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BRIEF COMMUNICATION

Discrimination against $^{13}CO_2$ in leaves, pod walls, and seeds of water-stressed chickpea

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Abstract

The rate of photosynthesis (P_N) in leaves and pods as well as carbon isotope content in leaves, pod walls, and seeds was measured in well-watered (WW) and water-stressed (WS) chickpea plants. The P_N, on an area basis, was negligible in pods compared to leaves and was reduced by water stress (by 26 %) only in leaves. WS pod walls and seeds discriminated less against $^{13}CO_2$ than did the controls. This response was not observed for leaves as is usually the case. Pod walls and seeds discriminated less against $^{13}CO_2$ than did leaves in both WW and WS plants. Measurement of carbon isotope composition in pods may be a more sensitive tool for assessing the impact of water stress on long-term assimilation than is the instantaneous measurement of gas exchange rates.

Additional key words: *Cicer arietinum*; gas exchange; photosynthesis; water potential; $\delta^{13}C$.

Chickpea (*Cicer arietinum* L.) is gaining importance as a pulse in South Western Australia and its seed filling often occurs during the onset of terminal drought when leaf photosynthesis decreases significantly (Davies *et al.* 1999). The effect on seed filling of a decreased leaf P_N could be partially redressed by photosynthate contributed by pods. While there is some information on P_N of detached pods in chickpea (Sheoran *et al.* 1987), there is no information on how the rate is affected by water stress. The pod morphology makes it difficult for measurement in most commercially-available portable gas exchange systems and this difficulty is exacerbated by low rates of gas exchange in pods (Leport *et al.* 1999). The objective of this study was to explore the usefulness of carbon isotope composition as a tool for assessing the long-term response of photosynthesis to water stress in pods and leaves of chickpea. This information is lacking for pods of any species. Our hypothesis was that, like in leaves, gas exchange of pods might be adversely affected during water stress. Furthermore, any changes in pod gas exchange may be reflected in isotopic composition of the pod dry matter in a similar way to which leaf gas exchange has been shown to affect leaf isotopic composition (Farquhar *et al.* 1982).

The chickpea cv. Sona was grown one plant per 7 000 cm^3 pot containing fertilised native soil from Merredin, Western Australia. A total of 114 plants were grown in a naturally-lit glasshouse located at the CSIRO, Floreat Park (31°57'S, 115°51'E), Western Australia. The day/night temperature was controlled at 22/15 °C. Seeds were sown on 19 January 1999 and at transplanting (26 January) the roots were inoculated with group N commercial *Bradyrhizobium*. Fifty days after sowing, water was withheld from half of the pots (water stressed, WS) while the other half was kept well watered (WW) and considered as controls. The gas exchange of fully-grown pods and their subtending leaves was measured at midday using a *CIRAS-1* portable gas

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exchange system (*PPS*, Hitchin, UK). Pod gas exchange was calculated on a projected area basis. Water potential (Ψ) of leaves at dawn was measured weekly using a pressure chamber (*Soil Moisture Equipment Corp.*, USA). Ψ of pods, measured at noon, was assumed to be the same as that of their covered subtending leaves (Shackel and Turner, unpublished). Determination of discrimination against $^{13}CO_2$ was carried out by sampling pods (separated into pod walls and seeds) and their subtending leaves 34 d after the water stress treatment started. All sampling was done at midday. Carbon isotope analyses were performed at the CSIRO Plant Industry, Canberra, Australia. A *Europa 20-20* isotope ratio mass spectrometer was used for the purpose. Due to discrimination against $^{13}CO_2$ during photosynthesis, the ratio of ^{13}C:^{12}C in plants is lower than that found in the atmosphere. Organic matter is often depleted in ^{13}C relative to the standard against which it is compared. Therefore values of $\delta^{13}C$ are negative as $\delta^{13}C = [(R_{sample}/R_{standard}) - 1) \times 1000]$, where R is the ratio of ^{13}C:^{12}C. $R_{standard}$ is for Pee Dee belemnite (Farquhar *et al.* 1982). Assuming isotopic composition of atmospheric CO_2 does not vary, then a less negative figure represents less discrimination (higher ^{13}C concentration in the tissue) against $^{13}CO_2$ during photosynthesis. *Microsoft Excel 97* was used for statistical analyses. Comparison between two means was done using a *t*-test and comparison among several means was carried out by analysis of variance.

Table 1. Water potential, Ψ [MPa] and net photosynthetic rate, P_N [μmol m^{-2} s^{-1}] of well watered (WW) and water stressed (WS) leaves and pods of chickpea. For leaves Ψ was measured before dawn and for pods at noon. Across the row, the same parameters followed by different letters are significantly different at $p<0.05$. Replications per treatment were 6 and 3 for, respectively, Ψ and P_N and the measurements were taken 31 d after the stress treatment started.

Organ	Ψ		P_N	
	WW	WS	WW	WS
Leaves	-0.68a	-1.68b	22.60a	16.70b
Pods	-1.66a	-2.27b	0.48a	0.56a

Stopping irrigation for 31 d significantly decreased Ψ (Table 1). The leaf photosynthetic rate was approximately 50 times higher than that of the pod in well watered plants. Water stress lowered leaf photosynthesis (by 26 %), but it did not affect pod photosynthesis. P_N (Table 1) was measured at a photosynthetically active radiation (PAR) of 1500 μmol m^{-2} s^{-1} at noon on a sunny day. Occasional chickpea measurements on overcast days showed no differences in P_N of leaves between treatments, and pods showed only respiration on these occasions. For example, at a PAR of 350 μmol m^{-2} s^{-1}, P_N of WW and WS leaves were, respectively, 3.2 and 2.3 μmol m^{-2} s^{-1}. The corresponding values for pods were: -0.3 and -0.2 μmol m^{-2} s^{-1}. The $\delta^{13}C$ values provide an integrated measure of physiolo-gical and environmental parameters influencing the performance of the plant throughout the experimental period and not subject to prevailing conditions at the time of sampling (Ehleringer *et al.* 1993). They may therefore provide a more reliable estimate of long-term water stress effect.

Differences in P_N were not reflected in the values of $\delta^{13}C$ which were the same in WW and WS leaves, but were less negative (by 1.13 ‰) in WS pods than in WW pods (Table 2). Presumably the value of $\delta^{13}C$ in WS leaves might have been higher if sampling had been from the leaves developed during the stress period. Our experimental protocol was to sample from leaves which were already fully expanded at the onset of stress treatment and were subtending the developing pods. It is also possible that the $\delta^{13}C$ values in WS leaves did not change because of an unchanged intracellular to ambient CO_2 concentration (C_i/C_a) ratio sometimes observed in long-term acclimation to water stress as reported for oilseed rape by Jensen *et al.* (1996).

Stomata on pod walls do not seem to be fully functional judged by the very low rates of transpiration we measured in pods (less than 0.5 mmol m^{-2} s^{-1} for pods compared to 2.5 for leaves) and stomata on pods are also sparse (Sheoran *et al.* 1987). Given the low P_N in pods (Table 1), it is logical to expect that $\delta^{13}C$ in pod is affected by that in the leaves which are the major sources of carbon for the pod. The $\delta^{13}C$ of leaves might vary according to age and environmental conditions which in turn would affect the $\delta^{13}C$ of pods. However, in our experiment, pods had a higher $\delta^{13}C$ than leaves by 3.14 ‰ for the control treatment and by 4.82 ‰ for the stress treatment (Table 2). This difference is greater than can be accounted for by the effect of water stress in leaves because Winter (1981) showed that in chickpea leaves water stress resulted in an increase in $\delta^{13}C$ by 2 ‰. It is therefore logical to expect that there was also a change of $\delta^{13}C$ in pods independent of carbon imported from the leaves, that may reflect fractionation during mobilisation of assimilates from the leaves. However, to our best knowledge, there is no report in the literature alluding to this possibility. Chickpea pod wall is rich in PEP-carboxylase (Sheoran *et al.* 1987) which discriminates less against ^{13}C than does ribulose-1,5-bisphosphate carboxylase/oxygenase (Farquhar 1983). This could be another reason for the higher values of $\delta\ ^{13}C$ in pods than in leaves (Table 2).

Respiration could have played a role in carbon isotope ratio of pods. Duranceau *et al.* (1999) showed

Table 2. Effect of water stress (WS) on $\delta^{13}C$ [‰] in leaves, pod walls, and seeds of chickpea. Each value is the mean of 10 replicates per treatment. LSD: $p<0.05 = 0.73$, $p<0.01 = 0.97$.

Organ	WW	WS
Leaves	-25.78	-26.33
Pod walls	-22.64	-21.51
Seeds	-21.07	-19.85

that in cotyledonary leaves of *Phaseolus vulgaris* respiration releases more $^{13}CO_2$ than $^{12}CO_2$. While the CO_2 respired from the seed is likely to be re-fixed by the internal pod wall, as reported for pea by Atkins and Pate (1977), the CO_2 respired by the external pod wall is lost to the atmosphere. We therefore expect more depletion of ^{13}C in the pod walls than in seeds leading to more negative values of $\delta^{13}C$ in pod walls than in seeds as observed in this experiment (Table 2). For WS pods, less diffusion of atmospheric CO_2 through their stomata or a higher respiration rate could be the reason for the higher $\delta^{13}C$ values than the control (Table 2). This diffusion difference may have been too small to be detectable in our gas exchange measurement (Table 1).

This research showed that P_N in chickpea pods is too low to contribute to seed filling in well watered and water stressed plants. It has also shown that for pods measurement of $\delta^{13}C$ gives a better reflection of water stress effects than do the gas exchange measurements. Future research should concentrate on the measurement of carbon isotope ratios in the CO_2 respired from pod walls and from seeds to explain the higher values of $\delta^{13}C$ in pods than in leaves.

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