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Evaluation of 13 Calendula (*Calendula officinalis*) Cultivars Response to Drought Stress

Maryam Zarghami Moghadam[1], Mahmoud Shoor[1*], Hossein Nemati[1] and Ahmad Nezami[2]

[1] Department of Horticultural Science and Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Agronomy Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

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Corresponding author's email: shoor@um.ac.ir

Abstract

Private gardens and public landscapes in urban areas have always been formed by bedding plants; therefore, managing the negative impacts of drought on landscape is deemed vital. Urban areas in arid expanses of land are dealing with the abovementioned issue through landscaping systems that consume low amounts of water; nevertheless, data concerning tolerance against regulated deficit irrigation and the subsequent reactions of many plants, particularly ornamental species such as herbaceous perennials and annuals, are next to nonexistent. An annual specie, Calendula officinalis L. (Asteraceae), is used for its medicinal properties all around the world, it also goes by the name calendula or marigold. This research was performed to find out and estimate the reaction caused by drought stress mainly at two different levels (100 and 50% FC) on 13 pots of marigold cultivars (Candyman Orange, Fiesta Gitana, Citrus Cocktail, Neon, Candyman Yellow, Fruit Twist, Sherbet Fizz, Oopsy Daisy, Pot Marigold, Greenheart Orange, Calendula Porcupine, Pink Surprise and Touch of the Red) with four replicates carried out in greenhouse environments. The morphological and biochemical responses/changes in characteristics of the plants in question were measured and analyzed at the final stage of the observation. Water stress (50% FC) lessened most of the assessed characteristics and raised proline content ($P<0.01$). Traits attributed to flowers such as diameter and flower that were also studied decreased while the expected index maintained unchanged. Ultimately, Neon and Candyman, two quality designated cultivars, were shown to resist the conditions the most. On the whole, it was evident that the chief factor that had the impact on growth and the subsequent flowering of annual bedding plants was water supply; moreover, during drought, the quantity and quality of the species fell dramatically. Examining resistance response and the relevant characteristics attributed to the specie under investigation will contribute to the implementation of sustainable landscape systems which require less input.

Keywords: Bedding plants, Future insights, Low input landscape, Ornamental annuals, Water scarcity.

INTRODUCTION

Some annual plants are mainly used the world over for their medical properties and ornamental uses; one kind of such plants is called *Calendula officinalis* L. (Asteraceae), which is identified as calendula or marigold. Also known as "pot marigold" in English, the specie has origins that could be traced back to West Asia and the Mediterranean region. Moreover, East Mediterranean region and Southern parts of Europe have also been mentioned as the original location in which this plant had been found (Omid Beygi, 2005; Borm and van Dijk, 1994).

Being ornamental, these plants are typically exposed to numerous strains resulting from the environment in which they are planted. Finally, influencing their visual traits negatively, these stresses affect the plants in several ways including their development and growth (Seki *et al*., 2003; Farooq *et al*., 2009, 2011). One environmental stress that damages the visual quality of the plants in the ornamental sense of the word most profoundly is drought (Lambers *et al*., 2008). So as to deal with the challenges mentioned earlier, it is crucial to fully study drought and its impacts on the ways through which these plants experience adaptations physiologically and morphologically (Yamaguchi-Shinozaki and Shinozaki, 2006).

Additionally, low-water consumption in landscaping is becoming more and more prevalent in dry and drought-stricken regions; nonetheless, there is not enough information about these plants' tolerance in the face of planned water stress and the resulting reaction shown by ornamental plants, in particular herbaceous perennials and annuals that are commonly planted in Iran. The primary causes leading to complications in the above-mentioned scenarios are as follows: overpopulation, sprawling of the cities, water quality and quantity limits, and excessive urbanization (Zekavati *et al*., 2018; Vahdati *et al*., 2017). Green irrigation water accounts for a huge bulk of the water consumed in semi-arid regions. In some areas, urban greenery consumes up to30-70% of the whole sum of water (Kjelgren *et al*., 2000). Consequently, water is increasingly turning into a scarcity in arid and semi-arid parts of Iran. These regions are classified as semi-arid/arid by a number of factors including poor annual rainfall, simmering summer months, and high exposure to solar radiation. The seasonal droughts that have recently occurred in Iran have stirred the state and local officials into action and this led to an enacting of rules and regulations concerning water conservation. On the other hand, population and urbanization boom have shown to be grave threats to sustainable natural resources.

Presently, groundwater resources are being exhausted at staggering rates and, being non-renewable, this shortage of water is deemed an alarming factor that leads to a critical reduction in agricultural yields and produce universally. This issue becomes specifically vital in semi-arid/arid regions (Kumar *et al*., 2012).A plant's ability to reproduce and accordingly have adequate growth in the face of drought is called water stress resistance, and its capability to gradually adapt its functional and structural features in accordance with the changes in the environment so as to survive the harsh conditions is known as drought acclimation (Emam *et al*., 2010).

Practically all aspects of a plant's growth are influenced by drought; nevertheless, the extent to which a plant responses to drought stress is basically bound to its stage of growth and exposure to the conditions, in particular the duration, scale, and severity. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. Drought stress is responsible for dehydration, stomatal closure, and limited gas exchanges that are subsequently followed by the inhibition of metabolism and photosynthetic rate in plants; this deteriorating process finally leads to plant death (Dunford and Vazquez, 2005). However, plant species, growth stage, duration, and intensity of water deficit are the variables that affect the durability of the plant and its ability to survive in tense environments (Dunford and Vazquez, 2005). Water stress induces different plant structural transformations, which are critical to react to drought stress; these changes range from morphological adaptations (decline in growth rate, deep rooting system, and modification of root to shoot ratio for desiccation avoidance) to physiological and metabolic reactions (stomatal closure, antioxidant accumulation, and expression of stress specific genes).

In urban planning and garden landscaping, bedding plants are an indispensable element; nevertheless, they are not irrigated adequately at all times and often undergo drought stress. Additionally, plants go through numerous biochemical and physiological alterations under water deficit conditions (Pattangual and Madore, 1999; Kidokoro *et al*., 2009). To this end, the effects of water regime on the growth, content of essential oil, and proline of *Calendula officinalis* L. plants were investigated. Water regimes augmented certain growth traits, that is, plant height, leaf area, flower diameter, and spike stem diameter by 75% FC (Metwally *et al*., 2013). Khalid (2006) reported that fresh and dry weights of *Ocimum* sp. were significantly decreased by water deficit. Also, Baher *et al*. (2002) claimed that water deficit reduced the fresh and dry weights of *Satureja hortensis* L. plants.

Aside from the effects on plants growth and overall appearance, planned methods for water management such as deficit irrigation contribute to the preservation of water resources without a considerable impact on the yield. In addition, this approach does not result in harsh damages to cultivated area. In this method, compared to standard irrigation, the consumption of water decreases in various levels (English, 1990).

In spite of the fact that stress management plays a pivotal role in modern landscaping, the knowledge about bedding annual ornaments' resistance against drought is not comprehensive enough, in particular when it comes to their use for landscaping purposes. This paper aims at evaluating drought stress reactions of 13 *Calendula officinalis* cultivars within greenhouse conditions for future urban greenery strategies.

MATERIALS AND METHODS

Experimental design and planning

This study was carried out in Ferdowsi University of Mashhad's research greenhouse at the faculty of agriculture in 2016-2017.The maximum and minimum temperatures ranged from 18-20 to 37-40 ºC, with 25-55% RH, and 9-10 hours of exposure to sunlight. In order to evaluate drought stress (100 and 50% FC) responses of 13 *Calendula officinalis* cultivars, namely Candyman Orange, Fiesta Gitana, Citrus Cocktail, Neon, Candyman Yellow, Fruit Twist, Sherbet Fizz, Oopsy Daisy, Pot Marigold, Greenheart Orange, Calendula Porcupine, Pink Surprise and Touch of the Red, a factorial experiment, based on a completely randomized design, was planned with four replicates under greenhouse conditions. The seed of the aforementioned cultivars were directly sowed into pots as high as 30 cm with the bed mixture of common horticulture soil and sand 50:50 w/w% after disinfection. Stress treatments were started at 4-6 leaf stage and by weight %FC method. In this method, first the soil is weighed and then it is watered fully. After the gravity runs off, the mixture (soil and pot) is at field capacity stage. At the end, the soil and pot are dried in an oven. It has to be taken into consideration that the difference between field capacity and dry stage is field capacity water need. Finally, the following traits were measured and evaluated at the end of the trial:

Chlorophyll content

Chlorophyll content was determined using Dere *et al*. (1998). 200 mg of fresh leaves was homogenized and extracted with 10 ml of methanol 99% (v/v %). After this process, absorbance was read at 666 and 653 nm and chlorophyll a and b were calculated using the equations below:

$$\text{Chl. a} = 15.65\ A_{666} - 7.340\ A_{653}$$
$$\text{Chl. b} = 27.05\ A_{653} - 11.21\ A_{666}$$
$$\text{Chl. t} = \text{Chl. a} + \text{Chl. b}$$

Leaf relative water content

The measurement of leaf relative water content (LRWC) was done via a method developed by Schonfeld *et al.* (1988). It starts by the removal of leaves from the stem. Secondly, the leaves are weighed in order to evaluate their fresh mass (FM) at the harvest stage. So as to pin point the turgid mass (TM), leaves are left floating in distilled water within an enclosed petri dish for about six hours. Afterwards, tissue paper is used to remove water from the surface of the leaf samples to be weighed. Next, they are placed in an oven at 80℃ for 48 hours to gain dry mass (DM). An analytical scale is used to measure mass, with a precision of 0.0001 g. Finally, values of FM, TM, and DM are applied to determine LRWC implementing the equation that follows:

$$LRWC\ (\%) = [(FM–DM)/(TM–DM)] \times 100$$

Leaf area

Leaf area was determined using a Li-3100 area meter (LI, Lincoln, Nebraska, USA).

Also, all flower characteristics, morphological, and biochemical traits were measured at the end of the experiment.

Proline content

The proline content was estimated by the method developed by Bates *et al*. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used to evaluate the proline content. The reaction mixture consisted of two ml of acid ninhydrin and two ml of glacial acetic acid, which was boiled at 100 ºC for one hour. After termination of reaction in an ice bath, the reaction mixture was extracted with six ml of toluene, and absorbance was read at 520 nm.

Statistical analysis

Data were analyzed as factorial ANOVA using JMP4. While significant ($P<0.05$) treatment effects were determined by ANOVA, data means were separated by the LSD test.

RESULTS AND DISCUSSION

Results showed significant differences in almost all measured traits for regulated deficit irrigation treatments ($P<0.01$ and $P<0.05$).Drought stress affected the measured traits in all 13 cultivars examined in this study. Morphological and biochemical traits were measured and analyzed at the end of the experiment. It was evident that deficit irrigation (50% FC) reduced most of the evaluated traits and increased proline content ($P<0.01$). Being the main index desired, flower characteristics were also observed. As a result, selected top cultivars among all the other species were Neon and Candyman; moreover, 100 %FC irrigation treatments obviously showed better results than 50% FC which were significantly different. Relative water content and total chlorophyll content did not change considerably within stress treatments, whereas noticeable variations were observed for cultivars.

Flower number

Regulated water stress affected flower number significantly (Table 1). According to the results, the highest (2.06 and 4.56) and lowest (2.20 and 1.00) flower numbers were observed in 100% FC Sherbet Fizz cultivar, and 50% FC Pink Surprise cultivar treatments, respectively (Table 2). On the other hand, interaction effects disclosed that 100% FC cultivar seven scores the highest (4.75) and 50% FC Pink Surprise and Pink Surprise cultivars were the lowest (1.00) (Fig. 1).

Table 1. Analysis of variance of the measured traits in experiment.

S.o.V	df	MS									
		Flower no.	Flower diameter	Flower fresh weight	Flower dry weight	Leaf area	Shoot fresh weight	Shoot dry weight	Proline content	Relative water content	Chlorophyll content
Stress (S)	1	0.47^{ns}	0.95^{**}	8.52^{**}	0.15^{**}	41620^{**}	297.00^{**}	1.35^{**}	0.104^{**}	0.82^{ns}	9.92^{ns}
Cultivar(C)	12	9.08^{**}	8.79^{**}	7.07^{**}	0.23^{**}	8575^{**}	55.58^{**}	1.09^{**}	0.031^{**}	130.28^{**}	56.60^{**}
S ×C	12	1.55^{**}	0.85^{**}	1.17^{**}	0.03^{**}	5511^{**}	26.78^{**}	0.21^{*}	0.040^{**}	39.81^{ns}	14.66^{**}
Error	78	0.26	0.11	0.2	0.01	316	8.12	0.1	0.002	35.18	5.19
CV (%)		23.47	6.19	23.53	26.32	12.57	18.66	12.92	17.6	8.14	19.6

*, ** and ^{ns}: significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 2. Drought response indices of *Calendula officinalis* within 13 cultivars.

	Flower no.	Flower diameter (mm)	Flower fresh weight (g)	Flower dry weight (g)	Leaf area (mm^2)	Shoot fresh weight (g)	Shoot dry weight (g)	Proline content (µmol)
Stress								
100% FC	2.06a	5.43a	2.16a	0.42a	161.42a	16.89 a	2.51 a	0.22b
50% FC	2.20a	5.24b	1.59b	0.34b	121.41b	13.51 b	2.29b	0.28a
Cultivar								
Candyman Orange	1.56ef	6.47ab	3.91a	0.73a	188.00b	17.65abc	2.58bc	0.38a
Fiesta Gitana	1.18fg	6.75a	1.30e	0.24fg	85.31f	10.69h	2.03ef	0.22ef
Citrus Cocktail	2.50c	3.86f	0.75f	0.18g	104.56e	13.02fgh	2.20def	0.24de
Neon	1.43efg	6.58ab	3.65a	0.68a	210.68a	19.34a	3.20a	0.27cd
Candyman Yellow	1.06fg	6.25b	1.41cde	0.29ef	139.87cd	16.22b-e	2.33cde	0.22ef
Fruit Twist	2.37cd	4.86cd	0.84f	0.18g	154.50c	16.34b-e	2.18def	0.27bcd
Sherbet Fizz	4.56a	3.86f	1.59b-e	0.37cde	150.62c	15.74c-f	2.41cd	0.19ef
Oopsy Daisy	2.68c	4.73de	1.32de	0.27efg	129.18d	13.59efg	2.05ef	0.30bc
Pot Marigold	2.37cd	4.88cd	1.94b	0.39bcd	142.25cd	18.64ab	2.81b	0.33b
Greenheart Orange	1.37fg	6.55ab	1.83bc	0.48b	142.56cd	14.79def	2.47cd	0.29bcd
Calendula Porcupine	1.98de	5.17c	2.03b	0.44bc	110.18e	13.67d-g	2.20def	0.20ef
Pink Surprise	1.00g	4.97cd	1.76bcd	0.32def	139.68cd	11.74gh	1.93f	0.17f
Touch of the Red	3.68b	4.47e	2.01b	0.40bcd	141.00cd	16.49bcd	2.83b	0.19f

*In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

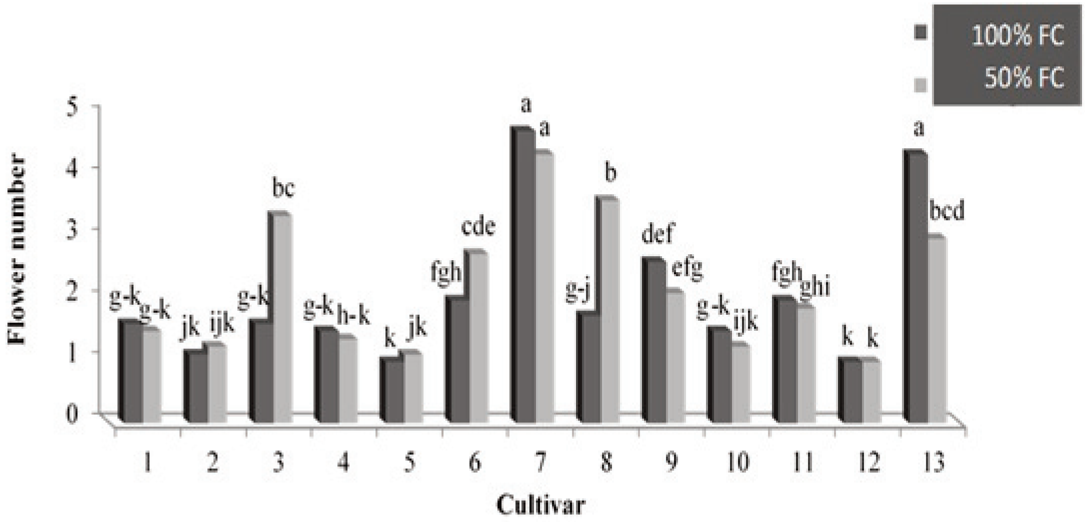

Fig. 1. Interaction effects of water stress and cultivars on flower number (P< 0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

Flower diameter

Regulated deficit irrigation stress affected flower diameter remarkably (Table 1). According to the results, highest (5.43 and 6.75mm) and lowest (5.24 and 3.86mm) flower diameters were observed in 100% FC Fiesta Gitana cultivar and 50% FC Citrus Cocktail cultivar treatments, respectively (Table 2). Interaction effects are shown in (Fig. 2). From this point of view, the combination of 100% FC and Neon cultivar presented the best results (7.2 mm) and 50% FC Citrus Cocktail cultivar demonstrated the lowest flower diameter (3.67 mm) (Fig. 2).

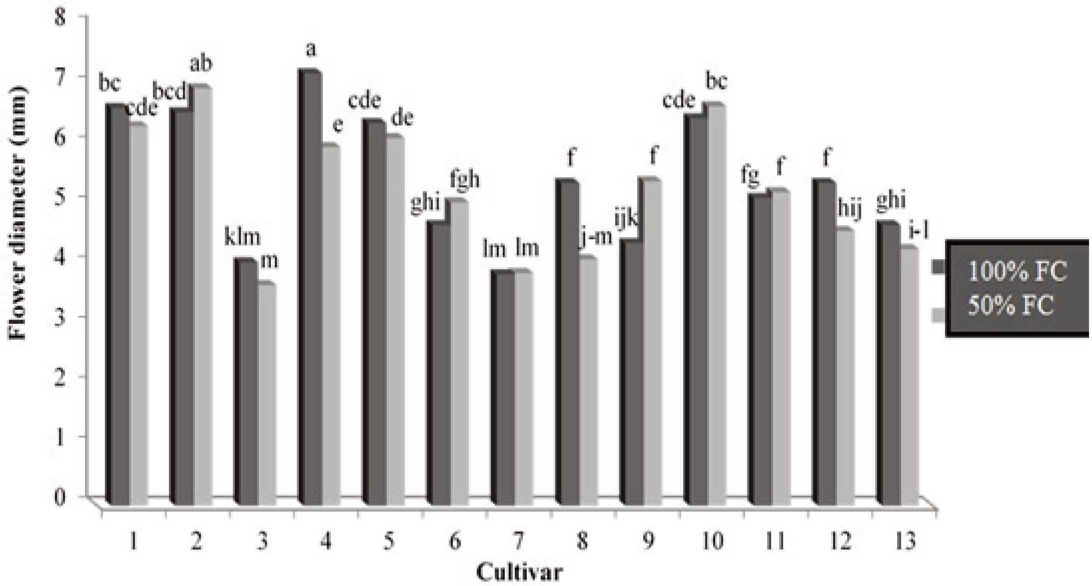

Fig. 2. Interaction effects of water stress and cultivars on flower diameter (P< 0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

Flower fresh and dry weight

Regulated deficit irrigation stress had a significant impact on flower fresh and dry weight (Table 1). According to the results, highest (2.16 and 3.91 g) and lowest (1.59 and 0.75 g) flower fresh weights were observed in 100 % FC Candyman Orange cultivar and 50% FC Citrus Cocktail cultivar treatments, in that order (Table 2). Highest interactions were shown in 100% FC Neon cultivar and the lowest in 50%FC Citrus Cocktail (Fig. 3a). On the other hand, the highest flower dry weight (0.42 and 0.73 g) was observed in 100% FC Candyman Orange cultivar and the lowest (0.34 g and 0.18 g) was marked in 50% FC Citrus Cocktail cultivar (Table 2). Highest interactions were exhibited in 100% FC Neon cultivar and the lowest in 50%FC Fruit Twist (Fig. 3 b).

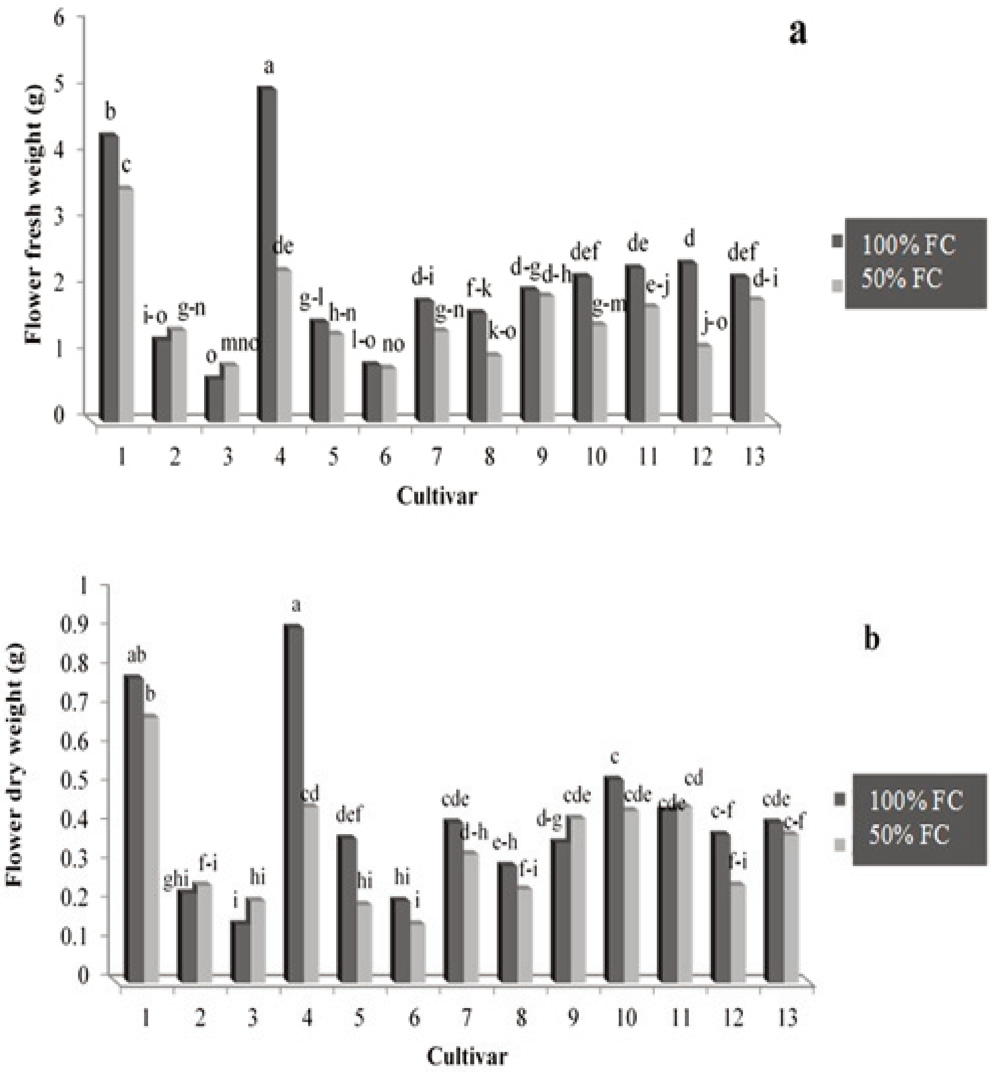

Fig. 3. Interaction effects of water stress and cultivars on flower fresh weight (a) and dry weight (b) (P<0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

Leaf area

Regulated deficit irrigation stress influenced leaf area considerably (Table 1). According to the results, the highest (161 and 210mm^2) and lowest (121and 85.3mm^2) leaf areas were observed in 100FC Neon cultivar and 50% FC Fiesta Gitana cultivar treatments, in turn (Table 2). On the other hand, regarding irrigation treatments within interactions, the highest (269mm^2) and lowest (69.75 mm^2) leaf are as were recorded in 100% FC Neon cultivar and 100% FC Fiesta Gitana cultivar, respectively (Fig. 4).

Shoot fresh and dry weight

Regulated deficit irrigation stress had a substantial impact on shoot fresh and dry weight (Table 1). According to the results, the highest (16.89 and 19.34g) and lowest (13.51 and 10.69 g) shoot fresh weights were noted in 100 % FC Neon cultivar and 50% FC Fiesta Gitana cultivar treatments, respectively (Table 2). The highest interactions (24.88 g) were shown in 100% FC Neon cultivar and the lowest (9.51 g) in 50% FC Fiesta Gitana (Fig. 5a).On the other hand, the highest shoot dry weight (2.51 and 3.20 g) was observed in 100% FC Neon cultivar and the lowest (2.29 g and 1.93 g) was marked in 50% FC Pink Surprise cultivar (Table 2). Highest interactions (3.68 g) were seen in 100% FC Neon cultivar and the lowest (1.84 g) in 50% FC Fiesta Gitana (Fig. 5 b).

Proline content

Regulated deficit irrigation stress affected proline content suggestively (Table 1).The results of the study demonstrated the highest (0.28 and 0.38µmol) and lowest (0.22 and 0.17 µmol) values to be observed in 100% FC Candyman Orange cultivar and 50% FC Pink Surprise cultivar treatments, correspondingly (Table 2). On the other hand, regarding irrigation treatments within interactions, the highest (0.58 µmol) and lowest (0.14 µmol) proline contents were recorded in 50% FC Candyman Orange cultivar and 100% FC Neon cultivar, in that order (Fig. 6).

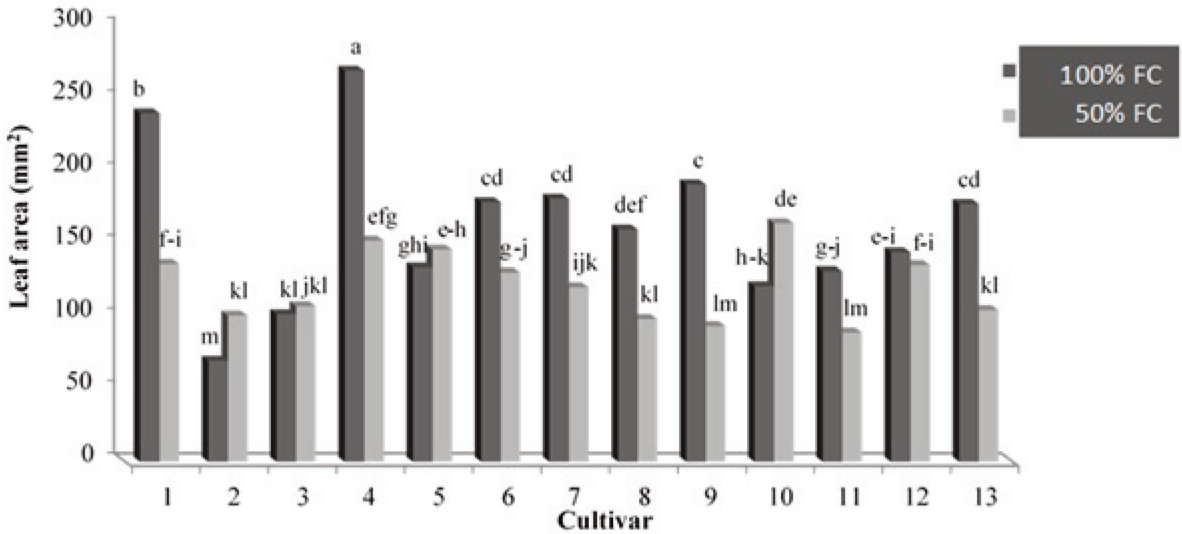

Fig. 4. Interaction effects of water stress and cultivars on leaf area (P< 0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

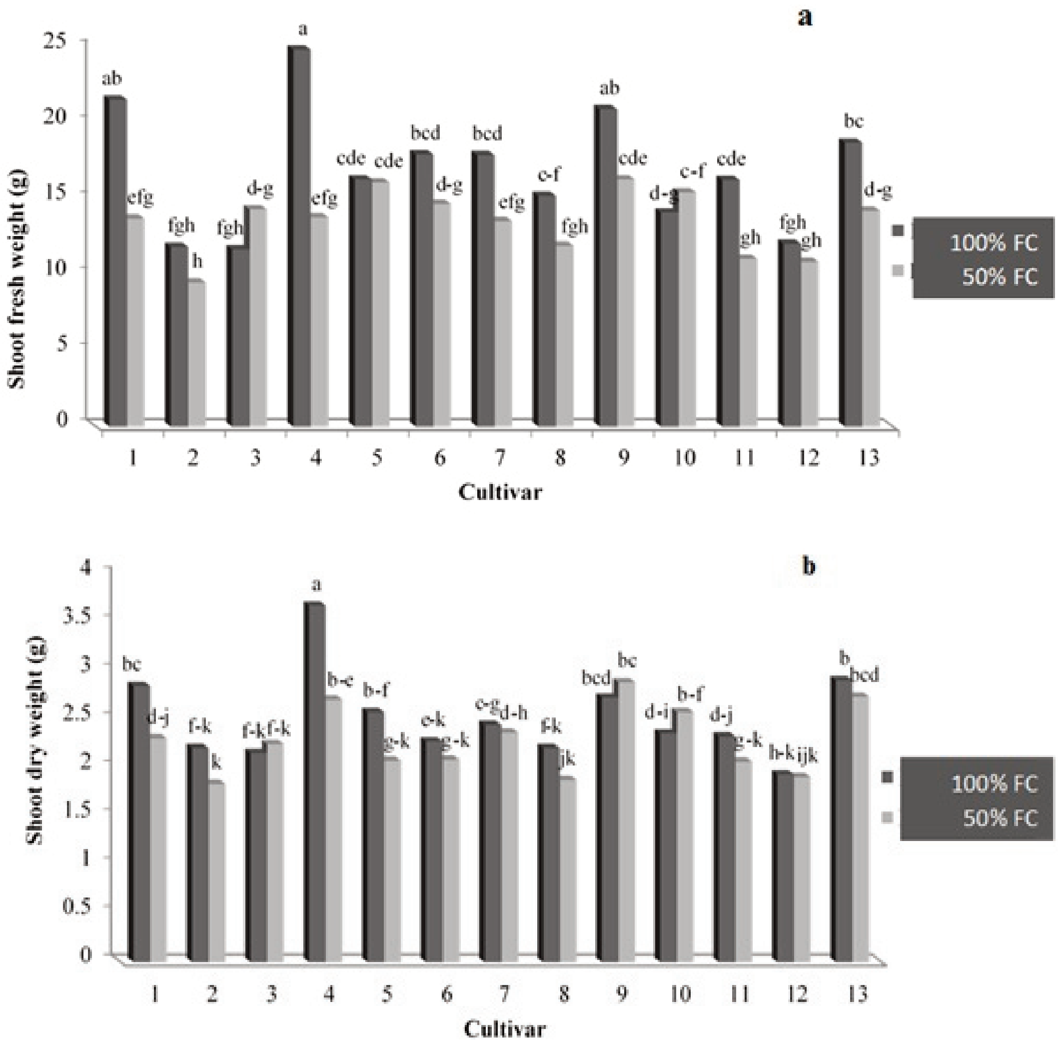

Fig. 5. Interaction effects of water stress and cultivars on shoot fresh weight (a) and dry weight (b) (P< 0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

Due to worldwide scarcity of water, trends in gardening are being modified and water saving techniques such as using alternate water sources and drought tolerant plant species, xeriscaping, and water wise and desert landscaping are gaining popularity. Annual precipitation reduction, urban population increase along with urban greenery shortage are all telltale signs of a grave issue in the near future which should be considered seriously. In recent years, water shortage has turned into an urgent question in Iran and it calls for optimized consumption of water in order to strike a balance between population demand and green space development.

Similarly, regular irrigation of urban greenery leads to an increase in public service expenditure and it does not follow the guidelines of sustainable landscaping regarding water consumption. ‘‘Water-wise urban’’ landscaping, that is, greenery that needs moderate amounts of water, is deemed vital and has to become a policy in order to sustain water resources in Iran, particularly in semi-arid/arid regions. Water-wise systems of landscaping are designed to preserve the urban

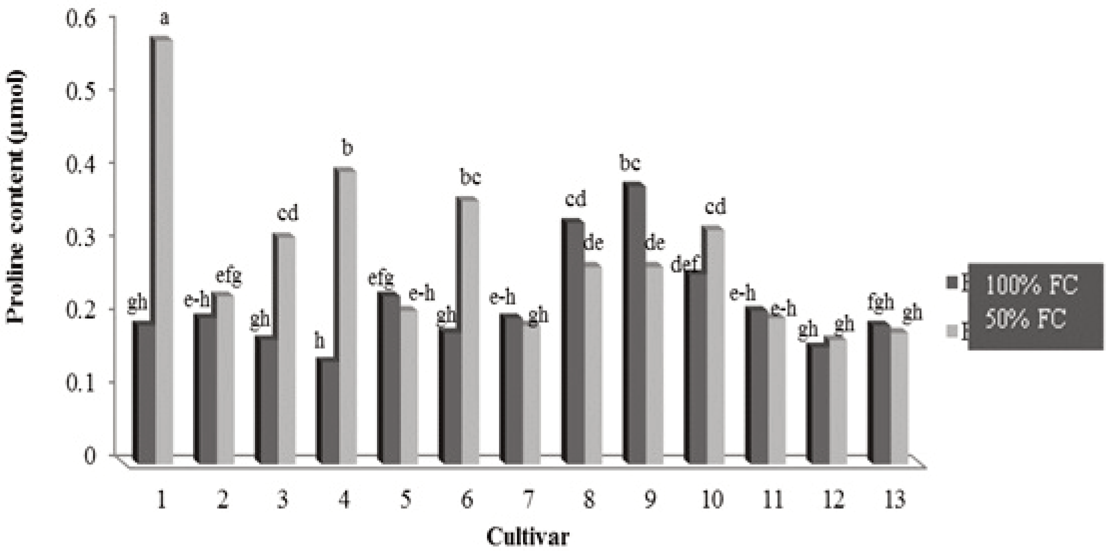

Fig. 6. Interaction effects of water stress and cultivars on proline content (P< 0.05).
(1: Candyman Orange, 2: Fiesta Gitana, 3: Citrus Cocktail, 4: Neon, 5: Candyman Yellow, 6: Fruit Twist, 7: Sherbet Fizz, 8: Oopsy Daisy, 9: Pot Marigold, 1: Greenheart Orange, 11: Calendula Porcupine, 12: Pink Surprise and 13: Touch of the Red).

appearance satisfactorily during long periods of dry weather. This goal is achieved by planting specific species of plants that are more tolerant against drought compared to turf grass. The water consumption level is suited to plant needs so as to achieve optimal conditions (Mee *et al*., 2003).

Equally important, data regarding water stress response and drought tolerance may contribute to the selection of proper types of plants for water-wise landscaping (Kjelgren *et al*., 2000). A wide variety of reactions of different species of plants in the face of drought could be observed and these responses are usually inter woven (Efeoglu *et al*., 2009) and their adaptability to the conditions in question may differ notice ably through various categories and types of landscaping plants(Sanchez-Blanco *et al*., 2004; Torrecillas *et al*., 2003). Certain species of plants in Mediterranean region displayed adaptations, both physiological and morphological, to water shortage stress (Dickson and Tomlinson, 1996); these modifications, however, ranged from the regulation of gas exchange (Moriana *et al*., 2002), osmotic adjustment (Chartzoulakis *et al*., 1999), development of leaf protective structures (i.e., hairs, thick cuticles andschlerenchymatic cells), leaf modifications (i.e., inclination variations, increased thicknesses and reduced surface areas) (Castro-Diez *et al*., 1998; Gratani and Bombelli, 2000) to more extensive root systems (Malinowski and Belesky, 2000).

Biotic and abiotic stresses adversely affect growth, metabolism and plant yields (YildizAktas *et al*., 2007), and prevent them from expressing their full genetic potentials (Zhu, 2002).Flower is oftentimes the single most important factor when it comes to plants used for ornamental purposes; accordingly, providing the plants with conditions that help them bloom for extended periods of time is of utmost importance. Basically, when these plants are exposed to stressful environments, a major reduction in flowering is expected due to modifications made by the plant for survival (Auge *et al*., 2003). Based on the findings by a number of researchers, recurrent growth in the root-shoot percentages in plants, which have been subject to water stress, is a normal phenomenon (Blum, 2005; Zwack and Graves, 1998); this alteration, nevertheless, may be attributed to adaptive strategies (Bargali and Tewari, 2004; Guo *et al*., 2006; Li *et al*., 2008) for the larger the roots become the more water the plant absorbs.

Furthermore, a wide range of crop plants has shown a fall in the Relative Water Content (RWC) when put under drought conditions (Lin and Kao, 1998; Clavel *et al*., 2005).Plants that are grown in areas that are naturally arid also displayed similar responses (Loik and Harte, 1997; Gratani and Varone, 2004); nonetheless, there is a lack of academic data shedding light on transformations that seasonal ornamental plants experience regarding changes in irrigation (Auge *et al*., 2003).

CONCLUSION

In the final analysis, based on the findings of this study, one may come to the conclusion that a certain number of morphological traits in *Calendula officinalis* experience transformations; this could be a good topic for future research. In this experiment, the two premium cultivars, namely Neon and Candyman, were ultimately selected as the most resistant to water stress. Due to herbaceous habit of annual bedding flowers, it seems that water availability and its continuous supply are two major factors affecting growth, and flowering. Additionally, the quality and quantity of the features decline in the face of drought noticeably, because these plants do not have a hard-wood structure or foliage type vision. Evaluation of these traits along with resistance response will make it possible for their use in breeding programs with low input landscapes in mind.

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