

¹Department of Agricultural Engineering, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

²Department of Horticulture, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

Effects of water stress and constitutive expression of a drought induced chitinase gene on water-use efficiency and carbon isotope composition of strawberry

Mahmoud Raeini-Sarjaz^{1*} and Vida Chalavi²

(Received December 18, 2010)

Summary

Recombinant DNA technology and tracer technique are being widely used for different purposes. Plant genetic transformation is a common practice for increasing crop tolerance to biotic and abiotic stresses and stable isotopes is a good tool to track the effects of environmental stresses on crop productivity. In this research, the effect of water stress on plant carbon isotopic composition ($\delta^{13}C$) and water use efficiency (WUE), as indicators of physiological processes and environmental factors, were evaluated for transgenic strawberry (*Fragaria × ananassa*) plants continuously expressing a chitinase gene and non-transgenic plants. To implement water stress, strawberry plants were grown in pots and were assigned to three different soil water contents (SWC) of well-watered, moderately-watered and water-stressed. Treatments were implemented for two months, in a growth chamber. At the final stage of the experiment, leaves were randomly collected from each experimental unit. After oven-drying and powdering, subsamples of 4 to 5 mg of plant materials were combusted under vacuum, using Vycor tubes containing cupric oxide and silver wire. Combustion took place at 520 °C for 5 hrs to produce carbon dioxide. Carbon isotope composition ($\delta^{13}C$) was measured relative to Vienna Pee Dee Belmnte (VPDB). Both SWC and the expression of chitinase gene in transgenic strawberry plants significantly affected $\delta^{13}C$ and WUE ($p < 0.001$). Highly significant ($p < 0.01$), but negative correlations were found between $\delta^{13}C$ and plant dry matter components, such as lead dry matter ($r = -0.92$; $p = 0.009$) and total dry matter ($r = -0.92$; $p = 0.008$). High correlations were found between $\delta^{13}C$ and water use per plant ($r = -0.93$; $p = 0.006$), and between $\delta^{13}C$ and leaf area per plant ($r = -0.93$; $p = 0.006$).

Introduction

Plant growth and productivity are based on capturing carbon dioxide molecules, which occurs through stomata. As a result, high crop production requires high stomatal conductance over time. Stomatal conductance (g) to CO₂ and water vapour are markedly influenced by environmental factors. Therefore, any change on stomatal conductance affects CO₂ capture and fixation, and water loss. A key factor which represents plant productivity under stressful conditions is the ratio of assimilated CO₂ to transpired water through stomata, which is termed water-use efficiency (WUE). This value (WUE) increases by environmental stresses, such as water stress and drought (RAEINI-SARJAZ et al., 1998; RAEINI-SARJAZ and CHALAVI, 2008; MONNEVEUX et al., 2006), salt stress (SHAHEEN and HOOD-NOWOTNY, 2005; MCCREE and RICHARDSON, 1987), and elevated CO₂ (MJWARA and BOTHA, 1997). Consequently, under stress conditions, either biotic or abiotic, the ratio of assimilated CO₂ to transpired H₂O increases.

Water-use efficiency could be measured in different ways. The traditional and tedious way is based on measurements of total dry matter and transpired water for whole crop life cycle, which is time

consuming, and due to water evaporation from soil and neglecting root dry matter in WUE calculation, can be misleading (FISCHER and TURNER, 1978). The second method is based on instantaneous leaf gas exchange (WUE_i), which does not extend to the whole plant life cycle. The third method, plant tissue carbon stable isotopes ratio, is used as an indirect indicator for WUE and is derived from measurement of carbon stable composition ($\delta^{13}C$), which is an integration of instantaneous water-use efficiency for the whole plant life cycle (ZHANG et al., 2009). It has been demonstrated (BRUGNOLI and FARQUHAR, 2000) that plant carbon isotope composition ($\delta^{13}C$) or carbon isotope discrimination ($\Delta^{13}C$) (JONES, 2004) is a consistent marker of water-use efficiency in C₃ plants. A strong positive correlation between $\delta^{13}C$ and WUE (WRIGHT et al., 1988; SARANGA et al., 1999), and a strong negative correlation between $\Delta^{13}C$ and WUE (RAEINI-SARJAZ et al., 1998) has been reported.

The mechanism of stable isotope technique is based on fractionation against heavy carbon dioxide molecule (¹³CO₂) during photosynthesis process. The chemical properties of ¹²CO₂ and ¹³CO₂ are identical, but as the latter is 2.3% heavier, C₃ plants discriminate more against ¹³CO₂. The average abundance of the two naturally occurring carbon dioxide stable isotopes, ¹²CO₂ and ¹³CO₂, in the atmosphere is 98.9 and 1.1 percent, respectively (FARQUHAR et al., 1989), while, the abundance of ¹³C in plant tissues is always less than that of the atmosphere (RUNDLE et al., 1989). These differences in abundance between source (air) and sink (plant tissues) carbon isotope ratios are due to slower diffusion of ¹³CO₂ through leaf boundary layer and stomata, and the activity of ribulose biphosphate carboxylase (*Rubisco*) enzyme, which favours ¹²CO₂ during carbon fixation (FARQUHAR et al., 1989; RAJABI et al., 2009).

The range of discrimination against heavier molecule varies within plant kingdom. It is a function of photosynthetic pathway, plant genetic potential and environmental factors (FARQUHAR et al., 1989; PYANKOV et al., 2000; EL-SHARKAWY and DE TAFUR, 2007; RAEINI-SARJAZ et al., 1998). Plant response to environmental stresses includes an array of physiological and biochemical changes. Almost all of these changes are the result of alteration of gene expression in plants, including induction of a large number of genes. Many plant species express a similar set of genes and produce common proteins in response to environmental stresses. Therefore, plants produce novel proteins or accelerate the accumulation of some already existing proteins. The precise function of most of these proteins is generally unknown. There is an array of such proteins in almost all plant species. Chitinases are proteins of 25-35 KDs molecular weight and their expression are induced in higher plants by environmental and biological stresses. Low levels of chitinases present in healthy plants and their expression during hypocotyl, leaves, roots and seed development may indicate that these proteins play some roles in plant normal growth and development (ROBINSON et al., 1997). Chitinases are also assumed to be involved in drought tolerance (YU et al., 1997), in freezing tolerance (YEH et al., 2000; HILOVAARA et al., 1999), and as a defence mechanism against fungi (KARASUDA et al., 2003).

In the present study, we hypothesized that transgenic strawberry plants constitutively expressing a drought induced chitinase gene

* Corresponding author

may have improved tolerance to water stress. Therefore, the aims of this study were to evaluate the effects of soil water contents and a transgenic strawberry line producing a chitinase protein at high level on carbon isotope composition of strawberry leaves.

Materials and methods

To detect the effect of water stress on transgenic strawberry plants, carbon isotope composition ($\delta^{13}\text{C}$) of strawberry leaves were measured under three soil water contents (SWC). Strawberry plants (cv. Joliette) were grown in pots under three SWC. Well-water plants were initially watered to 100% field capacity (FC) and re-watered when SWC dropped to 80%, moderately-watered and water-stressed plants were initially watered to 80 and 50% FC, respectively, and re-watered when SWCs dropped to 60 and 30% FC, respectively. During the experiment plants were uniformly fertilized by a 20-20-20 NPK fertilizer each week. A completely randomized design with four replications was employed for each treatment.

Individual potted plants leaf area (LA), leaf dry matter (LDM), petiole dry matter (PDM), root dry matter (RDM), total dry matter (TDM) and water used per plant (WU) were measured. Water-use efficiency (WUE) was calculated as total dry matter to total transpired water (WU). Leaves of different water treatments of strawberry plants were oven dried for 72 hrs at 60 °C. A Wiley mill was used to ground coarse samples to a fine powder. From each sample, 4 to 5 mg subsamples were combusted under vacuum using Vycor tubes containing silver wire and cupric oxide. To release CO_2 , combustion was carried out for 5 hrs at 520 °C.

Liquid N was used to cryogenically purify and collect carbon dioxide under vacuum. Purified CO_2 was injected to a ratio mass spectrometer (VG T50 GAS 903D Device, Middlewich, UK) and carbon isotope composition ($\delta^{13}\text{C}$) was measured relative to VPDB (Vienna Pee Dee Belemnite), a fossil belemnite Pee Dee (Ehleringer and Osmond, 1989). Pearson correlation coefficient was employed to calculate the relation between pooled data of $\delta^{13}\text{C}$ and total dry matter components of different water treatments. Proc GLM of SAS software was used to analyse the data, and Tukey post-hoc test was used to compare treatment means.

Results

The effect of soil water contents on $\delta^{13}\text{C}$ of strawberry leaves were highly significant ($p < 0.001$). Water stressed and moderately water stressed treatments caused 1.318‰ and 0.965‰ increase in $\delta^{13}\text{C}$ strawberry leaves compared to well-watered plants (Tab. 1). The mean leaf $\delta^{13}\text{C}$ of transgenic strawberry, along different SWC treatments, was significantly lower than that of non-transgenic strawberry (Tab. 1) and decreased by 0.564‰.

The effects of soil water content and transgenic strawberry on WUE were highly significant ($p = 0.001$) (Tab. 1). Soil water content

reduction caused WUE increase by 30 and 48 percent for moderate and stressed water treatments, respectively, relative to well-watered treatment. Transgenic strawberry also caused on average 24 percent increase in WUE relative to non-transgenic one. The trend of increase in WUE in transgenic strawberry, along SWCs of water stressed, moderately water stressed and well water treatments, relative to corresponding treatments for non-transgenic strawberry were 28.2, 24.4 and 14.9 percent, respectively, and not linear.

For strawberry pooled data, along different SWC treatments, highly significant ($p < 0.01$), but negative correlations were found between $\delta^{13}\text{C}$ and plant dry matter components, such as LDM ($r = -0.92$; $p = 0.009$), PDM ($r = -0.93$; $p = 0.006$), RDM ($r = -0.88$; $p = 0.02$) and TDM ($r = -0.92$; $p = 0.008$) (Fig. 1), and between $\delta^{13}\text{C}$ and leaf area per plant (LA) ($r = -0.93$; $p = 0.006$), and between $\delta^{13}\text{C}$ and water use per plant (WU) ($r = -0.93$; $p = 0.006$) (Fig. 2). No significant correlations were found between $\delta^{13}\text{C}$ strawberry pooled data ($r = 0.56$; $p = 0.24$) and WUE, but when correlations were made for transgenic and non-transgenic data separately, highly significant correlations were found between $\delta^{13}\text{C}$ and WUE ($r = 0.98$) for transgenic plants; and the same relations between $\delta^{13}\text{C}$ and WUE ($r = 0.99$) for non-transgenic plants (Fig. 3).

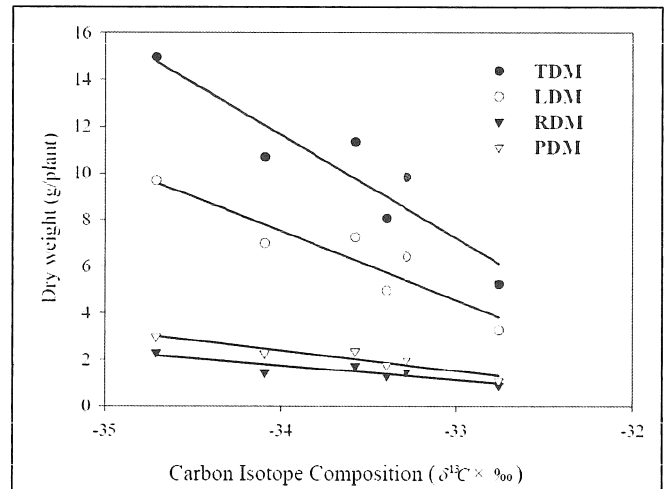


Fig. 1: Regression lines between strawberry dry matter components (TDM, LDM, RDM and PDM) and carbon isotope composition.

Discussion

The values of carbon isotope composition for leaves of 44 C_3 species from 43 genera and 23 botanical families of herb species reported an average value of around -28‰ for $\delta^{13}\text{C}$ (MARCHESE et al., 2006). A typical range of $\delta^{13}\text{C}$ for C_3 plants is between -25 to -29‰, which

Tab. 1: Mean water-use efficiency (WUE) (g/kg) and carbon isotope compositions ($\delta^{13}\text{C} \times \text{‰}$) as a function of soil water content for strawberry plants.

Water treatments	Water-use efficiency g kg^{-1}			Carbon isotope composition ($\delta^{13}\text{C}$, ‰)		
	Transgenic	Non-transgenic	Mean*	Transgenic	Non-transgenic	Mean*
Well	3.62±0.11	3.15±0.15	3.795 ^c	-34.703	-34.090	-34.396 ^b
Moderate	5.28±0.11	4.24±0.20	4.950 ^b	-33.578	-33.283	-33.431 ^a
Stress	6.00±0.43	4.68±0.24	5.630 ^a	-33.401	-32.755	-33.078 ^a
Mean**	4.98±0.51 ^a	4.02±0.36 ^b		-33.895 ^b	-33.311 ^a	

* Means that carry different letters in each column are significantly different at 5% level. ** Last row shows mean WUE and $\delta^{13}\text{C}$ of transgenic and non-transgenic strawberries across different water treatments.

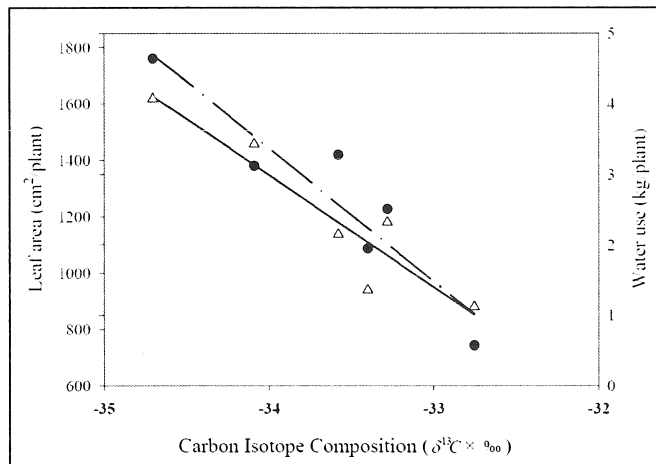


Fig. 2: Regression lines between strawberry leaf area (circle and dotted line) and water use (triangle and continuous line) with carbon isotope composition.

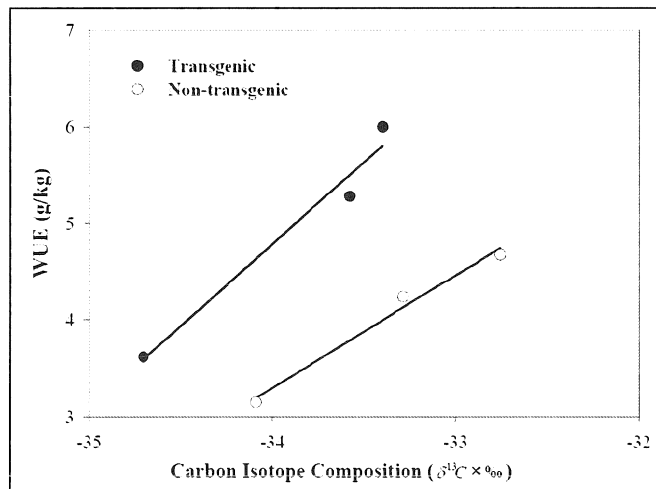


Fig. 3: Relations between transgenic and non-transgenic strawberry water-use efficiency and carbon isotope composition.

indicate co-limitation by carboxylation and diffusion processes (O'LEARY, 1993). The mean $\delta^{13}\text{C}$ value for this study is much lower than the above values. The higher the stomatal conductance the lower the $\delta^{13}\text{C}$ values (LAMBERS et al., 2008), therefore it seems lower $\delta^{13}\text{C}$ for strawberry, even for stress treatment, reveals higher limitation by carboxylation process in fixing $^{13}\text{CO}_2$ or more resistant to $^{13}\text{CO}_2$ diffusion by leaf boundary layer and stomata, due to leaf morphology (LI et al., 2006) or leaf anatomy (MARCHESE et al., 2005; CARELLI et al., 2003; JURIK et al., 1982) and stomatal cavity morphology (KOGAMI et al., 2001). It is speculated that the more negative values for $\delta^{13}\text{C}$ in transgenic strawberry could be a result of consecutive expression of chitinase gene.

Carbon isotope discrimination has been reported as an indirect indicator for soil water availability between different sites or time periods (STEWART et al., 1995; WANG, 2003; TSIALTAS and MASLARIS, 2006). It has been shown that $\delta^{13}\text{C}$ is an indicator of both integrated physiological processes and environmental properties, and also reveals how different species could adjust their gas exchange metabolisms in relation to their habitats (DAWSON et al., 2002; CHEN et al., 2007). The less negative $\delta^{13}\text{C}$ value, as a surrogate of integrated whole plant life WUE, for water-stressed strawberry plants is in agreement with reported WUE data for this species under water stress conditions (CHALAVI and RAEINI-SARJAZ,

2010) and other plants (RAEINI-SARJAZ and CHALAVI, 2008; RAJABI et al., 2009).

The mean leaf $\delta^{13}\text{C}$ of transgenic strawberry was significantly lower than that of non-transgenic plants (Tab. 1), which reveals a higher long term integrated biochemical and stomatal conductance to CO_2 for transgenic plants relative to control ones. It has been reported that chitinase, a drought induced gene, was involved in promoting vigorous growth (CHALAVI and RAEINI-SARJAZ, 2010), and enhancing fungal resistance in transgenic strawberries (ASAO et al., 2003; CHALAVI et al., 2003), by constitutive activation of gene responsible in enhancing tolerance. In agreement with our finding other researchers have reported genetic variation in carbon isotope composition for a variety of species (PENNINGTON et al., 1999; EL-SHARKAWY and DE TAFUR, 2007; JOHNSON et al. 2009). Therefore, not only chitinase was involved in resistance to water stress and enhancement of WUE, but also it caused vigorous growth in strawberry (CHALAVI and RAEINI-SARJAZ; 2010) and protection against gray mold (RICARDO et al., 2006). *Arabidopsis* as a model plant was employed to analyse the molecular bases of genetic variations in WUE. The results demonstrated significant genetic discriminations between *Arabidopsis* ecotypes in carbon isotope discrimination (MALSE et al., 1999), which was linearly correlated to p_i/p_a , the ratio of intercellular to ambient partial pressure of CO_2 . Genetic differences for carbon isotope discrimination have been reported for many C_3 plants (RAJABI et al., 2009; BONHOMME et al., 2009), as well as strawberry cultivars (JOHNSON et al., 2009). Consequently, it could be speculated that less ^{13}C enriched leaves of transgenic strawberry plants, as a partial function of chitinase gene constitutive expression, might have had more long term integrated stomatal conductance, which was translated to vigorous growth for chitinase-transgenic strawberry (CHALAVI and RAEINI-SARJAZ, 2010).

Positive correlations between $\delta^{13}\text{C}$ and WUE were found for individual transgenic and non-transgenic plants along different SWCs, in agreement with reports for other plants (RAEINI-SARJAZ and CHALAVI, 2008; RAEINI-SARJAZ et al., 1998). The slopes of regression lines for $\delta^{13}\text{C}$ and WUE for transgenic and control plants were similar, but had different intersections. Therefore, failure of significant correlations between $\delta^{13}\text{C}$ and WUE for pooled data of strawberry plants indicates that $^{13}\text{CO}_2$ fixation process by transgenic and non-transgenic plants had different thresholds. Therefore, some parameters, such as downstream fractionations associated with metabolism (CONDON et al., 2002), and genetic capacity, especially chitinase consecutive expression, could have caused these differences. JOHNSON et al. (2009) also reported that carbon isotope composition of leaves, fruits and crowns showed an increase in WUE in water-limited strawberry plants, with a significant difference between cultivars.

Positive correlations have been reported between yield and carbon isotope discrimination for both rainfed and full-irrigated wheat (MISRA et al., 2010; MISRA et al., 2006; MONNEVEUX et al., 2006), and bean lines (ZACHARISEN et al., 1999). Therefore low ^{13}C enrichment could be an indicator of high productivity, which is in agreement with our findings of high correlations between strawberry leaf $\delta^{13}\text{C}$ and yield components. These findings indicate that the higher the stomatal conductance the higher could be dry matter production, and $\delta^{13}\text{C}$ could be used as an indirect indicator for crop dry matter components and yield.

High and negative correlations between $\delta^{13}\text{C}$ and cumulative plant water use, and between $\delta^{13}\text{C}$ and plant total leaf area make known that $\delta^{13}\text{C}$ could be an excellent indicator of both plant water use and leaf area. There are reports on high linear correlations between water use and total biomass yield (QUARRIE et al., 1999), and between water use and leaf area (RADERSMA et al., 2006), which indirectly supports our findings.

References

- ASAO, H.G., ARAI, S., NISHIZAWA, Y., 2003: Environmental risk evaluation of transgenic strawberry expressing a rice chitinase gene. *Seibutsu Kogakkaishi* 81, 57-63 (English Abstract).
- BONHOMME, L., MONCLUS, R., VINCENT, D., CARPIN, S., CLAVEROL, S., LOMENECH, A.M., LABAS, V., PLOMION, C., BRIGNOLAS, F., MORABITO D., 2009: Genetic variation and drought response in two *Populus x euramericana* genotypes through 2-DE proteomic analysis of leaves from field and glasshouse cultivated plants. *Phytochemistry* 70, 988-1002.
- BRUGNOLI, E., FARQUHAR, G.D., 2000: Photosynthetic fractionation of carbon isotopes. In: Leegood, R.C., Sharkey, T.D., von Caemmerer, S. (eds.), *Photosynthesis: physiology and metabolism*. Advances in Photosynthesis, Kluwer Academic Publishers, The Netherlands, 399-434.
- CHALAVI, V., RAEINI-SARJAZ, M., 2010: Enhanced growth in transgenic strawberry plants. *Agro2010*. Montpellier, France, 29 August - 3 September, 2010.
- CHALAVI, V., TABAEIZADEH, Z., THIBODEAU, P., 2003: Enhanced resistance to *Verticillium dahlia* in transgenic strawberry plants expressing a *Lycopersicon chilense* chitinase gene. *J. Amer. Soc. Hort. Sci.* 128, 747-753.
- CONDON, A.G., RICHARDS, R.A., REBETZKE, G.J., FARQUHAR, G.D., 2002: Improving intrinsic water-use efficiency and crop yield. *Crop Sci.* 42, 122-131.
- CARELLI, L.M.C., QUEIROZ-VOLTAN, R.B., FAHL, J.I., TRIVELIN, P.C.O., 2003: Leaf anatomy and carbon isotope composition in *Coffea* species related to photosynthetic pathway. *Braz. J. Plant Physiol.* vol.15 no.1. Londrina.
- CHEN, S., BAI, Y., LIN, G., HUANG, J., HAN, X., 2007: Isotopic carbon composition and related characters of dominant species along an environmental gradient in Inner Mongolia, China. *J. Arid Environ.* 71, 12-28.
- DAWSON, T.E., MAMBELLI, S., PLAMBOECK, A.H., TEMPLER, P.H., TU, K.P., 2002: Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* 33, 507-559.
- EHLERINGER, J.R., OSMOND, C.B., 1989: Stable Isotopes. In: Pearcy, R.W., Ehleringer, J.R., Moodney, H.A., Rundel, P.W. (eds.), *Plant Physiological Ecology, Field Methods and Instrumentation*, 280-300. Chapman and Hall, London.
- EL-SHARKAWY, M.A., DE TAFUR, S.M., 2007: Genotypic and within canopy variation in leaf carbon isotope discrimination and its relation to short-term leaf gas exchange characteristics in cassava grown under rain-fed conditions in the tropics. *Photosynthetica* 45, 515-526.
- FARQUHAR, G.D., EHLERINGER, J.R., HUBICK, K.T., 1989: Carbon Isotope Discrimination and Photosynthesis. *Ann. Rev. Plant Physiol. Plant Biol.* 40, 503-537.
- FISCHER, R.A., TURNER, N.C., 1978: Plant productivity in the arid and semi-arid zones. *Ann. Rev. Plant Physiol.* 29, 277-317.
- HIILOVAARA-TEIJO, M., HANNUKALA, A., GRIFFITH, M., YU, X.M., PIHAKASKI-MAUNSBACH, K., 1999: Snow-mold-induced apoplastic proteins in winter rye leaves lack antifreeze activity. *Plant Physiol.* 121, 665-673.
- JONES, H., 2004: What is water use efficiency? In: Bacon, M.A. (ed.), *Water use efficiency in plant biology*, 27-41. Blackwell, Victoria, Australia.
- JOHNSON, A.W., GRANT, O.M., DAVIES, M.J., JAMES, C.M., PASSEY, A.J., SIMPSON, D.W., 2009: Investigating the response of strawberry to water deficit conditions. *Acta Hort.* (ISHS) 838, 169-174.
- JURIK, T.W., CHABOT, J.C., and CHABOT, B.F., 1982: Effect of light and nutrients on leaf size, CO₂ exchange, and anatomy in wild strawberry (*Fragaria virginiana*). *Plant Physiol.* 70, 1044-1048.
- KARASUDA, S., TANAKA, S., KAJIHARA, H., YAMAMOTO, Y., KOGA, D., 2003: Plant chitinase as a possible biocontrol agent for use instead of chemical fungicides. *Biosci. Biotech. Biochem.* 67, 221-224.
- KOGAMI, H., HANBA, Y.T., KIBE, T., TERASHIMA, I., MASUZAWA, T., 2001: CO₂ transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. *Plant, Cell & Environ.* 24, 529-538.
- LAMBER, H., CHAPIN, F.S., PONS, T.L., 2008: *Plant Physiological Ecology*. Springer, Utrecht University, The Netherlands.
- LEAVITT, S.W., LONG, A., 1985: Stable-carbon isotopic composition of maple sap foliage. *Plant Physiol.* 78, 427-429.
- LI, C., ZHANG, X., LIU, X., LUUKKANEN, O., BERNINGER, R., 2006: Leaf Morphological and Physiological Responses of *Quercus aquifolioides* along an Altitudinal Gradient. *Silva Fennica* 40, 5-13.
- MARCHESE, J.A., MING, L.C., DUCATTI, C., BROETTO, F., DA SILVA, E.T., LEONARDO, M., 2006: Carbon isotope composition as a tool to control the quality of herbs and medicinal plants. *Photosynthetica* 44, 155-159.
- MARCHESE, J.A., BROETTO, F., MING, L.C., DUCATTI, C., RODELLA, R.A., VENTRELLA, M.C., GOMES, G.D.G., DE FRANCESCHI, L., 2005: Carbon isotope composition and leaf anatomy as tool to characterize the photosynthetic mechanism of *Artemisia annua* L. *Braz. J. Plant Physiol.* 17, 187-190.
- MASLE, J., WONG, S.C., SHIN, J.S., FARQUHAR, G.D., 1999: Improving crop growth and water-use efficiency by raising carbon dioxide levels. *Rural Industries Research and Development Corporation*. Publication No 99/25. Kingston, Australia.
- MCCREE, K.J., RICHARDSON, S.G., 1987: Salt increases the water use efficiency in water stressed plants, *Crop Sci.* 27, 543-547.
- MISRA, S.C., SHINDE, S., GEERTS, S., RAO, V.S., MONNEVEUX, P., 2010: Can carbon isotope discrimination and ash content predict grain yield and water use efficiency in wheat? *Agr. Water Management* 97, 57-65.
- MISRA, S.C., RANDIVE, R., RAO, V.S., SHESHAYEE, M.S., SERRAJ, R., MONNEVEUX, P., 2006: Relationship between carbon isotope discrimination, ash content and grain yield in wheat in the Peninsular Zone of India. *J. Agron. Crop Sci.* 192, 352-362.
- MJWARA, J.M., BOTHA, C.E.J., 1997: Water use efficiency in *Phaseolus vulgaris* exposed to elevated CO₂. *ISHS Acta Hort.* 449, 439-448.
- MONNEVEUX, P., REKIKI, D., ACEVEDO, E., MERAH, O., 2006: Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. *Plant Sci.* 170, 867-872.
- O'LEARY, M.H., 1993: Biochemical basis of carbon isotope fractionation. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (ed.), *Stable isotopes and plant carbon-water relations*, 19-28. Academic Press, New York.
- PENNINGTON, R.E., RISCHLER, C.R., JOHNSON, H.B., POLLEY, H.W., 1999: Genetic variation for carbon isotope composition in honey mesquite (*Prosopis glandulosa*). *Tree physiol.* 19, 583-689.
- PYANKOV, V.I., GUNIN, P.D., TSOOG, S., BLACK, C.C., 2000: C4 plants in the vegetation of Mongolia: their natural occurrence and geographical distribution in relation to climate. *Oecologia* 123, 15-32.
- QUARRIE, A.S., STOJANOVIC, J., PEKIC, S., 1999: Improving drought resistance in small-grained cereals: A case study, progress and prospects. *Plant Growth Regul.* 29, 1-21.
- RADERSMA, S., ONG, C.K., COE, R., 1997: Water use of tree lines: importance of leaf area and micrometeorology in sub-humid Kenya. *Agroforestry Systems* 66, 179-189.
- ROBINSON, S.P., JACOBS, A., DRY, I.B., 1997: A class IV chitinase is highly expressed in grape berries during ripening. *Plant Physiol.* 114, 771-778.
- RAEINI-SARJAZ, M., CHALAVI, V., 2008: Effects of diverse microclimates and soil water contents on water-use efficiency and carbon isotope discrimination for bush bean. *JAST* 10, 43-53.
- RAEINI-SARJAZ, M., BARTHAKUR, N.N., ARNOLD, N.P., JONES, P.J.H., 1998: Water stress, water use efficiency, carbon isotope discrimination and leaf gas exchange relationships of the bush bean. *J. Agron. Crop Sci.* 180, 173-179.
- RAJABI, A., OBER, E.S., GRIFFITHS, H., 2009: Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugar beet. *Field Crops Res.* 112, 172-181.
- RICARDO, V.G., RICCI, J.C.D., HERNANDEZ, L., CASTAGNARO, A.P., 2006:

- Enhanced resistance to *Botrytis cinerea* mediated by the transgenic expression of the chitinase gene ch5B in strawberry. *Transgenic Res.* 15, 57-68.
- RUNDLE, P.W., EHLERINGER, J.R., NAGY, K.A., 1989: Stable isotopes in ecological research. Springer-Verlag, London.
- SARANGA, Y., FLASH, I., PATERSON, A.H., YAKIR, D., 1999: Carbon isotope ratio in cotton varies with growth stage and plant organ. *Plant Sci.* 142, 47-56.
- SHAHEEN, R., HOOD-NOWOTNY, R.C., 2005: Effect of drought and salinity on carbon isotope discrimination in wheat cultivars. *Plant Sci.* 168, 901-909.
- STEWART, G.R., TURNBULL, M.H., SCHMIDT, S., ERSKINE, P.D., 1995: $\delta^{13}C$ natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Aust. J. Plant Physiol.* 22, 51-55.
- TSIALTAS, J.T., MASLARIS, N., 2006: Leaf carbon isotope discrimination relationships to element content in soil, roots and leaves of sugar beets grown under Mediterranean conditions. *Field Crops Res.* 99, 125-135.
- WANG, G.H., 2003: Differences in leaf $\delta^{13}C$ among four dominant species in a secondary succession sere on the Loess Plateau of China. *Photosynthetica* 41, 525-531.
- WRIGHT, G.C., HUBICK, K.T., FARQUHAR, G.D., 1988: Discrimination in carbon isotope of leaves correlated with water-use efficiency of field-grown peanut cultivars. *Aust. J. Plant Physiol.* 15, 815-825.
- YU, L.X., DJEBROUNI, M., CHAMBERLAND, H., LAPONTAINE, J.G., TABAEIZADEH, Z., 1997: Chitinase: Differential induction of gene expression and enzyme activity by drought stress in the wild (*Lycopersicon chilense* Dun.) and cultivated (*L. esculentum* Mill.) tomatoes. *J. Plant Physiol.* 155, 745-753.
- YEH, S., MOFFATT, B.A., GRIFFITH, M., XIONG, F., YANG, D.S.C., WISEMAN, S.B., SARHAN, F., DANYLUK, J., XUE, Y.Q., HEW, C.L., DOHERTY, A., LAJOIE, G., 2000: Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. *Plant Physiol.* 124, 1251-1263.
- ZACHARISEN, M.H., BRICK, M.A., FISHER, A.G., OGG, J.B., EHLERINGER, J.R., 1999: Relationships between productivity and carbon isotope discrimination among dry bean and F2 progeny. *Euphytica* 105, 139-250.
- ZHANG, C.Z., ZHANG, J.B., ZHAO, B.Z., ZHANG, H., HUANG, P., 2009: Stable isotope studies of crop carbon and water relations: A review. *Agr. Sci. in China* 8, 578-590.

Address of the author:

Mahmoud Raeini-Sarjaz, Department of Agricultural Engineering, Sari, Agricultural Sciences and Natural Resources University, PO-Box 578, Sari, Iran; e-mail: m.raeini@sanru.ac.ir, raeini@yahoo.com, phone: 0098-151-3822574-6 ext. 123, cell: 0098-911-152-2416