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Changes in Kaempferol content of Chicory (*Cichorium intybus* L.) under water deficit stresses and planting densities

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To investigate the beneficial impacts of plant density on drought resistance of chicory (*Cichorium intybus* L. var. 'Qazvin'), we conducted an experiment in the field by measuring certain features essentially related to yield characters. Our objective in this study was to assess the interactive effects of various plant densities (6, 9, 12 and 15 plants/m^2) and watering limitation. In the latter, water supply was determined by applying irrigation levels, which was adjusted by regulating water evaporation from an evaporation pan (50, 100 and 150 mm water evaporation). Yield compounds as well as kaempferol content, biological yield, stem yield, leaf yield, plant height and root diameter were all significantly higher when plants were cultivated at the highest density. Non-drought stress treatments showed a significant increase in the yield of compounds, lateral stem number, root length and pod number.

Key words: Biological yield, kaempferol content, leaf yield, plant densities, stem yield, water stress.

INTRODUCTION

The aim of the present study was to investigate the fate of kaempferol in chicory (*Cichorium intybus* L.) under drought stress and planting densities. Chicory (*C. intybus* L.) is perhaps best known for its root extracts used as an ingredient in 'coffee substitute' beverages. It is less well known as grazed forage for ruminants. Chicory was first mentioned in New Zealand literature as animal forage by Cockayne (1915), but a long period then elapsed before Lancashire (1978) reported its excellent value for forage production under rotational grazing in dry summer conditions (Barry, 1996). High plant density may increase relative humidity within the canopy and also increase the duration of leaf wetness by reducing air movement and sunlight penetration (Burdon and Chilvers, 1982; Tu, 1997).

Thus, plant density could have a significant impact on plant disease incidence (Burdon and Chilvers, 1982; Copes and Scherm, 2005). Plant density of red chicory (*C. intybus* L. var. *foliosum* Hegi) was studied at a field in Linares, in south-central Chile in which 4 or 5 plants/m^2 were planted in a single or double planting line/row (Carrasco et al., 1998). The distance between rows was 0.60 m. The treatments were 60,000, 80,000, 130,000 and 170,000 plants/ha. The average total fresh weight/plant, the marketable fresh weight/plant and head size were higher at the lowest plant density. The total yield was higher at the treatment with 4 plants/m^2 and a double planting line/row. The highest marketable and export quality yield was obtained with the treatment of 4 plants/m^2 in the single planting line/row. The lowest marketable yield was observed in the highest plant density treatment. The critical plant density was 0.2 m

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with a single row. Two experiments were conducted in Southern Italy with two cultivars of chicory, 'Cicoria da foglie' (leaf chicory) and 'Cicoria di Galatina' (asparagus chicory) grown at three plant densities (11.1, 5.6 and 3.7 plants/m^2) (Bianco et al., 1994). At maturity, the aerial part of the plant was excised (or not), with the closest spacing during the second year resulting in highest seed yield, stems per plant and germination percentage. Plants *in situ* resulted in faster germination while the excised plants showed a decrease in seed yield, seed per plant, 1000-seed weight, plant height and number of stems per plant. These studies indicate that planting density has a significant effect on chicory growth and yield characteristics.

Water deficit occurs when water potential in the rhizosphere is sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development (Aliabadi et al., 2008). Drought stress is especially important in countries where crop agriculture is essentially rain-fed (Boyer, 1982; Ludlow and Muchow, 1990). Drought stress causes an increase in solute concentration in the environment, leading to an osmotic flow of water out of plant cells. This in turn causes the solute concentration inside plant cells to increase, thus lowering water potential and disrupting membranes along with essential processes like photosynthesis. These drought-stressed plants consequently exhibit poor growth and yield. In worst case scenarios, the plants completely die. Certain plants have devised mechanisms to survive under low water conditions. These mechanisms have been classified as tolerance, avoidance or escape (Kramer and Boyer, 1995; Neumann, 1995). Drought stress reduced chicory dry matter by reducing the leaf area and plant height (Labreveux et al., 2002). The results of another study showed that drought stress reduced shoot yield, essential oil yield and internode length, and increased essential oil percentage of coriander (Aliabadi et al., 2008).

MATERIALS AND METHODS

This study was conducted on an experimental field of the Islamic Azad University of Takestan branch, Iran (36°04' N, 49°42' W; 1265 m above sea level) from the 10th May to 1st October 2006, in sandy soil (Table 1). The mean annual temperature was 20°C and rainfall in the study area was 250 mm. The experimental units were designed on a factorial basis in a completely randomized block design with four replicates. The studied factors included irrigation (50, 100 and 150 mm water evaporation from an evaporation pan) and plant density (6, 9, 12 and 15 plants/m^2). The soil consisted of 21% clay, 30% silt and 49% sand (Table 1). The soil bulk density was 1.4 g/cm^3 and the field was prepared in a 15 m^2 area (5 m □ 3 m). A total of 48 plots of chicory (*C. intybus* L. var. 'Qazvin') were used in this experiment. Initially, phosphorus and potassium were added by applying 100 kg/ha ammonium phosphate and 200 kg/ha K_2O at cultivation time.

Nitrogen fertilizer was added in two stages; 75 kg/ha urea in the beginning of the stem elongation stage and 75 kg/ha urea in the beginning of the flowering stage. In order to determine the flavonoid content of leaves at the maturity, selected number of leaves and were placed at electrical oven in 75°C for 48 hrs and then were powdered by an electric mill and 151 mg of leaf powder was refluxed with 8 ml methanol acid (7: 1 (v/v) mixture of methanol and hydro*chloric* acid) for 1 h. After the solution cooled, it was filtered by passing through filter paper and was dissolved with 10 ml methanol in a Balon Joje 250 ML. To determine the graphs of materials in the extract, 20 µl of solution was injected into an HPLC at a wavelength of 370 nm with 3 replications.

Further measurements were conducted by determining the flavonoids kaempferol content using HPLC (model: KNAUER and detector: UV2600) at a wavelength of 370 nm and column (C18) under the following conditions: Column: nucleosil 100 – C18 (125*4) 5 µm; Temperature: ambient; Injection amount: 20 µm; Flow rate: 1 ml/min; Mobile phase: methanol (A), 0/5% (v/v) orthophosphoric acid aqase 0/5% (v/v) (B). The kaempferol standard graph was also determined by using 0.7 mg kaempferol (weighed by an electrical scale) and was dissolved with 10 ml methanol in a Balon Joje 250 ML. 5, 10 and 15 µl of solution were then injected into the HPLC at a wavelength of 370 nm with 3 replications. After achieving the graphs, the means of the graphs levels and standard graph depicted were determined. Then the treatment graphs were compared to standard graph and the kaempferol content were determined. To determine the biological yield, stem yield, plant height, lateral stem number, leaf yield and root diameter, 10 plants were selected randomly from each plot at maturity there data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) computer software at $P < 0.05$ (SAS Institute, Cary, USA, 1988).

RESULTS AND DISCUSSION

Changes in kaempferol content of chicory

Drought stress (water deficit stress) significantly ($P<0.05$) affected kaempferol content (Tables 2 and 3) which indicates that the highest kaempferol content was (4.5 kg/ha) under irrigation according to 50 mm water evaporation from evaporation pan. In (Tables 2 and 3), the result showed that the planting density was significantly affected by the kaempferol content in $P\leq0.01$ and the highest kaempferol content was (5.5 kg/ha) under planting density of 15 plant/m^2. (Table 2) also showed that the interactions of drought stress (water deficit stress) and planting density significantly affecting the kaempferol content in $P\leq0.01$. In (Table 4), the highest kaempferol content (8.8 kg/ha) and biological yield (20789 kg/ha) was observed under conditions of irrigation by 50 mm water evaporation and planting density by 15 plant/m^2.

Recent studies showed that there should be increase in the amount of the essential oil percentage in certain medicinal plants which were exposure to drought stress. Its compositions may appear good but because of stress, therefore more metabolites are produced in the plants and substances are prevented from oxidization in the cells (Singh-Sangwan et al., 1994). Our findings showed that kaempferol percentage was increased under drought stress, while kaempferol content decreased under these conditions. As the interactions between the kaempferol percentage and biological yield was considered almost as key factors to determine the kaempferol content in this

Table 1. Results of soil analysis.

Soil texture	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (mg/kg)	Na (Ds/m)	EC (1: 2.5)	pH	Depth of sampling (cm)
Sa	49	30	21	147.2	6.2	34.7	0.04	0.19	8.1	0-15
Sa.c.L	56	25	19	124.3	3.7	28.2	0.03	0.16	7.9	15-30

Table 2. Analysis of variance.

Sources of variation	df	Mean square									
		Kaempferol content	Biological yield	Stemyield	Leafyield	Lateral stem number	Podnumber	Plant height	Rootlength	Leafnumber	Root diameter
Replication	3	6.625	320.501	3723.154	3905.433	406.167 **	13996.41	0.102	0.0001	2424.743 *	0.079
Water deficit stress	2	225.13 **	15028.722**	12168.416 **	202871.917 **	13113.188 **	1343165.688 **	0.032	0.012 *	4414.521 **	0.766
Error a	6	1.38	577.908	961.332	2283.641	25.438	74970.66	0.039	0.001	331.493	0.246
Planting density	3	25.708 **	307.714	19457.991 **	273400.739 **	8290 **	341060.41 **	0.021	0.005 **	11088.188**	0.297
Water deficit stress × Planting density	6	18.344 **	4588.082 **	158.784	44311.203 **	343.438 **	28559.576	0.036	0.0001 *	141.188	0.067
Error b	27	1.304	621.586	538.316	3977.761	40.995	46365.873	0.031	0.0001	266.984	0.111
CV (%)		5.58	16.37	15.1	16.02	7.13	19.8	18.48	4.95	10.13	17.2

* and ** : Significant at 5% and 1% levels.

Table 3. Means comparison of main treatments.

Treatments		Kaempferol content (kg/ha)	Biological yield (kg/ha)	Stem yield (kg/ha)	Leaf yield (kg/ha)	Lateral stem number (stem/plant)	Pod number (pod/plant)	Plant height (cm)	Root length (cm)	Leaf number (leaf/plant)	Root diameter (cm)
Water deficit stress	50 mm evaporation	4.5 a	19708 a	1841 a	5226 a	10.7 a	135 a	119.2 a	23 a	16.8 a	2.2 a
	100 mm evaporation	2.5 b	14133 b	1446 b	3431 b	9.4 b	92.1 b	100.3 b	20 b	15.6 b	1.8 b
	150 mm evaporation	2.2 b	14123 b	1304 c	3151 b	5.5 c	83.6 c	95.3 b	19 b	13.7 c	1.8 b
Planting density	6 plant/m^2	1.3 c	9480 d	1060 d	2407 d	10.9 a	171.6 a	92.3 c	25 a	22 a	1.8 b
	9 plant/m^2	1.6 c	13824 c	1375 c	3194 c	8.2 b	107 b	102.4 bc	23 a	16 b	1.8 b
	12 plant/m^2	3.8 b	18192 b	1733 b	4246 b	8 b	85 c	108.7 ab	19 b	13.7 b	1.9 ab
Water deficit stress	15 plant/m^2	5.5 a	21870 a	1978 a	5898 a	8.2 b	89.1 c	116.3 a	17 b	13.5 b	2.2 a

Means within the same column and rows and factors, followed by the same letter are not significantly difference (P<0.05).

Table 4. Means comparison of interaction.

Treatments		Kaempferol content (kg/ha)	Biological yield (kg/ha)	Stem yield (kg/ha)	Leaf yield (kg/ha)	Lateral stem number (stem/plant)	Pod number (pod/plant)	Plant height (cm)	Root length (cm)	Leaf number (leaf/plant)	Root diameter (cm)
50 mm evaporation	6 plant/m^2	2.2 cde	14594 c	2254 a	8733 a	26.1 a	217 a	148.7 a	26 a	37.4 a	2.4 a
	9 plant/m^2	3.3 bcd	16766 bc	1744 bc	4390 cd	11.5 bcd	144 bc	114.3 b	24 abc	20 c	2.2 ab
	12 plant/m^2	3.8 bc	18950 b	1480 cde	3563 cdef	6.7 efg	112.4 c	109 bc	21 bcde	12.7 def	1.9 ab
	15 plant/m^2	8.8 a	20789 a	1195 ef	2658 fg	4.5 hg	115 c	92.3 cd	17 def	8.8 fg	1.7 b
100 mm evaporation	6 plant/m^2	1.6 def	11807 d	2128 a	5514 b	23.1 ab	152.1 b	116.3 b	26 ab	33.1 b	2.3 ab
	9 plant/m^2	5.5 ef	13979 cd	1636 bcd	3739 cde	10 cde	101.3 cd	111 bc	24 abc	19.1 c	2 ab
	12 plant/m^2	4.1 b	16163 bc	1347 def	3774 def	6.4 efg	67.4 e	102 bcd	19 cdef	12 efg	1.9 ab
	15 plant/m^2	3.6 bc	18002 b	1037 fg	1935 g	3.3 hi	82.3 d	83.8 d	17 ef	8.6 g	1.6 b
150 mm evaporation	6 plant/m^2	2.2 f	11802 d	1936 ab	4571 c	19.8 abc	145.7 bc	114.5 b	26 ab	30.7 b	2.2 ab
	9 plant/m^2	1 ef	13974 cd	1590 bcd	3662 cdef	9.4 def	75.9 d	109 bc	22 abcd	17.6 cde	1.9 ab
	12 plant/m^2	3.4 bc	16158 bc	1295 def	3184 ef	6.1 fg	75.3 d	97.5 bcd	18 def	11.8 efg	1.8 b
	15 plant/m^2	4.2 b	17952 b	795.5 g	1811 g	2.5 i	70 e	38.2 e	15 f	8 g	1.6 b

Means within the same column and rows and factors, followed by the same letter are not significantly difference ($P<0.05$).

study, biological yield reduces under drought stress solely and great reduction in the kaempferol content, while it increased the kaempferol percentage as its forms.

Also, the highest biological yield was achieved under optimal plant density because photosynthesis increases by development of leaf area and increased kaempferol content. Consequently, plants under non-drought stress have higher kaempferol content in leafs than that in plants under drought stress conditions. Scalabrelli et al. (2007) evaluated the effect of water stress on changes in leaf flavonoid of two grapevine genotypes. The flavonoid to hydroxycinnamate ratio markedly increased passing from well-watered to drought-stressed plants, while the quercetin to kaempferol ratio slightly increased because of drought stress. Rahmani et al. (2008) showed that drought stress reduced flavonoid content of calendula solely but also increased the flavonoid percentage. The drought stress decreased essential oil content and also increased the essential oil percentage of coriander (Aliabadi et al., 2008), Mexican marigold (Mohamed et al., 2002), yarrow (Sharifi et al., 2005) and lemongrasses (Singh-Sangwan et al., 1994).

Also, The planting densities treatments in study of Morteza et al. (2009) on valerian were 40000, 80000 and 120000 plants/ha. Their results showed that planting density had significant effect on essential oil content and its compounds (camphen, bornyl acetate and valerenal). The highest amount of essential oil content and its compounds were obtained under 120000 plants/ha. High plant density increased essential oil content of sweet annie (Ram et al., 1997) and cumin (Hashemi et al., 2008). The results of researchers were in agreement with the observation of our results. Akbarinia (2000) evaluated the effect of drought stress on flavonoid content of ajowan.

He noticed that the drought stress treatments had no significant effect on flavonoidcontent. The essential oil content of mint is not significantly affected due to drought stress (Abbaszadeh et al., 2008).

The results of Akbarinia (2000) and Abbaszadeh et al. (2008) were in disagreement

with our findings.

Effects of water deficit stress and planting density on morphological and yield compounds

The results in (Table 2) showed that the water deficit stress significantly affects the leaf yield, stem yield, biological yield, leaf number, pod number and lateral stem number in P≤0.01 and root length in P<0.05. Root diameter and plant height were not significantly affected under the water deficit stress. In (Table 3), certain morphological features including the highest leaf yield (5226 kg/ha), stem yield (1841 kg/ha), biological yield (19708 kg/ha), root length (23 cm), leaf number (16.8 leaf/plant), pod number (135 pod/plant), lateral stem number (10.7 stem/plant), root diameter (2.2 cm) and plant height (119.2 cm) were achieved under irrigation by 50 mm water evaporation from evaporation pan. In (Table 2), the result also showed that planting density significantly affected leaf yield, stem yield, root length, leaf number, pod number and lateral stem number in P≤0.01.

Root diameter, biological yield and plant height were not significantly affected in response to planting density. Highest leaf yield (5898 kg/ha), stem yield (1978 kg/ha), biological yield (21870 kg/ha), root diameter (2.2 cm) and plant height (116.3 cm) were achieved under planting density by 15 plant/m^2. Whilst the highest root length (25 cm), leaf number (22 leaf/plant), pod number (171.6 pod/plant) and lateral stem number (10.9 stem/plant) were achieved under planting density by 6 plant/m^2 (Table 3). Interaction of water deficit stress and planting density significantly affected leaf yield, biological yield and lateral stem number in P≤0.01 and root length at P<0.05 in (Table 2). Whilst the highest biological yield (20789 kg/ha) was achieved under irrigation by 50 mm water evaporation and planting density of 15 plant/m^2 and highest lateral stem number (26.1 stem/plant), leaf yield (8733 kg/ha) and root length (26 cm) were achieved under irrigation by 50 mm water evaporation and planting density of 6 plant/m^2 (Table 4).

As it was shown in the results, water deficit stress had a negative effect on most of the chicory characteristics under study. In order to resist water deficit stress, the plant uses different ways for reduction of evapo-transpirational areas. In this response, a great reduction in the length and width of the leaf and corresponding reduction in the area of the leaf, plant height and lateral stem number indicate that plant use some strategies to adjust evaporational areas. Therefore, reduction in the produced dry matter is correspondent to the reduction in the plant's photosynthesis under water deficit stress. It is well known that under water deficit stress, stomata's become blocked or half-blocked and this leads to a decrease in the absorption of Co_2 and on the other hand, the plants consume a lot of energy to absorb water, these causes a reduction in producing photosynthetic matters. It was also seen that as the water deficit stress increases, it causes the plant height, root diameter and stem yield to decrease. Shoot reduction could be due to the reduction in the area of photosynthesis, causing a drop in the production level of chlorophyll and the rise of the energy consumed by the plant in order to take in water and to increase the density of the protoplasm and to change the respiratory paths and the activation of the path of phosphate pentose, or the reduction of the root deploy, etc. This fact indicates that exerting water deficit stress on the flowering shoot yield decreases the chicory which causes a reduction in kaempfelor content.

These results showed that highest leaf, pod and lateral stem numbers were achieved under planting density of 6 plant/m^2 because the plant increased its shoot for the increase of assimilation matters by increase of refulgence absorb for compensation of low density in this condition. Therefore were increased leaf and lateral stem numbers under planting density by 6 plant/m^2. Also pod number was increased under low density because flower reproductive cells increased in this condition by low rivalry between plants. The plant increases its root diameter for increase of water absorption under high density by high rivalry between plants, but root length increases under low density because the assimilation matters are enough for increase of root length. The increase of planting density causes increase in plant height and this debilitates stems by decrease of stem diameter. Abbaszadeh et al. (2009) evaluated the effect of water deficit stress on morphological characteristics of balm. Their results showed that drought stress had a negative effect on most of the morphological characteristics. A big reduction in the biological and essential oil yields, length and width of the leaf and following reduction in the area of the leaf, plant height and tiller number were the causes of drought stress. The drought stress decreased biological yield, plant height and tiller number of salvia (Bettaieb et al., 2009), safflower (Ludlow, 1986) and sorghum (Younis et al., 2000). Also, the study results of Bianco et al. (1994) showed that high plant density increased seed yield, seed per plant, 1000 seed weight, plant height and number of stems per plant of chicory. The findings of the experiments were similar to the data of our study. Also, the plant density of red chicory -"radicchio rosso"- (*C. intybus* L. var. *foliosum* Hegi) was studied by Carrasco et al. (1998).

The treatments were 60000, 80000, 130000 and 170000 plants/ha. The average total fresh weight/plant, the marketable fresh weight/plant and head size were higher at the lower plant density. The total yield was higher at the treatment with 4 plant/m and a double planting line/row. The highest marketable and export quality yield was obtained with the treatment by 4 plants/m. The lowest marketable yield was observed in the highest plant density treatment. The experimental data that was designed to compare the effect of soil water availability

conditions on chicory (*C. intybus* L.), showed that dry matter yield of chicory was not affected by the water treatments applied (Labreveux et al., 2002). The results of Carrasco et al. (1998) and Labreveux et al. (2002) were in disagreement with our findings.

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