Effect of Salinity on Carbon Isotope Discrimination of Shoot and Root of Four Sugar Beet (Beta vulgaris L.) Cultivars

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ABSTRACT

A factorial pot experiment based on randomized complete blocks design with four replications was carried out in order to evaluate salt tolerance of four sugar beet cultivars including Madison (British origin) and three Iranian cultivars (7233-P12, 7233-P21 and 7233-P29) using carbon isotope discrimination ($\Delta$). Plants were grown in sand culture medium in greenhouse conditions. Sugar beet cultivars were irrigated with saline water (tap water as control, 50, 150, 250 and 350 mM of NaCl and CaCl$_2$ in 5:1 molar ratio) from 4-leaf stage for 16 weeks. Carbon isotope discrimination significantly decreased with increasing salinity. Significant differences of $\Delta$ between shoot and root were observed in all cultivars and all levels of salinity. Madison cv. showed lower $\Delta$ in shoot and root than the other three cultivars at all levels of salinity except the control, but cv. 7233-P29 had significantly higher $\Delta$ values at saline conditions of 150 mM and above. Although the regression of $\Delta$ and $C_i/C_a$ was positive, the slope of regression line was different from the pattern predicted by theory, indicating an underestimation of $\Delta$ in these samples. Relatively higher $^{13}$C (lower $\Delta$) was found in root as compared with shoots.

Keywords: Carbon/ Nitrogen ratio, Photosynthesis, Salt stress, Stomatal conductance, Water use efficiency.

INTRODUCTION

Soil salinity is a major abiotic stress affecting plant growth and productivity, especially in arid and semi-arid areas such as Iran. Therefore, breeding for salinity tolerance can contribute significantly to crop yield in salt-affected areas.

Most plants exposed to salinity show less CO$_2$ uptake by their leaves than the same plants not exposed to salinity. The photosynthetic capacity of plants grown under saline conditions is depressed depending on type of salinity, duration of treatment, species and plant age (Hester et al., 2001; Dadkhah, 2011). Many studies have concluded that the reduction in photosynthesis in response to salinity is to some extent the result of reduced stomatal conductance and, consequently, restriction of the availability of CO$_2$ for carboxylation (Dadkhah and Griffiths 2004; Kafi et al., 2007). During the fixation of carbon by photosynthesis, the naturally occurring stable isotope $^{13}$C is discriminated against, because of fractionation of carbon stable isotopes ($^{12}$C and $^{13}$C) mainly by Rubisco. Plants, therefore, contain a lower ratio of $^{13}$C to $^{12}$C than the air that supplies them (Farquhar et al., 1989).

The most general model describing carbon isotope fractionation during photosynthesis in C$_3$ plants assumes that the major components contributing to the overall fractionation is the differential diffusion of CO$_2$ containing $^{12}$C and $^{13}$C across the stomatal pathway and the fractionation by Rubisco. According to Farquhar et al. (1982), when stomatal conductance is small in relation to the capacity for CO$_2$ fixation, intercellular partial pressure of CO$_2$ (C$_i$) is

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also small and carbon isotope discrimination (Δ) tends toward 4.4‰ (a). When conductance is comparatively large, \( C_i \) approaches atmospheric CO\(_2\) partial pressure (\( C_a \)) and Δ approaches net fractionation (b) between 27 to 30‰. Farquhar et al. (1982) suggested the following expression for Δ in leaves of \( C_3 \) plants:

\[
\Delta = \alpha + (b - \alpha) \frac{C_i}{C_a}
\]  

(1)

Since the carbon incorporated in leaves is assimilated over a considerable time and under a range of environmental conditions, measuring Δ provides a long-term average estimate of \( C_i/C_a \), and, therefore, is a long term indicator of plant metabolism.

Guy et al. (1986) found that increased salinity decreased Δ in Puccinella maritima and \( C_i/C_a \) was low as expected from theory. This discrimination will be least in those plants that have the greatest water use efficiency. A linear increase in soil salinity produced significant (\( P \leq 0.05 \)) and linear decrease in crop yield and Δ, suggesting that Δ is a useful indicator of yield potential in crop plants (Qingzhen et al., 2006). Farquhar et al. (1989) demonstrated that because of partial stomatal closure due to salinity, \( C_i/C_a \) might decrease with increasing stress. Therefore, based on Equation 1, Δ decreases with increase in salinity. Brugnoli and Lauteri (1991) reported that in both a salt tolerant (Gossypium hirsutum L.) and a salt sensitive species (Phaseolus vulgaris L.) Δ decreased with increasing salinity. However, Δ showed a more pronounced salt-induced decline in Phaseolus than Gossypium.

Assessment of the possibility of using carbon isotope discrimination as an alternative method for selecting sugar beet material might be time saving and beneficial. Therefore, the aims of present study were to investigate the effect of salinity on Δ of shoots and roots of four sugar beet cultivars and evaluate the relationships between Δ, photosynthesis, dry matter and different plant parts.

Relationships between stomatal conductance (gs), photosynthesis (A) and intercellular CO\(_2\) partial pressure in different salt treatments and cultivars were also studied.

**MATERIALS AND METHODS**

Four sugar beet cultivars differing in their salt sensitivity (Madison of British origin and Iranian cultivars, 7233-P\(_{12}\), 7233-P\(_{21}\) and 7233-P\(_{26}\)) were grown in greenhouse conditions. Five levels of salinity (0, 50, 150, 250, and 350 mol m\(^{-3}\) of NaCl and CaCl\(_2\) in 5:1 molar ratio) were imposed. Samples were taken after 16 weeks of salt treatment. Four plants of each cultivar in each level of salt treatment were harvested. Samples were placed in freezer at -87°C and then freeze dried for 72 hours. The freeze dried samples were ground by mortar and pestle to pass a 2mm sieve. Ground samples of 1.0 to 1.5 mg were placed in a tin foil cap, taking care not to touch the foil during preparation. Carbon and nitrogen isotope composition (\( \delta^{13}C \) and \( \delta^{15}N \), respectively) of the shoot and root samples were analyzed in Europa Automated Nitrogen Carbon Analysis Solids/Liquids ANCA-SL System (Europe Ltd, Crewe, UK). Carbon isotope discrimination was calculated as follows:

\[
\Delta = \frac{\delta^{13}C - \delta^{15}N}{1 + \delta^{15}N}
\]  

(2)

Where, \( \delta^{13}C \) and \( \delta^{15}N \) are the carbon isotope composition of source air and plant material, respectively, relative to the international standard Pee Dee Belemnite (PDB) (Hubick et al., 1986). The values of \( \delta^{13}C \) and \( \delta^{15}N \) were measured as described by Hubick et al. (1986). Isotope compositions are generally expressed as parts per thousand, either (x\times10\(^{-3}\)) or with the symbol (%).

Total carbon and total nitrogen of shoots and roots were then analyzed using a PDZ Europa Automated Nitrogen Carbon Analysis-Solids/Liquid ANCA-SL system and calculated as mg g\(^{-1}\) dry weight. Carbon nitrogen ratio (C:N) was also calculated by dividing the total amount of carbon to nitrogen in each sample from measurements associated with nitrogen isotope. Intercellular and ambient CO\(_2\) and net photosynthesis were measured by Combined
Infra Red Gas Analysis System (CIRAS-1 portable photosynthesis system.
This study was designed as factorial pot experiment based on randomized complete blocks design with four replications. The data were subjected to balanced analysis of variance by Statistical Analysis System (SAS) Software for Windows version 6.12.

RESULTS

Isotope discrimination decreased with increasing salinity (Figure 1). The average shoot $\Delta$ of the four cultivars was $22.803 \times 10^{-3}$ and $19.82 \times 10^{-3}$ for the control and 350 mM salt, respectively. Madison cv. showed lower $\Delta$ in shoot than the other three cultivars at all levels of salinity except the control, but cultivar 7233-P29 had significantly higher $\Delta$ values at saline conditions of 150 mM and above.

Figure 2 shows the relationship between $\Delta$ in shoots and $C/C_\infty$. A positive correlation was observed between $C/C_\infty$ and $\Delta$ in shoots. Carbon isotope discrimination values of samples were much higher than the theoretical values calculated based on Equation 1, but the slope of the observed line was less than that of the theoretical line (Figure 2). Plants in the control conditions showed a higher $C/C_\infty$, indicating higher stomatal conductance in these plants.

Analysis showed that roots in all cultivars were isotopically heavier as compared with shoot. Thus, the discrimination was less in the roots than the shoots (Figure 1-b). Madison and 7233-P21 cultivars significantly had lower $\Delta$ in control conditions, while 7233-P29 and 7233-P12 had the highest $\Delta$.
under the same condition. At saline conditions, cv. 7233-P29 had significantly higher root $\Delta$ than the others. At high salt concentration (350 mM) the cultivars 7233-P29 and Madison had the highest and the lowest root $\Delta$, respectively, and their differences were significant. Results of regression showed that, at all levels of salinity, $\Delta$ in shoot was a good predictor for $\Delta$ in the root because the relationship between $\Delta$ in shoot and root was positively linear ($r^2 = 0.79$) (Figure 3). Dry matter accumulation decreased with increasing salinity (data not shown) as $\Delta$ did. Therefore, there was a positive correlation

**Figure 3.** Best fit relationship between shoot and root carbon isotope discrimination ($\Delta \times 10^3$) of sugar beet cultivars in the presence of different levels of salinity. Each point is the average of four replications.

**Figure 2.** Relationship between shoot carbon isotope discrimination ($\Delta \times 10^3$) and $C_i/C_a$ ratio of sugar beet cultivars in the presence of different levels of salinity. Closed symbol shows observed carbon discrimination and open symbol shows predicted carbon discrimination calculated from equation 1 with $\alpha = 4.4$‰ and $b = 29$‰. Each point is the average of four measurements.

between dry matter accumulation and $\Delta$ in the presence of salinity (Figure 4). The best correlation, a parabolic regression line, was plotted between total dry weight (TDW) and $\Delta$ in shoot.

The relationship between photosynthesis ($A$) and $\Delta$ is shown in Figure 5-a. Shoot $\Delta$ increased with increasing photosynthesis in all cultivars. However, there was a stronger correlation between photosynthesis and shoot $\Delta$ in cv. Madison ($r^2 = 0.95$) than the other cultivars. Cultivar 7233-P29 showed a weaker correlation between photosynthesis and $\Delta$ ($r^2 = 0.75$). The relationship between $\Delta$ and stomatal conductance ($g_s$) is shown in Figure 6. Shoot $\Delta$ increased with increasing $g_s$ in all cultivars. However, cv. Madison in control condition showed a higher $A$ and $g_s$ as well as higher $\Delta$ than the others. Intrinsic water use efficiency ($WUE_i$) was negatively correlated with $\Delta$ (Figure 7). A stronger correlation was seen between $WUE_i$ and shoot $\Delta$ in Iranian cultivars than in Madison (Figure 7-a).

Salinity increased shoot nitrogen concentration ($N$). Plants grown in control conditions had significantly lower $N$ concentration, while plants grown in the low level of salinity (50 mM) had significantly higher $N$ concentration compared to the controls (Figure 8-a). In control conditions, Madison had markedly higher shoot $N$-content than the other cultivars and the increase due to salinity was proportionately smaller than in the other cultivars. At 250 and 350 mM, the $N$-content of Madison was...
significantly lower than that at 150 and 50 mM, but not significantly different from the control. There were no significant differences in shoot N concentration between low (50 mM) and high (350 mM) levels of salinity in cultivars 7233-P29 and 7233-P12. At high level of salinity, cultivars 7233-P29 and Madison had significantly the highest and lowest N concentration in their shoots, respectively.

Root N concentration significantly increased with increasing salinity in all cultivars (Figure 8-b). Amongst sugar beet cultivars, Madison significantly had the lowest root N concentration at all levels of salt treatments.

Shoot C:N ratio decreased with increasing salinity (Figure 9-a). This reduction was not only because of increase in N content, but also decreased carbon accumulation in the shoot. In different sugar beet cultivars, C:N ratio had significant differences either in the control or salt stressed plants. Madison plants had significantly lower C:N ratio up to 250 mM of salinity, but not significant at the highest salinity, while cv. 7233-P12 generally had the highest shoot C:N ratio. Lower C:N ratio in Madison could be attributed to higher rates of photosynthesis (carbon) and lower nitrogen uptake by root.

C:N ratio of root also followed the same trend of C:N in the shoot (Figure 9-b). However, lower values of C:N ratio in shoots compared to that of roots could be due to storage of large amounts of
carbohydrate as sucrose in roots and lower N content in roots (Figure 9-b).

Root C:N ratio differences between levels of salinity became more pronounced with increasing salinity. The main reason for this is greater influence of salinity on photosynthetic capacity and transportation of carbohydrate to the roots at high levels of salt treatment.

**DISCUSSION**

Shoot and root carbon isotope discrimination ($\Delta$) decreased with increasing salt concentration. The cultivar Madison showed significantly lower $\Delta$ than the other cultivars under saline conditions but not in the control condition, and cv. 7233-P29 showed significantly higher $\Delta$ under salinity treatments. Although carbon isotope discrimination tended to be reduced by salinity, reduction in $\Delta$ was markedly greater at highest salinity (350 mM) than the other salinity concentrations. The relationship of photosynthesis ($A$), $\Delta$, and $g_s$ shows that one of the main causes of reduction in $A$ at saline conditions is stomatal conductance, which led to reduced discrimination (Farquhar et al., 1989; Matzner et al., 2001). The lower values of $\Delta$ in salt stressed plants in comparison with the control suggest that reduction in CO$_2$ partial pressure of the intercellular air spaces results from reduced stomatal conductance ($g_s$). This was confirmed by the positive correlation ($r^2 = 0.68$) between stomatal conductance and $\Delta$ in the presence of salinity. As expected from theory, a positive correlation was observed between shoot $\Delta$ and the average of $C_i/C_a$ measured by gas exchange. In shoots, the slope of the line based on $\Delta$ measured in samples was less than that of a line drawn based on Equation 1. The scattering of data might be partly attributed to the measurements of $C/C_a$ for a short period of photosynthesis, while the $\Delta$ values reflect carbon assimilation over a longer period (Kafi et al., 2007). In other words, although $C/C_a$ spanned a wide range of values (from 0.2 to 0.8 across all treatments), measured organic $\Delta$ did not decrease to the values predicted from gas exchange. This is because gas exchange measurements were taken at one point in the experimental...
program, but organic $\Delta$ integrates the history of the plant. Accordingly, if most structural carbon had been synthesized prior to any long-term effects of salinity, then organic $\Delta$ values would be higher than those predicted. Additionally, because the absolute rate of assimilation was reduced to such an extent by the salinity treatment, only a small amount of carbon was available for export or new growth. The results of this experiment are in agreement with Brugnoli and Lauteri (1991), who found a high correlation between $\Delta$ and $C/C_a$ in cotton leaves ($r^2 = 0.87$). In the current experiment, the correlation between $\Delta$ and $C/C_a$ was linear ($r^2 = 0.72$). Shoot $\Delta$ was negatively correlated with water use efficiency (A/E) measured by gas exchange. Variation in instantaneous water use efficiency (WUEi) can occur due to either changes in stomatal aperture or changes in assimilation rate (both of which can change $C_i$) (Matzner et al., 2001; Rajabi et al., 2009). If stomatal conductance had the greatest effect on $C_i$, then the expected change from the highest level of salinity to the control would be a decrease in A/E and increase in $C_i$ and $\Delta$. If, however, photosynthesis had the greatest effect on $C_i$, then, the expectation would be an increase in A/E and a decrease in $\Delta$ and $C_i$ from the highest stress to normal condition. In this study, plants grown in high salt concentration had lower $C_i$ and $\Delta$ values and greater WUEi values compared with controls. Therefore, the indications are that stomatal conductance ($g_s$) had a greater effect than photosynthesis ($A$) on $C_i$. Cultivar 7233-P29 had significantly higher $\Delta$. 

Figure 8. Nitrogen concentration in shoots (a) and roots (b) of sugar beet cultivars in the presence of different salt concentration. Each point is the average of four replications. Vertical lines are standard error of the means.

Figure 9. C/N in shoots and roots of sugar beet cultivars in the presence of different salt concentrations. Each point is the average of four measurements. Vertical lines are standard error of the means.
than Madison at high salinity levels. Cultivar 7233-P29 did not show a higher \( g_s \) and \( C_v \) than Madison at saline conditions. Therefore, the higher \( \Delta \) in cv. 7233-P29 under saline condition might be attributed to the ability of this cv. to re-use \( CO_2 \) produced by respiration because when stomata are closed due to water deficiency, there is less chance (almost no chance) for \( CO_2 \), produced by respiration, to escape to the atmosphere, thus, the plant re-uses this \( CO_2 \). However, this type of \( CO_2 \) is less enriched in \( ^{13}C \) because it has already passed through several physiological and biochemical processes that deplete its \( ^{13}C \) (Dadkhah and Griffiths, 2004).

There was relatively more \( ^{13}C \) in roots than in shoots. Variations in \( \Delta \) among different components within the plant have been reported by several authors in many species (Brugnoli and Farquhar, 2000; Kafi et al., 2007) and have been attributed to different causes including fractionation during secondary metabolism. In general, secondary products are depleted in \( ^{13}C \) relative to primary compounds and catabolic reactions prefer the ‘light’ molecules, while the ‘heavy’ ones are involved in biomass formation. Schmid and Gleixner (1998) studied the isotopic correlations in wood as a system (cellulose and lignin). They also reported that secondary products in plants were depleted by 3-6‰ relative to primary compounds from the same source. Accordingly, the \( ^{13}C \)-depletion in organic matter found in the present work could be caused by fractionation during non-photosynthetic processes. Indeed, the observed \( \delta^{13}C \) in leaf material integrates different discrimination steps occurring during photosynthesis, photorespiration, dark respiration, and during export and translocation of photosynthetic products. Another possible reason for declining \( \Delta \) in root material could be due to the time of producing carbohydrate for storage in the roots because, with time, \( \Delta \) decreases in the presence of salinity (Kafi et al., 2007). Therefore, as most carbohydrates accumulated in the roots are produced at the last stage of the plant’s life, this might cause the reduction of \( \Delta \) in the root.

Under saline conditions, either a decrease or an increase in nitrogen content of plants is observed compared to the plants grown under non-saline conditions (Joshi, 1987). In the halophytes and in many salt tolerant crop species, no significant effect of salinity on nitrogen content has been observed by some researchers (i.e. Pessarakli and Tucker, 1985; Alam, 1994). Dubey and Pessarakli (1995) suggests that the effect of salinity on N content depends on the plant species, the organs studied and the type of salinity. In the current study, N content of shoots and roots grown in saline conditions were significantly higher than N content of non-stressed plants.

Halophytic plants grown in saline medium generally show induced uptake and accumulation of \( NO_3^- \) to added N. These plants intrinsically accumulate higher amount of nitrogen compared to glycophytes under saline condition. For instance, plant species like Atriplex and Salicornia show higher uptake of \( NO_3^- \) in saline soil compared to non-saline conditions (Flowers et al., 1977). Higher nitrogen uptake shown by halophytes under saline conditions is related to their intrinsic tendency to accumulate compatible solutes many of which are nitrogen-containing amino acids and quaternary ammonium compounds (Flowers et al., 1977).

C:N ratio in both shoots and roots of sugar beet cultivars significantly decreased with increasing salinity. This could be due to increased N content and also a reduction in carbon because of adverse effects of salinity on photosynthesis, in saline conditions. C:N ratio is commonly used as an index of nitrogen status and can be utilized as a measure of adaptation to stress (Chapin, 1980). In this study, plants were grown at fixed nutrient levels from seedling stage and C:N ratio of shoot can, therefore, be considered as a measure of nitrogen use efficiency. High nitrogen use efficiency indicates that more carbon has been accumulated per unit nitrogen (Lissner et al., 1999). Reduction in C:N ratio with
increasing salinity in this study showed that nitrogen use efficiency declined.

Based on the above mentioned results and works reported previously, carbon isotope discrimination might be applicable, as a useful tool, for study of salinity tolerance of sugar beet cultivars. It shows a remarkable correlation with photosynthesis apparatus and it is responsive to salt stress in cultivars with different salt tolerance. Therefore, if the mass spectrometer system is available, the measurement of $\Delta$ is easy and quick. It needs dried samples of either leaf, stem, or root, with minimal problem with storage and handling of samples and analysis of carbon isotopes could be used in salinity studies of sugar beet genotypes.

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تأثیر نتیجه‌گیری بر تغییر کربن ایزوتوپی بخش‌های هواوی و ریشه چهار واریتی

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چکیده

به منظور ارزیابی تحلیل به‌صورت چهار واریتی گند قند به‌طور خصوصی، این پژوهش با استفاده از تکنیک تغییر در ایزوتوپی کربن (۱۸C) آزمایش صورت گرفت. فاکتورهای قابل طرح نشان دهنده کامیابی تغییرات ایزوتوپی کربن (۱۸C) شامل شرایط فاکتور و اثر گیاه‌ها چندر قند بر هر میزان کیفیت شده و در شرایط غلخانه‌ای گله‌داری شده. گیاهان چندر در محیط‌های چهار واریتی به مدت شانه‌های هفت هفته تحت شرایط رطوبتی شامل شاهد (آبی‌های با آب لوله کیسه‌های)، ۵۰، ۱۵۰ و ۲۵۰ میلی‌مول در مخلوط نمک‌های کلروژن سدیم و کلروژن کلسیم به‌صورت یکپارچه نمایید. با استفاده از اندازه‌گیری غلظه‌هایی شامل تغییرات کربن ایزوتوپی (۱۸C) در محیط وارد شده، مقدار Ci/Ca به‌طور متوسط و ریشه‌های شاهد در تاریک واریتی گریزند در محیط‌های دارای منابع که اگر به‌صورت مشابه با شاهد در محیط شاهد بود، مقدار Ci/Ca به‌صورت مشابه با شاهد در محیط شاهد بود.