

Probing the responses of four chicory ecotypes by ethylene accumulation and growth characteristics under drought stress

Hossein Sadeghi, Kimiya Ghanaatiyan

Department of Natural Resources and Environmental Engineering, College of Agriculture, Shiraz University, Shiraz, Iran

Abstract

Water deficit is the largest limiting abiotic factor in agriculture and will increase in future. Evaluating the drought stress-induced changes in growth parameters as well as the leaves ethylene accumulation of medicinal plants to grow these in arid and semi-arid areas has particular importance. Chicory (*Cichorium intybus* L.) is a famous medicinal herbal plant which grows in most parts of Iran. A factorial greenhouse experiment was conducted to evaluate the effect of drought stress [100 (as a control), 80, 60 and 40% of field capacity (FC)] on morphophysiological parameters as well as the leaves ethylene accumulation of four chicory ecotypes. The results showed a significant effect of drought on plant height, leaf area, shoot moisture content and total dry matter production of chicory ecotypes which were reduced under drought stress. Under increasing drought level the Siyah Shiraz (Kh) ecotype performed better by maintaining more growth characters, thereby leading to more production of dry matter than the other ecotypes. Isfahan ecotype was the most affected by rising tensions and showed more reduction in growth traits. Drought stress also considerably changed leaf ethylene content, that made the leaf ethylene biosynthesis to be significantly higher under severe (60 and 40% FC) stress when compared to control (100% FC) and was significantly higher in drought-tolerant chicory ecotype (Kh). In general, it can be concluded that Kh was superior to other ecotypes in terms of growth and leaves ethylene accumulation, and can be suitable for cultivation in arid regions.

Correspondence: Hossein Sadeghi, Department of Natural Resources and Environmental Engineering, College of Agriculture, Shiraz University, 71441-65186, Shiraz, Iran.
Tel/Fax: +98-713-2287159.
E-mail: sadeghih@shirazu.ac.ir; sadeghi3007@gmail.com

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Introduction

The agricultural sector is the major consumer of water in arid and semi-arid regions of Iran. Arid and semi-arid regions in Iran are characterised by low erratic rainfall between 250 and 500 mm year⁻¹, a rainy season generally is comprised from November to May with periodic droughts and a dry season from June to October. These regions have different associations of vegetative cover, generally short grasses or shrub vegetation. Therefore, these ecosystems are exposed to rapid land cover change that results in reducing productivity and moisture conditions (Rahimzadeh Bajgiran *et al.*, 2009). Arid and semi-arid regions make up about 61% of Iran land surfaces. As the rains vary highly in time, space, amount and duration, water is the most important limiting factor for biological and agricultural activities (Modarres and Rodrigues da Silva, 2007). Over the last decade, Iran has experienced its most prolonged, extensive and severe drought for over 30 years (Keshavarz *et al.*, 2013). In general, drought stress has profound effects on plant physiology, productivity and growth. Water stress can affect the growth of plant organs differently, thereby resulting in the alteration of the morphological traits of the plants, such as plant height, leaf area and dry weight (Liu *et al.*, 2013; Sapeta *et al.*, 2013). Plants have developed a wide diversity of morphophysiological drought tolerance mechanisms (Blum, 1996).

Many abiotic stress conditions, such as drought, chilling and freezing, high temperatures, flooding, chemical damage, radiation, and mechanical perturbation, stimulate high levels of the leaves ethylene. The phytohormone ethylene with specific roles in regulating tolerance and adaptation to environmental stress has long been regarded as stress hormone (Zhang *et al.*, 2006; Javid *et al.*, 2011). Ethylene is involved in ion homeostasis under stress by regulating the H⁺-ATPase gene expression (Waters *et al.*, 2007; Wang *et al.*, 2009). In several species, a significant increase in ethylene production has been reported under drought (Young *et al.*, 2004) and salt stress (Wi and Park, 2002). Thus, identifying ethylene concentration is important in determining plant stress tolerance (Cao *et al.*, 2007).

Chicory (*Cichorium intybus* L.) is a herbaceous species belonging to the Asteraceae family, known as blue sailors, endive, succory, coffee weed and Kasni (Persian) (Mosaddegh *et al.*, 2012), and is native to the Mediterranean region, mid Asia and northern Africa. In ancient times, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, vegetable crop, and occasionally for animal forage (Munoz, 2004). Also, the Greeks and Romans began to grow chicory as a vegetable crop 4000 years ago (Plmuier, 1972). Today, chicory is widely cultivated with many commercial uses in Europe, Lebanon, some Arab countries and North America (Wang and Cui, 2011). Chicory roots are one of the earliest known and most widely used raw materials for the manufacturing of coffee substitutes

(Pazola, 1987). Multiple researches describe the phytochemical composition and several health properties of chicory including anti-diabetic, wound healing and antioxidant capacities of chicory grown in various European countries (Spina *et al.*, 2008; Azay-Milhou *et al.*, 2013; Carazzone *et al.*, 2013; Morales *et al.*, 2014).

In order to improve the agricultural productivity within the water-limited areas, it is important to ensure crop yields under drought stress. Considering the key importance of chicory plantations (the flowers and leaf of the chicory plant are used as an herbal medicine in Iran), profound physiological knowledge is needed to support breeding programs, selecting superior genotypes and optimising crop management. Therefore, the present research was undertaken to evaluate the drought stress-induced changes in growth parameters as well as the leaves ethylene accumulation in four chicory ecotypes collected in southern part of Iran.

Materials and methods

Plant materials and stress treatments

In this study, four ecotypes of chicory, including Sefid Shiraz, Siyah Shiraz, Sefid Isfahan and Siyah Isfahan were used. Uniform and healthy seeds of the test plants were purchased from Pakan Bazr Co. (Isfahan, Iran) and stored at 4°C. Mature seeds of the four mentioned ecotypes were collected from four wild populations in Kazeroon (Sefid Shiraz), Kharameh (Siyah Shiraz), Ardestan (Sefid Isfahan) and Nain (Siyah Isfahan) located in the Southern Iran (Figure 1). The geographic characteristics of the collected ecotypes are presented in Table 1. After incubation (stored at 4°C for 48 hours), ten seeds were planted in 5 L pots filled with a mixture of peat moss and soil (1:2). The physico-chemical properties of the pot soil are presented in Table 2. The plants were thinned to six per pot after emergence. Pots were transferred to the greenhouse at the College of Agriculture, Shiraz University (Shiraz, Iran) [(29°43' N and 52°35' W, altitude 1.810 m above sea level (ASL)]. The greenhouse was covered with double-layer acrylic glazing, oriented north to south, equipped with a pad-and-fan cooling system, with a bench misting system for humidification, and a natural-gas-forced hot air heating system. Day and night temperatures were maintained at 28/22±2°C, respectively, with natural lighting conditions. Pots were kept weed-free by hand-hoeing and were irrigated at field capacity (FC) till 10 days after sowing.

Drought stress was imposed on plants 10 days after sowing by withholding irrigation according to the four irrigation regimes: 100 (control), 80, 60, and 40% FC for 60 days.

Growth parameters

Ten seeds of each cultivar were sown in each pot with equal distance from each others and five numbers of plants were sampled. Plant growth parameters including: plant height, leaf area, shoot moisture content, root moisture content and total plant dry weight were measured at 70 days after planting.

The leaf area of each leaf was measured using a leaf area meter (Delta-T Devices). Total plant dry weight was determined on plant material dried at 80°C for 72 h in a forced-air oven. Root moisture content and shoot moisture content were determined using the ratio of root water mass to root dry weight and the ratio of shoot water mass to shoot dry weight, respectively (Guo *et al.*, 2013).

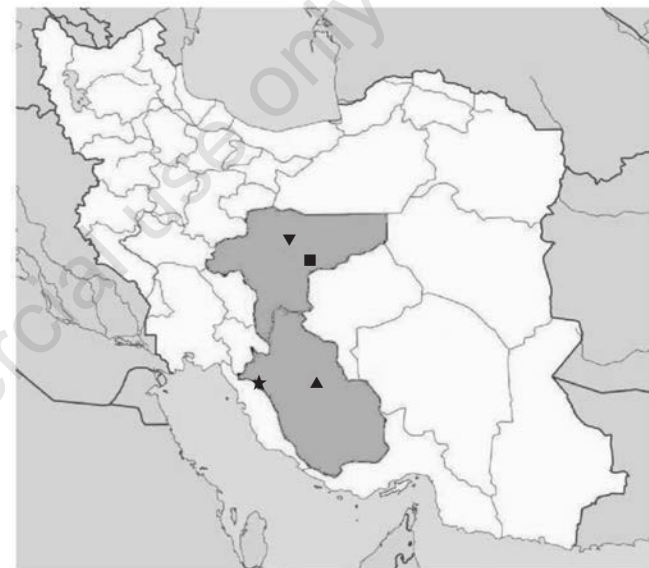


Figure 1. Locations of four *Cichorium intybus* ecotypes collected from Southern Iran. Star represents Kazeroon, triangle represents Kharameh, square represents Nain, inverted triangle represents Ardestan.

Table 1. Geographic and climatic characteristics of the locations where the *Cichorium intybus* ecotypes were collected.

Locations ecotypes	Kazeroon (Sefid Shiraz)	Kharameh (Siyah Shiraz)	Nain (Siyah Isfahan)	Ardestan (Sefid Isfahan)
Latitude (N)	29°35	29.29	32.52	33.22
Longitude (E)	51°40	53.18	53.05	52.21
Altitude (m)	950	1500	1549	1255
Geological substratum	Limestone	Limestone	Limestone	Limestone
Mean yearly temperature (°C)	23.1	18.7	16.8	18.2
Rainfall (mm.year ¹)	264	215	98	110

Table 2. Physico-chemical properties of the pot soil.

OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (dSm ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Total N (%)
0.82	7.1	7	66.7	26.3	Silty loam	0.04	16.5	476	0.08

OC, organic carbon; EC, exchangeable calcium.

Ethylene measurement

Leaf ethylene was measured from the five uppermost fully expanded leaves from each plant 70 days after planting, according to Amjad *et al.* (2014). Leaves (4-6) were detached, weighed and placed into 100 mL vessels in such a way to minimize the damage to leaves so that it would not affect the ethylene biosynthesis reading, sealed with special lids for collecting the gas samples and placed at room temperature for 48 h. To measure the ethylene concentration, about 1 mL of gas sample was collected with a syringe (Braun Melsungen AG) and samples were analysed by gas chromatography using ethylene standards of different concentrations. Final concentration of ethylene biosynthesis was presented as $\mu\text{mol g}^{-1}$ fresh weight (FW) h^{-1} (Singh *et al.*, 2010).

Statistical analysis

The experiment was carried out as a factorial arranged in completely randomized design (CRD) with four replications with standard error using SAS software (SAS Institute, 2004). The first factor was drought stress with four levels (100, 80, 60, and 40% FC). Different ecotype was the second factor, including Kn (Sefid Shiraz), Kh (Siyah Shiraz), An (Sefid Isfahan), and Nn (Siyah Isfahan). The number of plants per treatment was ten. Least significant difference (LSD) was used for mean comparison of main treatment factors and their interactions at the significant level of 5%.

Results

Plant height

Data analysis of variance (ANOVA) showed that the effect of Ecotypes and Drought and interaction (Ecotypes and Drought) had significant effect on plant height, leaf area, shoot moisture content, total dry weight and Leaf ethylene content ($P \leq 0.05$) and ($P \leq 0.01$) (Table 3). Plant height was significantly affected for all four chicory ecotypes with increased water deficit (Figure 2). The highest plant height of 35.7 cm was recorded for Kn ecotype under control (100% FC) and the lowest height of 14.6 cm for An ecotype under 40% FC treatment. There was no significant height difference between Kn and Kh ecotypes when irrigation was reduced from 100% to 80% FC; however, Kh ecotype was significant higher than Kn ecotype when water deficit was reduced from 60 to 40% FC.

Leaf area

Leaf area of the four chicory ecotypes was significantly affected by water deficit stress with the highest (104.2 cm^2) and lowest (64.2 cm^2) were obtained at 100 and 40% FC, respectively in Sefid Shiraz ecotype (Figure 3). Kn and Kh ecotypes significantly reduced their leaf areas at 80, 60 and 40% FC, with no significant difference between them. In contrary, An and Nn ecotypes reduced

their leaf areas (under water deficit irrigations) with significant differences between them.

Shoot and root moisture content

In general, root and shoot moisture contents significantly reduced with increased drought stress from 100 to 40% FC (Figure 4). The highest (70.2%) and lowest (62.86%) shoot moisture content were achieved at 100% FC in Kn ecotype and 40% FC in Nn ecotypes, respectively. In contrary, the highest (70.05%) and lowest (62.84%) root moisture content were obtained at 100% FC in Kh and 40% FC in ecotypes, respectively. The highest and lowest

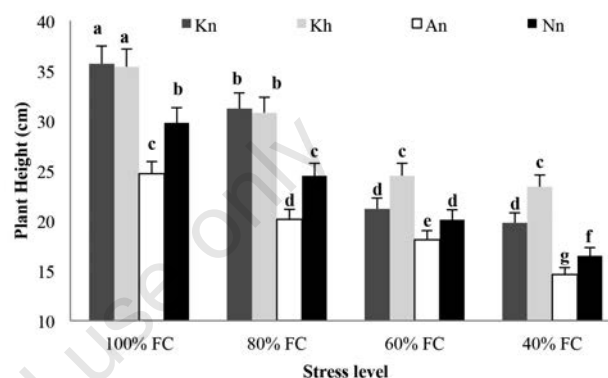


Figure 2. Variation of plant height of four chicory ecotypes under different stress levels. Means with the same letter(s) are not significantly different ($P \leq 0.05$). FC=field capacity.

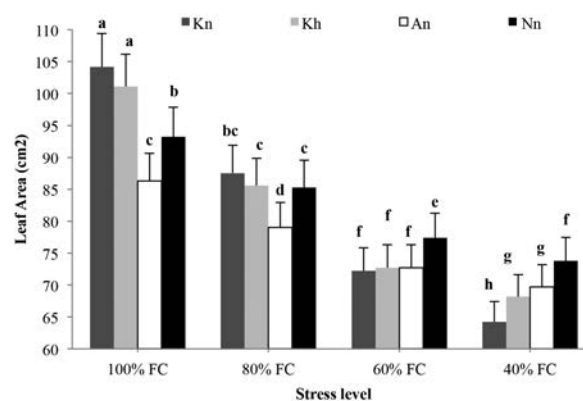


Figure 3. Variation of leaf area in four chicory ecotypes under different stress levels. Means with the same letter(s) are not significantly different ($P \leq 0.05$). FC=field capacity.

Table 3. Analysis of variance of the effect of drought on *Cichorium intybus* ecotypes traits.

Source of variance	Degree of freedom	Mean square				
		Plant height	Leaf area	Shoot moisture content	Leaf ethylene	Total dry weight
Ecotypes (E)	3	0.99*	0.01**	0.894**	0.788*	0.306*
Drought (T)	3	0.83*	0.004**	0.74**	0.822*	0.113*
E x T interaction	9	0.24**	0.02**	0.04*	0.02**	0.126*
Error	48	0.10	0.01	0.04	0.056	0.002
Coefficient variance (%)		11.8	13.6	14.5	15.46	13.42

* $P \leq 0.05$; ** $P \leq 0.01$.

reductions of shoot and root moisture contents (from 100 to 40% FC treatments) were recorded in Sefid Isfahan and Kn ecotypes, respectively (Figure 4).

Total plant dry weight

Total plant dry weight, as the most important parameter, reduced with increased drought stress levels in all chicory ecotypes. The highest (2.91 g) and lowest (1.91 g) dry matter production (per plant) were obtained at 100% FC in Kn and 40% FC in An ecotypes, respectively (Figure 5). There was a reduction in total plant dry weight at 80% FC in Kn ecotype with no significant difference with control (100%) FC; however, this difference became significant at 80 and 40% FC compared to control as well as each other. Kh ecotype reduced up to 80% FC of its total plant dry weight with no significant difference with control; however differences became significant at 60 and 40% FC (compared to control). Total plant dry weight was not significant at different drought treatments with to each other (except at 40% FC). Sefid Isfahan and Nn ecotypes reduced their total plant dry weight with a significant difference between different irrigation levels as well as control. The highest percentage of the total plant dry weight loss was recorded in Kn (27.07%) following with An (26.17%), Nn (21.8%) and Kh (15.26%) ecotypes (Figure 5).

There are highly positive and significant correlations between plant growth parameters including total dry plant weight (as the most important growth parameter) and plant height, leaf area, shoot and root moisture contents. The highest correlation coefficients were obtained between plant height and total plant dry weight (Table 4).

Leaf ethylene

Leaf ethylene synthesis significantly increased with increased drought stress levels in all four ecotypes (Figure 6). There was a significant difference between ecotypes. The highest (1.95 $\mu\text{mol g}^{-1}\text{FW h}^{-1}$) and lowest (0.53 $\mu\text{mol g}^{-1}\text{FW h}^{-1}$) ethylene production was obtained at 40% and 100% FC in Siyah Shiraz (Kh) and Sefid Shiraz (Kn) ecotypes, respectively. The basal level of ethylene synthesis as well as the slope of the ethylene production trend (from 100 to 40% FC) was noticeably higher than in Siyah Shiraz compare to Sefid Shiraz ecotype. Moreover, ethylene production was significantly higher at 60 and 40% FC than control in all ecotypes (Figure 6).

Discussion

One of the first responses to drought is stomatal closure which allows plants to limit transpiration, but it also limits CO₂ absorption, leading to decrease in photosynthetic activity (Nayyar and Gupta, 2006). Several studies have reported the reduction of plant height under water deficit, in several crops, cultivated pasture, desert and Mediterranean annual species (Bettaieb *et al.*, 2009; Ahmad Alhadi *et al.*, 1999; Khalid, 2006; Cabuslay *et al.*, 2002). Reduction of plant height under drought conditions is mainly due

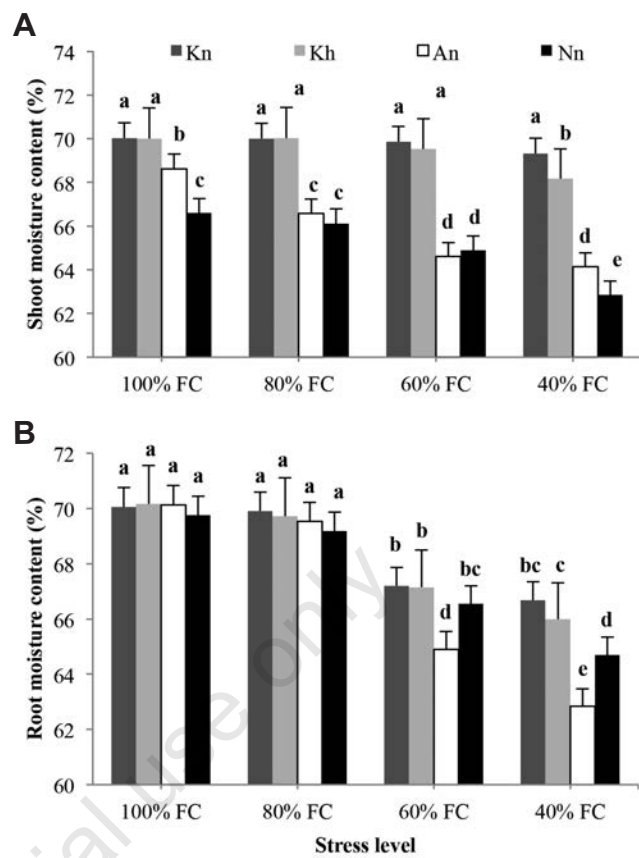


Figure 4. Variation of shoot (A) and root (B) moisture content in four chicory ecotypes under different stress levels. Means with the same letter(s) are not significantly different ($P \leq 0.05$). FC=field capacity.

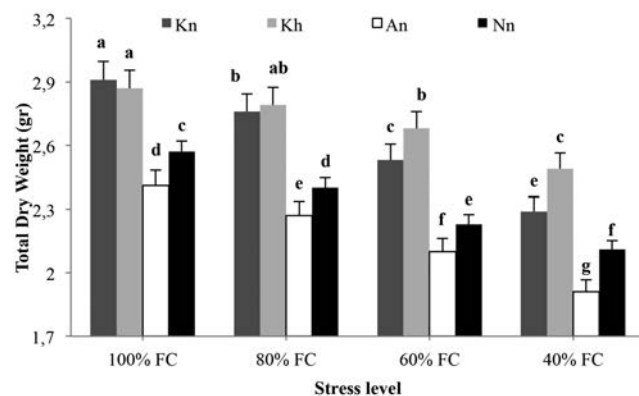


Figure 5. Variation of total dry weight in four chicory ecotypes under different stress levels. Means with the same letter(s) are not significantly different ($P \leq 0.05$). FC=field capacity.

Table 4. Correlation between total dry weight and other growth traits.

	Plant height	Leaf area	Shoot moisture content	Root moisture content
Total dry weight	0.94**	0.691**	0.803**	0.671**

** $P \leq 0.01$.

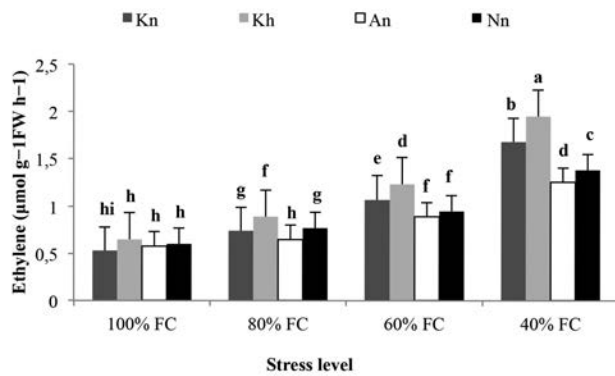


Figure 6. Leaves ethylene content in four chicory ecotypes under different stress levels. Means with the same letter(s) are not significantly different ($P \leq 0.05$). FC=field capacity.

to the decreasing turgor pressure and subsequent cell division and enlargement (Cabuslay *et al.*, 2002). Plant height can also be decreased through reduction in leaf area, photosynthetic activity, translocation of assimilates and finally through reduced plant growth.

Regarding the effect of water stress on leaf area, the different water stress levels resulted in a gradual decrease with increased stress in all chicory ecotypes. Alfredo *et al.* (2000) reported similar variation of cassava (*Manihot esculenta*) genotypes in response to water stress. In response to drought stress, plants were able to control transpired water loss by reducing the leaf expansion rate to prevent dehydration of leaf tissues (Nayyar and Gupta, 2006). The impact of water stress on leaf growth can be explained as a method of adaptation to water shortage condition to limit the rate of transpiration, to maintain the water supply in soil around plant roots to increase the chances of survival of the plant (Boutraa *et al.*, 2010).

The use of root and shoot moisture contents as an indicator of plant water status, especially turgor potential and plant drought tolerance is common (Boutraa *et al.*, 2010). In genotypes of sugarcane (*Saccharum* spp.), drought tolerant genotypes keep high moisture content when compared with drought-sensitive genotypes (Silva *et al.*, 2007). Siyah Shiraz and Sefid Shiraz ecotypes might be considered drought-tolerant when moisture content is being used as criteria in screening for drought tolerance. Dry weights decreased under various water stress levels. The dry weights of the plants significantly decreased due to exposure to injurious levels of drought (60 and 40% of FC). This could be a result of the fact that low soil water availability decreases photosynthesis, carbohydrate accumulation and total plant dry matter. The same results showed by Ekren *et al.* (2012), Boutraa *et al.* (2010) and Khalid (2006). Siyah Shiraz (Kh) was found to be more droughts tolerant than Sefid Shiraz (Kn) ecotype due to relatively lower dry weight reduction under water deficit conditions in Siyah Shiraz (Kh) ecotype.

Ethylene is regarded as a stress hormone, because it is involved in many plant stress morphophysiological responses (Cao *et al.*, 2007). Ethylene has an important role in many aspects of plant biology, from seed germination to dormancy, ripening and senescence, and the regulation of stomatal closure (by promoting NADPH oxidase-mediated reactive oxygen species (ROS) production in stomatal guard cells) as well as defences against biotic and abiotic stresses (Bartoli *et al.*, 2013). According to Achard *et al.* (2006), ethylene signalling pathways might be essential for survival under adverse environmental conditions and it is involved in

control of growth as well as stress tolerance. For example, drought induced the accumulation of the ethylene precursor and the activation of ethylene signalling leading to a reversible arrest of cell cycle (Bartoli *et al.*, 2013). In agreement with this, leaf ethylene biosynthesis was demonstrated to be significantly higher under severe (60 and 40% FC) stress compared to the control (100% FC) and it was significantly higher in drought-tolerant chicory ecotype (Siyah Shiraz) than the others. This has also been supported by the findings of Ben Hassine and Lutts (2010), who found enhanced ethylene production under drought stress in *Atriplex halimus*.

Conclusions

In explaining the endogenous mechanisms that confer stress, plant adaptation or tolerance is essential in providing insights into the potential of the plants in order to adapt to environmental change. In the present study, responses of some morphophysiological parameters were evaluated. They consisted of plant height and leaf area, total plant dry weight, moisture content and ethylene accumulation at four drought stresses (100, 80, 60 and 40% FC) in four chicory ecotypes from Iran. The results showed that Siyah Shiraz (Kh) performs better than other ecotypes under water deficit conditions, as indicated by higher moisture content, leaves ethylene accumulation in leaves and lower total dry biomass, leaf area and plant height reduction under different level of drought stress. A better understanding of the physiological basis of changes in drought resistance, after proper validation, could be used to select new cultivars of crops to obtain a higher yield under water deficit conditions.

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