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EFFECTS OF DIVERSE MICROCLIMATES AND SOIL WATER CONTENTS ON WATER-USE EFFICIENCY AND CARBON ISOTOPE DISCRIMINATION FOR BUSH BEAN

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Abstract

Environmental variables including soil water content (SWC) act as constraints to crop growth and productivity. Therefore, open air (E_0), perforated (E_1) and non-perforated (E_2) plastic housings were used with well-watered (W_0), moderately-watered (W_1) and water-stressed (W_2) bush bean plants to explore relationships between water-use efficiency (WUE), carbon isotope discrimination (Δ) and isotopic composition (δ_p), leaf assimilation rate (A) and leaf Kjeldahl nitrogen (N) under diverse environments. CO_2 concentration and the air carbon isotopic composition (δ_a) varied with the environment. The δ_a values were reduced by about 0.8×10^{-3} and 3.8×10^{-3} in E_1 and E_2, respectively, compared with that in E_0. SWC significantly affected WUE, Δ, δ_p in E_0 and E_1 but not in E_2. Decoupling of plants from the outside atmosphere might have contributed in maintaining the above quantities almost constant in E_2. The Δ-value increased by about 2.2×10^{-3} in E_0 and 1.7×10^{-3} in E_1 compared with E_2. Water stress reduced Δ-value by about 1.1×10^{-3}in both E_0 and E_1. WUE and Δ were significantly correlated in E_0 and E_1 (r = - 0.72, and - 0.75, respectively) whereas there was no definite relationship between WUE and Δ in E_2 indicating that stomatal conductance was almost independent of SWC. The N-content had little effect on Δ. Leaf N significantly increased in water-stressed plants depending upon the time of harvest and the environment. The mean leaf assimilation rate was significantly higher in E_0 than in either E_1 or E_2.

Keywords: water-use efficiency, carbon isotope discrimination, bush bean, plastic culture

1. Introduction

Two stable isotopes of carbon occur naturally in the atmosphere as $^{12}CO_2$ and $^{13}CO_2$ with their respective abundances of 98.9% and 1.1% (Fritz and Fontes, 1980). Although the isotope effect due to mass difference is usually a nuisance in radiotracer methodology, the same effect can be turned around and used as a tool especially in studying chemical reactions that proceed in tandem. Isotope effects occur in plant tissues due to differences in the diffusivities of $^{13}CO_2$ and $^{12}CO_2$ in the ambient air (Farquhar et al., 1989) and also in biochemical reactions involved in photosynthesis (Melander and Saunders, 1979). Since crops encounter different environments during their growth, the ^{13}C to ^{12}C ratio varies significantly in tissues of C_3 plants (Farquhar et al., 1982). This variation in the ratio of isotopes can be used to evaluate the effects of genetic and environmental factors on the yield performance of cultivars. Any environmental factor that affects stomatal conductance and enzymatic activity may result in changes in water-use efficiency (WUE) and ^{13}C discrimination (Δ) (Farquhar et al., 1982) as defined in Eq. (2).

The theory of isotope effect has been established through a linear relationship between Δ and the ratio C_i/C_a of the internal CO_2 concentration in the plant tissue, C_i, to that of the ambient air, C_a (Farquhar and Richards, 1984). Ambient CO_2 concentration (C_a) is almost constant in a wide range of environmental conditions, while internal CO_2 concentration (C_i) can vary as a photosynthetic response to environmental variables. Eq. (1) describes the relationship between WUE_i of a single leaf defined as the ratio of the instantaneous photosynthetic and transpiration rates (mol CO_2/mol H_2O) and C_i/C_a (Farquhar et al., 1989),

$$WUE_i = \frac{C_a(1 - C_i / C_a)}{1.6\nu} \quad (1)$$

where, v = water vapor pressure difference between the intercellular spaces and the ambient air. The factor 1.6 is the ratio of the diffusivity of water vapor to that of CO_2 in the air. A negative correlation was found between Δ and both the long-term transpiration efficiency, WUE_t, defined as the total dry matter per kg of water transpired (Farquhar

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and Richards, 1984; Ehdaie et al., 1991; Ismail and Hall, 1992; Raeini-Sarjaz et al., 1998) and WUE_i (Wright et al., 1988, 1994) for several crops. For legumes, Meinzer et al. (1990) reported simultaneous reductions in stomatal conductance and in Δ with increased WUE_t for water-stressed cowpea. Ehleringer et al. (1991) found a high correlation between C_i/C_a and Δ for common bean. Wright et al. (1994) demonstrated a significant effect of various water regimes on Δ for peanut. Ehdaie et al. (1991) obtained higher Δ-values for greenhouse-grown wheat than those grown in the field. Hall et al. (1990) observed an association between gas exchange and Δ when the roots of the same cowpea genotypes were subjected to varying environments. Rao and Wright (1994) showed a significant effect of location and water regime on Δ for cowpea. Johnson et al. (1995) observed a significant correlation between Δ and WUE_t for lentil. Hubick (1990) reported that low-N peanut plants accumulated less dry matter and used less water than the high-N plants.

The efficiency of water use can be increased if the major factors that influence water loss are evaluated. For example, the rate of transpiration from a canopy is mainly a function of stomatal and boundary layer conductances, water vapor pressure deficit (VPD), net radiation, wind speed, and temperature. The effect of each of these factors may depend on the canopy structure and the surrounding growing environment. The canopy boundary layer conductance may have a crucial impact on the relation between water use and isotope discrimination of plants inside plastic housing. In the open air, wind increases canopy conductance and enhances mass and heat transfer to the atmosphere, while in enclosed environments, the boundary layer conductance is relatively small which lowers the heat and mass transfer processes (Jarvis and McNaughton, 1986; Jones, 1992). The open air canopy is well-coupled to the atmosphere and transpiration is mostly controlled by stomatal conductance, while inside a plastic housing the canopy is decoupled from the outside air and energy input becomes the governing factor for transpiration (Jones, 1992). The CO_2 concentration under enclosed environments such as plastic tunnels and greenhouses might be higher than open air. Long- term enhanced CO_2 concentrations will lead to stomatal conductance reduction (Jones and Jongen 1996; Pospíšilová and Èatský, 1999), therefore, will tend to reduce leaf transpiration rate and increase WUE (Jones and Jongen, 1996; Saralabai *et al*. 1997). Hence, the increase of water use efficiency might be the positive effect of environmental elevated CO_2 (Pospíšilová and Èatský 1999).

Although ^{13}C discrimination literature is extensive in plant breeding, plant physiology, eco-physiology and other fields (Ehleringer et al., 1993), the interaction of crop growth and soil moisture on Δ needs to be examined for diverse environments so that a suitable growing environment can be determined for a particular crop. The physical environment can be modified to create favorable microclimate for optimum plant growth. For example, plastic culture is being increasingly used, especially in temperate climates, to promote early-season vegetable production and to reduce the detrimental effects of low air and soil temperatures. Plant physiological responses, especially long term stomatal conductance, to these artificial microclimates are not well known. ^{13}C discrimination, as a long term indicator of C_i/C_a, therefore stomatal conductance, and also a probe of long-term WUE (Farquhar et al., 1989; Condon et al., 1990; Hubick, 1990; Brugnoli and Farquhar, 2000) may provide a tool to assess responses of plants to changes in the growing environments.

Thus, the objectives of this study were to examine whether decoupling of plants from the outside atmosphere (closed plastic housings), or providing facilities for re-coupling through holes (perforated plastic enclosures) in combination with soil moisture availability may influence WUE_t, leaf N, and Δ of the bush bean.

2. Materials and methods

Well-watered (W_0), moderately-watered (W_1), and water-stressed (W_2) plants were used in combination with the experimental environments of the open air (E_0), a plastic housing perforated uniformly with 400 holes m^{-2} each of 0.5 cm diameter (E_1), and a closed plastic cover (E_2). The housings were made of clear polyethylene plastic sheets which were transformed into tunnels of 1 m in width, 10 m in length, and 0.8 m in height. The plastic sheets were 87% transparent (Raeini-Sarjaz and Barthakur, 1997) to photosynthetic photon flux density. Bush bean seeds (*Phaseolus vulgaris* L. cv. Provider) were germinated in the greenhouse as described previously (Raeini-Sarjaz and Barthakur, 1997). The one-week old seedlings were transferred from the greenhouse to the sites E_0, E_1, and E_2 when ambient temperature was not harmful for open air plants, on May 15, Macdonald Campus, Experimental Farm, McGill University ($45^{o}25'45''$N and $73^{o}56'00''$W). All plants were initially watered to 100% field capacity (FC). Soil water content (SWC) of W_0 plants was kept at 100% FC, while for W_1 and W_2 plants water was supplied to 100% FC only when SWC reached 50% and 30% FC, respectively. Two control pots with an identical amount of soil but without plants were

watered and weighed similarly to monitor non-plant evaporative losses. The transpiration rate was calculated from the difference between added and lost water.

Water was replenished each day and the loss was measured with a balance. CO_2 concentrations were measured instantaneously at each site using a photosynthesis system (LI-6200, LI-COR Inc., Lincoln, NE, USA). During growth and development, plants were fertilized uniformly each week with 20-20-20 NPK. To reduce leaf sunburns during sunny and hot days, the tunnel ends were opened at noon for three hours of ventilation. At mid-June with increasing air temperature during the pre-flowering stage (Day 35), the plastic covers were removed. Shelter was provided whenever there was rain or a risk of rain. In order to evaluate the effects of crop growth conditions on yield and WUE, half of the plants were harvested after the removal of the plastic cover (HT1 = 35 d), and the rest were kept in ambient air until pod harvest time (HT2 = 50 d).

For each harvest, five pots from each treatment were selected randomly, and the plant parts were put into paper bags. The contents were dried in an oven at 60°C for 72 h to determine the total dry matter (TDM) with an electronic balance of ± 0.001 g precision, and WUE_t was calculated. Leaf N at both harvest times was measured on 0.2 ± 0.005 g of subsamples of ground leaf tissue using Automated Ion Analyzer (Lachat Instruments, model Quikchem AE, USA).

To evaluate the effect of previous soil moisture conditions on leaf gas exchange (LGE) in each experiment, plants were transferred to the greenhouse before measurements. The LGE measurements were made with a steady-state LI-6200 photosynthesis system (LI-COR, Inc., Lincoln, NE, USA). The measurements were made on newly developed trifoliate leaves of recently watered (100% FC) plants of all water regime treatments between 11:00 and 14:00 local time on bright sunny days. The mean air temperature (37°C) and relative humidity (56%) were constant during the LGE measurements.

2.1 Carbon Isotope Discrimination

Trifoliate leaves at harvest times HT1 and HT2 were dried at 60°C for 72 h and then ground to a fine powder using a Wiley mill. From each sample 4 to 5 mg subsamples of leaf tissues were combusted under vacuum using Vycor tubes containing silver wire and cupric oxide. Combustion took place at 820°C for 5 h to release CO_2. Combusted samples were left at room temperature for 12 h prior to the liquid N cryogenic purification of CO_2. Eq. (2) was used to calculate carbon isotope discrimination (Δ) from the measurements of $\delta_p = (R_p/R_s - 1)$, where R_p is the $^{13}C/^{12}C$ ratio in the plant and R_s that of Pee Dee Belemnite (PDB) standard (Ehleringer and Osmond, 1989).

$$\Delta = \frac{(\delta_a - \delta_P)}{(1 + \delta_P)} \quad (2)$$

To measure carbon isotopic composition of the air, δ_a, air at each site was collected in special aluminum bags (Mil-B-131H, Ludlow Corp.) and air CO_2 was immediately purified cryogenically. The isotopic composition measurements for duplicated samples were made by an isotope ratio mass spectrometer (VG T50 GAS 903D Device, Middlewich, UK). The mean of the duplicated samples of δ_p is reported along with Δ.

2.2 Statistical analysis

Experiments were conducted on a complete random design model. Variances of experiments were found homogeneous using Bartlett's test. A combined analysis of variance (ANOVA) was employed for the entire data of three experiments using SAS software (SAS Institute Inc., Cary, NC, 1990), where sources of variations were environment (E), soil water content (SWC), harvest time (HT), and their interactions. An ANOVA was performed on data of each experiment separately, where SWC was the source of variation. For LGE analysis, leaf temperature and photon flux density were employed as covariates in the model. For determining correlations, Pearson correlation procedure was employed, and unpaired Cochran's t-test was used to compare harvest time results within each experiment.

3. Results

The mean CO_2 concentrations were 453, 732 and 1478 μmol mol^{-1} within E_0, E_1 and E_2 environments, respectively. The measured δ_a values were: - 8×10^{-3} in E_0, - 8.8×10^{-3} in E_1, and - 11.8×10^{-3} in E_2. The mean daytime air temperature and relative humidity during the last two weeks of May were 24.6, 32.4, 34.5°C and 65, 75, 95%, respectively.

The result of the combined ANOVA statistics showed the environmental diversity to have highly significant effects on N, Δ, δ_p , WUE_t, and assimilation rate (A). Therefore, each environmental data was analyzed separately by ANOVA. Leaf N of W_2 plants were significantly higher than those of W_0 in E_0 at HT1; and E_2 environments regardless of harvesting times (**Table 1**). The previous SWC history had little effect on *A*, and there was no significant interaction between SWC and

environment. Therefore, pooled data of *A* for different SWC were run to test the effect of various environments. E_0 significantly increased the assimilation rate compared with E_1 and E_2. The mean *A*-values increased in the order of $E_0 > E_1 > E_2$ environments (**Table 1**). A definite relationship did not emerge between N and Δ except showing a correlation at the time of HT1 harvest in E_0 ($r = - 0.81$).

There were significant differences in both ^{13}C enrichment (δ) and ^{13}C discrimination (Δ) values at both HT1 and HT2 in E_0 and E_1 for different water regimes, while such differences almost disappeared in E_2. WUE_t generally increased with reduction of soil water contents in both E_0 and E_1 at both HT1 and HT2 stages, while WUE_t remained almost constant along different SWC in E_2 SWC (**Table 1**). WUE_t and Δ relationships were linear and significant in both E_0 ($r = - 0.76$, $p < 0.02$) and E_1 environments ($r = - 0.75$, $p < 0.02$), while a significant relationship failed to show ($r = 0.18$, $p > 0.6$) in E_2 (Fig. 1). The t-test showed significant differences ($p \leq 0.04$) in Δ-values between HT1 and HT2 for E_1 and E_2, but not for E_0. The δ_p values showed significant differences along soil water contents in E_0 and E_1 and decreased with increasing SWC (**Table 1**).

5. Discussion

Although WUE_t enhancements for water-stressed bean plants in E_0 and E_1 environments may seem to be unlikely at first thought, yet several authors (Nobel, 1991; Ismail and Hall, 1992; Raeini-Sarjaz and Barthakur, 1997; Raeini-Sarjaz et al., 1998) concurred with the present finding. An explanation for this apparent anomaly can be found in decreased biomass production and a drastic reduction in the rate of transpiration for the plants growing in these environments. The plants were decoupled from the outside atmosphere and a high relative humidity and temperature prevailed in E_2 (Raeini-Sarjaz and Barthakur, 1997), which might have prevented from obtaining a similar enhancement of WUE_t in E_2. For example, the mean daytime air temperature and relative humidity during the last two weeks of May were 24.6, 32.4, 34.5°C and 65, 78, 95% in E_0, E_1 and E_2 environments, respectively.

The less negative δ or lower Δ values in plant tissues corresponds to the lesser discrimination against ^{13}C and is an indicator of imposed environmental stresses on stomatal conductance. To compare these values the source (air) CO_2 should have the same enrichment in ^{13}C, δ_a. The less negative mean δ values in E_0 and E_1 environments (-26.57×10^{-3} and -26.95×10^{-3}, respectively) compared with that of E_2 environment (-28.29×10^{-3}) can be speculated as a more imposed stress condition on E_0 and E_1 plants, while lower Δ values on plants of E_2 environment compared with those of other environments contradicts the δ values. The Δ values increased in E_0 and E_1 environments by about 2.2×10^{-3} and 1.7×10^{-3}, respectively, compared with E_2 at HT1. As Δ values were adjusted against the source isotopic composition, therefore reduction of Δ values of E_2 must be due to other environmental imposed stresses on stomatal behavior other than water stress alone. Ehdaie et al. (1991) reported higher Δ-values for greenhouse-grown plants in higher humidity than those of field-grown crops in less humid air. Our results were not in agreement with the above finding perhaps due to the variation in δ_a values inside the growing environments. In microclimates, source CO_2 enrichment in ^{13}C might be different from ambient air, which mostly is influenced by soil respiration and spatial variation in δ_a (Jones, 1992). The above authors assumed the δ_a value to remain constant in both open air and in the greenhouse. If we make the same assumption our findings agree with their data. The validity of this assumption needs to be tested in the future. Within each growth environment, water stress reduced Δ-value by about 1.1×10^{-3}, except in E_2 where Δ changed little. The reduction in Δ-values from water stress in our experiments (E_0 and E_1) agreed fairly well with the results of previous authors on other species (Meinzer et al., 1990; Ismail and Hall, 1992; Wright et al., 1994). Thus, stomatal conductance and carbon isotope discrimination decreased with water stress in a similar fashion, except when plants were not coupled with the ambient air, as was the case for E_2 environment. Well-watered plants growing in an atmosphere of high relative humidity had high stomatal conductance (Comstock and Ehleringer, 1993). The essentially constant value of Δ in E_2 environment across different soil water contents might be attributed to an imposition of a less restriction expected for stomatal conductance and enzymatical activity of water-stressed plants compared with those of W_0 plants. The soil water depletion is expected to be less for plants in an atmosphere of high relative humidity. Photosynthesizing plants in enclosures are exposed to low-velocity air movements. This might contribute to a depletion of air CO_2 due to photosynthesis and also release of CO_2 from the decaying organic matters in the soil. The increased CO_2 concentrations reported in E_1 and E_2 environments indicates that the latter process to be dominant. The δ_a values varied with the growing environment, which was due to decomposing organic matters of C_3 plants residues (Boutton, 1991). Photosynthesizing C_3 plants discriminate against $^{13}CO_2$ which increases canopy δ_a. However, CO_2 originated from decaying C_3 plant organic matters is not enriched in

$^{13}CO_2$ (Farquhar et al., 1989) and will reduce δ_a.

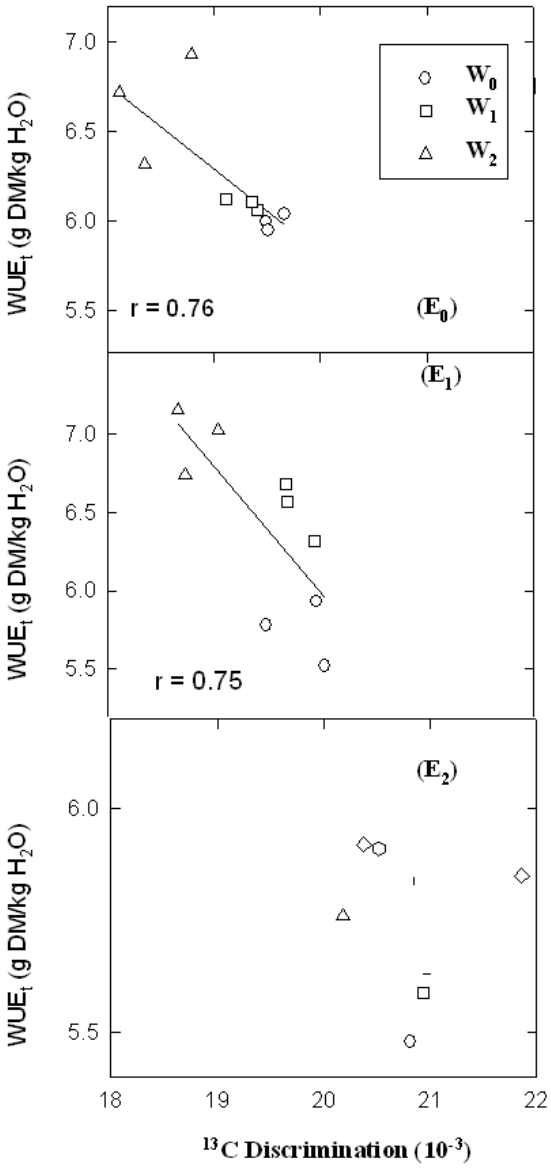

Figure 1. Relations between carbon isotope discrimination (Δ) and water-use efficiency (WUE_t) in open (E_0), perforated (E_1) and closed (E_2) growth environments. Legends: circle, rectangular and triangle represent well-watered (W_0), moderate (W_1) and stress-watered (W_2) plants, respectively.

Our measurements of lower δ_a values inside the enclosures compared with the open air indicated the contribution of CO_2 from decaying organic matters. The intermediate δ_a value in the perforated housing showed that air movement through the holes facilitated the exchange between the enriched $^{13}CO_2$ of the open air with the low enriched $^{13}CO_2$ inside the enclosure.

Harvest time had significant effect on mean Δ-values. After harvest HT1 plants of all environments were kept in the open air, which experienced increasingly higher air temperature as the summer season approached, while the relative humidity was reduced. The increased Δ-values in E_1 and E_2 environments at HT2 could result from a higher availability of $^{13}CO_2$ in the ambient air ($\delta_a \approx -8 \times 10^{-3}$) compared with those under plastic covers ($\delta_a < -8 \times 10^{-3}$) and also coupling of stomatal movement with open air conditions. The negative relationships and significant correlations between WUE_t and Δ in E_0 and E_1 environments of our experiment agreed not only with the theory of the isotope effect but also with the experimental works of previous authors (Wright et al., 1994; Johnson et al. 1995; Raeini-Sarjaz et al., 1998).

Although the relationship between Δ and WUE_t in E_2 was not significant, the decoupling of plants and its surrounding from outside air (Jarvis and McNaughton, 1986; Jones, 1992) may have contributed to practically no change in WUE_t across soil water contents**.** Therefore, the poor mixing condition of E_2 might have reduced plant water use, resulting in an almost no variation in WUE_t and Δ. Under closed environments such as E_2, canopy transpiration is mainly controlled by energy input (radiation) rather than stomatal conductance (Jarvis and McNaughton, 1986). The driving force for transpiration from stomatal cavity to boundary layer is the gradient of vapor saturation deficit (VPD). In an isothermal condition under an enclosed environment, transpiration from a canopy increases the relative humidity toward saturation point, which in turn reduces the driving force of VPD gradient towards zero. Further transpiration occurs via energy input to the environment that increases air temperature, and thus increases VPD which in turn enhances transpiration. The overall leaf-to-air VPD in E_2 was lower than those of the other environments, and no air movement was registered inside E_2 (Raeini-Sarjaz and Barthakur, 1997). Therefore, the absence of an air mixing mechanism combined with a low leaf-to-air VPD in E_2 resulted in a relatively small leaf boundary layer conductance and relatively low transpiration. This is expected to reduce the driving force for mass transfer. The likelihood of any increase in WUE or a reduction of Δ from low soil water content might be due to the stomatal activity. Although decoupling of plants inside the E_2 caused little change in WUE in plants across different soil water contents, but the modification of plastic housing through perforations (E_1) increased WUE and reduced Δ of water-stressed plants. Mass and heat transfer were

facilitated by the presence of holes, so that the plants were coupled actively with the outside environment. Linear regression analyses showed different slopes and y-intercepts for Δ versus WUE_t relationships for different environments. This indicated that each growth environment affected WUE_t and Δ relationship somewhat differently. The absence of a relationship between Δ and WUE_t in E_2 indicated that stomatal conductance in the closed tunnel was almost independent of soil waiter contents.

Table 1. Mean leaf N (μg/mg), Δ (× 10^{-3}), δ (x 10^{-3}), WUE_t (g DM/ kg H_2O) and leaf assimilation rate, A (μmol m^{-2} s^{-1}) with harvest time (HT), environment (E) and soil water content (SWC) for bush bean.

HT	E	SWC	N	Δ	δ	WUE_t	A
		W_0	36.44[b]	19.55[a]	-27.03 [b]	5.99[b]	15.75
1	E_0	W_1	37.93[b]	19.29[a]	-26.78 [b]	6.09[b]	16.10
		W_2	48.05[a]	18.42[b]	-25.94 [a]	6.65[a]	16.61
Mean			40.81	19.08	-26.57	6.24	16.15 [x]
		W_0	33.20	19.01[a]	-27.29 [b]	5.68[b]	14.13
1	E_1	W_1	32.21	18.95[a]	-27.24 [b]	6.52[a]	12.75
		W_2	34.55	17.99[b]	-26.32 [a]	6.98[a]	15.75
Mean			33.32	18.65	-26.95	6.72	14.21 [y]
		W_0	30.80b	17.13	-28.51	5.99	12.58
1	E_2	W_1	34.61b	16.90	-28.28	5.80	15.48
		W_2	41.76a	16.69	-28.09	5.98	13.50
Mean			35.72	16.91	-28.29	5.92	13.85 [y]
		W_0	50.53	19.56[a]	-27.03 [b]	3.55 [b]	
2	E_0	W_1	55.58	18.59[b]	-26.09 [a]	3.63 [b]	
		W_2	52.33	18.58[b]	-26.11 [a]	4.64 [a]	
Mean			52.81	18.91	-26.41	3.94	
		W_0	48.38	20.34[a]	-27.78 [b]	3.67 [b]	
2	E_1	W_1	46.52	20.01[ab]	-27.47 [ab]	3.64 [b]	
		W_2	43.78	19.42[b]	-26.90 [a]	4.12 [a]	
Mean			46.22	19.92	-27.38	3.81	
		W_0	43.85b	19.95	-27.41	3.29 [b]	
2	E_2	W_1	50.59a	19.61	-27.04	3.51 [b]	
		W_2	53.85a	19.57	-27.09	3.77 [a]	
Mean			49.43	19.71	-27.18	3.52	

Different letters of [a] and [b] show significant differences across different SWC ($p < 0.05$), while [x] and [y] show significant differences between different environments.

Since all plants received the same amount of fertilizer, N content was expected to be depended on the rate of growth. Plants with a low growth rate contained more leaf N. The results on Δ and N were in fair agreement with those reported by Ehleringer (1990). The higher average assimilation rates for plants in E_0 than those of E_1 and E_2 supported the findings of Comstock and Ehleringer (1993) who reported leaf N to increase photosynthetic rates. The increased assimilation rates of W_2 compared with those of W_0 plants, although not significant, indicated the effect of leaf N on photosynthesis.

The present results showed that carbon isotope discrimination is a valuable tool in exploring transpiration efficiency and growing environment relationships of a C_3 plants. This type of research is expected to be increasingly important in view of the expected environmental changes and their influence on plant growth.

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