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Photosynthesis, water use efficiency, and $\delta^{13}C$ in two rice genotypes with contrasting response to water deficit

A.J. PIETERS[*,***] and M. NÚÑEZ[**]

Laboratorio de Ecofisiología Vegetal, Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Apartado 20632, Caracas 1020-A. Venezuela[*]
Departamento de Fisiología y Bioquímica Vegetal, Instituto Nacional de Ciencias Agrícolas, Gaveta Postal No. 1., San José de las Lajas, C.P. 32700, La Habana, Cuba[**]

Abstract

The effects of water deficit and re-irrigation were studied in glasshouse-grown rice plants (cvs. Cimarrón and Fonaiap 2000) which differ in their susceptibility to water deficit. Relative water content decreased from >90 to 67−69 % and recovered to pre-stress values within 24 h after re-irrigation. The irradiance-saturated rate of photosynthesis (P_{sat}), transpiration rate (E), and stomatal conductance (g_s) decreased with water deficit. E and g_s decreased similarly in both cultivars, but P_{sat} was more strongly inhibited in Cimarrón than in Fonaiap 2000. Water deficit increased water use efficiency (WUE_T) over 2-fold in Fonaiap 2000 and by 1.5-fold in Cimarrón. The ratio of intercellular to ambient CO_2 concentration (C_i/C_a) decreased in Fonaiap 2000 during mild stress but increased at severe stress. Contrarily, Cimarrón did not change C_i/C_a with water deficit. After re-irrigation Fonaiap 2000 recovered P_{sat} to *ca.* 80 % of control values 24 h after re-irrigation, whereas Cimarrón recovered to 60 % of control values 48 h after re-irrigation. E and g_s recovered to a lesser extent (50 %) than P_{sat}, after 48 h of re-irrigation in both cultivars. Total aboveground and green (live) biomass were unaffected by water deficit in Fonaiap 2000 but were reduced by 21 and 40 % in Cimarrón, respectively. Dead biomass increased in stressed plants of both cultivars but to a larger extent in Cimarrón than in Fonaiap 2000. Water deficit increased $\delta^{13}C$ in Fonaiap 2000, whereas Cimarrón was unaffected by water deficit showing lower values than those of Fonaiap 2000. $\delta^{13}C$ was highly and linearly correlated to the ratio C_i/C_a. WUE_T was also significantly correlated to $\delta^{13}C$.

Additional key words: dry mass; intercellular CO_2 concentration; *Oryza sativa*; relative water content; stomatal conductance; transpiration rate.

Introduction

Water deficit is probably the most important stress factor affecting plant growth and crop production worldwide (Passioura 1996) and photosynthesis is one of the main metabolic processes impaired by this stress (for a review see Chaves 1991). Maintenance of comparatively high rates of CO_2 assimilation during water deficit is a key factor for drought tolerance, as it would represent a source of extra carbon that can extend plant growth in stressful environments. Under stress conditions growth and yield are more limited by photosynthesis than they can be in optimal environments (Araus *et al.* 2002). However, water deficit affects leaf expansion (and therefore growth) earlier than photosynthesis (Boyer 1970, Hsiao 1973).

The relation between growth and photosynthesis under water deficit is complex and may vary according to the occurrence of other stresses, plant species, *etc.* Thus, understanding of factors regulating growth and development, as well as the relationship between processes taking place at the organ (photosynthesis) and those occurring at the whole plant levels (biomass accumulation) under water deficit are of major relevance for the selection of secondary traits for stress tolerance.

Water use efficiency (WUE), is an important trait associated with adaptation to water deficit (Martin and Thorstenson 1988, Condon *et al.* 2002, Rebetzke *et al.* 2002). However, accurate assessment of WUE has proved difficult. Even under glasshouse conditions proper estimations of WUE are complicated due to the difficulty of discriminating between water transpired by the plant

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[***] Author for correspondence; fax: ++58 212 5041088, e-mail: apieters@ivic.ve

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and evaporation. Instead, the ratio between leaf irradiance-saturated net photosynthetic rate (P_{sat}) and the corresponding transpiration rate (E), *i.e.* WUE_T, has been extensively used (Condon *et al.* 2004). Nevertheless, the instantaneous nature of WUE_T, although extremely useful, does not allow an integrated view of processes that link carbon gain by photosynthesis to longer term carbon fixation into plant dry mass (Martin and Thorstenson 1988). Specifically, the association between WUE_T and biomass production and yield should be carefully analysed.

The ratio of $^{13}C/^{12}C$ in plant tissue expressed relative to the PDB value ($\delta^{13}C$) is related to WUE in crop species (Farquhar and Sinclair 1984) and represents a tool to analyse plant responses to water deficit. Farquhar *et al.* (1982) demonstrated theoretically that $\delta^{13}C$ is closely correlated with the ratio of intercellular and ambient CO_2 concentration (C_i/C_a) and WUE_T. Experimental evidence has shown the validity of Farquhar's model (Brugnoli *et al.* 1988, Farquhar *et al.* 1989). Therefore, $\delta^{13}C$ under water deficit is a good surrogate of WUE_T and offers a way to relate instantaneous measurements with longer term carbon fixation into plant dry mass. However, data on the relation between $\delta^{13}C$ and yield or biomass production is inconsistent (Condon *et al.* 2004).

Rice (*Oryza sativa* L.) is a major crop providing a large proportion of the caloric intake for nearly two thirds of the world's population. Rice is a luxurious water consuming crop and is particularly sensitive to periods of water deficit, mainly in rain fed lowland and upland areas when dry periods can be unpredictable and of variable intensity and duration. Intra-specific variation in tolerance to water deficit has been reported in a variety of crops including rice (Lilley and Fukai 1994, Pieters and El Souki 2005). However, tolerance mechanisms to water deficit can be genotype-dependent making the process of detailed characterisation of genotypes with differential responses to water deficit, a primary objective to incorporate secondary traits in breeding programmes for drought tolerance.

We analysed the response of two rice genotypes with differential susceptibility to water deficit grown in the glasshouse. Biomass production and gas exchange were examined to evaluate the relation between maintenance of leaf photosynthesis and plant growth under water deficit. Carbon isotope composition was determined to establish the association between this parameter and the tolerance to water deficit and the relationship between carbon isotope discrimination, gas exchange parameters, and biomass during the development of water deficit.

Materials and methods

Plants and growth conditions: Plants of two rice cultivars with different genetic background (Cimarrón and Fonaiap 2000) were selected (Arnao *et al.*, unpublished): Fonaiap 2000 was released in the year 2000 by the National Institute of Agricultural Research (INIA), Venezuela and has previously shown tolerance to water deficit (Pieters and El Souki 2005). Cv. Cimarrón was released in 1988 by INIA and is still commercially cultivated due to its yield and good performance against diseases, and has also been used as a parental line in several crosses leading to the release of new cultivars.

Seeds of both cultivars were surface sterilized and germinated in Petri dishes with distilled water. Seven days after germination, seedlings were transferred to pots of 22 cm diameter and 25 cm height (1 seedling per pot) filled with soil, and grown in the glasshouse under natural light supplemented with sodium vapour lamps providing a minimum PPFD of 350 μmol m^{-2} s^{-1}. Maximum PPFD at the top of the canopy was 1 500 μmol m^{-2} s^{-1}. Mean temperatures were 34.7/22.3 ºC and relative humidity was 67/89 %, day/night. A 2-cm deep water lamina was maintained in each pot and plants were fertilized once with commercial fertilizer 15 : 15 : 15 (N : P : K). Six weeks after transplant, 10 pots of each cultivar were drained to eliminate the water lamina (day 0) and further irrigation was stopped. Another 10 plants of each cultivar were kept irrigated throughout as controls, and measured at the end of the experiment to detect any ageing effect. At this stage plants of both cultivars had 3−4 stems bearing 5−6 leaves per stem. Measurements started on day 0 and continued over 17−18 d (depending on the cultivar). At this point plants were re-irrigated to soil capacity and the recovery was followed over 48 h. For measurements, the youngest fully expanded leaf (5th and 6th leaf counting from the base of the plant) was used.

Relative water content (RWC) was determined in leaf segments taken at 08:00 h (local time) as RWC = (FM − DM)/(TM − DM), where FM is the leaf fresh mass, TM is the turgid mass after 6 h of re-hydration in distilled water, and DM is the dry mass of the leaf segment after being oven-dried at 60 ºC for 48 h.

Gas exchange: Leaf gas exchange was measured between 09:00 and 11:00 h (local time) with an infrared gas analyser (model *CIRAS 2*, *PP-Systems*, Hitching, Herts., UK), connected to a *PLC6* automatic universal leaf cuvette. P_{sat} was measured at a CO_2 concentration, leaf temperature, relative humidity, and PPFD of 376.0±0.3 μmol mol^{-1}, 25.3±0.19 ºC, 70 %, and 2 000±5.3 μmol m^{-2} s^{-1}, respectively.

Plant biomass: Stressed and well irrigated plants were harvested at the end of the water deficit treatment. Aboveground material was separated into green (live) and dead tissue, dried in an oven at 60 ºC for 48 h, and weighed.

Carbon isotope determination: Stable-isotope analyses were conducted in a Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS) on dried plant samples previously ground in a ball mill. Approximately 5.0 mg of plant samples were weighed in tin cups and combusted in a *Eurovector* Elemental Analyzer (Milan, Italy) at 1 050 ºC. Resulting gases were separated and analysed in an *Isoprime* isotope ratio mass spectrometer (*GV*, Manchester, England). Stable-isotope ratios were expressed as deviations from PDB standard: $\delta^{13}C$ [‰] = $[(R_{sample}/R_{standard}) - 1] \times 1\,000$, where R represents the ratio of heavy to light isotope for the sample and standard, respectively.

Statistics: Biomass and $\delta^{13}C$ were analysed by a two-way ANOVA and a Tukey test for multiple comparisons. Statistical significance was assumed when $p<0.05$. Statistical analysis was performed using *SigmaPlot* version *11.0*.

Results

RWC and gas exchange: RWC changed little during the first 13 d of water deficit and accelerated thereafter. By day 17 Cimarrón had reached a RWC of *ca*. 67 %, whereas Fonaiap 2000 showed a comparable value one day later (Fig. 1*A*). After re-watering, RWC recovered to nearly control values within 24 h and 48 h later, plants of both cultivars had fully recovered.

Despite small changes in RWC during the first 13 d of treatment, Cimarrón had decreased P_{sat} by day 4 of water deficit and by day 13 it reached rates below 2.0 μmol m^{-2} s^{-1} (Fig. 1*B*). Contrarily, Fonaiap 2000 showed a significant depression of P_{sat} by day 7 of treatment and by day 13 it still had rates of 8 μmol m^{-2} s^{-1}. After 48 h of re-irrigation, Cimarrón recovered P_{sat} by 60 %, whereas Fonaiap 2000 had restored it by nearly 80 % within 24 h of re-irrigation. Values of irrigated plants of both cultivars measured on days 17 (Cimarrón) and 18 (Fonaiap 2000) of experiment were undistinguishable from those measured on day 0 of treatment.

Transpiration rate (*E*) and stomatal conductance (g_s)

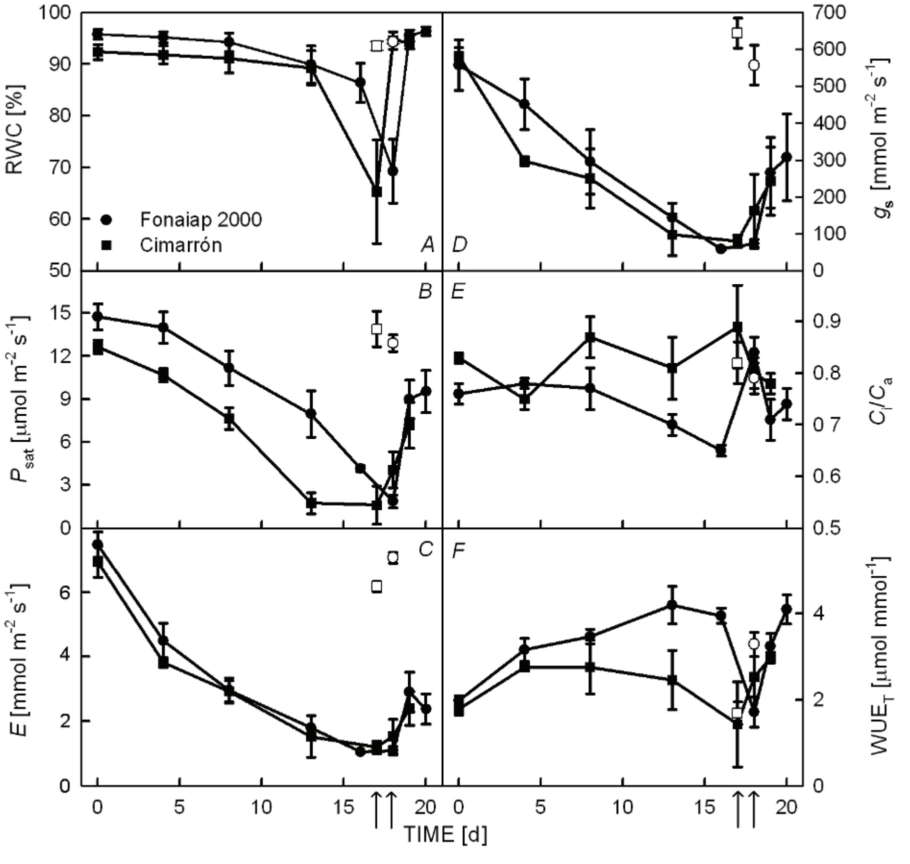

Fig. 1. Time course of (*A*) relative water content, RWC, (*B*) irradiance saturated rate of photosynthesis, P_{sat}, (*C*) transpiration rate, *E*, (*D*) stomatal conductance, g_s, (*E*) intercellular/ambient CO_2 concentrations, C_i/C_a, and (*F*) water use efficiency, WUE_T (P_{sat}/E) in plants of rice genotypes Fonaiap 2000 and Cimarrón subjected to water deficit by suspension of irrigation and re-watered. *Open symbols* are plants kept irrigated throughout and measured at the end of the experiment. *Arrows* indicate the time of re-watering for each cultivar. Means ± SE of five different plants.

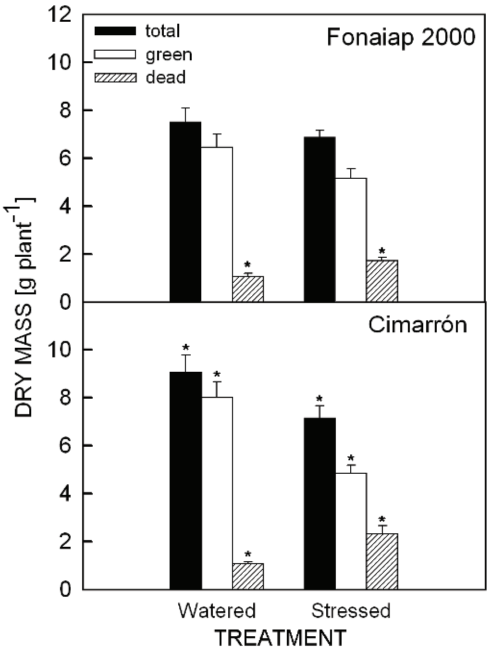

Fig. 2. Total, green (live), and dead plant biomass of rice genotypes Fonaiap 2000 and Cimarrón subjected to water deficit by suspension of irrigation for 17−18 d and plants kept irrigated throughout. Means ± SE of five different plants. Comparisons were made for each organ between water regimes. *Bars with asterisks* indicate statistically significant at $p<0.05$.

decreased similarly in both cultivars and also recovered to similar values after re-watering (Fig. 1*C,D*), although Cimarrón took 48 h to attain E and g_s values similar to those of Fonaiap 2000 24 h after re-watering. As with P_{sat}, plants kept irrigated and measured at the end of the water deficit treatment were similar to those observed before irrigation was withheld.

In Cimarrón, C_i/C_a was maintained as in control plants during water deficit (Fig. 1*E*). Conversely, in Fonaiap 2000 C_i/C_a decreased by day 13 of water deficit, when a significant drop in P_{sat} was observed, and then increased largely under severe water deficit (day 18 of treatment). Re-watering returned C_i/C_a to pre-stress values in both cultivars.

Four days after the stress was imposed, WUE_T increased in both cultivars (Fig. 1*F*). However, further increases in Cimarrón were marginal whereas in Fonaiap 2000 WUE_T continued to increase (up to 2-fold of control plants) until severe stress, when it decreased to pre-stress values in both cultivars. Re-watering increased WUE_T to values even higher than those of unstressed plants in both cultivars.

Biomass: Total aboveground biomass of Fonaiap 2000 was unaffected by water deficit (Fig. 2). The same result was observed in green biomass, whereas dead biomass increased significantly under water deficit. Cimarrón decreased its total aboveground biomass by 21 % after 17 d of water deficit and the reduction was associated to the decrease in green biomass (Fig. 2) resulting in a 2.5-fold larger dead biomass in stressed plants than in the controls. We found no changes in biomass accumulation in either cultivar 48 h after water supply was resumed (data not shown).

Table 1. Carbon isotope composition ($\delta^{13}C$) [‰] in bulk plant material of rice genotypes Fonaiap 2000 and Cimarrón subjected to water deficit or kept irrigated. Means of 5 replicates. Means followed by the same letter are not significantly different after a two-way ANOVA, $p<0.05$.

Cultivar	Water regime Watered	Water deficit
Fonaiap 2000	–28.8a	–28.3b
Cimarrón	–30.4c	–30.3c

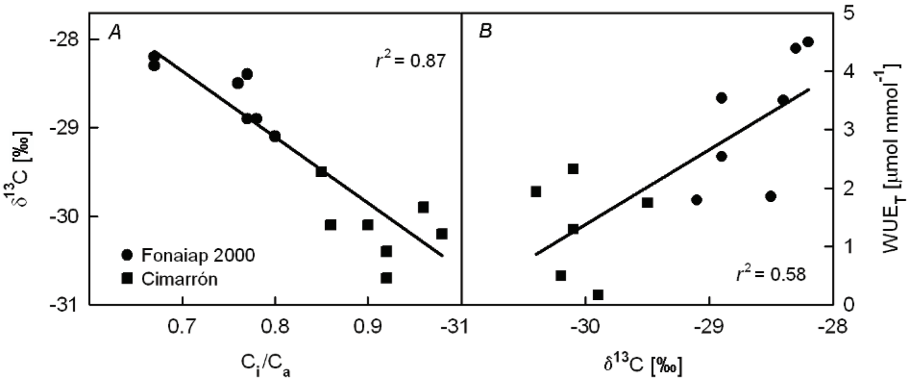

Fig. 3. Regression analysis between (*A*) carbon isotope composition ($\delta^{13}C$) and the ratio C_i/C_a, and (*B*) between water use efficiency (WUE_T) and $\delta^{13}C$ of rice genotypes Fonaiap 2000 and Cimarrón. Values were obtained from measurements made at the end of the experiment from plants either kept irrigated or water stressed for 17−18 d.

Carbon isotope composition: Fonaiap 2000 showed the highest values of $\delta^{13}C$, regardless of the water regime (Table 1). Differences in $\delta^{13}C$ between cultivars were on average over 1 ‰. The effect of water deficit was to increase $\delta^{13}C$, being statistically significant only in Fonaiap 2000. The plot of $\delta^{13}C$ *vs.* C_i/C_a resulted in a strong negative linear relation (Fig. 3*A*). On the other hand, WUE_T and $\delta^{13}C$ were positively correlated (Fig. 3*B*).

Discussion

In rice cultivar differences are often studied (*e.g.* Kumagai *et al.* 2007). Maintenance of higher P_{sat} in Fonaiap 2000 than in Cimarrón, despite similar time courses of stomatal closure, suggests that different mechanisms regulated the photosynthetic response to water deficit in the cultivars analysed. The initial decrease in C_i/C_a with the onset of water deficit observed in Fonaiap 2000 clearly indicated that diffusion limitations were predominant until severe stress (day 18) is reached. These results are in agreement with a previous report on this cultivar in which D1 protein decreased by 20 % only during severe water deficit (Pieters and El Souki 2005), similarly as did ribulose-1,5-bisphosphate carboxylase/oxygenase content (Pieters and El Souki, unpublished). Contrarily, Cimarrón maintained C_i/C_a close to that of unstressed plants even under relatively mild water deficit (day 7), suggesting an early non-stomatal limitation to photosynthesis. The view that photosynthesis was limited by metabolism in Cimarrón much earlier during water deficit than in Fonaiap 2000, is also supported by the slower recovery of P_{sat} observed in Cimarrón after re-irrigation. This suggests that other processes involving gene induction and protein synthesis that could take longer to attain pre-stress functioning are operating earlier and to a much larger extent in Cimarrón than in Fonaiap 2000.

A higher mesophyll activity during water deficit, as observed in Fonaiap 2000, could be linked to a higher tolerance to water deficit. Inoue *et al.* (2004) reported for two wheat cultivars with contrasting responses to water deficit that the tolerant cultivar showed smaller reductions in yield and growth under water deficit, associated to larger rates of photosynthesis and a decrease in C_i under non-irrigated conditions. Contrarily, the susceptible cultivar increased C_i early under water deficit and showed the largest inhibition of photosynthesis, which resulted in a much lower yield and growth under water deficit as compared to the tolerant cultivar (Inoue *et al.* 2004).

We showed that growth and senescence were differentially affected by water deficit in Fonaiap 2000 and Cimarrón. In the former cv., water deficit only accelerated senescence as dead biomass increased under stress but green biomass remained unchanged (see Fig. 2). Leaf senescence is normally associated to remobilisation of carbon and N reserves to actively growing tissue since N absorption is drastically reduced as the soil dries out. Probably, a more effective re-translocation of N to younger leaves took place in Fonaiap 2000 under water deficit, as has been shown earlier under non-stressful conditions (Pieters *et al.* 2006). A more efficient N re-translocation could have contributed to a higher P_{sat} under water deficit and to a faster recovery of this cultivar after re-watering, as P_{sat} and leaf N are closely linked. The lack of growth inhibition in Fonaiap 2000 under water deficit can thus be attributed to a more positive carbon balance during stress, because of higher P_{sat}. In Cimarrón accelerated senescence (larger than in Fonaiap 2000) and inhibition of growth were observed. These results support the idea that maintenance of higher rates of photosynthesis under water deficit can extend growth during stress events compared to cultivars with an earlier and stronger inhibition of CO_2 assimilation. Our results also support the view that under water deficit growth is more closely linked to leaf photosynthesis than under non-stressful conditions (Araus *et al.* 2002).

WUE_T has become a selection criterion for tolerance to water deficit and high yields under water scarcity (Condon *et al.* 2004). High WUE_T can be achieved by decreasing C_i/C_a (Condon *et al.* 2004). Low C_i/C_a values can arise from either decreases in E through stomatal closure or maintenance of P_{sat} at relatively high values for a given g_s. The increased WUE_T observed in Fonaiap 2000 was the result of higher photosynthetic rates under water deficit, which decreased C_i/C_a because stomata limited photosynthesis during most of the water deficit treatment. Contrarily, in Cimarrón the increase in WUE_T resulted mainly from reductions in E as P_{sat} decreased very early and reached lower values than in Fonaiap 2000 under water deficit. This suggests that selection for WUE_T alone can be misleading, unless factors determining WUE_T under water deficit are evaluated. Genotypes showing increased WUE_T, due to maintenance of P_{sat} and reductions in transpiration (Fonaiap 2000), are more likely to maintain growth and consequently yield will be less affected under water deficit. Contrarily, genotypes with increased WUE_T due solely to reductions in E will show reduced growth as a result of a diminished capacity for CO_2 assimilation (Udayakumar *et al.* 1998).

Plants of Fonaiap 2000 kept irrigated throughout the experiment grew smaller than those of Cimarrón despite showing higher $\delta^{13}C$ and WUE_T. Such apparent contradiction has been reported previously in well irrigated wheat and barley (Cooper *et al.* 1987, Condon *et al.* 1993). One explanation is that in cereals high $\delta^{13}C$ genotypes tend to have “conservative growth” in the absence of water deficit, if genotypes with high $\delta^{13}C$ and WUE_T are also genotypes with low g_s and low photosynthetic capacity (Condon *et al.* 2004). However, in our case this alternative can be ruled out as g_s and P_{sat} were very similar in Fonaiap 2000 and Cimarrón under irrigation. A possible reason for this is that plant growth is not directly related to leaf photosynthetic capacity in the absence of water deficit as high photosynthetic capacity in cereals has been achieved by concentrating N in less leaf area intercepting fewer photons (Nelson 1988). Thus, if full photon interception is not achieved, the advantage of a high photosynthetic capacity is not realised in terms of growth. This lack of positive association between crop growth and P_{sat} could explain why Fonaiap 2000 grew smaller under irrigation, despite showing higher WUE_T and δ^{13}C than Cimarrón.

The strong correlation found in this study between δ^{13}C and WUE_T (Fig. 3*B*) is in agreement with previous theoretical and experimental reports (Farquhar *et al.* 1982, Dingkhun *et al.* 1991), but contrasts with those of Scartazza *et al.* (1998) on rice, who found that carbon isotope discrimination in bulk plant material was weakly correlated to WUE and reflected an integration period of over 20 d (Scartazza *et al.* 1998). This discrepancy can be ascribed to differences in the development stage in which samples were taken in both reports. In the study of Scartazza *et al.* (1998) plants were sampled during grain filling when isotope composition of plant material is the result of several processes including recently assimilated carbon, carbon skeletons previously formed, and carbon translocated from reserve organs such as stems and leaf sheaths (Setter *et al.* 1996), therefore "diluting" the relation between C_i/C_a and plant tissue isotope composition. In our study, plants were sampled during the most active vegetative growth stage (*ca.* 45 d after transplant), when recently assimilated carbon by photosynthesis is readily incorporated into newly synthesised plant material, resulting in a strong correlation between bulk plant isotope composition and transient estimations of C_i/C_a from gas exchange techniques.

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New Hart's Rules. – Oxford University Press, New York 2005. ISBN 978-0-19-861041-0. 417 pp., £ 12.99.

More than 100 years ago, H.H. Hart, Printer to the University of Oxford and Controller of the University Press, prepared and in 1893 published a 24-page booklet. This started a series of books on rules for editors and authors. The book was modified many times and presented under different titles (*e.g.* The Oxford Guide to Style). Its format has often been changed; the content and number of pages have increased. The last, completely rewritten edition returns partly to the original name (Hart's Rules for Compositors and Readers at the University Press, Oxford) and brings broad information prepared by 15 editors and contributors (helped by another acknowledged 15 persons). Pocket size of the book helps its transport and the clear style of explanation helps its use.

Twenty chapters of the book bring general information on preparing manuscripts and editorial work, explain principles of spelling, hyphenation, punctuation, and capitalization, show how to refer to people and place names, treat italic, roman, and other types, give reference to work titles (*e.g.* to books of the Bible or Shakespeare plays), present quotations and direct speech, abbreviations and symbols, numbers and dates, include material written in foreign languages (*e.g.* Arabic, German Fraktur, Greek, Hebrew, and Russian alphabets, the often used abbreviations, and the respective transliterations, peculiarities of Slavonic languages), give law and legal references. Special chapters solve questions of writing in science (including SI units, biological nomenclature, medical terminology, and table of chemical elements), mathematics (list of mathematical symbols), and computing, arranging lists of information and tables, preparing illustrations, adding notes and references, arranging bibliographies (also website and other electronic data), preparing indexes, and in addition give information of copyright and other responsibilities. The reader finds in the chapters information on paper and book sizes, differences of British and American spelling, principles of word division, use of comma, definite and indefinite articles, *etc*. The appendices present proof-reading marks according to the British Standard, a very useful glossary of printing and publishing terms, and a detailed subject index.

The book will certainly help all authors and editors of scientific articles and books, especially those whose mother tongue is not English.

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