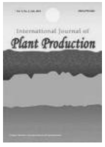

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Evaluation of four chamomile species under late season drought stress

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Abstract

To examine the genetic variation for drought stress in chamomile, eight accessions belonging to four species were evaluated under both field and greenhouse conditions using normal and late season drought stress. In the field experiment drought stress were initiated with irrigation cease at the commencement of flowering time. In greenhouse experiment, irrigation carried out at flower initiation when soil moisture reached to 80% and 50% of field capacity in control and drought stress treatments respectively. Orthogonal comparison showed high inter-and intra-species variation for all studied traits. In both experiments, drought stress caused significant reduction of flowering period, maturity time, flower number per plant, flower diameter, flower fresh and dry weight per plant, plant fresh and dry weight and plant relative water content. In field condition under both control and drought stress Gorgan1 accession (*Anthemis alltissima*) showed the highest flower fresh and dry weight. In greenhouse experiment, Isfahan (*Matricaria chamomilla*) and Mashhad (*Tripleurospermum sevance*) accessions produced greater flower yield and total dry mater under control and drought stress conditions, respectively. Minimum reduction due to drought stress for this two traits in the field experiment was for Ilam2 (*Anthemis psedocotula*) and in greenhouse experiment was for Ilam1 (*Anthemis psedocotula*) accessions. The maximum reduction rate due to drought stress in most genotypes was related to plant and flower fresh and dry weight. *Tripleurospermum sevance* species showed less reduction in fresh and dry flower weight when encountered to drought stress, hence it is more drought tolerant as compared to other investigated chamomile species.

Keywords: Chamomile; Drought stress; Inter and intra species variation.

Introduction

Chamomile is one of the major medicinal plants used across the world, and has been mentioned in at least in 26 pharmacopoeias of different countries for curing various diseases (Salamon, 1992). Various species of the genus *Matricaria*, *Tripleurospermum* and *Anthemis* are known as chamomile. This annual plant belongs to the composite family is very aromatic (Grdiner, 1999). The main useable part of this plant is its capitol (flowers) which is located at the apex of stem (McGuffin et al., 1996; Grdiner, 1999).

Chamomile has been known for centuries in Iran and is well established as traditional folk medicine used in the form of chamomile tea. Several pharmaceutical products are produced recently from chamomile in Iran under license of Ministry of Health (Baghalian et al., 2008). Chamomile naturally grows almost in many parts of Iran; however, it is frequency more in west, northwest and southern part of the country. Chamomile production in field and greenhouse has been increased considerably in recent years. Today in some parts of the world including the central parts of Iran this plant is being cultivated (Nazaralipour and Safedkon, 2003).

Drought stress is the lack or deficiency of humidity in susceptible stages of plant growth. It is the most important environmental stress and the main factor for yield reduction in plants (Singh, 2005; Patil and Khanna-chopra, 2006). Response of plants to drought stress depends on time and place, and especially in field conditions can be mixed with the effects of the other stresses such as high temperature (Yordanov et al., 2003; Cattivelli et al., 2008). Furthermore, responses to drought stress are extremely different according to the plant genetic background. Inter-and intra-species variations in drought tolerance are almost well known (Rampino et al., 2006).

Depending on the plant growth stages, drought stress influences morphology, anatomy, physiology and biochemistry of plants (Upadhyaya and Panda, 2004). Generally drought stress at the end of the growth season by disturbing water status and reducing the ripening period result in early maturity and affects other characteristic with final reduction of biomass (Johnston and Fowler, 1992).

Despite considerable studies on the effect of drought stress on field crops the information on the behavior of the aromatic and medicinal plants has not been well investigated under water deficiency. Even though these plants frequently develop their adaptation with the unfavorable environmental conditions such as drought stress but it is clear that for understanding yield evolution of medicinal plants and their existence and tolerance to dry regions, the determination of improved conditions for their planting is required (Letchamo and Gosselin, 1996). Razmjoo et al. (2008) has reported that water deficiency inhibited various growth parameters of *Matricaria chamomila* to various degrees. Wild type German chamomile (*Chamomilla recutita* L.) plants are better adjusted to desiccation than the cultivated plants of similar growth pattern (Baczek-Kwinta et al., 2009). Hornok (1992) introduced optimum irrigation as the main term for producing chamomile with a high flower yield. Pirzad et al. (2006) showed that drought stress in field conditions significantly affects the flower dry weight in *Matricaria chamomilla*. Afzali et al. (2007) showed that increased drought stress on *Matricaria chamomilla* under the greenhouse conditions leads to the decrease in dry flower weight. Significant differences for flower fresh and dry weights of chamomile were reported after imposing drought stress (Lebaschy and Ashoorabadi, 2004).

In other medicinal plants such as sweet basil, drought stress significantly affected fresh and dry weight of plant, root length and stem length (Khalid, 2006). Using four accessions belonging to wild and local genotypes of *Matricaria chamomilla*, Frantz and Holzl (1978) observed significant differences among genotypes in the amount of tiller and flowering time. Due to lack of sufficient knowledge on chamomile response to drought stress, this experiment was carried out to assess the tolerance of four chamomile species to late season drought stress.

Materials and Methods

Eight accessions of chamomile were collected from different areas in Iran. They included two accessions of *Anthemis alltissima* from Gorgan, two accessions of *Anthemis psedocotula* from Ilam, three accessions of *Tripleurospermum sevance* from Mashhad, Zabol and Fars, and one accession of *Matricaria chamomilla* from Isfahan. The drought stress tolerance of these accessions were evaluated in both field and greenhouse conditions.

In the field experiment the accessions were planted in a split-block design on randomized complete blocks with three replications under the control and end season drought stress conditions in a field located in Mobarekeh Isfahan. Planting was carried out on March 15, 2007. Between and within row spaces were considered 30 and 20 centimeters respectively. In each plot six rows each of 3 meters length were planted and recording was carried out from the four middle rows. Drought stress treatment exercised with ceasing the irrigation at the flowering time for each experimental unit.

In greenhouse experiment the mentioned accessions and two levels of stress (control and end season drought stress) were applied in a factorial experiment with complete randomize block design at three replications in the research greenhouse of Shahrekord University. The planting was carried out on August 30, 2007. In this experiment pots with the height and diameter of 25 cm were used. Each pot was filled with an equal ratio of clay, sand and animal manure. To supply the cold requirement of the plants, at the beginning of winter during the rosette period the pots were placed outside the greenhouse (with the mean day and night temperature of 12 and 5 °C respectively) for one month. To accelerate flowering, in stem formation plants were irradiated with synthetic light from a normal fluorescent lamp with 800 Lux light for 13 hours per day. In greenhouse experiment, from the start of flowering time, irrigation applied for the control treatment at relatively 80% of field capacity and for the stress treatment at relatively 50% of field capacity which determined by weighing of pots every 3 days.

The studied traits were consisted of flowering time; the number of days from planting till start of flowering, maturity time; number of days from planting date to complete flowering when harvest is done, flowering period; the time length between flowering and maturity dates, number of tillers grown from crown, flower diameter (as determined by measuring the middle part of the capitol without tongue florets using digital Collis in 0.01 mm scale), number of flowers per plant, fresh and dry weights of the above ground plant parts, flower wet and dry weight per plant and relative water content (RWC) of shoot. Dry total shoot weight was determined by placing plants in dry oven at 80 ^{o}C for 24 hours. The flower dry weight was determined after drying the flowers in shadow at a mean temperature of 25 ^{o}C for 7 days and later they were weighed. Shoot relative water content was measured according to Liu and Staden (2001).

The rate of changes in percent due to drought stress was calculated using the following formula:

$$\text{Rate of Change} = \frac{\text{value of trait in drought stress condition - value of trait in control condition}}{\text{value of trait in control condition}} \times 100$$

Statistical calculations were performed by SAS Statistical Program, (SAS Institute, 1999) and significant means comparisons were made by LSD Fisher. The significance of different mean comparisons of species and genus was determined by orthogonal comparisons. Linear regression analysis and coefficient of determination were used to determine the relation of each character under drought stress and control conditions and also field and greenhouse conditions.

Results and Discussion

The separate results of the analysis of variance for field and greenhouse experiments showed that the effect of accessions is significant ($P<0.01$) for all traits (data not shown). Analysis of variance also showed that drought stress except flowering time and number of tiller in both field and greenhouse experiments had significant effect on other traits. Since stress had been applied after tiller and flowering development, therefore the lack of difference for these two traits between drought stress and control conditions was expectable.

Mean values of traits for accessions under and rate of changes due to drought stress which had occurred in field and greenhouse experiments are shown in tables 1 and 2, respectively. Regarding phenological traits under field conditions Ilam1 accession (*Anthemis psedocotula*) and Isfahan accession (*Matricaria chamomilla*) both formed flower 67 days earlier than other accessions, and the former accession had the earliest maturity in both control and drought stress conditions after 92 and 85 days, respectively (Table 1). In this experiment the length of flowering period i.e. the flowering start time till flower completion, varied in control conditions between 15.7 days for Ilam2 (*Anthemis psedocotula*), and to 35 days for Fars (*Tripleurospermum sevance*). Drought stress decreased the length of this period on average by 7.4 days (25.7%). The maximum of this reduction was for the accession of Mashhad (*Tripleurospermum sevance*), which was 12 days or 38.3%. In the greenhouse experiment due to autumn cultivation, plants were in the rosette period within the winter and compared to field experiment, flowering time and maturity time increased and reached to approximately 190 and 235 days respectively, under control conditions (Table 2). Furthermore, different growing conditions in the two experiments can be the main reason of this difference. Isfahan (*Matricaria chamomilla*), in this experiment entered to flowering earlier than other accessions again. This accession in both control and drought conditions was also the accession of the earliest maturity, whereas the latest maturity accessions in greenhouse experiment were from different species. Drought stress in this experiment caused early maturity for different accessions from 4 days (*Anthemis psedocotula*, Ilam1) up to 12 days (*Matricaria chamomilla*, Isfahan). In fact with regard to the importance of complete existence of reproductive stage, probably the plant will try to accelerate reproductive stage with the optimum usage of available water. Therefore the length of flowering period would be decreased. This strategy has been observed in a numbers of other annual plants (Fox, 1990; Aronson et al., 1992; Rajakaruna et al., 2003). In other species the phenological changes under drought are reported. Fukia et al., (1999) reported that in all screening method for drought tolerance flowering time should be considered.

Table 1. Mean comparisons of accessions and rate of change (%change) due to drought stress compared to control in field experiment.

Accession	Treatment	Flowering time (day)		Maturity time (day)		Flowering period (day)		Tiller Number (nu)		Flower diameter (mm)		flower number /plant (nu)	
		Mean	%Change	Mean	%Change	Mean	%Change	Mean	%Change	Mean	%Change	Mean	%Change
Tripleurospermum sevance, Fars	Control	94.0^{c}	0.0	129.0^{a}	-6.5	35.0^{a}	-23.8	5.9^{de}	-7.9	8.47^{ef}	-6.3	159.5^{de}	-37.6
	Drought stress	94.0^{c}		120.7^{b}		26.7^{cd}		5.5^{e}		7.93^{f}		99.6^{f-j}	
Tripleurospermum sevance, Zabol	Control	96.7^{ab}	-1.4	129.0^{a}	-8.8	32.3^{a}	-30.9	8.8^{a-c}	4.5	8.53^{ef}	-2.3	205.9^{cd}	-41.6
	Drought stress	95.3^{bc}		117.7^{b}		22.3^{def}		9.2^{a}		8.33^{ef}		120.1^{ef}	
Tripleurospermum sevance, Mashhad	Control	99.0^{a}	0.0	130.3^{a}	-7.6	31.3^{ab}	-38.3	8.7^{a-c}	-1.1	8.10^{f}	4.9	250.2^{bc}	-55.5
	Drought stress	99.0^{a}		120.3^{b}		19.3^{fg}		8.6^{a-c}		8.50^{ef}		111.4^{e-h}	
Anthemis alltissima, Gorgan1	Control	82.3^{d}	0.0	107.0^{c}	-2.8	24.7^{cde}	-12.2	8.7^{a-c}	-5.3	17.20^{a}	-9.1	101.3^{f-i}	-34.5
	Drought stress	82.3^{d}		104.0^{cd}		21.7^{ef}		8.3^{a-d}		15.63^{b}		66.9^{g-j}	
Anthemis alltissima, Gorgan2	Control	95.3^{bc}	0.0	129.0^{a}	-8.5	33.7^{a}	-32.7	7.5^{a-e}	6.3	8.27^{ef}	-4.4	213.3^{c}	-45.6
	Drought stress	95.3^{bc}		118.0^{b}		22.7^{def}		7.9^{a-e}		7.90^{f}		116.1^{e-g}	
Anthemis psedocotula, Ilam1	Control	66.7^{e}	0.0	91.7^{f}	-6.9	25.0^{cde}	-25.3	9.8^{a}	-8.8	9.96^{d}	-3.7	108.1^{f-h}	-48.3
	Drought stress	66.7^{e}		85.3^{g}		18.7^{fg}		8.9^{ab}		9.59^{de}		55.8^{ij}	
Anthemis psedocotula, Ilam2	Control	82.0^{d}	-1.2	97.7^{e}	-3.1	15.7^{gh}	-12.8	6.2^{c-e}	-9.7	13.63^{c}	0.2	66.1^{h-j}	-22.5
	Drought stress	81.0^{d}		94.7^{ef}		13.7^{h}		5.6^{e}		13.67^{c}		51.3^{j}	
Matricaria chamomilla, Isfahan	Control	67.3^{e}	-0.49	102.0^{d}	-7.2	34.7^{a}	-20.2	7.5^{a-e}	-13.3	7.49^{f}	-2.0	428.5^{a}	-35.1
	Drought stress	67.0^{e}		94.7^{ef}		27.7^{bc}		6.5^{b-e}		7.34^{f}		278.2^{b}	
Mean	Control	85.4^{a}	-0.4	114.5^{a}	-6.8	29.0^{a}	-25.7	7.9^{a}	-3.8	10.21^{a}	-2.9	191.6^{a}	-41.4
	Drought stress	85.1^{a}		106.7^{b}		21.6^{b}		7.6^{a}		9.86^{b}		112.3^{b}	

Continued Table 1.

Accession	Treatment	Plant fresh weight (gr)		Plant dry Weight (gr)		Flower fresh weight /plant (gr)		Flower dry weight /plant (gr)		RWC (%)	
		mean	%Change	mean	%Change	mean	%Change	mean	%Change	mean	%Change
Tripleurospermum sevance, Fars	Control	194.3^{ab}	-58.5	68.2^{a}	-44.2	25.2^{d-g}	-37.0	9.7^{c-e}	-26.3	65.2^{bc}	-19.2
	Drought stress	80.7^{cd}		38.1^{bc}		15.9^{g-i}		7.2^{ef}		52.6^{f}	
Tripleurospermum sevance, Zabol	Control	185.9^{ab}	-46.3	66.7^{a}	-36.2	32.7^{b-d}	-39.7	11.9^{bc}	-34.4	64.1^{b-d}	-10.7
	Drought stress	99.8^{c}		42.6^{b}		19.7^{e-i}		7.8^{ef}		57.3^{e}	
Tripleurospermum sevance, Mashhad	Control	238.5^{a}	-56.9	76.7^{a}	-43.3	40.4^{a-c}	-42.8	15.9^{a}	-42.8	67.1^{b}	-14.1
	Drought stress	102.9^{c}		43.5^{b}		23.1^{d-h}		9.8^{c-e}		57.6^{e}	
Anthemis alltissima, Gorgan1	Control	166.2^{b}	-47.5	42.6^{b}	-23.1	50.8^{a}	-43.9	17.0^{a}	-31.6	74.3^{a}	-15.8
	Drought stress	87.3^{cd}		32.7^{b-}		28.5^{d-f}		11.6^{cd}		62.6^{cd}	
Anthemis alltissima, Gorgan2	Control	236.7^{a}	-59.0	80.9^{a}	-48.9	41.4^{ab}	-55.9	15.6^{ab}	-48.0	65.5^{bc}	-12.0
	Drought stress	97.0^{c}		41.3^{b}		18.3^{e-i}		8.1^{d-f}		57.6^{e}	
Anthemis psedocotula, Ilam1	Control	78.0^{cd}	-63.8	26.7^{b-}	-57.9	19.6^{e-i}	-59.2	9.0^{c-e}	-66.2	65.8^{bc}	-8.5
	Drought stress	28.3^{d}		11.2^{e}		8.0^{i}		3.0^{g}		60.3^{de}	
Anthemis psedocotula, Ilam2	Control	91.3^{c}	-36.2	22.7^{cd}	-15.9	17.4^{e-i}	-29.3	10.3^{c-e}	-23.2	73.9^{a}	-10.1
	Drought stress	58.3^{cd}		19.1^{de}		12.3^{hi}		7.9^{d-f}		66.4^{bc}	
Matricaria chamomilla, Isfahan	Control	90.2^{c}	-47.5	20.2^{de}	-20.7	29.1^{c-e}	-41.6	7.4^{ef}	-39.5	77.6^{a}	-14.7
	Drought stress	47.3^{cd}		16.0^{de}		17.0^{f-i}		4.5^{fg}		66.2^{bc}	
Mean	Control	160.1^{a}	-53.0	50.6^{a}	-39.6	32.1^{a}	-44.4	12.1^{a}	-38.0	69.2^{a}	-13.2
	Drought stress	75.2^{b}		30.6^{b}		17.9^{b}		7.51^{b}		60.1^{b}	

A- Means followed by the same letter in each column are not significantly different in LSD Fisher test at the 5% level.

Table 2. Mean comparisons of accessions and rate of change (%change) due to drought stress compared to control in greenhouse experiment.

Accession	Treatment	Flowering time (day)		Maturity time (day)		Flowering period (day)		Number tiller		Flower diameter (mm)		Flower Number /plant	
		Mean	%change	Mean	%Change	Mean	%Change	Mean	%Change	Mean	%change	Mean	%change
T. sevance, Fars	Control	204.7^{ab}	0.5	245.7^{a}	-3.3	41.0^{d}	-22.0	8.7^{bc}	-34.62	11.33^{de}	-3.2	61.0^{cd}	-33.3
	Drought stress	205.7^{ab}		237.7^{bc}		32.0^{ef}		5.7^{de}		10.97^{de}		40.7^{ef}	
T.sevance, Zabol	Control	208.7^{a}	-1.0	235.7^{c}	-3.5	27.0^{f}	-23.5	7.3^{cd}	0.00	11.08^{de}	-1.1	42.3^{ef}	-33.9
	Drought stress	206.7^{ab}		227.3^{d}		20.7^{g}		7.3^{cd}		10.96^{de}		28.0^{hi}	
T. sevance, Mashhad	Control	203.7^{b}	0.8	246.0^{a}	-3.3	42.3^{d}	-22.8	6.7^{cd}	5.00	10.70^{de}	-1.5	46.3^{d-f}	-54.0
	Drought stress	205.3^{ab}		238.0^{b}		32.7^{e}		7.0^{cd}		10.54^{e}		21.3^{ij}	
A. alltissima, Gorgan1	Control	190.0^{d}	0.4	244.3^{a}	-3.3	54.3^{a}	-16.0	10.7^{ab}	6.25	16.33^{b}	-3.4	45.0^{ef}	-54.1
	Drought stress	190.7^{d}		236.3^{bc}		45.7^{cd}		11.3^{a}		15.77^{b}		20.7^{j}	
A. alltissima, Gorgan2	Control	192.7^{cd}	-0.5	245.0^{a}	-3.4	52.3^{ab}	-14.0	8.7^{bc}	-3.85	10.87^{de}	-3.8	51.7^{c-e}	-27.1
	Drought stress	191.7^{d}		236.7^{bc}		45.0^{cd}		8.3^{bc}		10.46^{e}		37.7^{fg}	
A. psedocotula, Ilam1	Control	162.3^{e}	-1.2	213.3^{e}	-1.9	51.0^{ab}	-3.9	7.3^{cd}	-13.64	12.39^{c}	-6.6	62.3^{c}	-36.4
	Drought stress	160.3^{e}		209.3^{f}		49.0^{bc}		6.3^{c-e}		11.57^{cd}		39.7^{ef}	
A. psedocotula, Ilam2	Control	196.3^{c}	-2.4	246.3^{a}	-3.8	50.0^{abc}	-9.3	5.0^{de}	-20.00	17.34^{a}	-5.2	29.3^{gh}	-34.1
	Drought stress	191.7^{d}		237.0^{bc}		45.3^{cd}		4.0^{e}		16.44^{ab}		19.3^{j}	
M. chamomilla, Isfahan	Control	161.3^{e}	-0.2	203.0^{g}	-5.9	41.7^{bc}	-28.0	6.3^{c-e}	0.00	6.30^{f}	-1.5	180.3^{a}	-42.3
	Drought stress	161.0^{e}		191.0^{h}		30.0^{ef}		6.3^{c-e}		6.20^{f}		104.0^{b}	
Mean	Control	190.0^{a}	-0.5	234.9^{a}	-3.5	45.0^{a}	-16.5	7.6^{a}	-7.1	12.04^{a}	-3.5	64.8^{a}	-39.9
	Drought stress	189.1^{a}		226.7^{b}		37.5^{b}		7.0^{a}		11.64^{b}		38.9^{b}	

Continued Table 2:

Accession	Treatment	Plant fresh weight (gr)		Plant dry Weight (gr)		Flower fresh weight /plant (gr)		Flower dry weight /plant (gr)		RWC (%)	
		Mean	%Change	Mean	%change	Mean	%change	Mean	%change	Mean	%Change
T. sevance, Fars	Control	34.2^{bc}	-32.2	15.9^{b}	-25.4	8.4^{b}	-50.0	3.3^{b}	-57.5	52.8^{d-f}	-7.5
	Drought stress	23.2^{f-i}		11.9^{cd}		4.2^{ef}		1.4^{d}		48.8^{ef}	
T. sevance, Mashhad	Control	46.4^{a}	-37.3	19.6^{a}	-27.7	6.9^{bc}	-64.0	3.3^{b}	-52.2	57.5^{cd}	-10.8
	Drought stress	29.1^{c-e}		14.2^{bc}		2.5^{g}		1.6^{d}		51.3^{d-f}	
T. sevance, Zabol	Control	34.7^{b}	-29.8	14.7^{b}	-21.9	5.1^{de}	-20.4	2.9^{bc}	-46.5	57.6^{cd}	-3.7
	Drought stress	24.4^{e-g}		11.5^{cd}		4.0^{ef}		1.5^{d}		55.5^{cd}	
A. alltissima, Gorgan1	Control	23.4^{f-h}	-37.6	15.1^{b}	-32.4	5.6^{cd}	-67.7	4.8^{a}	-65.9	35.4^{g}	-14.9
	Drought stress	14.6^{j}		10.2^{de}		1.8^{h}		1.6^{d}		30.1^{g}	
A. alltissima, Gorgan2	Control	29.4^{b-e}	-39.2	15.5^{b}	-38.3	8.3^{b}	-38.3	4.8^{a}	-51.9	47.4^{ef}	-1.6
	Drought stress	17.9^{ij}		9.5^{de}		5.1^{de}		2.3^{c}		46.6f	
A. psedocotula, Ilam1	Control	28.0^{d-f}	-35.9	9.1^{ef}	-24.5	6.5^{b-d}	-36.4	2.7^{bc}	-38.2	68.4^{ab}	-9.3
	Drought stress	17.9^{h-j}		6.8^{fg}		3.4^{f}		1.7^{d}		62.2^{bc}	
A. psedocotula, Ilam2	Control	21.2^{g-i}	-40.9	9.4^{de}	-39.0	6.1^{cd}	-47.8	3.2^{b}	-48.2	55.5^{c-d}	-2.7
	Drought stress	12.5^{j}		5.8^{g}		3.2^{fg}		1.7^{d}		54.0^{d-e}	
M. chamomilla, Isfahan	Control	30.9^{b-d}	-42.1	8.4^{ef}	-33.4	15.5^{a}	-47.7	3.3^{b}	-50.2	72.6^{a}	-5.9
	Drought stress	17.9^{ij}		5.6^{g}		8.1^{b}		1.6^{d}		68.3^{ab}	
Mean	Control	31.0^{a}	-36.5	13.5^{a}	-30.5	7.8^{a}	-48.1	3.5^{a}	-52.5	55.9^{a}	-6.8
	Drought stress	19.7^{b}		9.3^{b}		4.0^{b}		1.7^{b}		52.1^{b}	

A- Means followed by the same letter in each column are not significantly different in LSD Fisher test at the 5% level.

In both experiments for other traits, inter- and intra-specific variations also existed and the drought stress caused reduction of their means as well (Tables 1 and 2). The intensity of reduction caused by drought stress was dependent on the experimental conditions (field or greenhouse), kind of trait and the accession. In field conditions the maximum flower diameter in both control and drought stress was related to *Anthemis alltissima*. The flower diameter in Gorgan1 (*Anthemis alltissima*) was approximately two times of Gorgan2 (Table 1). In this experiment Isfahan (*Matricaria chamomilla*) had the least flower diameter in control (7.49 mm) and drought stress (7.34 mm) conditions, but showed the maximum number of flowers per plant in both control and drought stress conditions i.e. 428 and 278, respectively. The number of flowers produced in this accession was more than 5 times of the accession Ilam2 (*Anthemis psedocotula*). The number of flowers in all accessions under stress condition was reduced significantly so that the number of flowers in some accessions such as Mashhad (*Tripleurospermum sevance*) was less than 55% of the control condition. Meanwhile Ilam2 (*Anthemis psedocotula*) showed the least change caused by drought stress for the number of flowers per plant (22.5%).

Plant fresh weight under field conditions in the control varied from 78 gram (*Anthemis psedocotula*, Ilam1) to 238.5 gram (*Tripleurospermum sevance*, Mashhad) on average showed maximum reduction (53%) due to drought stress among the traits being measured (Table 1). Among accessions, the maximum change in plant fresh weight was related to Ilam1 (*Anthemis psedocotula*), with the rate of 64%. This accession also showed the maximum reduction due to drought stress for shoot dry weight (58%). Compared to the other accession of this species, Ilam2 (*Anthemis psedocotula*), showed the minimum reduction due to drought stress in both fresh and dry plant weight with 36.2% and 15.9%, respectively. These are in agreement with the results of Lebaschy and Ashoorabadi, (2004) and Razmjoo et al. (2008) who reported drought reducedgrowth of chamomile. Razmjoo et al. (2008) reported that the greatest reduction occurred in flower dry weight of *Matricaria chamomilla*. Baczek-Kwinta et al. (2009) also reported plant fresh weight reduction in another species of chamomile (*Chamomilla recutita* L.) caused by drought stress in control condition. On the whole, plant produces the maximum biomass under favorable humidity and drought stress can cause remarkable reduction of biomass production (Ercoli et al., 2008). This matter has also occurred for the examined chamomile accessions, even though the rate of this reduction varied among them. It is considered that besides other factors, stability in biomass production is a drought tolerance criterion (Purcell et al., 2000). In this regard, Ilam2 (*Anthemis psedocotula*) had the most stability in shoot production.

The weight of flower produced by chamomile is very important due to economic reasons (Pirzad et al., 2006). In the field condition in the control treatment, the maximum flower fresh and dry weight per plant was 50.8 and 17 gram, respectively in Gorgan1 (*Anthemis alltissima*) (Table 1). Although the reduction rate in this accession caused by stress was more than others but due to its high primary production it showed still the maximum fresh and dry flower weight per plant under stress condition. Due to drought stress, Ilam2 (*Anthemis psedocotula*) and Ilam1 (*Anthemis psedocotula*), showed the minimum and maximum rate of change respectively, for both fresh and dry flower weight.

Therefore, in field conditions these two accessions could be considered as the most tolerant and sensitive accessions respectively, for flower production. Furthermore little amounts of biomass and flower weight reductions due to drought in accessions of *Anthemis psedocotula* species could mention the importance of intra species variations for drought tolerance in chamomile.

Generally under greenhouse conditions flower diameter was more than field conditions (Table 2). In control and drought stress conditions, again Gorgan1 (*Anthemis alltissima*) and Isfahan (*Matricaria chamomilla*) respectively had the maximum and minimum flower diameter, and the Isfahan (*Matricaria chamomilla*) in both conditions produced the maximum number of flowers. But, in most accessions under both control and drought stress flower number, plant fresh and dry weights, flower fresh and dry weights reduced significantly in both greenhouse and field conditions. Different environmental conditions of field and greenhouse, for instance, limited soil volume and nutrients in pots, and the limitation of growth and root distribution in pots are factors of these variations (Letchamo and Marquard, 1993; Rajakaruna et al., 2003). Applying the drought stress in greenhouse experiments also led to a significant reduction in most of the traits including plant and flower fresh and dry weights, even though the intensity of the rate of change caused by drought stress was different with respect to accessions. Flower dry weight and flower fresh weight with the average of 52.5% and 48.1% respectively, showed the maximum reduction due to drought stress (Table 2). Traits such as flower number per plant, plant fresh weight and plant dry weight with the average rate of change of 39.9%, 36.5% and 30.5% respectively, were at the next rank of reduction due to drought stress. Generally in chamomile, flowering continued till the end of growth. Probably due to the optimum usage of available water, after applying stress the plant will be inhibited from producing new flowers and will consume the available water leading to a significant reduction of flower number. This reduction can also be caused by reduction of available nutrients (Fukaia et al., 1999; Pantuwan et al., 2004; Ercoli et al., 2008). Gorgan1 (*Anthemis alltissima*) with 67.7% flower fresh weight and 65.9% flower dry weight had the maximum rate of reduction caused by drought stress. Under greenhouse conditions, Zabol (*Tripleurospermum sevance*), for plant fresh and dry weight and flower fresh weight, and Ilam1 (*Anthemis psedocotula*) for its flower dry weight had more stability than other accessions after drought. Negative effect of drought stress on flower weight in *Matricaria chamomilla* under field and greenhouse conditions has been reported (Pirzad et al., 2006; Afzali et al., 2007). Salinity also decreased fresh flower weight, dry flower weight and dry weight of aerial stems in Chamomile (Baghalian et al., 2008).

Change in plant relative water content (RWC) is one of the effects of drought stress (Rampino et al., 2006; Cattivelli et al., 2008). In this experiment, the water content of chamomile accessions exposed to drought stress showed significant reduction (Tables 1 and 2) and was related to growth conditions (field or greenhouse) in agreement with former studies on *Chamomilla recutita* L (Baczek-Kwinta et al., 2009).

Species' mean orthogonal comparisons of traits (Tables 3 and 4) indicated that the four species are almost different for all recorded traits except for tiller number in field conditions. In both experiments *Tripleurospermum sevance* and *Anthemis alltissima* had the latest maturity and flowering time and *Matricaria chamomilla*, was matured approximately 26 and 44 days before than *T. sevance* in field and greenhouse conditions, respectively. Regarding the long growth period of *Tripleurospermum sevance* in the field it can benefit the most available facilities to produce more plant fresh and dry weight compared to other species. Thus, in field conditions *Tripleurospermum sevance* produces approximately 2 and 3 times plant fresh and dry weight, respectively compared to *Matricaria chamomilla*. It seems that flower fresh and dry weight in studied species is mostly related to the length of flowering period so that species like *Tripleurospermum sevance* and *Anthemis alltissima* which had significantly longer flowering period produced more flower fresh and dry weights. The least and the most number of flower per plant was belong to *Anthemis psedocotula* and *Matricaria chamomilla* species respectively, in both experiments. Overall variation between species' was better shown in field experiment compared to greenhouse experiment for all studied traits. The relative water content was significantly higher in shoot of *Matricaria chamomilla*. Although *Anthemis psedocotula* had the lowest range of plant and flower fresh weight production in both experiments, *Matricaria chamomilla* had the lowest plant and flower dry weight among other species. It is important to note that *Matricaria chamomilla* produced significantly the maximum number of flowers compared with other species, however it had a smaller flower diameter than others. The variation in traits such as tiller number and flowering time among wild and native species of *Matricaria chamomilla* have been reported (Frantz and Holzl, 1978). Orthogonal comparison of species reduction in fresh and dry flower weight due to drought stress in the both experiments (data not shown) indicated that *Tripleurospermum sevance* less reduction; and hence are more drought tolerant chamomile species.

One of the main questions in any stress breeding programme is performance expectation of the traits in stress conditions based on the control condition (Houshmand et al, 2005). Regression equation fitted for each of the traits under field and greenhouse conditions to distinguish the possibility of estimation of the studied trait under stress on the basis of incidence of that trait under the control condition (Table 4). Significant linear relationship with high coefficient of determination (R^2) for performance in stress and control conditions was observed for most of the traits in both field and greenhouse experiments. It seems that in chamomile reliable performance expectation in stress condition is possible for the traits with the high coefficient of determination such as flower diameter or flowering period. In contrast, low coefficients for flower dry weight in both experiments, equal to 0.393 (in greenhouse experiment) and 0.734 (in field experiment) show that flower yield in the control condition can not be a suitable predictor for this trait in a condition with drought stress through end of growth period. Therefore, the evaluation of accessions for flower yield under each condition separately is necessary. Similar results were also observed in other plants (Spechta et al., 2001; Houshmand et al., 2005).

Table 3. Mean comparison of traits in four chamomile species in field condition.

Species and comparison	Flowering time (day)	Maturity time (day)	Flowering period (day)	Number tiller (nu)	Flower diameter (mm)	Flower Number /plant (nu)	Plant fresh weight (gr)	Plant dry Weight (gr)	Flower fresh weight /plant (gr)	Flower dry weight /plant (gr)	RWC (%)
Tripleurospermum sevance	96.33	124.17	27.84	7.79	8.31	157.79	150.36	55.96	26.19	10.40	60.64
Anthemis alltissima	88.83	114.50	25.67	8.10	12.25	124.13	146.82	49.38	34.48	13.10	65.01
Anthemis psedocotula	74.08	92.33	18.25	7.63	11.71	70.33	63.96	19.92	14.35	7.59	66.61
Matricaria chamomilla	67.17	98.33	31.16	7.03	7.41	353.35	68.78	18.14	23.08	5.95	71.86
T. sevance vs *A. alltissima*	7.50^{**}	9.67^{**}	2.17^{*}	0.31^{ns}	3.94^{**}	33.66^{**}	3.54^{ns}	6.57^{*}	8.29^{**}	2.70^{**}	4.37^{**}
T. sevance vs *A. psedocotula*	22.25^{**}	31.83^{**}	9.59^{**}	0.16^{ns}	3.40^{**}	87.47^{**}	86.40^{**}	36.04^{**}	11.85^{**}	2.81^{**}	5.97^{**}
T. sevance vs *M. chamomilla*	29.17^{**}	25.83^{**}	3.32^{**}	0.76^{ns}	$.90^{*}$	195.56^{**}	81.58^{**}	37.82^{**}	3.11^{ns}	4.45^{**}	11.23^{**}
A. alltissima vs *A. psedocotula*	14.75^{**}	22.17^{**}	7.42^{**}	0.47^{ns}	0.54^{ns}	53.81^{**}	82.86^{**}	29.47^{**}	20.14^{**}	5.51^{**}	1.59^{ns}
A. alltissima vs *M. chamomilla*	21.67^{**}	16.17^{**}	5.49^{**}	1.07^{ns}	4.84^{**}	229.22^{**}	78.04^{**}	31.25^{**}	11.40^{**}	7.15^{**}	6.85^{**}
A. psedocotula. vs *M. chamomilla*	6.92^{**}	6.00^{**}	12.91^{**}	0.60^{ns}	4.30^{**}	283.03^{**}	4.82^{**}	1.78^{ns}	8.74^{ns}	1.64^{ns}	5.26^{**}

A- Contrasts values are the absolute value of difference between mean species.
B- Presence of significant means are determined based on orthogonal comparison in analysis of variance.
C- ns, * and ** : Means differences are not significant and significant at P>0.05 and P>0.01 respectively.

Table 4. Mean comparison of traits in four chamomile species in greenhouse condition.

Species and comparison	Flowering time (day)	Maturity time (day)	Flowering period (day)	Number tiller (nu)	Flower diameter (mm)	Flower Number /plant (nu)	Plant fresh weight (gr)	Plant dry Weight (gr)	Flower fresh weight /plant (gr)	Flower dry weight /plant (gr)	RWC (%)
Tripleurospermum sevance	205.78	238.39	32.61	7.11	10.93	39.94	32.01	14.51	5.18	2.34	53.92
Anthemis alltissima	191.25	240.58	49.33	9.75	13.36	38.75	21.35	12.56	5.21	3.39	39.88
Anthemis psedocotula	177.67	226.50	48.83	5.67	14.44	37.67	20.63	7.77	5.00	2.30	59.97
Matricaria chamomilla	161.17	197.00	35.83	6.33	6.25	142.17	24.42	7.04	11.80	2.46	70.45
T. sevance vs *A. alltissima*	14.53^{**}	2.19^{ns}	16.72^{**}	2.64^{**}	2.42^{**}	1.19^{ns}	10.67^{**}	1.96^{**}	0.03^{ns}	1.05^{**}	14.04^{**}
T. sevance vs *A. psedocotula*	28.11^{**}	11.89^{**}	16.22^{**}	1.44^{*}	3.51^{**}	2.28^{ns}	12.11^{**}	6.75^{**}	0.38^{ns}	0.04^{ns}	6.06^{**}
T. sevance vs *M. chamomilla*	44.61^{**}	41.39^{**}	3.22^{*}	0.78^{ns}	4.68^{**}	102.22^{**}	7.60^{**}	7.47^{**}	6.62^{**}	0.11^{ns}	16.53^{**}
A. alltissima vs *A. psedocotula*	13.58^{**}	14.08^{**}	0.50^{ns}	4.08^{**}	1.08^{**}	1.08^{ns}	1.44^{ns}	4.79^{**}	0.41^{ns}	1.08^{**}	20.10^{**}
A. alltissima vs *M. chamomilla*	30.08^{**}	43.58^{**}	13.50^{**}	3.42^{**}	7.11^{**}	103.42^{**}	3.07^{ns}	5.52^{**}	6.59^{**}	0.93^{**}	30.57^{**}
A. psedocotula vs *M .chamomilla*	16.50^{**}	29.50^{**}	13.00^{**}	0.67^{ns}	8.19^{**}	104.50^{**}	4.51^{ns}	0.73^{**}	7.01^{**}	0.15^{ns}	10.48^{**}

A- Contrasts values are the absolute value of difference between mean species.
B- Presence of significant means are determined based on orthogonal comparison in analysis of variance.
C- ns, * and ** : Means different no significant and significant at P>0.05 and P>0.01 respectively.

Table 5. Regression equation and coefficient of determination (R^2) between stress and control conditions in field and greenhouse experiments for the studied traits in eight chamomile accessions.

Traits	field		Greenhouse	
	Equation	R^2	Equation	R^2
Flowering time	0.067+0.995x	0.998	-3.422+1.013x	0.988
Maturity time	4.819+0.577x	0.755	-10.81+1.075x	0.913
Flowering period	11.98+0.827x	0.975	-10.83+1.011x	0.984
Number tiller	-0.5396+1.026x	0.868	-0.891+1.046x	0.713
Flower diameter	0.802+0.887x	0.981	0.405+0.930x	0.996
Number flower	-4.874+0.611x	0.937	1.936+0.570x	0.970
Weight of fresh flower	1.728+0.502x	0.846	-0.029+0.522x	0.806
Weight of fresh plant	13.23+0.387x	0.855	-1.439+0.681x	0.946
Weight of dry plant	5.924+0.487x	0.888	-0.643+0.742x	0.944
Weight of dry flower	-0.354+0.648x	0.734	0.950+0.206x	0.393
Relative water content	4.868+0.797x	0.761	-1.6+0.960x	0.965

Conclusion

The main aim of this study was the evaluation of eight accessions belonging to four chamomile species and their reaction to late season drought stress in control and field conditions. Remarkable inter and intra species variations were shown for the investigated traits in field and greenhouse experiments under both control and drought stress conditions. Drought stress at the late growth season led to a significant reduction in the observed characteristics except the traits that were developed before applying stress including flowering time and tiller number. Biomass production including fresh and dry weight of shoot and flower were affected mostly under drought stress. Diversity in the rate of changes caused by drought stress in accessions indicates their tolerance rate variations when facing drought stress. Based on this parameter the most tolerance accession was Ilam2 (*Anthemis psedocotula*). Because breeding programs aim to improve features desirable for cultivation, it may lead to the loss of some adaptive traits of chamomiles species (Baczek-Kwinta et al., 2009), hence the tolerance accession could be used in the breeding programs. On the other hand, means orthogonal comparison showed that the studied species have potential in different traits. For example *Matricaria chamomilla*, *Tripleurospermum sevance* and *Anthemis alltissima* showed the high flower number per plan, plant dry weight and flower dry weight per plant respectively. Combinations of a series of the favourable traits by crossing of the species will enable the pyramiding of traits and to detect better genotypes. In this way crossability of the species should be considered. Fitted equations between control and stress conditions indicated the possibility of assessment of the traits in stress conditions based on control data in the chamomile species. However for the economic traits such as flower yield, separate evaluation of accessions under each condition is necessary.

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