Full Length Research Paper

Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L.

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The effect of water stress, excess water and water deficit, on the leaf relative water, amounts of proline, water soluble sugars and total chlorophyll and chlorophyll a, b were examined, to characterize the involvement of these components in the adaptive processes. An experiment was carried out with four irrigation levels (W1, W2, W3 and W4 consisting 100, 85, 70 and 55% of field capacity, respectively) arranged in randomized complete block design with five replications. Potted seedlings of *Matricaria chamomilla* were used as a test medicinal plant. Leaf relative water content, percentage of proline and total soluble sugar were not affected by irrigation regimes. But, irrigation had significant effect on amount of total chlorophyll and chlorophyll a, b. The maximum amount of chlorophyll a (19 mg/g), chlorophyll b (16 mg/g) and total chlorophyll (35 mg/g) obtained from W2 and W3. The minimum amount of chlorophyll a (15 mg/g), chlorophyll b (11 mg/g) and total chlorophyll (26 mg/g) obtained from W1, W4, W4, respectively. The values of total chlorophyll and chlorophyll a, b were the same in irrigation, at 100 and 55% of field capacity.

Key words: Drought stress, irrigation, *Matricaria chamomilla*, osmolytes, proline, soluble sugar .

INTRODUCTION

Water availability, one of the most limiting environmental factors affecting crop productivity, is a well known fact that, crop growth is frequently subjected to water stress during the course of its life time. Stress imposed during these periods drastically affects crop growth, ultimately leading to a massive loss in yield and quality (Govindarajan et al., 1996; Hudak and Patterson, 1996; Moreshet et al., 1996). Water stressing or droughting can be intensified by gradually lengthening the drought period that accumulates gradually and may persist over a long period of time, making it difficult to determine when a drought has begun or when it has ended. Water deficit is very common in the production of most crops and numerous studies have indicated that they can have substantial negative impacts on plant growth and development (Carrow, 1996; Crasta and Cox, 1996; Dean et al., 1996; Faver et al., 1996; LeCoeur and Sinclair, 1996).

There is some evidence that roots are the primary sensors of water deficit in the soil, causing the observed physiological and biochemical perturbations in the stems and the decline in growth to be generally interconnected with changes in plant nutrition, carbon dioxide balance and water relations. Plant nutrient elements and available water are absorbed by plant roots in independent processes but they are closely related to one another

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(Levitt, 1980). The reductions in uptake and transpiration are usually associated with a reduction in the water content of the shoots and stomatal aperture, suggesting that water stress has developed in the leaves (Gerakis et al., 1975). Under environmental stress conditions (drought, high and low light), plants show the ingenious adaptations at physiological level, accompanied with the change of various gene expressions. For example, 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 (Ishitani et al., 1995; Yoshiba et al., 1995; Ueda et al., 2001). Proline is accumulated in many plant species under various stress conditions (Delauney and Verma, 1993). Therefore, it is considered that proline concentration should be properly regulated, according to environmental condition (Ueda et al., 2001).

In particular, in regard to its use to monitor peroxidative damage due to water deficit conditions, we must consider that thiobarbituric acid may react with several oxidized products of amino acids, among which proline and carbohydrates are known to accumulate for osmotic adjustment (Reddy et al., 1993). Many solutes may be used in osmotic adjustment. Inorganic ions such as Na^+, K^+ and Cl^-, accounted for most of the osmotic potential in several species (Ford and Wilson, 1981) while sugars and amino acids, especially proline (Ruban et al., 1996; Montane et al., 1997) are the major osmo-regulators in vascular plants (Adamska, 1997). The reason is probably the convenience of osmolyte storage in large, osmotically inactive molecules, such as starch or protein, which may serve several functions and from which they can be retrieved under conditions of stress. It appears that neither the synthesis of new compounds nor biochemical pathways are involved during osmotic adjustment (Joshi et al., 1994).

The water stress resulted in significant decreases in chlorophyll content and the leaf relative water content. Total chlorophyll content in high water stress was reduced by 55% compared to the control (Kirnak et al., 2001).

Based on our knowledge, information about the effects of water stress on leaf relative water content (LRWC), proline, total soluble carbohydrate and chlorophyll changes in this species are scarce. The main aims of the present study were to find out the effect of irrigation regime on the amounts of LRWC, proline, total soluble carbohydrate and chlorophyll in German chamomile leaves.

MATERIALS AND METHODS

In order to study the effect of different irrigation regimes on the leaf relative water content (LRWC), total chlorophyll and chlorophyll a, b, proline and total soluble sugars as osmosis regulators, an experiment was conducted in the greenhouse conditions at Urmia University, West Azarbayjan province, Iran, from May to August,

2004. The soil physical and chemical properties were presented in (Table 1).

Seeds of *Matricaria chamomilla* L. tetraploid variety Bodegold, a chamazulene-high mixed type with a high content of bisabolol, obtained from Germany, were used in the present study. The seeds were sown in pots in spring 2004. Four levels of irrigation including 100 (W1), 85 (W2), 70 (W3) and 55% (W4) of field capacity were considered as treatments and arranged in randomized complete block design with five replications. The leaf relative water content was determined in the fully expanded topmost leaf of the main shoot. The fresh weight of the sample leaves was recorded and the leaves were immersed in distilled water in a Petri dish. After 2 h, the leaves were removed, the surface water was blotted-off and the turgid weight recorded. Samples were then dried in an oven at 70°C to constant weight. Leaf relative water content was calculated using the following formula (Turner, 1981):

$$LRWC\ (\%) = [(F.W - D.W) / (T.W - D.W)] \times 100 \qquad (1)$$

Where: F.W., Fresh weight; D.W., Dry weight; T.W., Turgid weight.

In order to measure the chlorophyll content, 0.25 g of complete leaves were ground in cool water in darkness and adjusted to volume 25 ml by distilled water. Then 0.5 ml of this solute was mixed with 4.5 ml acetone 80% and centrifuged 3000 rpm for 10 min. The upper zone of this solution was taken for spectrophotometery, at 645 and 663 nm wavelengths. To estimate total chlorophyll and chlorophyll a, b by spectrophotometery, the following equations were used (Gross, 1991; Turner, 1981):

$$Chlorophyll\ a\ (g/l) = (0.0127 \times OD663) + (0.00269 \times OD645) \qquad (2)$$

$$Chlorophyll\ b\ (g/l) = (0.0229 \times OD645) + (0.00468 \times OD663) \qquad (3)$$

$$Total\ chlorophyll\ (g/l) = (0.0202 \times OD645) + (0.0082 \times OD663) \qquad (4)$$

OD645 and OD662 present the absorption in 645 and 663 nm wavelengths, respectively. To determine the amount of leaf proline and total carbohydrate, 0.5 g of complete leaves were ground in 5 ml ethanol 95%. Its upper zone was washed with ethanol 70% twice, centrifuged at 3500 rpm for 10 min (Irigoyen et al., 1992) and measured by spectrophotometer at 515 nm wavelength for proline (Paquin and Lechasseur, 1979) and 625 nm for total soluble carbohydrate (Irigoyen et al., 1992). Statistical analysis was performed using MSTATC software. Mean comparisons were carried out using Student-Neuman Keul's test (SNK).

RESULTS AND DISCUSSION

Leaf relative water content (LRWC), proline and total soluble sugar were not affected by irrigation levels (Table 2). Irrigation has significant effect on the amount of total chlorophyll ($P < 0.01$) and chlorophyll a ($P < 0.05$), b ($P < 0.01$) (Table 2). The maximum amount of chlorophyll a (19 mg/g), chlorophyll b (16 mg/g) and total chlorophyll (35 mg/g) were obtained from W3 and W2 (irrigation at 70 and 85% of field capacity, respectively). The minimum amount of chlorophyll a (15 mg/g) obtained from W1, irrigation at 100% of field capacity whereas chlorophyll b (11 mg/g) and total chlorophyll (26 mg/g) were obtained from W4. The values of chlorophyll were the same at 100 and 55% of field capacity (Figure 1).

Chlorophyll content was reduced at high water stress

Table 1. Some physical and chemical properties of the studied soil.

Soil depth (cm)	Soil texture	Silt- clay-sand (%)	Field capacity (%)	ρ (g/cm^3)	Organic mater (%)	Organic carbon (%)	N (%)	P (mg/kg)	K (mg/kg)	EC (ds/m)	pH
0 - 30	Clay-Loam	28 - 33 - 40	22.5	1.51	2.0	0.88	0.20	21	450	0.46	7.6
30 - 60	Clay-Loam	29 - 36 - 36	22.5	1.57	1.3	0.60	0.18	6.5	336	0.63	7.6

Table 2. Analysis of variance of some water relation parameters of *M. chamomilla* L. as affected by irrigation regimes.

Source of variation	Df.	Leaf relative water content (LRWC)	Chlorophyll			Proline	Total soluble carbohydrate
			a	b	Total		
Replication	4	47.3 ^{ns}	22.8 *	13.0 *	67 **	0.121 ^{ns}	0.021 ^{ns}
Irrigation levels	3	27.1 ^{ns}	25.7 *	24.0 **	98 **	0.116 ^{ns}	0.005 ^{ns}
Error	12	24.1	4.7	3.6	12	0.054	0.014
Coefficient of variation (%)		6.05	13.00	14.25	11.68	6.53	5.20

ns, *, and ** , non-significant, significant at P < 0.05 and P < 0.01, respectively. Df. Degree of freedom.

(W1, water excess and W4, water deficit) as compared to well irrigated plants (W2 and W3). In low water stress, chlorophyll a, b and total chlorophyll contents increased while by increasing stress pressure, their values reduced to the lowest amount. Beeflink et al. (1985) reported increase in chlorophyll in onion under drought stress. The above mentioned results are in accordance with this result confirming increase in chlorophyll a, b and total, under drought stress. Our results are in agreement with the findings of Bradford and Hsiao (1982) and Chartzoulakis et al. (1993). The adverse effect of water stress on chlorophyll concentration has previously been shown for young peach trees by Steinberg et al. (1990). Kirnak et al. (2001), Dhindsa et al. (1981), Chen et al. (1991) have associated the increased electrolyte leakage to reductions in chlorophyll concentrations (due to leaf senescence) while Premachandra et al. (1992) and McDonald and Archbold (1998) have shown that reductions in water use affect electrolyte leakage.

Under our experimental condition, LRWC had no changes in range of our treatments. Despite being same, LRWC in all treatments were in high amounts, showing a high level maintenance of water in water deficit and excess water conditions. Thus, for reducing the amount of water in German chamomile, one should have a strength water deficit. The rate of RWC in plants with high resistance against drought is higher than others. In other words, plant having higher yields under drought stress should have high RWC. Under water deficit, the cell membrane is subjected to changes such as increase in penetrability and decrease in sustainability (Blokhina et al., 2003). Microscopic investigations of dehydrated cells revealed damages, including cleavage in the membrane and sedimentation of cytoplasm content (Blackman et al., 1995).

Our study showed any change of osmotic adjustments (proline and total soluble carbohydrates) in all irrigation regimes. Damaging cell membrane by water stress probably reduced osmotic adjustment ability (Blackman et al., 1995; Mayer and Boyer, 1981). Having high level maintenance of water in all treatments (water deficit and excess water), prevents accumulation of osmotic adjustments, like proline and total soluble sugars. Therefore, reduction of osmolytes in German chamomile should have the needed strength water deficit.

The accumulation of osmolyte compounds in the cells, as a result of water stress is often associated with a possible mechanism to tolerate the harmful effect of water shortage. The contribution of sugars as an osmotic solute in expanded and partly expanded sunflower leaves was studied by Jones and Turner (1980). They found that contents of sugars did not change in
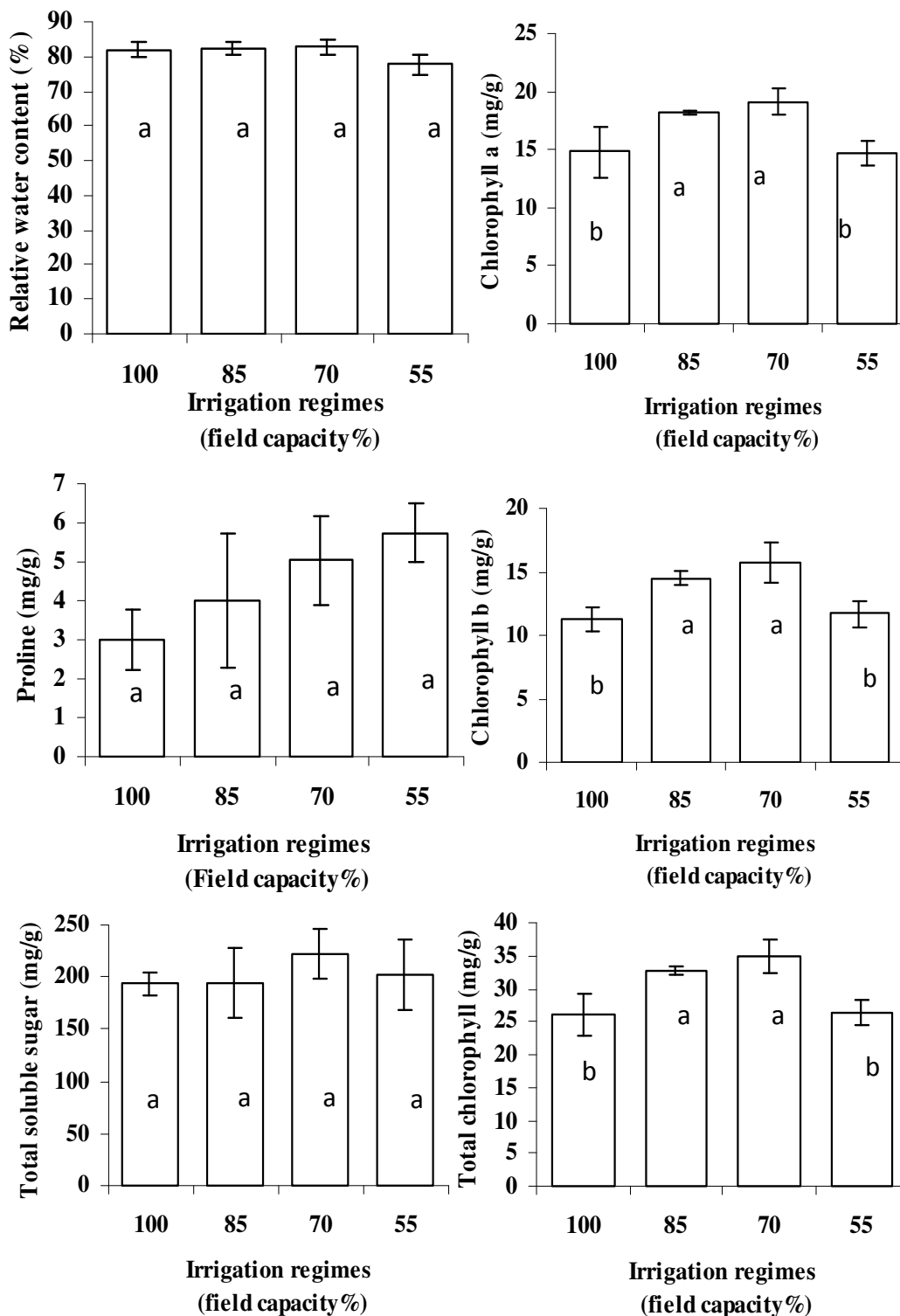
Figure 1. Comparisons of means of leaf relative water content (LRWC), proline, total soluble sugar, total chlorophyll and chlorophyll a, b at different irrigation regimes. Same letters have no significant differences.

fully expanded leaves. In addition to sugars, some plants also accumulate other low molecular mass compounds, such as proline (Gzik, 1996; Bajji et al., 2001). One of the most studied solutes is the amino acid proline and high

proline content in plants under water stress is frequently observed in several species (Clifford et al., 1998; Bajji et al., 2001) and may act as a regulatory or signaling molecule to activate multiple responses that are part of the adaptation process (Maggio et al., 2002; Claussen, 2005). In this experiment, leaves of plants had similar proline content in all levels of watering (Table 1). After rewatering, the synthesis of proline in leaves of all treatments returned to the initial content. In fact, a linear relationship could be observed between the proline content and the hydric deficit produced on the plants at different times of this process (Díaz et al., 2005). This result is easy to explain considering that proline accumulation may result from both induction of proline biosynthesis and/or inhibition of its oxidation (Hong et al., 2000). Moreover, there are previous reports that activation of pyrroline-5-carboxylate synthetase (an enzyme from proline biosynthesis) as well as inhibition of proline dehydrogenase (an enzyme from proline degradation), are both faster in fast drought than in slow drought (Sanada et al., 1995).

Water deficit can destroy the chlorophyll and prevent making it (Montagu and Woo, 1999; Nilsen and Orcutt, 1996). Mensha et al. (2006) found that subjecting sesame to drought stress caused leaf chlorophyll was increased and then remained unchanged. Overall, from the results of this experiment, it can be concluded that water stress (excess water and water deficit) significantly decreases leaf chlorophyll (chlorophyll a, b and total chlorophyll) concentrations. But there were any changes in amounts of LRWC, proline and total soluble carbohydrates at the experimental range of water stress.

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