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Carbon isotope discrimination and water use efficiency relationships of alfalfa genotypes under irrigated and rain-fed organic farming

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ABSTRACT

Carbon isotope discrimination (Δ) has been proposed as a method for evaluating water use efficiency (WUE) in C_3 plants and as a precise technique for screening plants with higer tolerance under water deficit conditions. In this research, 18 alfalfa genotypes from different geographical origins were evaluated under irrigated and rain-fed conditions in organically managed fields in Austria. Significant differences were found amongst harvests for Δ-shoot under both conditions while genotype by harvest interaction was only significant under irrigated condition. Drought stress under rain-fed condition reduced the overall mean of water use efficiency and carbon isotope discrimination responses(up to 34%), but the ratios of reduction differed for characters and genotypes. Narrow ranges were found for all traits especially for WUE-TBY (total biomass yield) (0.78 kg m^{-3}) and Δ-shoot (0.53‰) based on genotype means over locations and years, although variation and ranges were higher under irrigated condition. Regarding the variable and low correlations, simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure the selection of superior genotypes and minimize potential biomass reductions that may result from using Δ-shoot as the only selection criterion to improve WUE. Sitel was the most water use efficient genotype(2.79 and 4.48 kg m^{-3} based on shoot dry matter and total biomass,respectively) across two condition (widely adapted genotype) followed by Mohajeran, Fix232 and Verko under irrigated condition (as specific adapted genotypes) and Vlasta, Sanditi, Ghara-aghaj under rain-fed condition.

1. Introduction

Due to steadily increasing demands for water supply and global climate changes, breeding programs for water use efficiency improvement in all crops will play an important and vital role in the future. Carbon isotope discrimination (Δ) has been proposed as a method and technique for evaluating and improving water use efficiency (WUE) in C_3 plants like alfalfa (Ehleringer et al., 1993; Johnson and Tieszen, 1994; Martin et al., 1999; Raeini-Sarjaz et al., 1997; Pietsch et al., 2007).

The physiological basis of the association between Δ and WUE is well established. WUE may be estimated as the ratio of dry matter accumulation over time to amount of water transpired (transpiration efficiency, TE) or as the ratio of CO_2 assimilation to stomatal conductance or transpiration (WUE of gas exchange or instantaneous WUE). In C_3 species, the isotopic ratio of heavy isotope of carbon (^{13}C) to ^{12}C in plant materials is less than the isotopic ratio of ^{13}C to ^{12}C in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis which leads to a depletion of the plant dry matter in ^{13}C. This process depends on the ratio of the intercellular to atmospheric CO_2 concentration (C_i/Ca) which is linked to stomatal conductance (Farquhar et al., 1982; Jones, 2007). Increasing CO_2 assimilation or decreasing stomatal conductance results in increasing WUE and declining of leaf intercellular CO_2 (C_i) and consequently Δ. Therefore, there should be a negative relationship between WUE and Δ due to the independent relation between C_i and Δ or WUE (Farquhar et al., 1982; Farquhar and Richards, 1984; Teulat et al., 2001). For breeding programs, the

Abbreviations: Δ, carbon isotope discrimination; WUE, water use efficiency; TE, transpiration efficiency; FDR, frequency domain reflectometry; SAW, soil available water; SHDM, shoot dry matter; STDM, stubble dry matter; RODM, root dry matter; TBY, total biomass yield; ET, evapotranspiration.

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Table 1
Some properties of the experimental soil at two field trials.

Texture	Gross-Enzersdorf (irrigated) Silty loam	Raasdorf (rain-fed) Silty loam
Organic carbon content (%)		
0–30 cm	1.5	2.0
30–60 cm	1.4	0.7
Depth of A horizon	45–50 cm	25–35 cm
Bulk density (g cm^{-1})	1.4–1.6	1.3–1.4

variation in the CO_2 assimilation to stomatal conductance or water transpiration ratio can be exploited in indirect selection for WUE via Δ. Johnson and Tieszen (1994) reported significant differences among 18 alfalfa accessions and a significant negative association between Δ and water-use efficiency in them. However, positive correlation between Δ and dry matter yield has been reported among nine alfalfa germplasms under irrigated condition (Ray et al., 1998) and also among 30 elite half-sib families grown in non stress and drought stress conditions (Ray et al., 1999a, 1999b). However, selection for low Δ has been suggested as a method to indirectly evaluate WUE and as a criterion to improve WUE in alfalfa breeding programs.

The study of Δ in different plant parts might help breeders to choose the part which would maximize the correlation between Δ and WUE and its stability in different environments.

Stable and consistent genotype rankings for Δ in different shoot parts (Johnson and Rumbaugh, 1995) and also different environments (Johnson and Tieszen, 1994; Ray et al., 1998) in alfalfa suggest that any plant part can be sampled for evaluating Δ under either favorable or unfavorable conditions. Johnson and Rumbaugh (1995) reported that although plant parts (stem, upper leaves, bottom leaves and entire shoot) differed in Δ, there were similarities in Δ response across tested clones and water levels in plant parts. However, the most of these few studies on Δ in different plant parts have been focused on different parts of above-ground harvestable biomass in C_3 plants such as Johnson and Rumbaugh (1995) in alfalfa, Hannachi et al. (1996) in bread wheat and Hafsi et al. (2000) in durum wheat.

In this study two major groups of genotypes, Iranian ecotypes as adapted genotypes to semi-arid condition and European cultivars as improved and suitable ones for locations uder study, were used. The objectives of this survey were (i) to estimate WUE and Δ in different alfalfa genotypes under irrigated and rain-fed conditions, (ii) to determine the effect of drought stress on WUE and Δ under rain-fed condition, (iii) to study interrelationships between biomass productions, WUE and Δ in both conditions, and (iv) to study variation and consistency of Δ in harvestable (shoot) and non-harvestable (stubble and root) parts of plant biomass under irrigated and rain-fed conditions.

2. Materials and methods

2.1. Experiment description and design

In order to assess carbon isotope discrimination (Δ), relationship between WUE and Δ and the effect of drought stress on it in eighteen alfalfa genotypes (Table 2), this study was carried out in two separate trials, namely, irrigated (no water stress) and rain-fed (water stress) at two different organically managed fields, Gross-Enzersdorf (48°12′ N, 16°33′ E) and Raasdorf (48°15′ N, 16°37′ E), respectively, of the research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria during 2006–08. The farm management was organic and stockless, no organic manures were applied. The soil in both fields is a Calcaric Phaeozem (WRB) from loess with a silty loam textur (Table 1). The amount of precipitation and applied irrigation water and the average temperate from March to September in 2007–08 are shown in Fig. 1.

Table 2
Name of tested genotypes and their origin.

No.	Name	Origin	No.	Name	Origin
1	Mohajeran	Iran-West	10	Verko	Hungry
2	Khorvande	Iran-West	11	Vlasta	Czech Republic
3	Famenin	Iran-West	12	Monz42	Slovakia
4	Gharghologh	Iran-Northwest	13	Fix232	Slovakia
5	Ordobad	Iran-Northwest	14	NS- Banat	Serbia
6	Shorakat	Iran-Northwest	15	Sanditi	Netherlands
7	Ghara-aghaj	Iran-Northwest	16	Alpha	Netherlands
8	Hokm-abad	Iran-Northwest	17	Plato	Germany
9	Sitel	Netherlands	18	Niva	Czech Republic

Both trials were hand seeded in May, 2006. The first experimental year (i.e. 2006) was considered as establishment year. The seeding density was 25 kg ha^{-1} which was adjusted by the germination rate of the cultivars. The experimental design was an α-lattice design with two replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six experimental alfalfa plots. Each genotype was seeded in 12 rows, each 2 m long, in the rain-fed trial at Raasdorf and 8 rows, each 1.5 m long, in the irrigated trial at Gross-Enzersdorf. Spacing between rows in both trials was 12.5 cm.In the irrigated trial, soil moisture content was monitored weekly by four FDR (Frequency Domain Reflectometry, ThetaProbe ML2x, UMS GmbH, München, Germany) probes in 15, 40, 80 and 120 cm soil depths; these devices were installed in one plot in each incomplete block including cultivars of 1, 9 and 18 in each replication while, in rain-fed trial, probes of SENTEK diviner 2000. FDR system were installed in 2 plots (one tube in each plot) in each incomplete block including cultivars 1, 5, 9, 11, 14 and 18 to a soil depth of 120 cm. Irrigation would be started at 50% depletion of soil available water (SAW) content (SAW = water content difference between field capacity and permanent wilting point) based on FDR probe readings in 15 cm soil depth in the irrigated trial. The amount of applied irrigation water was calculated for 0–30 cm depth based on soil water content up to field capacity. Plots were irrigated by a drip irrigation system.

2.2. Data collection

Plots were hand clipped three times at 30–40% of flowering using a garden scissor to a 5-cm stubble height every year (2007–08). Shoot (SHDM) and stubble (STDM) yield data (t ha^{-1}) were adjusted to a dry matter basis by sub-sampling approximately 200 and 50 g of fresh shoot and stubble, respectively, from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 48 h. Annual shoot dry matter production was determined by summing the yield data over the harvests within each year. Root dry matter (RODM) (t ha^{-1}) was determined using a soil corer with 9 cm diameter. Two samples were taken per plot down to 30 cm depth, and fresh roots after washing were dried at 60 °C for 48 h. Root dry matter and stubble dry matter were recorded only at the third harvest in each year.

Water use efficiency (WUE) was calculated based on both shoot dry matter (WUE-SHDM) and total biomass yield (WUE-TBY) as follows:

WUE = Dry matter production/total water use [kg m^{-3} H_2O]

Total water use during vegetation period was calculated according to an estimate of the actual evapotranspiration (ET) using the climatic water balance equation (Pietsch et al., 2007) as follows:

$N + B = T + E + A + S + \Delta R$

Where N, B, T, E, A, S and ΔR are precipitation, irrigation, transpiration, evaporation, surface runoff, leaching and change in the water content of the soil profile (0–90 cm), respectively. Surface

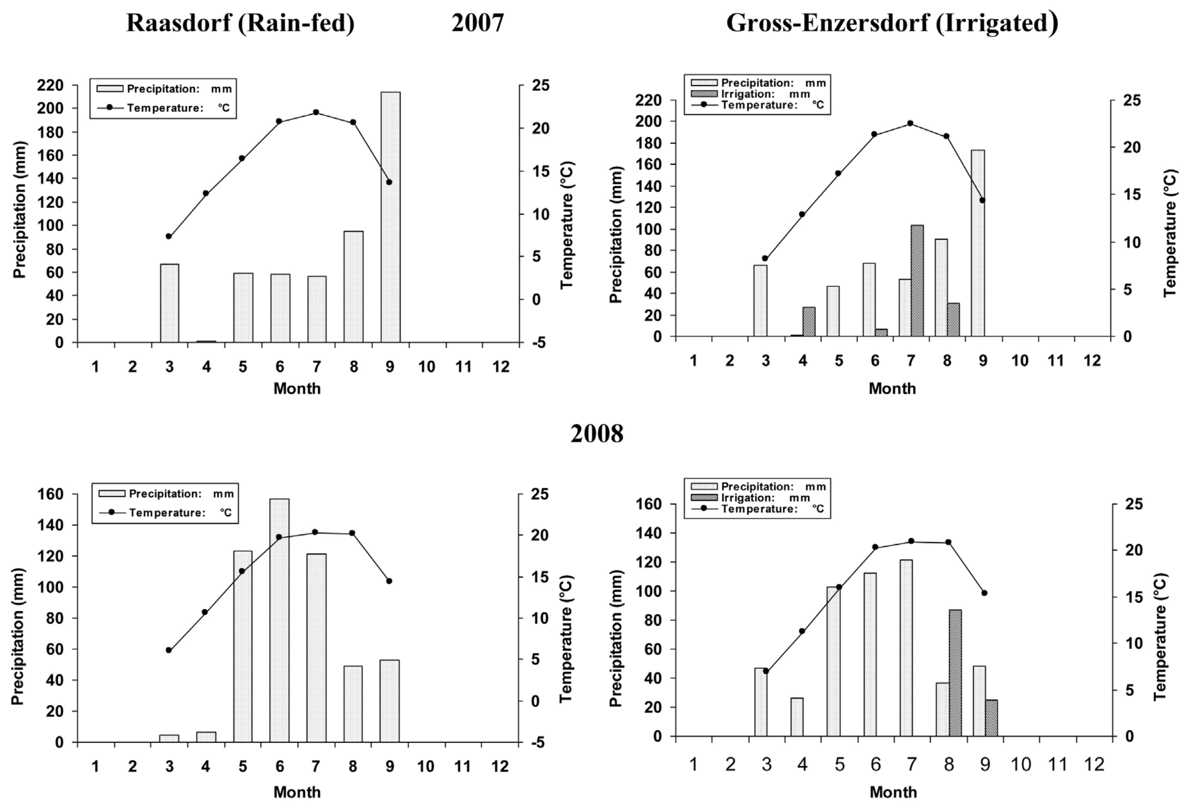

Fig. 1. Monthly precipitation, average temperature and applied irrigation water from March to September 2007 and 2008.

runoff (A) and leaching (S) were considered as zero during the growth period. The change in the soil water content (ΔR) was calculated by weekly data recording from FDR probes under both conditions. The average of calculated ΔR in 2 plots of each incomplete block under rain-fed condition and calculated ΔR in single plot in each incomplete block under irrigated condition were used to adjust total water use of plots within an incomplete block. The total amount of applied water was determined for the rain-fed trial based on total precipitation and for irrigated trial based on summing up total precipitation and applied irrigation water during the vegetation period. Finally, the following simplified equation was used for calculating WUE:

$$T + E = N + B - \Delta R$$

Carbon isotope discrimination (Δ) in the various plant parts (shoot, stubble and root) was determined at harvest time each year. Each sample was sequentially ground in a 1-mm sieve and mixed extensively. The Δ values (‰) were determined with an isotope ratio mass spectrometer (IRMS-Thermo Quest Finnigan DELTA plus) in the laboratory of the Department of Chemical Ecology, University of Vienna, according to procedures of Farquhar et al. (1989):

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}$$

where $\delta^{13}C$ is the value of stable isotope ratio (air or plant) which is expressed as the $^{13}C/^{12}C$ ratio (R_{sample}) relative to the PeeDee belemnite standard ($R_{standard}$) (Craig, 1957):

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{standard} - 1}\right) \times 1000$$

$$R_{sample} = \frac{^{13}C}{^{12}C},\ R_{standard} = -8\ ‰$$

2.3. Statistical analysis

Two years and locations data of WUE-SHDM, WUE-TBY, Δ-stubble, Δ-root and average of Δ-shoot over harvests per year were analyzed using repeated measure analysis of variance based on an alpha-lattice design. ANOVA was done by PROC MIXED in SAS software (SAS Institute, 2004). Location (L), replication (Rep) and genotype (G) were considered as fixed effects, while incomplete block into replication [iblock (rep)] and year (Y) were considered as random effect and repeated measure, respectively. The analysis was done using two different covariance structures, the unstructured (UN) and the first-order autoregressive [AR (1)]. The Akaike Information Criterion (AIC) was used to find the best model describing the covariance structure. Difference between Iranian ecotypes and European cultivars was evaluated by contrast using CONTRAST statement. Mean comparisons were adjusted for the p-values ($\alpha = 0.05$) using ADJUST = SIMULATION option.

2.4. Phenotypic correlation

Phenotypic correlations were calculated between WUE-SHDM, WUE-TBY and Δ-shoot, Δ-stubble and Δ-root based on adjusted means of genotypes over years in each location to examine the usefulness of Δ of different plant parts as a selection tool for WUE improvement under different conditions.

3. Results

3.1. ANOVA and mean comparisons

The results of repeated measure analysis of variance for characters under study are shown in Table 3. The first order autoregressive (AR1) model of covariance structure was selected for WUE- SHDM and WUE- TBY based on the value of Akaike Information Criterion

Table 3
Significance level for the fixed effects and their interactions in combined ANOVA of two locations.

Trait Source	WUE-SHDM kg m^{-3}	WUE-TBY kg m^{-3}	Δ-shoot ‰	Δ-stubble ‰	Δ-root ‰
Location (L)	***	***	***	***	**
Year (Y)	***	***	***	***	**
Genotype (G)	***	***	*	ns	ns
Iranian vs. European	***	*	ns	ns	ns
L*Y	ns	ns	***	***	ns
L*G	***	***	ns	ns	ns
Y*G	**	**	ns	ns	ns
L*Y*G	***	**	ns	ns	ns

The significance levels 0.001, 0.01, 0.05 and non significant are indicated with ***, **, *and ns, respectively. WUE: water use efficiency; SHDM: shoot dry matter; TBY: total biomass yield; Δ: carbon isotope discrimination.

(AIC), while it was the unstructured model for Δ-shoot, Δ-stubble and Δ-root.

3.1.1. Main effects

Locations (i.e. irrigated and rain-fed) and years showed highly significant differences in all characters.Average of WUE-SHDM and WUE-TBY was 3.0 and 4.8 kg m^{-3} under irrigated condition and 1.9 and 3.5 kg m^{-3} under rain-fed condition, respectively, showing a 34.4% and 27.2%-reduction due to drought stress effect under rain-fed condition (Table 5). Drought stress under rain-fed condition also decreased the average values of Δ for shoot, stubble and root by −4.5, −4.0 and −5.3%, respectively.

During 2007,Average of WUE-SHDM, WUE-TBY, Δ-shoot, Δ-stubble and Δ-root was 1.9 (kg m^{-3}), 3.2 (kg m^{-3}), 21.6 (‰), 20.7 (‰) and 20.5 (‰) while in 2008, it was 3.0 (kg m^{-3}), 5.1 (kg m^{-3}), 22.5 (‰), 22.5 (‰) and 21.0 (‰), respectively showing 36.7, 37.3, 4.0, 7.9 and 2.6% reduction in 2007 due to a different rainfall pattern and a higher intensity of drought stress.

Significant differences among genotypes were found for WUE-SHDM, WUE-TBY and Δ-shoot, while no significant differences were observed for Δ-stubble and Δ-root (Table 3). However, phenotypic variation among genotypes was higher for WUE-SHDM and WUE-TBY than for Δ-shoot. Sitel had the highest WUE-SHDM (2.79 kg m^{-3}) and WUE-TBY (4.48 kg m^{-3}) across locations and years while Khorvande had the lowest mean with 1.82 and 3.70 kg m^{-3} for both characters, respectively (Table 4). Ghargologh, Hokmabad and Plato ZS had the lowest Δ-shoot (21.87‰), Δ-stubble (21.11‰) and Δ-root (20.48‰) means over locations and years (Table 4). The overall mean for WUE-SHDM, WUE-TBY, Δ-shoot, Δ-stubble and Δ-root was 2.45 (kg m^{-3}), 4.14 (kg m^{-3}), 22.07 (‰), 21.57 (‰) and 20.77 (‰) with a range of 0.96 (kg m^{-3}), 0.78 (kg m^{-3}), 0.53 (‰), 0.80 (‰) and 0.56 (‰), respectively (Table 4).

Iranian and European genotypes did not show any significant difference for Δ in different plant parts while they differed significantly in WUE-SHDM and WUE-TBY (Table 3).

3.1.2. Genotype by location Interaction (GLI) effects

GLI showed highly significance differences for WUE-SHDM and WUE-TBY. Mohajeran with 3.4 kg m^{-3} under irrigated condition and Sitel with 2.3 kg m^{-3} under rain-fed condition exhibited the highest WUE-SHDM, and Khorvande with 2.2 kg m^{-3} under irrigated and 1.5 kg m^{-3} under rain-fed condition had the lowest WUE-SHDM (Table 5). The drought stress reduced overall the mean of WUE-SHDM by 34.4% under rain-fed condition. Mohajeran with – 47.2% and Sanditi with −23.3% had the greatest and the lowest reductions, respectively.

Fix 232 with 5.5 kg m^{-3} and Vlasta with 4.1 kg m^{-3} exhibited the highest WUE-TBY under irrigated and rain-fed conditions, respectively, while Sanditi with 4.3 kg m^{-3} and Khorvande with 3.0 kg m^{-3} had the lowest WUE-TBY (Table 5). The overall mean of WUE-TBY was decreased by 27.2% under rain-fed condition. Fix 232 with −39.6% and Vlasta with −11.9% exhibited the greatest and the smallest reduction, respectively (Table 5).

There was no significant difference in Δ-shoot, Δ-stubble and Δ-root among genotypes within each location (Tables 3 and 5). Ghargologh and Niva with 22.3‰ under irrigated and Mohajeran, Famenin and Ghargologh with 21.4‰ under rain-fed condition had the lowest Δ-shoot responses. For Δ-stubble, the lowest values belonged to Ghargologh with 21.4‰ under irrigated and Ordobad with 20.5‰ under rain-fed condition. Ghargologh with 20.9‰ and Hokmabad with 19.9‰ had the lowest Δ-root responses among genotypes under irrigated and rain-fed conditions, respectively.

Table 4
Comparisons of genotype means over years and locations for studied characters.

Trait	SHDM		WUE-SHDM		TBY		WUE-TBY		Δ-shoot		Δ-stubble		Δ-root	
Cultivar	t ha^{-1}		kg m^{-3}		t ha^{-1}		kg m^{-3}		‰		‰		‰	
Mohajeran	13.9	de	2.59	ef	22.8	cd	4.28	cde	22.02	ab	21.76	a	20.94	a
Khorvande	9.6	a	1.82	a	19.5	a	3.70	a	22.12	ab	21.41	a	21.05	a
Famenin	12.9	bcd	2.42	bce	21.4	ac	4.05	ae	21.90	a	21.69	a	20.72	a
Ghargologh	11.7	bc	2.23	bc	22.5	bcd	4.25	cde	21.87	a	21.21	a	20.51	a
Ordobad	11.7	bc	2.21	bd	21.2	ac	3.97	abd	22.09	ab	21.25	a	20.73	a
Shorakat	13.1	be	2.46	bf	23.0	cd	4.30	cde	22.14	ab	21.85	a	21.02	a
Ghara-aghaj	13.5	ce	2.56	cf	21.9	ac	4.12	ae	22.11	ab	21.69	a	20.83	a
Hokmabad	11.4	ab	2.15	ab	21.1	ac	3.96	abd	22.05	ab	21.11	a	20.62	a
Sitel	14.9	e	2.79	f	23.6	c	4.48	e	22.13	ab	21.75	a	20.94	a
Verko	13.6	ce	2.56	cdf	21.8	ac	4.11	ae	22.01	ab	21.82	a	20.68	a
Vlasta	14.0	de	2.64	ef	23.4	cd	4.37	de	22.21	ab	21.84	a	20.75	a
Monz 42	12.3	bcd	2.30	bce	21.0	ad	3.95	ad	22.21	ab	21.54	a	20.58	a
Fix 232	13.9	de	2.62	ef	23.2	cd	4.39	de	22.40	b	21.91	a	20.91	a
NS-Banat	13.5	ce	2.55	cdf	23.5	c	4.42	be	22.13	ab	21.69	a	21.05	a
Sanditi	13.5	ce	2.53	cdf	21.3	ac	4.02	ae	22.03	ab	21.65	a	20.61	a
Alpha	13.1	be	2.48	bf	20.3	ab	3.85	ac	22.06	ab	21.59	a	20.79	a
Plato ZS	13.9	de	2.61	ef	22.1	bcd	4.14	ae	21.95	ab	21.15	a	20.48	a
Niva	13.8	de	2.63	ef	21.9	ac	4.20	cde	21.91	a	21.41	a	20.70	a
Mean	13.02		2.45		21.97		4.14		22.07		21.57		20.77	
Range	5.3		0.96		4.1		0.78		0.53		0.80		0.56	
SE	0.4		0.09		0.6		0.12		0.10		0.25		0.20	

There is no significant difference at 0.05 level between genotypes with a common letter in the same column. SE:Standard error of mean; WUE: water use efficiency; SHDM: shoot dry matter; TBY: total biomass yield; Δ: carbon isotope discrimination.

Table 5
Genotype means over years in each condition including the percent of change for each genotype under rain-fed condition.

Cultivar	WUE-SHDM (kg m^{-3})				Change	WUE-TBY (kg m^{-3})				Change	Δ-shoot				Change	Δ-stubble				Change	Δ-root				Change
	IR		RN		%	IR		RN		%	IR		RN		%	IR		RN		%	IR		RN		%
Mohajeran	3.4	e	1.8	ae	−47.2	5.1	bcd	3.4	ab	−33.6	22.6	a	21.4	a	−5.3	22.3	a	21.3	a	−4.6	21.8	a	20.1	a	−7.5
Khorvande	2.2	a	1.5	a	−31.1	4.4	ab	3.0	a	−31.9	22.5	a	21.7	a	−3.6	21.8	a	21.1	a	−3.3	21.3	a	20.8	a	−2.4
Famenin	2.9	bce	1.9	ae	−33.3	4.7	ad	3.4	ac	−28.0	22.4	a	21.4	a	−4.7	21.8	a	21.6	a	−1.0	21.2	a	20.3	a	−4.3
Gharghologh	2.6	ab	1.9	ae	−26.6	4.8	ad	3.7	ac	−23.5	22.3	a	21.4	a	−4.2	21.4	a	21.0	a	−1.7	20.9	a	20.1	a	−3.5
Ordobad	2.8	bcd	1.6	abd	−42.4	4.8	ad	3.1	ab	−35.2	22.6	a	21.6	a	−4.6	22.0	a	20.5	a	−6.8	21.4	a	20.0	a	−6.5
Shorakat	3.0	bce	1.9	ae	−36.4	4.8	ad	3.8	bc	−20.9	22.8	a	21.5	a	−5.7	22.4	a	21.3	a	−4.9	21.8	a	20.2	a	−7.1
Ghara-aghaj	2.9	bce	2.2	ce	−25.6	4.5	ab	3.8	bc	−15.6	22.6	a	21.6	a	−4.7	21.8	a	21.6	a	−1.0	21.4	a	20.3	a	−5.0
Hokm-abad	2.7	ac	1.6	ac	−39.5	4.7	ac	3.2	ab	−30.7	22.5	a	21.6	a	−4.1	21.6	a	20.7	a	−4.3	21.3	a	19.9	a	−6.6
Sitel	3.2	de	2.3	e	−28.4	5.1	bcd	3.8	bc	−25.3	22.7	a	21.6	a	−4.8	22.1	a	21.4	a	−3.4	21.5	a	20.4	a	−5.3
Verko	3.2	ce	1.9	ae	−42.4	5.0	ad	3.2	ab	−34.7	22.4	a	21.6	a	−3.4	22.5	a	21.2	a	−5.8	21.4	a	20.0	a	−6.4
Vlasta	3.1	bce	2.2	ef	−28.3	4.7	ac	4.1	c	−11.9	22.7	a	21.7	a	−4.0	22.1	a	21.5	a	−2.8	21.1	a	20.4	a	−2.9
Monz 42	2.9	bce	1.7	abcf	−40.5	4.7	ac	3.2	ab	−31.9	22.7	a	21.7	a	−4.6	22.1	a	20.9	a	−5.5	21.2	a	20.0	a	−5.5
Fix 232	3.2	de	2.0	cde	−36.5	5.5	d	3.3	ab	−39.6	22.9	a	21.9	a	−4.7	22.3	a	21.5	a	−3.5	21.4	a	20.4	a	−4.6
NS_Banat	3.0	bce	2.1	cde	−29.8	5.3	cd	3.6	ac	−32.5	22.7	a	21.5	a	−5.2	22.1	a	21.2	a	−4.0	21.7	a	20.4	a	−6.0
Sanditi	2.9	bce	2.2	ef	−23.3	4.3	a	3.7	ac	−14.3	22.6	a	21.5	a	−4.9	22.1	a	21.2	a	−4.3	21.2	a	20.0	a	−5.4
Alpha	3.2	ce	1.8	ae	−42.6	4.5	ab	3.2	ab	−30.3	22.6	a	21.5	a	−4.7	22.3	a	20.9	a	−6.2	21.3	a	20.3	a	−4.6
Plato ZS	3.1	ce	2.1	cde	−34.0	4.7	ac	3.6	ac	−24.2	22.4	a	21.5	a	−3.8	21.5	a	20.8	a	−3.0	21.0	a	20.0	a	−4.7
Niva	3.1	ce	2.1	be	−32.0	4.8	ad	3.6	ac	−24.9	22.3	a	21.5	a	−3.3	22.0	a	20.9	a	−5.1	21.4	a	20.0	a	−6.6
Mean	3.0		1.9		−34.4	4.8		3.5		−27.2	22.6		21.6		−4.5	22.0		21.1		−4.0	21.3		20.2		−5.3
Range	1.2		0.8			1.1		1.1			0.7		0.5			1.1		1.1			0.9		0.9		
SE	0.13					0.17					0.14					0.35					0.28				

IR: irrigated; RN: rain-fed; SE: standard error of mean; WUE: water use efficiency; SHDM: shoot dry matter; TBY: total biomass yield; Δ: carbon isotope discrimination. Genotypes with a common letter in same column do not differ significantly at 0.05 probability level.

3.2. Correlation coefficients

There was a positive and significant correlation between WUE-SHDM and WUE-TBY based on genotype means under irrigated condition (r = 0.50*), rain-fed condition (r = 0.83**) and for the overall mean (r = 0.74**) (Table 6). Low to intermediate positive correlations were observed between WUE-SHDM and Δ-shoot (r = 0.25), WUE-SHDM and Δ-root (r = 0.37), WUE-TBY and Δ-shoot (r = 0.36) and WUE-TBY and Δ-root (r = 0.47*) under irrigated condition. Negative correlations occurred between WUE-SHDM and Δ-shoot (r = −0.06) and WUE-TBY and Δ-shoot (r = −0.19), and negligible positive between WUE-SHDM and Δ-root (r = 0.05) and WUE-TBY and Δ-root (r = 0.02) under rain-fed condition (Table 6). There were positive and mostly significant correlations between Δ-stubble and WUE-SHDM and Δ-root and WUE-TBY under both conditions (Table 6). The correlations between Δ in different plant parts were positive and especially significant under irrigated conditions.

4. Discussion

Although there was no large difference in the amount of precipitation between two locations and years, mid- and late-season stresses were imposed on genotypes under rain-fed condition during the first and second year of the study, respectively (Fig. 1).

4.1. WUE and Δ in different genotypes and conditions

Sitel and Khorvande were the best and the worst genotypes based on WUE-SHDM and WUE-TBY. However, the ranks of some top yielding genotypes such as Plato ZS and Niva and also low yielding genotypes such as Shorakat and Gharghologh changed when considering the total biomass, harvestable and non-harvestable, in estimation of WUE (WUE-TBY) compared with WUE-SHDM, indicating the importance of considering non-harvestable biomass, particularly roots, in a precise evaluation and selection of genotypes.

In spite of significant differences among genotypes (Table 3) and their diverse origin (Iranian and European genotypes), narrow ranges were found for all traits especially for WUE-TBY (0.78 kg m^{-3}) and Δ-shoot (0.53‰) based on genotype means over locations and years (Table 4). Increasing variation and ranges among genotypes for Δ and WUE under irrigated condition (Table 5) suggested more opportunities to improve WUE via Δ in this condition compared to rain-fed environment. The average values of WUE-SHDM,WUE-TBY, Δ-shoot and Δ-stubble were higher and those of Δ-root were lower for European than for Iranian genotypes.

4.2. Δ in different plant parts

Johnson and Rumbaugh (1995) reported that stems had the lowest Δ followed by entire shoot, upper leaves and bottom leaves. In our study, the stubbles as a short terminal part of stems showed lower Δ values than the entire shoot. The difference between below ground (roots) and above ground (shoots and stubbles) plant parts for Δ showed a general trend of an enrichment in ^{13}C in roots (Figs. 2 and 3). Zhao et al. (2004) reported enrichment in ^{13}C and lower Δ in roots and grains compared to flag leaves and stems in two cultivars of upland rice during different developmental stages under three water regimes. Differences in chemical composition and anatomical characteristics between these plant parts can be caused by fractionating in export and partitioning of photosynthates among various plant parts (Brugnoli and Farquhar, 2000; Cole et al., 1970; Raza et al., 2013).

Table 6
Simple correlation between water use efficiency and Δ in different plant parts.

		WUE SHDM	WUE TBY	Δ Shoot	Δ Stubble
WUE-TBY	IR	0.53*			
	RN	0.83**			
	Mean	0.73**			
Δ-shoot	IR	0.25	0.36		
	RN	−0.06	−0.19		
	Mean	0.04	0.15		
Δ-stubble	IR	0.57*	0.36	0.56*	
	RN	0.55*	0.49*	0.11	
	Mean	0.58*	0.46*	0.33	
Δ-root	IR	0.37	0.47*	0.48*	0.64**
	RN	0.05	0.02	0.34	0.52*
	Mean	0.06	0.26	0.36	0.55*

IR: irrigated; RN: rain-fed; mean: overall mean; WUE: water use efficiency; SHDM: shoot dry matter; TBY: total biomass yield; Δ: carbon isotope discrimination.
* Indicates significant correlations at 0.05 level ($n = 18$).
** Indicate significant correlations at 0.01 level ($n = 18$).

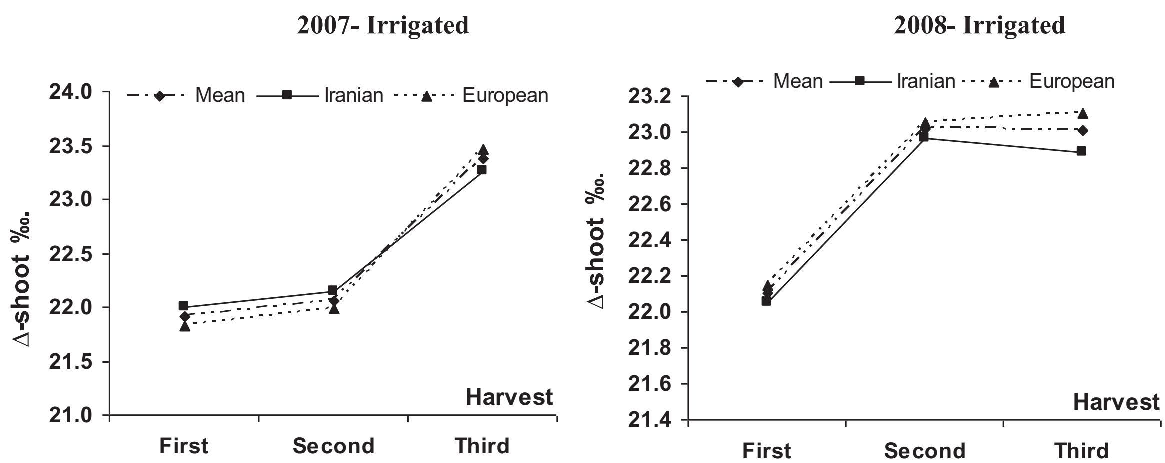

Fig. 2. Δ-shoot values for Iranian, European and grand mean of genotypes at different harvests under irrigated condition during 2007–08.

The difference among genotypes for Δ-shoot was dependent on harvest time and stress condition. One reason for lower Δ-shoot values under rain-fed than under irrigated conditions (Tables 5 and 6) can be less opening of stomata under rain-fed compared to irrigated conditions due to drought stress which resulted in a lower Δ-shoot under the rain-fed condition. Johnson and Rumbaugh (1995) reported lower values for Δ in the drought than in wet treatments in alfalfa clone experiments.

Regarding Δ-shoot values and ranges at different harvests, it can be concluded that the first harvest is the appropriate time to evaluate Δ-shoot response of genotypes for selection purpose under irrigated condition while under rain-fed condition, the suitable time should coincide with water stress which was the second harvest-2007 and the third harvest-2008.

4.3. *Interrelationships between biomass productions, WUE and Δ*

The relation of SHDM and TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) with Δ responses of genotypes (shoot, stubble and root) were variable depending on plant part

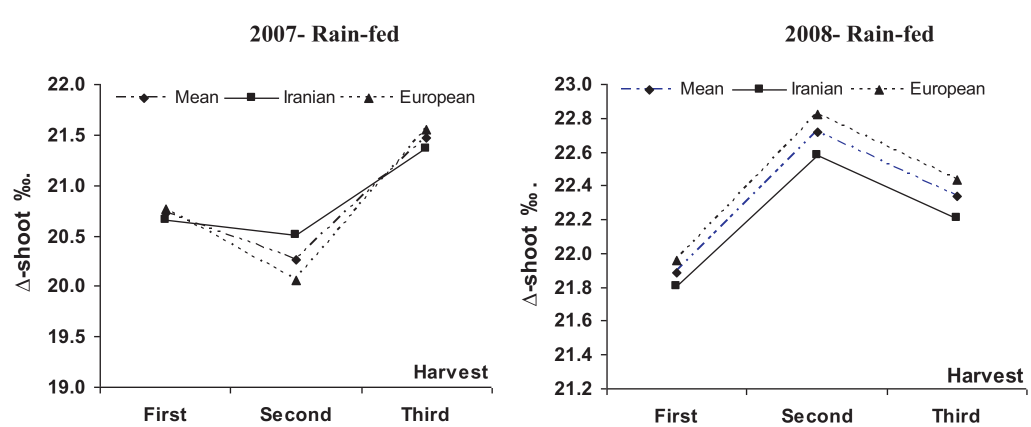

Fig. 3. Δ-shoot values for Iranian, European and grand mean of genotypes at different harvests under rain-fed condition during 2007–08.

and environmental conditions (irrigated and rain-fed) (Table 6). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Some high-yielding and water efficient genotypes such as Sitel, Fix 232, Vlasta (Tables 4 and 5) had high values of Δ-shoot reflecting greater assimilation and carbon fixation rate and consequently greater stomatal conductance in these genotypes than others. Johnson and Tieszen (1994) reported a significant negative correlation ($r = -0.63$ to -0.73) between shoot WUE and Δ among 18 alfalfa genotypes. Ray et al. (1998) found positive and significant correlation ($r = 0.64$, $P < 0.10$) between Δ and alfalfa shoot dry matter yield under irrigated condition. Ray et al. (2004) reported that higher yielding populations tended to have higher Δ in a diallel analysis among nine alfalfa germplasms. Read et al. (1991) in crested wheatgrass and Condon et al. (2004) in wheat reported a positive correlation between Δ and dry matter production, but Raeini-Sarjaz et al. (1998) found a negative correlation ($r = -0.88$ to $r = -0.92$) between WUE and Δ in bush bean.

Regarding the positive correlation between different plant parts for Δ (Table 6), relationships between Δ responses and biomass production and water use efficiency under different conditions (Table 6), and the required time and labor to assess individual plant parts, the Δ-shoots values may be the simplest and most economical single criterion to characterize alfalfa genotypes for high WUE via Δ in alfalfa breeding programs. However, using Δ-shoot as the only selection criterion to improve WUE may cause some reductions in SHDM or TBY and consequently in WUE-SHDM or WUE-TBY depending on selected genotypes. The simultaneous assessment of genotypes for Δ-shoot and biomass production, however, can ensure selection of superior genotypes and minimize potential biomass reductions.

4.4. Drought stress, WUE and Δ

Significant difference between two locations and years for WUE and Δ indicated the magnitude effect of drought stress under rain-fed condition (for source of location) and of different pattern of precipitation between two growing seasons (for source of year) on studied traits. Due to stress effects, the overall mean of water use efficiency and carbon isotope discrimination responses, reduced. The effect of drought stress varied depending on genotypes under rain-fed condition. Some genotypes with high water use efficiency under irrigated condition such as Mohajeran, Fix 232 and Verko could not retain their superiority under rain-fed condition. In contrast, some genotypes with low water use efficiency under irrigated condition Such as Sanditi, Vlasta and Ghara-aghaj relatively improved their efficiency under rain-fed condition. In addition, the rank of some genotypes such as Sitel, Plato ZS, Niva, Khorvande, Hokmabad and Monz 42, either with high or low water use efficiency, was more or less the same under both conditions.

Regarding the genotype means for WUE-SHDM and WUE-TBY under both conditions (Table 5), Sitel was the most water use efficient genotype under both conditions (widely adapted genotype), followed by Mohajeran, Fix232 and Verko under irrigated condition (as specific adapted genotypes) and Vlasta, Sanditi, Ghara-aghaj under rain-fed condition. The lower reduction of WUE-TBY than WUE-SHDM due to drought stress under rain-fed condition (Table 5) implies that the non harvestable plant parts, particularly roots, were affected less by unfavorable conditions than the harvestable part (shoot). A higher correlation between WUE-SHDM and WUE-TBY under rain-fed condition (Table 6) can be regarded as a closer relation between harvestable and non-harvestable biomass under this condition.

The decrease of the overall mean of Δ under rain-fed condition was relatively similar in different plant parts (Table 5). However, the effect of drought stress on genotypes was more uniform for Δ-shoot than for Δ-stubble and Δ-root (Table 5). Jefferies and Mackerron (1997) reported that values for Δ-tuber in potato was consistently lower than stem and leaf and decreased more rapidly. Regarding the Δ response of genotypes in different plant parts, Iranian genotypes like Gharghologh had lower Δ values than European genotypes in most of the cases. The relation between Δ-root and Δ-shoot was closer than the relation with Δ-stubble under both conditions (Table 6).

5. Conclusion

The entire shoots were the best and additionally simplest and most economical single criterion rather than stubble and root to characterize alfalfa genotypes for high WUE via Δ in this study. Genotypes differed for Δ-shoot depending on harvest time and conditions indicating inconsistent differences in Δ-shoot. The rank correlations between different harvests for Δ-shoot were low and mostly insignificant under both conditions. Meanwhile, the rank correlation between two locations or years was positive and mostly non-significant for Δ in all plant parts in spite of non significant $L \times G$, $Y \times G$ and $L \times Y \times G$ interactions. First year of our study (2007) was the proper time to assess genotypes for Δ-shoot response under both conditions. In addition, the first harvest was the appropriate time to evaluate Δ-shoot response of genotypes for selection purpose under irrigated condition while under rain-fed condition, the suitable time should coincide with water stress. The association between SHDM and TBY and consequently water use efficiency (WUE-SHDM and WUE-TBY) with Δ responses of genotypes (shoot, stubble and root) were variable based on plant part and condition of study (irrigated and rain-fed). Correlations between Δ-shoot and shoot dry matter, total biomass yield and their relevant water use efficiency were positive under irrigated condition, while they were negative under rain-fed condition. Thus, simultaneous assessment of genotypes for Δ-shoot and biomass production can ensure selection of superior genotypes and minimize potential biomass reductions that may occur when using Δ-shoot as only selection criterion to improve WUE. Sitel was the most water use efficient genotype across two condition (widely adapted genotype) followed by Mohajeran, Fix232 and Verko under irrigated condition (as specific adapted genotypes) and Vlasta, Sanditi, Ghara-aghaj under rain-fed condition.

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