Scientia Iranica, Vol. 14, No. 4, pp 327–335
© Sharif University of Technology, August 2007

SCIENTIA
IRANICA

Correlation Between the Carbon Isotope Composition (δ^{13}C) of *Puccinellia ciliata* and Balansa Clover in Duel Stresses of Waterlogging and Salinity (NaCl)

A. Mostajeran* **and Z. Rengel**[1]

Theoretically, plants growing under stress conditions, such as in saline or waterlogged conditions, should behave with $^{13}CO_2$ discrimination and have different δ^{13}C values. Therefore, this experiment was conducted to evaluate the effect of factors such as salinity and hypoxia in affecting delta values of *Puccinellia ciliata* and Balansa clover (*Trifolium michelianum* Savi. Var balansae boiss). This study reveals that these two species (C_3 plants) behave differently and B. clover has more positive δ^{13}C values (7.6%) compared to *P. ciliata*. The overall average of δ^{13}C were -28.85 and -26.66 $^0/_{00}$, respectively, for *P. ciliata* and B. clover. The δ^{13}C values for the shoots and roots of the two species also have different values (more positive values in roots compared to shoots). Waterlogging and salinity have a significant affect on $^{13}CO_2$ discrimination and, statistically, these effects on δ^{13}C values are significant ($p < 0.001$). This means that environmental factors, such as salinity and waterlogging, create a situation for plants in which the discriminate rate against δ^{13}C would be changed (less discrimination for hypoxia and more for salinity conditions). The combined effects on species and plant parts of waterlogging and salinity are not statistically insignificant on δ^{13}C values, however, each factor separately has a significant effect on δ^{13}C values. *P. ciliata* was grown in different seasons (summer and winter) and showed significant differences in δ^{13}C values (by almost 4%). The carbon content (%) of the two species was different, but, were not related to δ^{13}C, respectively.

INTRODUCTION

Elements can exist in both stable and unstable (radioactive) forms. Most elements of biological interest (including C, H, O, N and S) have two or more stable isotopes, with the lightest of these present in much greater abundance than the others. Among stable isotopes, the most useful as biological traces are the heavy isotopes of carbon. This element, found in the earth, the atmosphere and all living things, has a heavy isotope (^{13}C) with a natural abundance of 1% or less and a light isotope (^{12}C) that makes up all of the remainder (carbon also has a radioactive isotope, ^{14}C) [1].

Studies examining stable isotopes at or near natural abundance levels are reported as delta, a value given in parts per thousand or per mil ($^0/_{00}$). Delta values are not absolute isotope abundances but differences between sample readings and one or another of the widely used natural abundance standards which are considered to be $\delta^{13}\mathrm{C}$ = zero (e.g., for C, at %^{13}C = 1.1112328, based on Pee Dee Belemuite, PDB) [2]. Absolute isotope ratios (R) are measured for sample and standard, and the relative measure, δ^{13}C, is calculated as follows:

$$\delta^{13}\mathrm{C}\ ^0/_{00}\mathrm{vs.\ std} = \left(\frac{R_{\mathrm{sample}} \quad R_{\mathrm{std}}}{R_{\mathrm{std}}}\right) 1000\ \delta^0/_{00},$$

where $R = [\mathrm{at}\%^{13}\mathrm{C}/\mathrm{at}\%^{12}C]$.

The isotopic ratio of ^{13}C to ^{12}C in plant tissue is less than in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis. Terrestrial plants fix atmospheric CO_2 by two main photosynthesis reaction pathways: The Calvin-Benson, or C_3 and the Hatch-Slack, or C_4. C_3 plants convert

---

*. *Corresponding Author, Department of Biology, Faculty of Science, University of Esfahan, I.R. Iran.*

[1] *Department of Soil Science and Plant Nutrient, University of Western Australia, WA, Australia.*

atmospheric CO_2 to a phosphoglycerate compound with three-C atoms, while C_4 plants convert CO_2 to dicarboxylic acid, a four-C compound. Carbon isotopes are strongly fractionated, with the result that C_4 plants have higher $\delta^{13}C$ values, ranging from -17 $^0/_{00}$ to -9 $^0/_{00}$, with a mean of -13 $^0/_{00}$ relative to PDB [2], while C_3 plants show delta values ranging from -32 $^0/_{00}$ to -20 $^0/_{00}$, with an average value of -27 $^0/_{00}$.

Variation in discrimination against $^{13}CO_2$ during photosynthesis is due to both stomata limitations and enzymatic processes [3,4].

In C_3 plants, the total tissue $\delta^{13}C$ value is largely determined by discrimination against $^{13}CO_2$ by ribulosebisphosphate carboxylase, the expression of which is dependent upon the diffusion gradient of CO_2 into the leaf [5]. The rate of diffusion of $^{13}CO_2$ across the stomata pore is lower by a factor of 4.4 $^0/_{00}$. Additionally, there is an isotope effect caused by the preference of ribulose bisphosphate carboxylase (Rubiso) for $^{12}CO_2$ over $^{13}CO_2$ by a factor of 27 $^0/_{00}$. In both cases, the processes discriminate against the heavier isotope [6,7], thus, within limits, the $\delta^{13}C$ values of C_3 plants do vary [8].

In C_4 plants, Carbonic Anhydrase (CA) facilitates both the chemical and isotopic equilibration of atmospheric CO_2 and bicarbonate (HCO_3) in the mesophyll cytoplasm. The CA-catalyzed reaction is essential for C_4 photosynthesis and the model of carbon isotope discrimination ($\delta^{13}C$) in C_4 plants predicts that changes in CA activity will influence $\delta^{13}C$ [9].

A strong correlation between discrimination or $\delta^{13}C$ and different factors, such as water use efficiency, salinity and temperature etc., has been reported for numerous crop and tree species [10-13]. For example, Zhang et al. [14] found that discrimination was significantly ($P =< 0.0001$) correlated with transpiration efficiency ($R =\ 0.85$) and instantaneous water use efficiency ($R =\ 0.7$) in Larix occidentalis Nutt.

It has been reported that large amounts of salt induced shifts in $\delta^{13}C$ values of two C_3 plant halophytes, native to salt sloughs in western Canada [15]. The greatest effects were seen in the graminaceous glycohalophyte (i.e., non-salt-accumulating) *Puccinellia nuttalliana* (Schults) Hitch., in which a close correlation between $\delta^{13}C$ and water-use efficiency was demonstrated by gas exchange analysis [8]. A less dramatic response was evident in the succulent euhalophye (i.e., salt-accumulating) *Salicornia europaea* L. ssp. Rubra (Nels.) Brietung. Similar results have been reported for *Bisphyma austrate* [16] and Spinach [17].

P. ciliata is well suited to saline-waterlogged conditions; in contrast, the B. clover (*Trifolium michelianum* Savi. Var *balansae* boiss) is less tolerant to waterlogging and salinity [18]. Although they are two of the best known and most widely used legumes and grasses in Australia, there has been a small amount of research undertaken on these plants [18]. Therefore, these two species (C_3 plants) have been selected to compare for (a) discrimination against $^{13}CO_2$ using $\delta^{13}C$, (b) to correlate these values with plant carbon content and (c) to evaluate the effects of salinity and waterlogged conditions on $\delta^{13}C$.

METHODS AND MATERIALS

Two experiments were conducted at different seasons in a glasshouse (temperature: Day, 22±2, night 18±2), at UWA, Australia. In the first experiment, *Puccinellia ciliata* and Balansa clover (*Trifolium michelianum* Savi. Var balansae boiss) were grown in summer 2002. Special pots (40 cm in height and 10 cm in diameter) were filled with 3 kg of Lancilin sandy soil collected from Lancilin in Western Australia (soil was sieved and then the basal nutrient was added to the soil in each pot). The final number of plants per pot, before salinization, was 5 and 3 for *P. ciliata* and B. clover, respectively (after having been tined twice to have uniform plants). A factorial experiment, using a split-split plot design, was conducted, using waterlogging as the main plot and salinity as the sub plot, with 3 replicates. Waterlogging treatments were aerated (controlled), versus 10 and 20 days of waterlogging for *P. ciliata* and 7 and 14 days of waterlogging for B. clover. Sodium chloride was used as the salinity treatment at different rates for different species (0, 0.87, 1.75, 3.5 g NaCl kg ^{1} soil, respectively, for *P. ciliata* and 0, 0.58, 1.17, 2.34 g NaCl kg ^{1} soil, respectively, for B. clover).

The application of NaCl started gradually on day 30 (for *P. ciliata*) and day 25 (for B. clover), mixed with DI water as daily needed water. Waterlogging was applied to the pots from the bottom at day 50 for *P. ciliata* and day 40 for B. clover, using the N_2 bubbling procedure for deoxygenating the water. The waterlogged condition was continued for 10 days in the *P. ciliata* pots and for 7 days in the B. clover pots and was then stopped for half of the waterlogged plants (water was drained from the bottom of these pots). The *P. ciliate* and B. clover plants in the drained pots were taken care of for 10 or 7 days more, respectively, under aerated conditions (or recovery time). The collected water was reused during recovery days in the related pots.

When 20 days (*P. ciliata*) or 14 days (B. clover) of waterlogging were ended in the second portion of the waterlogged pots, the plants were harvested for the two species (*P. ciliata* at day 70 and B. clover at day 55).

During the experiment, the redox potential (Eh, mv) was measured as the O_2 indicator in the waterlogged pots and, at the same time, the EC of the waterlogged pots were measured using an EC meter.

In winter 2003, the second experiment was conducted, using higher NaCl levels for the salinity treatment and *Puccinellia ciliata* as the plant species. The B. clover was eliminated because of less tolerance to higher salinity. In this experiment, *P. ciliata* was grown as mentioned in the first experiment, except that the level of salinity was increased to 4.67 g NaCl kg ^{1} soil. The salinity levels were 0, 1.17, 2.34, 3.51 and 4.67 g NaCl kg ^{1} soil (this is almost equal to the range of ECs of 1.5 to 24 dS m ^{1} in the waterlogged pots). Plant samples were obtained (the whole plant) at four different stages of growth, namely: Before salinity commenced, before waterlogging was applied (after salinization finished), after the first 10 days of waterlogging (before recovery time started) and at the end of the growing season.

At harvest time, the whole plants were removed and then the roots were washed carefully. The plant parts (shoot and root) for each treatment were oven dried separately for 6 days at 65°C and then ground to a fine powder using a ball mill grinder. Then, almost 5 mg of shoot or root were weighed into tin capsules (5×9 mm) and sealed for further analysis. Isotope Ratio Mass Spectrometry (Robopreb and Tracer Iron Mass Spectrometer, Manufactured-Europa PDZ) was used for ^{13}C and total C analysis of the plant parts. The δ^{13}C values were calculated according to:

$$\delta^{13}\mathrm{C}\ {}^{0}/_{00}\ \text{vs. std} = \left(\frac{R_{\text{sample}}\quad R_{\text{std}}}{R_{\text{std}}}\right)1000,$$

the results are expressed per mill ($\delta^{0}/_{00}$), relative to the PDB standards.

Carbon Isotope discrimination (Δ) can be calculated as:

$$\Delta = (\delta a \quad \delta p)/(1+\delta p),$$

where δa and δp are the isotope compositions of air and plant material, respectively, relative to PDB. The average values reported for δa were -8.4 ($^{0}/_{00}$) for the glasshouse [7,19].

RESULTS

Symptom

Observed symptoms of salt-waterlogging damage in B. clover began with chlorosis and necrosis (usually marginal) of the older leaves, which spread all over the leaves after waterlogging started. The chlorosis was soon followed by the death of the old leaves and damage to the growing tip in some plants under high saline-waterlogged treatment. No symptoms were observed in *P. ciliata* plants up to the end of waterlogging, neither in the first experiment (grown in summer) nor in the second experiment (grown in winter).

Carbon Content (% DW) of the Species

The amount of carbon content (% dry weight) of the shoots and the roots of *Puccinellia ciliata* and B. clover grown in summer are presented in Tables 1 and 2. The results indicate that, although the carbon content in the shoots of the two species shows higher values than for the roots, B. clover contains less carbon (by 5.1%) in its plant parts compared to *Puccinellia ciliata.* The difference comes from the carbon content of the shoots of the two species (*P. ciliata*, 43.53% DW and B. clover, 40.82% DW). The roots have almost the same amount of carbon in dry weight (37.13% DW and 37.76% DW, respectively). From a statistical point of view, the difference in the mean values of the carbon content of the roots is not statistically significant for the two species, but, the difference in the shoots of the two species is statistically significant ($p =< 0.001$).

Although there are some differences in the mean values of the carbon content under the saline or waterlogged treatments in *P. ciliata*, these differences are not statistically significant for the shoots or the roots. However, the differences under aerated vs. waterlogged conditions for the roots are statistically significant ($p =< 0.05$).

The clover shoot shows significant differences in carbon content, due to a hypoxic condition, but, the difference is not significant for the salinity effect. The carbon content of the clover shoots in a hypoxic condition (14 days) was 8.9% lower, compared to those in the aerated pots. In contrast, the waterlogged roots of the clover had 5.1% more carbon content than those under aerated conditions.

The *P. ciliata* grown in the winter (Table 3) had a lower carbon content in the shoots than in the plants that were grown in the summer. This is true for aerated or waterlogged treatments. In contrast, the roots had a higher total carbon content.

Not only did the amount of carbon content change in different seasons in *P. ciliata*, but also, the stage of growth affected carbon content. The carbon content of the shoots of *P. ciliata* grown in winter was 42.2% DW at an early stage of growth (day 30) which, then, reduced to an average of 39.5% DW, due to salinity, and which, finally, reached an average of 42.5% DW in aerated pots at day 60. However, root carbon content at the early stage of growth was lower (35.6% of DW) and which, then, increased by application of NaCl and/or waterlogging to almost 40% DW.

Discrimination Against $^{13}CO_2$

The overall mean δ^{13}C of the two species grown in summer was -28.82 and -26.73 $^{0}/_{00}$ for *P. ciliata* and B. clover, respectively, which means that less discrimination (by 7.2%) occurred in B. clover against

Table 1. Carbon content (% dry weight) for *Puccinellia ciliata* shoots and roots grown in summer in controlled conditions (temperature: day 22 ± 2 and night 18 ± 2°C). Waterlogging treatments were aerated, 10 days of hypoxia plus 10 days of recovery and 20 days of hypoxia.

	Shoot			**Root**		
g NaCl kg ^{1} Soil	**Aerated**	**Waterlogged**		**Aerated**	**Waterlogged**	
		10 Days	**20 Days**		**10 Days**	**20 Days**
0	43.64	43.64	43.63	37.24	39.29	37.62
0.87	43.06	42.83	42.69	35.73	37.59	36.18
1.75	44.75	42.80	42.67	37.40	36.85	35.73
3.51	45.07	42.82	44.71	34.22	39.13	38.55

Table 2. Carbon content (% dry weight) of the Balansa clover shoots and roots grown in summer under controlled conditions (temperature: day 22 ± 2 and night 18 ± 2°C). Waterlogging treatments were aerated, 7 days hypoxia plus 7 days of recovery and 20 days of hypoxia.

	Shoot			**Root**		
g NaCl kg ^{1} Soil	**Aerated**	**Waterlogged**		**Aerated**	**Waterlogged**	
		7 Days	**14 Days**		**7 Days**	**14 Days**
0	43.79	39.26	41.52	36.26	37.45	38.46
0.58	42.68	41.92	41.94	35.14	35.52	37.61
1.17	43.59	40.20	39.32	38.24	40.36	39.75
2.34	43.51	36.81	35.31	37.58	37.83	38.88

Table 3. Carbon content (% dry weight) for *Puccinellia ciliata* shoots and roots grown in winter in glasshouse under controlled conditions (temperature: day 22 ± 2 and night 18 ± 2°C). Waterlogging treatments were aerated, 10 days of hypoxia plus 10 days of recovery and 20 days of hypoxia.

	Shoot				**Root**			
g NaCl kg ^{1} Soil	**Day 30***	**Day 50***	**Day 60***		**Day 30**	**Day 50**	**Day 60**	
			Aerated	**10 Days**			**Aerated**	**10 Days**
0	42.21	39.80	43.38	40.29	35.56	39.47	40.64	38.90
1.17		40.28	45.09	39.45		40.03	41.06	39.07
2.34		38.68	41.52	40.43		40.68	40.22	38.52
3.51		39.10	41.62	39.43		40.65	41.80	38.67
4.67			41.04	40.58			41.19	39.55

* Day 30: Salinization started; day 50: Waterlogging started and day 60: The first 10 days of waterlogging terminated.

$^{13}CO_2$, compared to *P. ciliata* (Tables 4 and 5). The difference is statistically significant ($p =< 0.001$) and might be due to less tolerance of B. clover to salinity and waterlogging compared to *P. ciliata*. Lichtfouse et al. [10] reported that grasses growing near a major highway in Paris, France, have strikingly depleted $\delta^{13}C$ values, averaging -35.08 $^{0}/_{00}$, versus rural grasses that show an average of -30.59 $^{0}/_{00}$.

The shoots and roots of each species also have different $\delta^{13}C$ values. The difference in the mean values of $\delta^{13}C$ of the shoots and roots for both species are statistically significant ($p =< 0.001$). Not only are the $\delta^{13}C$ values of plant parts different in different species, but also, the values of $\delta^{13}C$ in the roots are lower, compared to the shoots, by almost 2%. When a linear regression was plotted for shoot $\delta^{13}C$ values vs. root $\delta^{13}C$ values (Figure 1), the result indicated that a linear relation exists between root $\delta^{13}C$ and shoot $\delta^{13}C$ ($R = 0.968$). In other words, as the plant leaves absorbed more $^{13}CO_2$, more of this component was expected in the root, however the rate of discharge into the roots is less compared to the shoots in different species under different conditions. Guy and Reid [8] reported that the roots were about $1^{0}/_{00}$ more positive than shoots in *P. nuttalliana*. Kao and Tsai [20] indicated that, regardless of water treatment, the leaves of *Glycine soja* always had significantly more negative $\delta^{13}C$ values than those of *G. tomentella* and *G. tabacina* ($p < 0.05$). However, there is no significant difference between *G. tomentella* and *G. tabacina* in $\delta^{13}C$ values, during

Table 4. Mean δ^{13}C ($^{0}/_{00}$) values for *Puccinellia ciliata* shoots and roots grown in summer in glasshouse under controlled temperature (18 ± 2 night and 22 ± 2 day) and humidity. Waterlogging treatments were aerated, 7 days hypoxia plus 7 days of recovery and 20 days of hypoxia.

	Shoot			**Root**		
g NaCl kg ^{1} Soil	**Aerated**	**Hypoxia**		**Aerated**	**Hypoxia**	
		10 Days	**20 Days**		**10 Days**	**20 Days**
0	-29.32	-29.33	-29.72	-28.58	-28.68	-29.24
0.78	-29.12	-29.34	-29.62	-28.69	-28.51	-28.83
1.5	-29.08	-28.85	-29.60	-28.12	-28.33	-28.69
3.0	-28.55	-28.79	-28.59	-27.78	-27.91	-27.93

Table 5. Mean δ^{13}C ($^{0}/_{00}$) values for Balansa clover shoots and roots grown in summer in glasshouse under controlled temperature (18 ± 2 night and 22 ± 2 day) and humidity. Waterlogging treatments were aerated, 7 days hypoxia plus 7 days of recovery and 20 days of hypoxia.

	Shoot			**Root**		
g NaCl kg ^{1} Soil	**Aerated**	**Hypoxia**		**Aerated**	**Hypoxia**	
		7 Days	**14 Days**		**7 Days**	**14 Days**
0	-27.92	-27.41	-27.11	-27.20	-27.02	-26.86
0.68	-27.47	-27.01	-26.88	-26.82	-26.73	-26.37
1.32	-26.46	-26.65	-26.48	-26.23	-25.95	-26.33
2.5	-26.06	-26.02	-26.24	-26.08	-25.83	-25.77

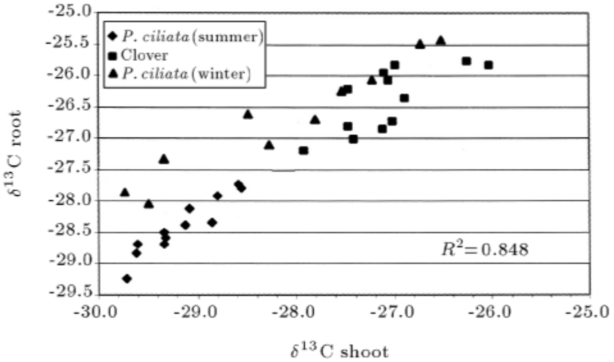

Figure 1. The relation between δ^{13}C ($^{0}/_{00}$) of the root versus shoot in two species (*Puccinellia ciliata* and Balansa clover) grown in different seasons under controlled conditions (temperature: Day 22 ± 2 and night 18 ± 2°C).

either water treatment ($p => 0.05$).

The δ^{13}C values of shoots and roots in each species were affected significantly by salinity and waterlogging. As salinity increases, the discrimination against $^{13}CO_2$ decreases, but, with different severity for different species. The difference between the overall mean of the δ^{13}C values of the shoot and root of B. clover is statistically significant ($p =< 0.001$) and affected (more positive), due to salinity, by 3.13% and 4.07%, respectively. These differences were less (more discrimination) in *P. ciliata* shoots and roots ($p = 0.01$) compared to B. clover, due to salinity, by 2.6% and 2.8%, respectively. All of the above differences are statistically significant ($p =< 0.001$).

It has been reported that *P. ciliata* is highly tolerant to salinity (can tolerate sea water salinity) and, therefore, it can be concluded that there was less salinity stress for this species, due to the application of 3.5 g NaCl Kg ^{1} soil (almost 24 dS/m in waterlogged pots). Guy and Reid [8] also reported that, at typical salinities, δ^{13}C changed linearly with salinity in *P. nuttaliana*, however, trends in δ^{13}C with salinities were the same in all plant parts. Poss et al. [11] proposed stable carbon isotope discrimination as an indicator of cumulative salinity and boron stress in *Eucalyptus camaldulensis*.

The waterlogged condition affects the two species differently. When the δ^{13}C values for waterlogged pots (14 or 20 days) were compared with those for aerated conditions, the *P. ciliata* showed more discrimination against $^{13}CO_2$ under waterlogged conditions (more negative δ^{13}C), than B. clover which, in contrast, showed less discrimination against $^{13}CO_2$ under the same conditions. It can be concluded that the plants, which are less sensitive to waterlogged conditions, may have more discrimination against $^{13}CO_2$. *P. ciliata* grown in waterlogged conditions had higher dry matter production compared to that of aerated pots, however, B. clover's dry matter was less in waterlogged conditions, compared to that of aerated pots. There are different conclusions related to water limitation in plant response. However, plants grown under water deficit behave differently in regard to water use efficiency and δ^{13}C values [12,13,20-24].

Comparison between the δ^{13}C values of *P. ciliata* grown at different seasons (summer, -28.65 and winter, -26.95 $^{0}/_{00}$) under aerated conditions (Tables 4 and 6) shows that the difference (5.93%), due to different seasons, is also statistically significant. The important point is that, although δ^{13}C values for the control pots (aerated and non-saline) are the same in different seasons, the values of δ^{13}C belonging to different levels of salinity or waterlogging, either in shoots or roots, in winter, are more positive (less discrimination against $^{13}CO_2$) compared to summer. Consistently, despite "identical" growth conditions, values were more positive in the winter than in the summer. This is an indication of the effect of environmental factors, such as daylight and amount of radiation etc. rather than temperature and relative humidity. This conclusion is not the same as indicated for *P. nuttalliana* [8].

As salinity increases from 0 to 4.67 g NaCl per kg soil, the discrimination against $^{13}CO_2$ severity decreased in the aerated pots (9.39% in shoot, 5.26% in root) compared to summer (decreased by 0.77% in shoot, 2.8% in root). This means that one may have an interaction of season (temperature, day length, etc) and salinity. This is one of the differences in the behavior of *P. ciliate*, with regard to the summer or winter season. However, in plants grown under controlled conditions, the dry matter production in winter is lower compared to summer.

Although the difference in season has a significant affect on $^{13}CO_2$ discrimination, the stage of growth also has a significant effect on δ^{13}C values. At an early stage of growth (day 30), the δ^{13}C values of the shoot and root of *P. ciliata* in the aerated pots, which were -30.09 and -28.5 $^{0}/_{00}$, respectively, changed to -28.25 and -26.62 $^{0}/_{00}$ (day 50) and, then, again, changed to -29.49 and -28.04 $^{0}/_{00}$. These changes indicated that δ^{13}C varies due to changes in environment factors. These variations, due to environmental factors, especially temperature, have been reported by [8] for *P. nuttalliana.*

When δ^{13}C values of the roots or shoots of *P. ciliata* were correlated linearly vs. plant parts, such as shoot, root, total dry weight and shoot to root ratio, the results indicated (Table 7 and Figure 2) that the shoot's δ^{13}C values linearly correlated ($R = 0.749$) with the shoot and root dry weight in *P. ciliata.* This is the same for the roots of *P. ciliata* (root's δ^{13}C values correlated only with shoot dry weight).

The linear regressions for shoot or root's δ^{13}C values for B. clover vs. plant parts (Table 8) indicated that shoot's δ^{13}C values were linearly correlated significantly with plant part indexes ($p =< 0.001$). In contrast, none of the linear regressions for the root δ^{13}C values and other plant parts were statistically significant. This might be an indication of less discrimination

Table 6. Mean δ^{13}C ($^{0}/_{00}$) values for *Puccinellia ciliata* shoots and roots grown in winter in glasshouse under controlled condition temperature (18 ± 2 night and 22 ± 2 day). Waterlogging treatments were aerated, 7 days hypoxia plus 7 days of recovery and 20 days of hypoxia.

	Shoot				Root			
g NaCl kg ^{1} Soil	**Day 30***	**Day 50***	**Day 60***		**Day 30**	**Day 50**	**Day 60**	
			Aerated	**10 Days**			**Aerated**	**10 Days**
0	-30.09	-28.25	-29.49	-29.73	-28.5	-26.62	-28.04	-27.86
1.17		-27.56	-27.81	-29.34		-26.16	-26.69	-27.33
2.34		-26.62	-27.23	-28.27		-25.87	-26.05	-28.61
3.51		-26.47	-26.52	-28.49		-25.47	-25.43	-26.10
4.67		-26.25	-26.73	-27.53		-25.22	-25.48	26.25

* Day 30: Salinization started, day 50: Waterlogging started and day 60: The first 10 days of waterlogging terminated.

Table 7. Linear regression analysis for *Puccinellia ciliata* δ^{13}C of the shoot and root vs. plant index.

δ^{13}C ($^{0}/_{00}$) of the Shoot vs. Plant Index			**δ^{13}C ($^{0}/_{00}$) of the Root vs. Plant Index**		
Plant Index	***R***	***P***	**Plant Index**	***R***	***P***
Shoot dry weight	0.75^{*}	0.050	Shoot dry weight	0.93^{*}	0.001
Root dry weight	0.25^{ns}	0.435	Root dry weight	0.28^{ns}	0.380
Total dry weight	0.26^{ns}	0.413	Total dry weight	0.35^{ns}	0.264
Shoot: root ratio	0.36^{ns}	0.255	Shoot: Root ratio	0.46^{ns}	0.133

Table 8. Linear regression analysis for B. clover δ^{13}C of the shoot and root vs. plant index.

δ^{13}C ($^0/_{00}$) of the Shoot vs. Plant Index			**δ^{13}C ($^0/_{00}$) of the Root vs. Plant Index**		
Plant Index	***R***	***P***	**Plant Index**	***R***	***P***
Shoot dry weight	0.897^{**}	0.001	Shoot dry weight	0.67^{ns}	0.017
Root dry weight	0.827^{**}	0.001	Root dry weight	0.59^{ns}	0.043
Total dry weight	0.885^{**}	0.001	Total dry weight	0.65^{ns}	0.210
Shoot: Root ratio	0.827^{**}	0.001	Shoot: Root ratio	0.61^{ns}	0.360

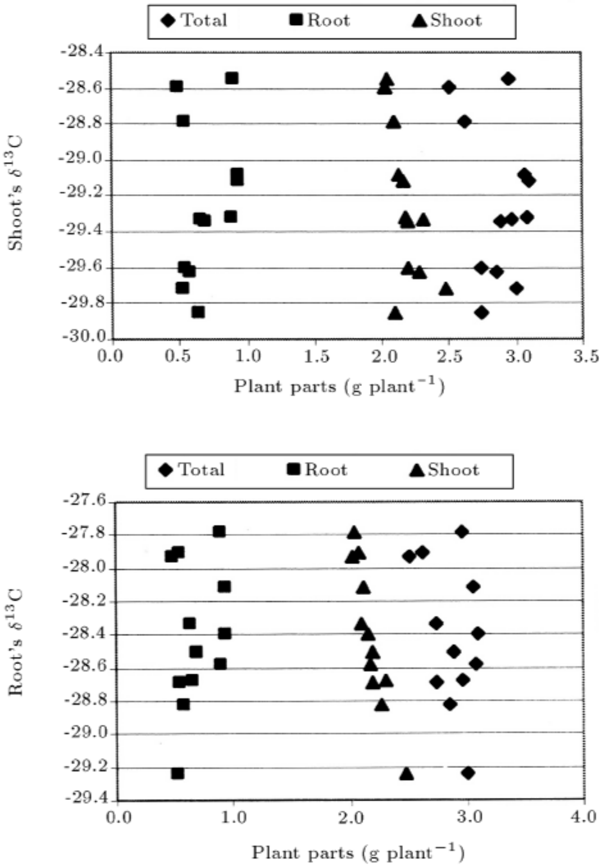

Figure 2. δ^{13}C ($^0/_{00}$) values of *P. ciliata* shoots and the roots vs. plant dry matter (g plant ^{1}).

against $^{13}CO_2$ and less allocation of $^{13}CO_2$ into the roots of B. clover.

CONCLUSION

Although the mangroves *Avicennia marina* and *Aegiceras corniculatum* do not show a clear δ^{13}C response to chronic salinity stress [5], *P. ciliata* and B. clover presented different δ^{13}C values under saline or waterlogged conditions. The authors results are similar to other reports, which indicate changes in δ^{13}C for different species under different environmental conditions [8,10,25-29].

The CO_2 is absorbed by leaves, synthesized and, then, the product moves to stems and roots. Therefore, any type of CO_2 absorbed by the leaves may stay in the shoots or move to the roots. Regarding the rate of photosynthesis, the allocation of photosynthetic products into roots or shoots may be different for ^{12}C and ^{13}C components. This would create different δ^{13}C values for the roots and shoots of plant species (Figure 3). In this experiment, the allocation of the C-isotope into roots and shoots were different in *P. ciliate* and B. clover, however, *P. ciliate* moves less ^{13}C-compounds into roots compared to B. clover.

Although the depression of growth by NaCl was similar for the two species, B. clover displayed a greater shift in δ^{13}C, relative to controls. The difference can be concluded on the basis of less tolerance of B. clover to salinity compared to *P. ciliata*. If this is the case, one may expect lower changes in δ^{13}C values (less discrimination) of the species, which is less tolerant to saltiness.

At a high concentration of NaCl, Na^+ can displace Ca^{2+} from the plasma membrane of root hair (e.g. cotton), resulting in a net positive charge in plasma membrane permeability that can be detected as a leakage of K^+ from the cell [30]. Under non saline conditions, the cytosol of higher-plant cells contains 100 to 200 mM K^+ and 1 to 10 mM Na^+, an ionic environment, in which many enzymes function optimally. An abnormally high ratio of Na^+ to K^+ and high concentrations of total salts and inactivated

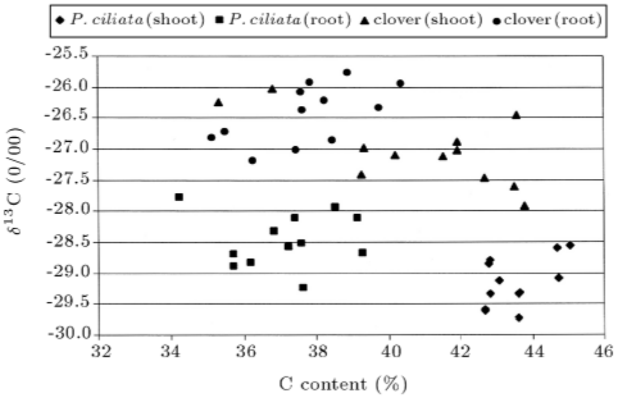

Figure 3. Relation between C content (%) and δ^{13}C ($^0/_{00}$) of the shoots and roots of two species (*Puccinellia ciliate* and Balansa clover.

enzymes may inhibit protein synthesis [30]. Change in Na content of leaves and, also, the high Na: K ratio of the shoot of B. clover compared to *P. ciliata* can be the reason for optimal enzyme activity, such as ribulosebisphosphate carboxylase and, also, the diffusion gradient of CO_2 into the leaf [5].

Salinity is reported that has an effect on δ^{13}C values. In *Phaseolus vulgaris* L., salinity changes the δ^{13}C, due to stomata conductivity, diffusion limitation and activity of ribulosebisphosphate carboxylase [4,31]. Poss et al. [29] proposed to use δ^{13}C values for evaluation of the salinity effect in *Eucalyptus camaldulensis.* Poss et al. also reported that salinity decreases the discrimination against $^{13}CO_2$. However, different cultivars of *Pistashio* behave differently, relating to irrigation with different salinity, some cultivars discriminate less against $^{13}CO_2$ compared to others [32]. Poss et al. also reported that differences due to the time of measurement are not statistically significant. Although roots and shoots discriminate differently against $^{13}CO_2$, in some cultivars, the difference in discrimination rates is not significant.

Waterlogged conditions affect the δ^{13}C values of different species, differently. B. clover is less tolerant to waterlogged conditions and, therefore, the values of δ^{13}C are more positive (less discrimination against $^{13}CO_2$). However, *P. ciliata*, which are not sensitive to waterlogged conditions, presented more negative values. This may be because of an increase in stomata conductance of *P. ciliata.* The magnitude of the CO_2 diffusion gradient can be increased by either a lower stomata conductance or a greater capacity for carboxylation (or any combination of the two). More positive salt-induced shifts in δ^{13}C values are largely due to decreased stomata conductance and, under waterlogged conditions, are largely because of increases in stomata conductance.

When the supply of oxygen is insufficient for aerobic respiration, roots begin to ferment pyruvate (formed in glycolysis) and to lactate through the action of lactate dehydrogenase (LDH). The accumulation of lactic acid lowers the cellular pH. As the intercellular pH drops, fermentation switches from the production of lactic acid to ethanal because of the different pH optima of the cytosolic enzymes involved [30]. In healthy cells, the vacuole contents are more acidic (pH 5.8) than the cytoplasm (pH 7.4). But, under conditions of extreme O_2 deficiency, protons gradually leak from the vacuole into the cytoplasm, adding to the acidity generated in the initial burst of lactic acid fermentation.

The difference in δ^{13}C values at different stages of growth indicated that *P. ciliata*, at an early stage of growth and under no stresses, such as salinity or hypoxia, discriminates more against $^{13}CO_2$. Later, the effect of different stresses caused higher values. Poss et al. [30] also reported the same results.

ACKNOWLEDGMENTS

This work was supported by the University of Esfahan, Iran, and the University of Western Australia, Australia. I would like to thank the Laboratory of UWA for the analysis of Carbon Isotopes in plant samples.

REFERENCES

1. Farquhar, G.D. and Richards, R.A. "Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes", *Aust. J. Plant Physiol.*, **11**(6), pp 539-552 (1984).
2. Craig, H. "Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide", *Geochem. Acta.*, **12**(1&2), pp 133-149 (1957).
3. Ehleringer, J.R. "Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans", *Plant Physiol.*, **93**, pp 1422-1425 (1990).
4. Zhang, J.W. and Marshall, J.D. "Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga Menziesii* and *Pinus Ponderosa* in different environments", *Functional Ecology*, **9**(3), pp 410-412 (1995).
5. Farquhar, G.D., O'Leary, M.H. and Berry, J.A. "On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in the leave", *Aust. J. Plant Physiol.*, **9**(2), pp 121-137 (1982).
6. Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T. "Carbon isotope discrimination and photosynthesis", *Ann. Rev. Plant Physiol.*, **40**, pp 503-537 (1989).
7. Hubick, K.T., Farquhar, G.D. and Shorte, R. "Correlation between water use efficiency and carbon isotope discrimination in diverse Peanut (Arachis) germplasm", *Aust. J. Plant Physiol.*, **13**(6), pp 803-816 (1986).
8. Guy, R.D. and Reid, D.M. "Factors affecting $^{13}C/^{12}C$ ratios of inland halophytes. I. Controlled studies on growth and isotopic composition of Puccinellia Nuttalliana", *Can. J. Bot.*, **64**(11), pp 2693-2699 (1986).
9. Cousins, A.B., Badger, M.R., von Caemmerer, S. "Carbonic anhydrase and its influence on carbon isotope discrimination during C_4 photosynthesis. Insights from antisense RNA in flaveria bidentis", *Plant Physiol.*, **141**(1), pp 232-242 (2006).
10. Lichtfouse, E.M., Lichtfouse, M. and Jaffrezic, A. "Delta^{13}C values of grasses as a novel indicator of pollution by fossil-fuel-derived greenhouse gas CO_2 in urban area", *Environ. Sci. Technol.*, **37**(1), pp 87-89 (2003).

11. Poss, J.A., Gratton, S.R., Suarez, D.L. and Grieve, C.M. "Stable carbon isotope discrimination: An indicator of cumulative salinity and boron stress in *Eucalyptus Camaldulensis*", *Tree Physiol.*, **20**(16), pp 1121-1127 (2000).

12. Warren, C.R., McGrath, J.F. and Adams, M.A. "Water availability and carbon isotope discrimination in conifer", *Oecologia*, **127**(4), pp 476-486 (2001).

13. Wright, G.C., Nageswara Rao, R.C. and Farquhar, G.D. "Water-use-efficiency and carbon isotope discrimination in peanut under water deficit conditions", *Crop. Sci.*, **34**(1), pp 92-97 (1994).

14. Zhang, J.W., Feng, Z., Cregg, B.M. and Schumann, C.M. "Carbon isotopic composition, gas exchange and growth of three populations of ponderosa pine differing in drought tolerance", *Tree Physiology*, **17**(7), pp 461-466 (1997).

15. Guy, R.D., Reid, D.M. and Krouse, H.R. "Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions", *Oecologia*, **44**(2), pp 241-247 (1980).

16. Neales, T.F., Fraser, M.S. and Roksandic, Z. "Carbon isotope composition of the halophyte disphyma clavellatum (Haw), chinnock (Aizoceae), as affected by salinity", *Aust. J. Plant Physiol.*, **10**(5), pp 437-444 (1983).

17. Downton, W.J.S, Grant, W.J.R. and Robinson, S.P. "Photosynthetic and stomata responses of spinach leaves to salt stress", *Plant Physiol.*, **77**, pp 85-88 (1985).

18. Rogers, M.E., Craig, A.D., Munns, R.E., Colmer, T.D., Nichols, P.G.H., Malcolm, C.V., Barrett-Lennard, E.G., Brown, A.J, Semple, W.S., Evans, P.M., Cowley, K., Hughes, S.J., Snowball, R., Bennett, S.J., Sweeney, G.C., Dear, B.S. and Ewing, M.A. "Corrigendum to: The potential for development of fodder plants for the salt-affected areas of southern and eastern Australia", *Aust. J. Expt. Agric.*, **46**(12), pp 1665-1665 (2003).

19. Brugnoli, E., Hubick, K.T., Caemmerer, S. von, Wong, S.C. and Farquhar, G.D. "Correlation between carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressure of carbon dioxide", *Plant Physiol.*, **88**, pp 1418-1424 (1988).

20. Kao, W.Y. and Tsai, T.T. "Tropic leaf movements, photosynthetic gas exchange, leaf delta 13C and chlorophyll a fluorescence of three soybean species in response to water availability", *Plant, Cell Environ.*, **21**(10), pp 1055-1064 (1998).

21. Condon, A.G., Richards, R.A. and Farquhar, G.D. "The effect of variation in soil water availability, vapor pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat", *Aust. J. Agric. Res.*, **43**(5), pp 935-947 (1992).

22. Hall, A.E., Ismail, A.M. and Menendez, C.M. "Implications for plant breeding of genotypic and drought induced differences in water-use-efficiency, carbon isotope discrimination, and gas exchange", In *Stable Isotopes and Plant Carbon-Water Relations*, Ehleringer, Jr Hall, A.E. and Farquhar, G.D., Eds., Academic press, San Diego, CA, USA (1993).

23. Ismail, A.M., Hall, A.E. and Bray, E.A. "Drought and pot size effects on transpiration efficiency and carbon isotope discrimination of cowpea accessions and hybrids", *Aust. Plant Physiol.*, **21**(1), pp 23-35 (1994).

24. Livingston, N.J. and Spittlehouse, D.L. "Carbon isotope fractionation in tree rings early and late wood in relation to the intra-growing season water balance", *Plant, Cell Environ.*, **19**(6), pp 768-774 (1996).

25. Ansari, R., Naqvi, M.S., Khanzada, N.A. and Hubick, K.T. "Carbon-isotope discrimination in wheat under saline conditions", *Pak. J. Bot.*, **30**, pp 87-93 (1998).

26. Arndt, S.K. and Wanek, W. "Use of decreasing foliar carbon isotope discrimination during water limitation as a carbon tracer to study whole plant carbon allocation", *Plant, Cell Environ.*, **25**(5), pp 609-616 (2002).

27. Condon, A.G., Farquhar, G.D. and Richards, R.A. "Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat leaf gas exchange and whole plant studies", *Aust. J. Plant Physiol.*, **17**(1), pp 9-22 (1990).

28. Francy, R.J., Gifford, R.M., Sharkey, T.D. and Weir, B. "Physiological influences on carbon isotope discrimination in huon pine (*lagarostrobos Franklinii*)", *Oecologia*, **66**(2), pp 211-218 (1985).

29. Poss, J.A., Suarez, C.M., Grieve, C.M., Shannon, C.M. and Gratton, S.R. "Carbon, isotope discrimination and transpiration efficiency in eucalyptus under salinity and boron stress", *Acta Horticult.*, **537**, pp 215-222 (2000).

30. Taiz, L. and Zeiger, E., *Plant Physiology*, Sinauer Ass. Inc: Sounderland (1998).

31. Peuke, A.D., Glaab, J., Kaiser, W.M. and Jeschke, W.D. "The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L.; IV. Flow and metabolism of nitroganic nitrogen and malate depending on nitrogen nutrition and salt treatment", *J. Exp. Bot.*, **47**(3), pp 377-385 (1996).

32. Hokmabadi, H. "Response of different pistachio rootstocks to sodium chloride and boron excess in irrigation water", *PhD Thesis*, University of Tarbiat Modaress, Tehran, Iran (2003).