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Effect of increasing severity of drought stress on leaf physiological and morphological characters in *Calendula officinalis* L.

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ABSTRACT

To evaluate effect of water deficit stress on some physiological and morphological characters of *Calendula officinalis* L., an experiment was conducted as split plot at the Research Farm of Faculty of Agriculture Urmia University (latitude 37.53°N, 45.08°E, and 1320 m above sea level), Urmia-Iran in 2011. Treatments were irrigation (irrigation after 30, 60, 90 and 120 mm evaporation from class A pan) as main plots and gradual rise intensification of water deficit (increasing the irrigation intervals after first irrigation cycle amounted 0, 5, 10 and 15 mm evaporation) as sub plots. Data analysis of variance showed the significant interaction between irrigation and stress strength on single leaf area, leaf width, length and weight,, the number of leaves per plant, leaf area index (LAI), specific leaf area (SLA) and leaf area ratio (LAR). Means comparison indicated that the maximum single leaf area, leaf width, length, dry weight, the number of leaves per plant, LAI, SLA and LAR (38.14 cm^2, 3.32 cm, 13.24 cm, 0.22 g, 13.24, 3.26, 85.82 cm^2/g, 19.37 cm^2/g, respectively) were obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The maximum proline (0.01 mg/l) and soluble carbohydrate (0.52 mg/l) were obtained from irrigation after 120 mm evaporation as the most sever water deficit stress.

KEYWORDS

Calendula officinalis;
Irrigation;
Leaf;
Osmolyte;
Water stress.

INTRODUCTION

Marigold (*Calendula officinalis* L.) belonged to Asteracea family and native to Mediterranean region, is an annual herb with pinnately divided leaves and flowers which are used as a decorative plant in horticultural industry; Calendula grows up to 60 cm in height and produces large yellow or orange flowers. The flowers are the part of the herb used medicinally[7,22,34].

Limited water supply is also another major environmental constraint in productivity of crop and medicinal plants. Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis[9]. Drought stress is considered to be one of the most important abiotic factors limiting plant growth and yield in many areas[21]. Drought impacts include growth, yield, osmotic adjustment water relations, and photosynthetic activity[2,26]. Against this stress, plants adapt themselves by different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical processes. Adaptation to this stress is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, betaines and praline[10,11,37]. Biosynthesis of proline, a well-known osmo-protectant, is triggered by drought stress and the expression level of the gene encoding pyrroline-5-carboxylate synthetase (P5CS), a component of proline synthetic pathway, is also increased[17,36,38]. The other ability to resist drought and cope with arid environments through conserving water can be achieved either by decreasing water loss or by increasing water absorption and the morphological and physiological adaptations are the tools by which plant can achieve this task. Reducing leaf area leads to limiting water loss through transpiration rate from the plant. Leaf area may be reduced due to drought through inhibiting leaf initiation[14,20] or decreasing leaf size[15]. Reddy et al.[28] reported that low yielding genotypes showed the least reduction in leaf area per plant and total dry matter production due to moisture stress. The main aims of the present study were to find out the effect of irrigation regime on the amounts of leaf traits, proline and total soluble carbohydrate in *Calendula officinalis* leaves.

MATERIALS AND METHODS

Experimental site

To investigate the effect of irrigation intervals and increasing water deficit stress on leaf morpho-physiological characteristics of *Calendula officinalis*, a field experiment was carried out as split plot based on complete blocks design with three replications. The experiment was conducted at Research Farm of Urmia University (latitude of 37.53°N, 45.08°E and 1320 m above sea level) in 2011. Experimental units in each replication composed of 8 line of 2 m long. Inter-row and inter-plant spacing was 0.3 and 0.05 m, respectively. Water stress applied on the 4-5 leaf stage of plant growth. The field was kept weed free by hand weeding. Treatments were irrigation regimes (irrigation after 30, 60, 90 and 120 mm evaporation from class A pan) as main factor allocated to main plots and (0, 5, 10 and 15 mm evaporation from class A pan) increase to main factors as sub factor, allocated to subplots.

Measurements

Osmolytes (proline and total soluble carbohydrate)

To measure leaf proline and total soluble carbohydrate, 0.5 g of complete leaves were ground in 5 ml 95% ethanol followed by 70% ethanol. Then, upper zone of this extract centrifuged at 3500 rpm for 10 min[16] and measured by spectrophotometer at 515 nm wave lengths for praline[27] and at 625 nm wave lengths for total soluble carbohydrate[16].

Physiological characteristics of leaf

The single leaf area (in four nodal of steam) was determined by leaf area meter (Area Ueter AM 200). Leaf area index (LAI) was measured by LAI meter (model LP-80). Specific leaf area (SLA) and leaf area ratio (LAR) were calculated using the following relationships:

SLA (cm^2/g) = Total leaf area (cm^2) / leaf dry weight

LAR = total leaf area / total dry weight.

Statistical analysis

Analysis of variance (ANOVA) of data was performed using the general linear model (GLM) procedure in the SAS 9.1 software[30]. The student-Neuman Keul's test (SNK) was applied to compare treatments means using the MSTATC software package.

RESULTS

Results of analysis of variance (ANOVA) showed the significant effect of irrigation on the proline and soluble carbohydrates ($P \leq 0.01$), and significant effect of stress strength on the proline ($P \leq 0.01$). However, there was significant interaction effect between irrigation and increasing stress strength on single leaf area, leaf width, leaf length, leaf weight, the number of leaves, leaf area index (LAI), specific leaf area (SLA) and leaf area ratio (LAR) ($P \leq$ 0.01) (TABLE 1).

Means comparison indicated that the maximum single leaf area (38.14 cm^2) was obtained from plants grown under irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The minimum single leaf area (8.48 cm^2) was obtained from

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TABLE 1 : Analysis of variance for effects of irrigation and increasing severity of drought stress on physiological and morphological characteristics of Marigold (*Calendula officinalis* L.) leaves.

Source of variation	df	Mean square (Ms)				
		Single leaf area	Leaf width	Leaf length	Leaf weight	Leaf area index (LAI)
Replication	2	5.34	0.014	0.29	0.0003	0.02850625
Irrigation (A)	3	1080.27**	6.61**	114.12**	0.036**	13.98875764**
Error	6	0.73	0.0022	0.02	0.0002	0.00665347
Stress strength (B)	3	88.82**	0.118**	4.105**	0.0029**	0.50671875**
A × B	9	3.39**	0.016**	0.29**	0.0005**	0.05149282**
Error	24	0.34	0.0004	0.00998	0.00008	0.00221389
Coefficient of variance (%)	2.68	4.69	1.13	9.41	3.196738	

Source of variation	df	Mean square (Ms)				
		Specific leaf area (SLA)	leaf area ratio (LAR)	Number of leaves	Proline	Soluble carbohydrates
Replication	2	21.90216	1.138502	0.013	0.00000002	17.824827
Irrigation (A)	3	7717.80328**	343.920389**	128.83**	0.0000478**	716.182852**
Error	6	3.33876	0.167833	0.001	0.00000003	18.994494
Stress strength (B)	3	324.11655**	16.601039**	0.55**	0.0000011**	10.903591^{ns}
A × B	9	18.01115**	0.936883**	0.03**	0.00000003^{ns}	17.583434^{ns}
Error	24	1.59244	0.074064	0.001	0.00000007	20.698544
Coefficient of variance (%)		2.914646	2.686764	0.40	3.41	14.36

*** and ** Significant at $P \leq 0.05$, $P \leq 0.01$, respectively; df, degree of freedom.**

irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 1).

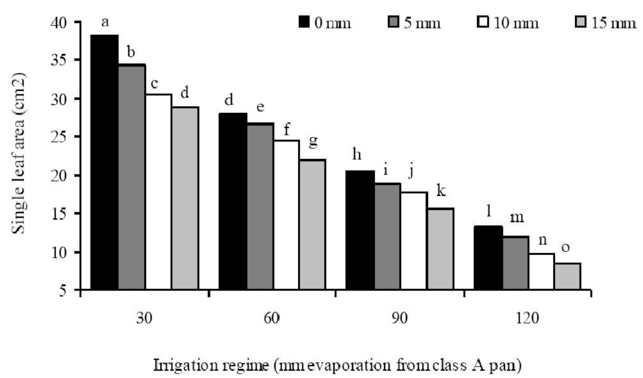

Figure 1 : Means comparison of single leaf area in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The widest leaf (3.32 cm) was observed at irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The minimum leaf width (1.39 cm) belonged to irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 2).

The longest leaf (13.24 cm) belonged to irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The shortest leaf (5.11 cm) was obtained from irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 3).

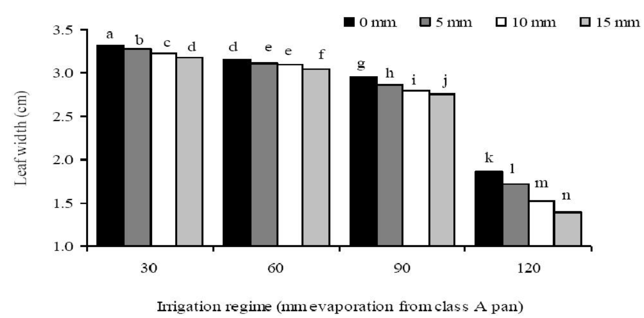

Figure 2 : Means comparison of leaf width in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

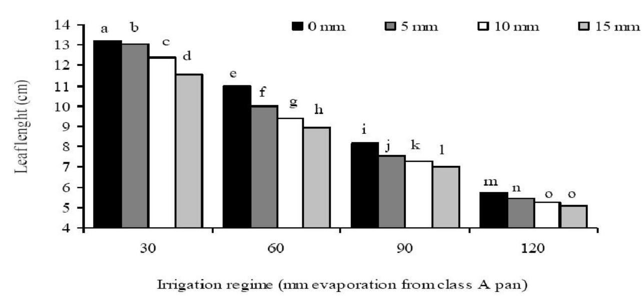

Figure 3 : Means comparison of leaf length in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The greatest single leaf dry weight (0.22 g) belonged to irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation) and the smallest single leaf dry weight (0.03 g) belonged to irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 4).

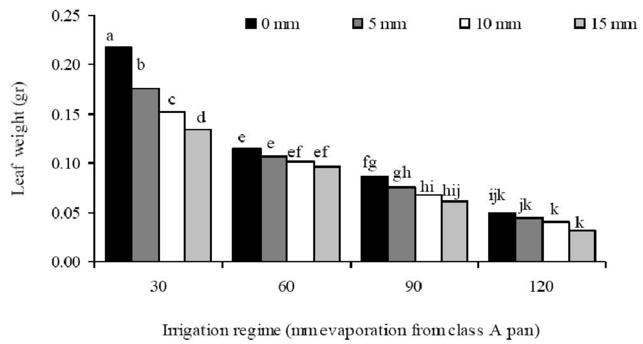

Figure 4 : Means comparison of leaf weight in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The maximum leaf area index values (3.36) belonged to irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The minimum leaf area index values (0.29) belonged to irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle, that had no significant difference with irrigation after 120 mm and 10 mm additive evaporation per each irrigation cycle (Figure 5).

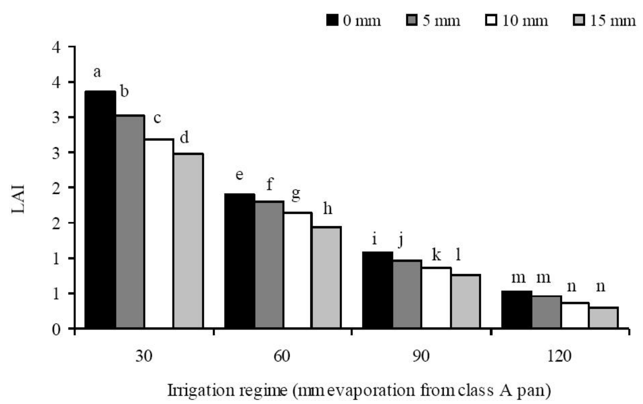

Figure 5 : Means comparison of leaf area index (LAI) in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The maximum specific leaf area (85.82cm^2/g) belonged to irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The minimum specific leaf area (13.31cm^2/g) belonged to irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle, that had no significant difference with irrigation after 120 mm and 10 mm additive evaporation per each irrigation cycle (Figure 6).

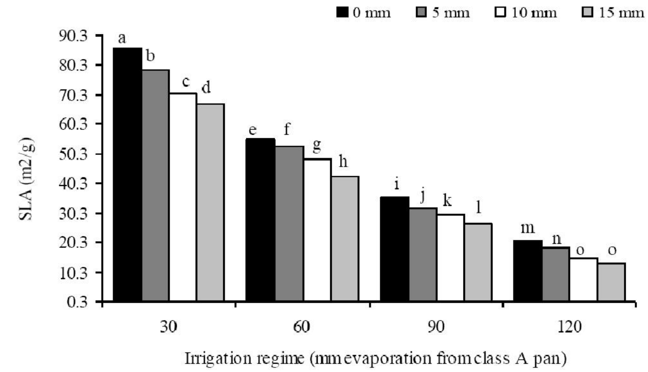

Figure 6 : Means comparison of specific leaf area (SLA) in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The maximum leaf area ratio (19.37cm^2/g) belonged to irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation). The minimum leaf area ratio (3.77cm^2/g) belonged to irrigation after 120 mm and 5 mm additive evaporation per each irrigation cycle, that had no significant difference with irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 7).

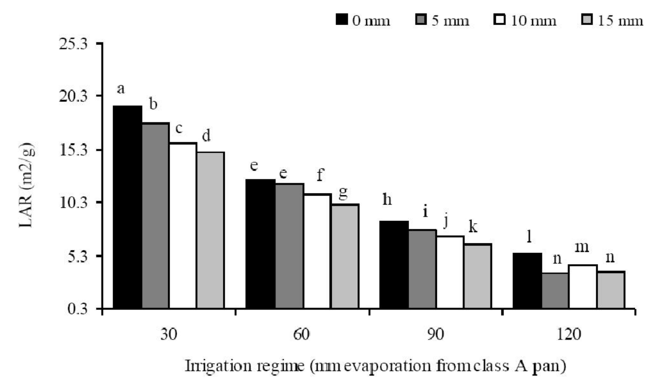

Figure 7 : Means comparison of leaf area rate (LAR) in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The maximum numbers of leaves per plant (13.2) was obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation), that had no significant difference with irrigation after 30 mm and 5 and 10 mm additive evaporation per each irrigation cycle. The minimum numbers of leaves per plant (5.19) was obtained from irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 8).

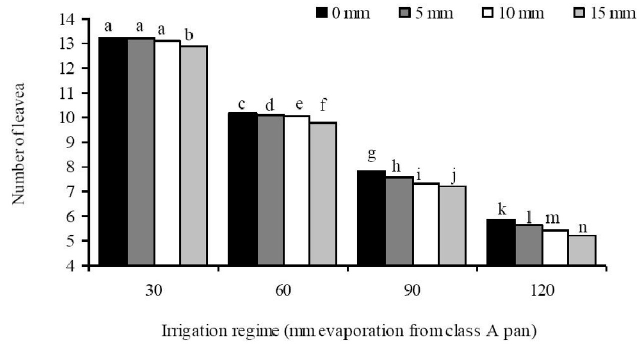

Figure 8 : Means comparison of the number of leaves in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The highest leaf proline content (0.01 mg/l) was obtained from irrigation after 120 mm and the lowest leaf proline (0.005 mg/l) was obtained from irrigation after 30 mm. The highest leaf proline content (0.008 mg/l) was occurred at plants irrigated after 15 mm additive evaporation per each irrigation cycle. The minimum value of leaf praline (0.0072 mg/l) was observed at plants of control treatment (0 mm evaporation) (Figure 9).

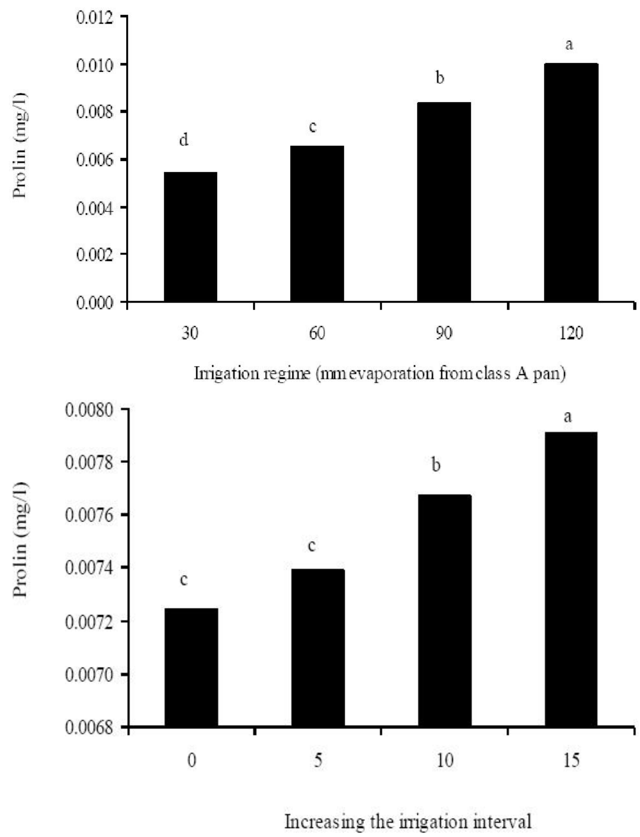

Figure 9 : Means comparison of proline in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

The maximum total soluble carbohydrate (0.52 mg/l) was obtained from irrigation after 120 mm evaporation from pan, and the minimum one (0.31 mg/l) was obtained from plants irrigated after 30 mm evaporation (Figure 10).

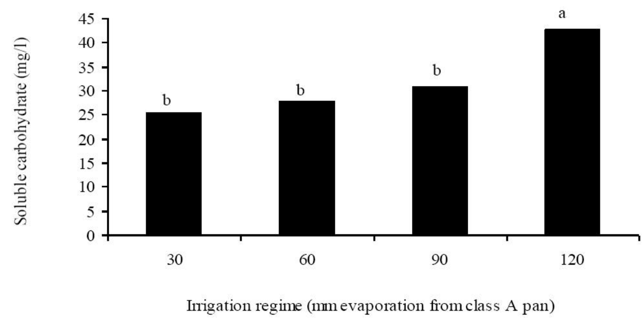

Figure 10 : Means comparison of soluble carbohydrates in *Calendula officinalis* L. under different irrigation regime. The same letters show non significant differences.

DISCUSSION

The maximum single leaf area, leaf width, leaf length, leaf weight, the number of leaves, leaf area index (LAI), specific leaf area (SLA) and leaf area ratio (LAR) were observed at plants irrigated after 30 mm and control treatment of water deficit strength (0 mm evaporation). The maximum proline and total soluble carbohydrates were observed at irrigation after 120 mm evaporation. Results indicated that the severe water deficit stress decreased single leaf area, leaf width, leaf length, leaf weight, the number of leaves, leaf area index (LAI), specific leaf area (SLA) and leaf area ratio (LAR). But water deficit stress caused to raise up amounts of leaf proline and total soluble carbohydrates. Cell growth is the most important process and is affected by water stress. Plant size is indicated by a decrease in height or smaller size of leaves when there is a decrease in the growth of cells[13]. When leaf size is smaller, the capacity to trap light decreases too and the capacity of total photosynthesis decreases, i.e. Photosynthesis is restricted in water shortage conditions, with a subsequent reduction in plant growth and performance[13]. Plant size, like area and weight of leaf, length and width of leaf, is in accordance with leaves size[24]. Leaf dry weight was increased significantly by increasing the availability of soil moisture, and water stress also reduced leaf area[8]. Tollenaar[35] found that LAI values generally range from

2 to 6 in maize under water stress conditions. Other studies suggest that a water shortage during the growing period reduces the leaf area[1,18,33]. Pandey et al.[25] reported that the highest corn LAI was obtained under well-irrigated conditions. The lowering LAR under water stress was facilitated by the reduction of total leaf area and leaf thickness. Because a decreased LAR commonly associated with a high tissue density and total non-structural carbohydrate content in leaves under drought conditions[6]. In our study the leaf thickness decrease was also accompanied by increased SLA. Small cells can withstand turgor pressure better than large cells, and can contribute to turgor maintenance more effectively under drought conditions[5,32]. Growth arrest, as would be caused by the water deficit treatments, is a possibility to preserve carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery after stress relief[12,19,23,31]. Hendawy and Khalid[12] showed that sugars and proline contents showed a pronounced increased by increasing the water stress levels of *Salvia officinalis* L. plants. These results agree with those of Slama et al.[31] and Blum and Ebercon[3], who indicated that proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues under water deficit.

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