F.D.: Flower Diameter, $D.W_f$: Dry Weight of Flower, $F.W_p$: Fresh Weight of Plant, $D.W_p$: Dry Weight of Plan

Table 5 Biochemical parameters of *A. millefolium* L. under drought stress condition

Drought stress	R.W.C. (%)	Pro. (µg/g f_w)	Chl. (mg/g f_w)	CAR. (mg/g f_w)	CAT. (µMol/g f_w.min)	PROX. (µMol/g f_w.min)	I.L. (%)
25%	80.56 a	183.8 d	2.21 a	1.97 a	0.017 c	0.12 c	5.75 b
50%	70.54 ab	221.6 c	2.20 a	1.67 ab	0.020 c	0.14 c	5.97 b
75%	58.31 b	258.2 b	1.50 b	1.38 b	0.032 b	0.24 b	7.02 ab
100%	37.75 c	327.1 a	1.31 b	1.32 b	0.042 a	0.31 a	8.37 a

*Similar letters show a non-significant difference at the 5% level of significance on the basis of Duncan test.
R.W.C.: Relative Water Content, Pro.:Proline, Chl.: Chlorophyll, CAR.: Carotenoid, CAT.: Catalase, PROX.: Peroxidase, I.L.: Ion Leakage

The effect of drought stress on morphological characteristics was significant ($p < 0.001$) and the interaction effect of drought stress and time on all parameters except shoot and flower number was significant ($p < 0.001$).

Recorded data for physiological characteristics showed that with increasing levels of drought stress, the amount of relative water content, chlorophyll and carotenoid decreased and the amount of proline, catalase, peroxidase and ion leakage was increased. The highest amount of relative water content (80.56%), chlorophyll (2.21 mg/g f_w) and carotenoid (1.97 mg/g f_w) were observed in the 25% drought stress and the highest amount of proline (327.1µgr/g f_w), catalase (0.042 µMol/g f_w.min), peroxidase (0.31µMol/g f_w.min) and ion leakage (8.37) was observed in the 100% drought stress (Table 5). The effect of drought stress on all physiological characteristics was significant

DISCUSSION

A. millefolium is a drought-tolerant plant with perfect foliage and flowers. So, it can be used in urban landscaping in order to save water. Seeds of yarrow have low germination due to their dormancy. Applying GA3 treatment could increase germinating parameters and the highest amount of germination percentage, speed of germination, vigour index and seedling length achieved in 500 ppm GA3 treatment. Our results were in agreement with Rawat *et al*. [17] in Abies pindrow and Picea smithiana, Chauhan *et al*. [18] in Galium tricornutu and Gashi *et al*. [19] in Ramonda serbica and Ramonda nathaliae, which showed same results about the effect of GA3 on seeds germination. Gibberellic acid could be used as a replacement for cold treatment and our results showed that with increasing GA3 concentration, germinating parameters improved. Therefore, dormancy of yarrow seeds might be due to physiological reasons including prematurity of seeds embryo or existence of retardants in the seeds or both of them [20].

The growth and development of plants are directly affected by different stresses. Stresses are the most important factor in the reduction of plants yield [21]. In our research also, the mean-variance of recorded data showed that a high level of drought stress (75% and 100%) caused a decrease in all the morphological parameters of the plants. Although, stress is so important in photosynthesis, leaves and stems growth and development [26]. Plants that are in the dry areas of the world are usually exposed to drought stress. Water shortage causes disorders in the physiological process of plants, such as leaves growth, photosynthesis, stomata function, metabolism variation, drying and eventually death of plants [23]. Riaz *et al*. [24] studied the effect of drought stress on tagetes sp. and reported that in 70% drought stress, the height of plants was reduced. Saremirad and Mostafavi [25], Eyni Nargeseh *et al*. [26], Bagheri *et al*. [27], Karimi *et al*., [28], Jorenush and Rajabi [29] also reported the same results on the effect of drought stress on the plants. Although in all the cases higher irrigation doesn't mean a higher yield of the plants. In our research, plant morphological parameters in the drought stress of 50% showed better function in comparison with the 25% drought. Our result is in agreement with Yuan *et al*. [30], who reported that high irrigation can't be a reason for better plant performance. They showed that the better yield of tobacco plants was achieved in irrigation with 155 liter water, while with a higher volume of water (165 liters), plant yield and also morphological characteristics were reduced. One of the first symptoms of water scarcity is a decrease in turgor pressure and a reduction in the growth and development of plant cells. With the reduction of cell growth, the growth of different parts of plants will be limited. Therefore, the lower morphological features of the plants are the first symptoms of water stress on the plants [31]. In addition, absorption of nutrients reduces in the water stress condition, therefore growth and development of leaves and photosynthesis will be reduced and plant yield decreases [31, 32]. This reaction of the plant causes plant resistance in the drought stress condition [21]. By reduction of size in different plant organs, the wet and dry weight of plants will decrease [33, 34]. Our results showed that all morphological parameters of plants including plant height, plant coverage, shoot No., leaf No., leaf area, flower No., flower diameter, flower dry weight, plant wet and dry weight were reduced at high level (75% and 100%) of drought stress.

The relative water content of the plant is an important parameter, which is related to stomata behavior and root system in the plant. Saving the internal moisture of a plant needs a deep root system for water absorption [35]. Due to drought stress conditions, the relative water content of plants decreases, roots send ABA signals to close stomata, the entrance of CO2 decreases and finally photosynthesis will reduce [36, 37]. Chlorophyll is the most important content of chloroplast for the photosynthesis process [38]. A decrease in the amount of chlorophyll in drought stress conditions happens due to oxidation of

pigments and degradation of chlorophyll [39, 8, 40]. Xiao *et al*. [41] reported that the reason for the decrease in the amount of chlorophyll in drought stress in corn was the low speed of synthesis or rapid decomposition of chlorophyll. We showed that by increasing the level of drought stress from 25% to 100%, the amount of relative water content, chlorophyll and carotenoids was decreased. In the drought stress condition, one of the ways to increase the drought resistance is by moderating the osmotic potential in the plant cells. Proline is one of the amino acids which its amount increases in stress conditions. Proline helps plant cells to balance osmotic potential in the cells, directly or indirectly and increases plant resistance in stress conditions [41]. The highest amount of proline was recorded in 100% drought stress which is in agreement with Koc *et al*. [42]. They showed an increase in the amount of proline in pepper in the drought stress condition.

During drought stress, accumulation of H2O2 happens in the plant, which leads to oxidative damage in protein, lipids and DNA [43]. CAT enzyme converts H2O2 to H2O and 1/2 O2 [44]. Our findings show that CAT increased during drought stress. Dacosta and Huang [45] reported that in the drought condition, CAT and SOD activities, also lipid peroxidation was increased in the bent grass leaves.

POD has a major role in environmental stresses such as water stress. It removes the excess H2O2. An increase in antioxidant enzymes activity is associated with drought tolerance of plants [46]. Tatari *et al*., [47] also reported that drought stress caused an increase in CAT and POD in Agropyron desertorum and Poa pratensis. Chopra and Selote [12] showed that APX, CAT and POD activity in tolerant wheat was more than that of susceptible types. When an increase in POD activity happens in the plants, which can maintain a higher amount of water in their leaf. Our results also show that with increasing drought stress levels, POD was increased.

The important parameter in resistance to drought stress is the stability of the cell membrane. In conditions with increasing drought stress, electrolyte leakage will also increase [49, 50, 51]. Therefore, because of the fast response to water reduction, electrolyte leakage is the most important indicator in the drought stress condition.

Finally, we concluded that *A. millefolium* is a wild plant, which can be domesticated easily. Beside of its long flowing duration and green covering foliage, it has a high tolerance to water deficit. Therefore, it can be introduced for urban landscaping of dry and semi-dry regions.

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