Alternate partial root-zone irrigation reduces bundle-sheath cell leakage to \( \text{CO}_2 \) and enhances photosynthetic capacity in maize leaves

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Abstract

The physiological basis for the advantage of alternate partial root-zone irrigation (PRI) over common deficit irrigation (DI) in improving crop water use efficiency (WUE) remains largely elusive. Here leaf gas exchange characteristics and photosynthetic \( \text{CO}_2 \)-response and light–response curves for maize (\( \text{Zea mays} \) L.) leaves exposed to PRI and DI were analysed under three N-fertilization rates, namely 75, 150, and 300 mg N kg\(^{-1}\) soil. Measurements of net photosynthetic rate (\( A_n \)) and stomatal conductance (\( g_s \)) showed that, across the three N-fertilization rates, the intrinsic WUE was significantly higher in PRI than in DI leaves. Analysis of the \( \text{CO}_2 \)-response curve revealed that both carboxylation efficiency (CE) and the \( \text{CO}_2 \)-saturated photosynthetic rate (\( A_{\text{sat}} \)) were significantly higher in PRI than in DI leaves across the three N-fertilization rates; whereas the N-fertilization rates did not influence the shape of the curves. The enhanced CE and \( A_{\text{sat}} \) in the PRI leaves was accompanied by significant decreases in carbon isotope discrimination (\( \Delta^{13}\text{C} \)) and bundle-sheath cell leakiness to \( \text{CO}_2 \) (\( \Phi \)). Analysis of the light–response curve indicated that, across the three N-fertilization rates, the quantum yield (\( \kappa \)) and light-saturated gross photosynthetic rate (\( A_{\text{max}} \)) were identical for the two irrigation treatments; whilst the convexity (\( \kappa \)) of the curve was significantly greater in PRI than in DI leaves, which coincided with the greater CE and \( A_{\text{sat}} \) derived from the \( \text{CO}_2 \)-response curve at a photosynthetic photon flux density of 1500 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \). Collectively, the results suggest that, in comparison with the DI treatment, PRI improves photosynthetic capacity parameters CE, \( A_{\text{sat}} \), and \( \kappa \) of maize leaves and that contributes to the greater intrinsic WUE in those plants.

Key words: Alternate partial root-zone irrigation, bundle-sheath cell leakage, carbon isotope discrimination, maize, photosynthetic capacity.

Introduction

Shortage of irrigation water constrains crop production worldwide, and with the projected climate change its impacts will become more significant in the near future (WRI, 2005). To cope with the water shortage, it is necessary to develop water-saving agriculture countermeasures, thereby producing more crops per drop. Deficit irrigation (DI) and alternate partial root-zone irrigation (PRI) are water-saving irrigation strategies being widely used in arid and semi-arid regions (Jensen et al., 2010). DI is a method that irrigates the entire root zone with an amount of water less than the potential evapotranspiration, and the mild stress has minimal effects on the yield (English and Raja, 1996). PRI is a further development of DI; it involves irrigating only part of the root zone, leaving the other part to dry to a pre-determined level before the next irrigation (Kang and Zhang, 2004). By alternately wetting
and drying part of the root zone, PRI allows the induction of the abscisic acid (ABA)-based chemical signalling from the drying roots to regulate growth and water use of the shoots, thereby increasing water use efficiency (WUE; Loveys et al., 2000). Accordingly, DI and PRI treatments are expected to trigger different water deficit stress mechanisms, consequently causing different plant physiological and growth responses (Dodd, 2007; Liu et al., 2009).

Recently, two meta-analyses have been carried out to examine the relative advantage of PRI over DI in terms of improving crop WUE (Dodd, 2009; Sadras, 2009); although the conclusions of the two analyses are somewhat different, both authors have indicated that PRI is superior to DI in enhancing crop WUE across several crop species. During recent years, in order to illustrate the mechanisms underlining the agronomic advantage of PRI over DI, a number of studies have been carried out to reveal the differences in the two types of irrigation in influencing ABA signalling (Dodd et al., 2008, 2009), root growth (Mingo et al., 2004; Liu et al., 2006), and crop N nutrition (Kirda et al., 2005; Wang et al., 2009, 2010a, b). Most of the studies showed that, under a similar degree of water saving, PRI plants possess significantly stronger ABA signalling, larger root systems, and greater N accumulation than do the DI plants (Mingo et al., 2004; Dodd et al., 2008; Wang et al., 2010a), and all of these responses might have contributed to the higher WUE in those plants.

In maize (Zea mays L.), PRI has shown a great potential to save water and increase crop WUE (Kang et al., 1998; Kirda et al., 2005; Li et al., 2007; Hu et al., 2009). Both Kirda et al. (2005) and Li et al. (2007) have reported that, compared with DI treatment, PRI could also improve fertilization-N use efficiency in maize plants. Most recently, studies on C3 plants including potato and tomato have been carried out to reveal the differences in the conclusions of the two analyses are somewhat different, thereby increasing water use efficiency (WUE; Loveys et al., 2000). Accordingly, DI and PRI treatments are expected to trigger different water deficit stress mechanisms, consequently causing different plant physiological and growth responses (Dodd, 2007; Liu et al., 2009).

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Materials and methods

Plant material and growth conditions

The experiment was conducted from 1 April to 24 June 2010 in a climate-controlled greenhouse at the experimental farm of the Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark. At the fifth leaf stage, maize (Z. mays L.) seedlings were transplanted into 19.6 l pots (25 cm diameter and 40 cm deep). The pots were evenly divided into two vertical compartments by plastic sheets, such that water exchange between the two compartments was prevented. The pots were filled with 20.2 kg of naturally dried soil with a bulk density of 1.14 g cm\(^{-3}\). The soil was classified as sandy loam, having a pH of 6.7, total C 12.9 g kg\(^{-1}\), total N 1.4 g kg\(^{-1}\), NH\(_4\)-N 0.7 mg kg\(^{-1}\), and NO\(_3\)-N 19.1 mg kg\(^{-1}\). The soil was sieved by it passing through a 2 mm mesh and it had a volumetric soil water content (SWC) (% vol.) of 30.0% and 5.0% at water holding capacity and permanent wilting point, respectively. The average SWC of the soil was monitored by a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp., Goleta, CA, USA) with probes (33 cm in length) installed in the middle of each soil compartment. The climate conditions in the greenhouse were set at: 26/20±2 °C day/night air temperature, 15 h photoperiod, and >500 μmol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density (PPFD) supplied by sunlight plus metal-halide lamps. The concentration of CO\(_2\) in the greenhouse remained almost stable throughout the experiment, approximately equal to the concentration in the outside air (i.e. 380 μl l\(^{-1}\)).

N-fertilization and irrigation treatments

Three N-fertilization rates, namely low N (N1, 75 mg N kg\(^{-1}\) soil), medium N (N2, 150 mg N kg\(^{-1}\) soil), and high N (N3, 300 mg N kg\(^{-1}\) soil), were included in the experiment. The N fertilizer supplied as NH\(_4\)NO\(_3\) was mixed thoroughly with the soil before filling the pots. In addition, P and K were also applied as KH\(_2\)PO\(_4\) (380 mg kg\(^{-1}\) soil) and K\(_2\)SO\(_4\) (130 mg kg\(^{-1}\) soil) to the soil to meet the nutrient requirement for plant growth. The maize plants were well watered in the first 10 d after transplanting. Thereafter, the plants were exposed to two deficit irrigation regimes: (i) PRI in

quantifies the efficiency of the CO\(_2\)-concentrating mechanism (Farquhar, 1983). Therefore, it has been frequently used as an indicator for evaluating the C\(_4\) photosynthetic efficiency (Williams et al., 2001; Ubierna et al., 2011). Studies in maize, sorghum (Sorghum bicolor L.), and other C\(_4\) grasses have shown that severe drought stress could significantly increase Φ (Bowman et al., 1989; Williams et al., 2001, and references therein). However, until now it has remained unknown whether Φ is influenced by water-saving irrigation regimes where the plants are exposed to moderate drought stress.

Here data are presented on gas exchange, photosynthetic CO\(_2\)-response and light–response curves, carbon isotope discrimination (Δ\(^{13}\)C), and bundle-sheath cell leakiness to CO\(_2\) (Φ) of maize leaves subjected to PRI and DI treatments. In addition, three N-fertilization rates were included in the study in order to clarify the interactive effect between the irrigation regimes and plant N levels. The purpose was to explore the mechanisms by which the photosynthetic capacity of maize leaves is influenced by different water-saving irrigation strategies, and to examine if such effects could account for the superiority of PRI compared with DI in improving WUE.

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which one soil compartment was watered daily to SWC 28% (vol.) while the other was allowed to dry to ~10 d, then the irrigation was shifted to the other compartment; and (ii) DI in which the same amount of water as used for the PRI plants was used to irrigate the whole pot evenly. The experiment was a complete factorial design comprising nine treatments and each treatment had four replicates. The irrigation water was tap water with negligible concentrations of nutrients. The irrigation treatments lasted for 8 weeks, during which each soil compartment of the PRI plants had experienced six drying/wetting cycles. The average SWC in the pots from 29 to 51 d after onset of treatment (DAT) was calculated to reveal the degree of soil water deficits under the two irrigation regimes.

Leaf gas exchange, chlorophyll content index, water potential, and stable carbon isotope signatures

Diurnal gas exchange [net photosynthetic rate (\(A_a\)) and stomatal conductance \(g_s\)] measurements were made on five sunny days (i.e. 29, 30, 31, 44, and 51 DAT) with a Li-6200 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA) on the first fully expanded leaf, which is the fourth leaf counted from the top of the shoot. The intercellular to ambient CO\(_2\) concentration ratio \(\frac{C}{C_a}\) obtained from the gas exchange measurements were used in the calculations of the bundle-sheath cell leakiness to CO\(_2\) (\(\Phi\)).

The chlorophyll content index (CCI) was taken using a CCM-200 (Opti-Science, Tyngsboro, MA, USA) 44 DAT from the uppermost fully expanded leaf. The CCI values are closely correlated with the \(Chl_a \) and \(Chl_b\) and total \(Chl\) contents in the leaves and, therefore, have been used as a good indicator for photosynthetic capacity of the leaves (Richardson et al., 2002). All CCI readings were taken midway between the stalk and the tip of the leaf; values of each leaf were the mean of five readings around the same position. Midday leaf water potential \(\Psi_l\) was measured 45 DAT with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on the leaves just below those used for gas exchange measurements from 10:30 h to 13:00 h.

The plants were harvested on 24 June (51 DAT). The biomass of leaves used for \(\delta^{13}\)C measurements were determined after drying an oven at 70 °C for 72 h. Dry samples of leaves were ground to a fine powder for \(\delta^{13}\)C analysis using the Dumas dry combustion method in a system consisting of an ANCA-SL Elemental Analyser coupled to a 20-20 Tracer Mass Spectrometer (Europa Scientific Ltd, Creve, UK).

The \(\delta^{13}\)C composition of \(\delta^{13}\)C of leaf dry biomass was calculated as:

\[
\delta^{13}C = 1000 \left[ \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right]
\]

where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are the \(\delta^{13}\)C/\(\delta^{12}\)C ratios of the leaf sample and Pee Dee Belemnite (PDB) standard, respectively.

Carbon isotope discrimination \(\Delta (\delta^{13}\text{C})\) of the leaf samples was calculated using Equation 2:

\[
\Delta^{13}C = \frac{\delta_a - \delta_p}{1 + \delta_p}
\]

where the subscripts \(a\) and \(p\) refer to air and the plant, respectively (Farquhar et al., 1989). The \(\delta_a\) value for the ambient atmosphere was taken as –7.7%o.

Leakiness of the bundle-sheath cells to CO\(_2\) (\(\Phi\))

\(\Phi\) was estimated using the equations derived by Farquhar et al. (1989) for \(C_4\) photosynthesis. The instantaneous values of \(C_{i}/C_a\) from the 5 d leaf gas exchange measurements were combined with time-integrated values of \(\Delta^{13}\text{C}\). Using this approach, \(\Phi\) was estimated as:

\[
\Phi = \frac{\Delta^{13}C - a + (a - b)C_i/C_a}{\beta_3 - s}C_i/C_a
\]

\(\Delta^{13}C\) is the fractionation occurring during diffusion of CO\(_2\) into the leaf; \(b\) (–5.7%o) is the combined fractionation due to PEPC (2.2%o) and the activity of carbonic anhydrase in the mesophyll, \(b_3\) (30%o) is the fractionation by Rubisco, and \(s\) (1.8%o) is the fractionation associated with leakage of CO\(_2\) from the bundle sheath to the mesophyll (von Caemmerer et al., 1997).

**Determination of the photosynthetic CO\(_2\)-response curve**

The photosynthetic CO\(_2\)-response curve was determined using a Ciras-II Portable Photosynthesis System (Ciras-II, PP Systems, UK) with a PPFD level of 1500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) from 40 to 48 DAT on the same leaves used for gas exchange measurements. Measurements were taken at CO\(_2\) levels of 650, 250, 100, 50, 700, 1000, and 1500 \(\mu\)l l\(^{-1}\). \(A_p\) values were plotted against the respective intercellular CO\(_2\) concentrations \(C_i\) to produce a response curve. The model of \(C_p\) photosynthesis developed by von Caemmerer and Furbank (1999) was used to simulate the CO\(_2\) response curves. This model was expressed as:

\[
A_n = a(1 - e^{-bx}) + c
\]

where \(A_n\) is the net photosynthetic rate and \(x\) is \(C_i\). Using this equation, the CO\(_2\)-saturated photosynthetic rate \(\left(A_{sat}\right)\) was calculated as \(a + c\) and the carboxylation efficiency (CE) as the slope at \(A_n = 0\) (calculated as \(b[a+c]\)).

**Determination of the photosynthetic light–response curve**

During the same days on which the photosynthetic CO\(_2\)-response curves were measured, the photosynthetic light–response curves were developed by measuring \(A_n\) at different PPFD levels using the Ciras-II Portable Photosynthesis System at a CO\(_2\) concentration of 400 \(\mu\)l l\(^{-1}\). Measurements were taken at PPFD levels of 1000, 1500, 2000, 700, 500, 300, 200, 100, 50, and 0 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). The photosynthetic light–response curve was modelled by a non-rectangular hyperbola. Photosynthetic parameters derived from the light–response curves were determined according to the method described by Richardson et al. (2002). The model was expressed as:

\[
A_n = \frac{\alpha Q + A_{max} - \sqrt{(\alpha Q + A_{max})^2 - 4\alpha\alpha Q A_{max} - R_d}}{2\alpha}
\]

where \(A_n\) is the rate of net photosynthesis (\(\mu\)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)); \(Q\) is the PPFD (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)); \(A_{max}\) is the dark respiration rate (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)); \(\alpha\) is the maximum apparent quantum yield of CO\(_2\) (\(\mu\)mol CO\(_2\) mol\(^{-1}\) photons); and \(\kappa\) is a dimensionless convexity term (0 < \(\kappa\) < 1)).

**Statistical analysis**

The non-linear regression procedure of the statistical programme SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA) was applied to fit photosynthetic CO\(_2\)–response and light–response curves for individual plants. Linear regressions and analysis of covariance (ANCOVA; SPSS) were used to test the effects of irrigation regimes on \(A_n\) and \(g\) across the three N-fertilization rates. Two-way analyses of variance (ANOVA; SPSS) were made to determine the effects of the irrigation regimes and N-fertilization rates on measured SWC, CCI, \(\Phi\), \(C_i/C_a\), \(\Delta^{13}\text{C}\), \(\Phi\), and the parameters derived from the CO\(_2\)-response and light–response curves.
Results

Relationship between stomatal conductance and photosynthetic rate

Across the three N-fertilization rates, there were significant linear relationships between $A_n$ and $g_s$ for both the DI and PRI plants (Fig. 1). The slopes of the two regression lines, namely the intrinsic WUE, were significantly greater in the PRI than in the DI leaves ($P < 0.05$) (Fig. 1).

Soil water content, chlorophyll content index, leaf water potential, carbon isotope discrimination, intercellular to ambient CO$_2$ concentration ratio, and the bundle-sheath cell leakage to CO$_2$

Figure 2a and Table 1 show that, across the three N-fertilization rates, SWC was identical for DI and PRI pots, whereas SWC was significantly affected by N-fertilization rates, and there was a significant interactive effect between irrigation regimes and N-fertilization rates on SWC (Table 1). In DI pots, SWC was the highest under N1, intermediate under N2, and the lowest under N3; whereas in PRI, SWC was similar across the three N-fertilization rates. Comparing the two irrigation regimes, SWC of DI was significantly higher than PRI under N1, and the opposite was true under N3; while under N2, SWC was similar for the two irrigation regimes.

Figure 2b shows that both N-fertilization rates and irrigation regimes had significant effects on CCI; across the two irrigation regimes, N2 and N3 leaves had significantly higher CCI than the N1 leaves; whereas, when

analysed across the three N-fertilization rates, it was found that PRI plants had significantly higher CCI than DI plants.
However, there were no significant interactions between N-fertilization rates and irrigation regimes on CCI in maize leaves (Table 1).

Across the N-fertilization rates, there was no significant difference for leaf water potential ($\Psi_1$) under PRI and DI (Fig. 2c, Table 1); whereas across the irrigation regimes, $\Psi_1$ was significantly higher in N2 than in N1 and N3 (Fig. 2c).

Irrigation regimes had a significant effect on $\Delta^{13}C$ and $\Phi$ in maize leaves (Table 1), which is especially true under N1 and N2 treatments (Fig. 2e, f). When analysed across the N levels, it was found that both $\Delta^{13}C$ and $\Phi$ were significantly less in PRI than in DI leaves (Fig. 2e, f, Table 1). However, $C/C_a$ was influenced neither by the N-fertilization rates nor the irrigation regimes (Fig. 2d, Table 1). As both $C/C_a$ and $\Phi$ could affect $\Delta^{13}C$, $\Delta^{13}C$ was plotted separately against $C/C_a$ and $\Phi$ (Fig. 3). It can be seen that only $\Phi$ was significantly correlated with $\Delta^{13}C$ (Fig. 3b) and there was no clear relationship between $C/C_a$ and $\Delta^{13}C$ (Fig. 3a), indicating that $\Phi$ was the major factor influencing $\Delta^{13}C$ in maize leaves (Fig. 3).

### Photosynthetic CO$_2$–response curve

The model of $C_4$ photosynthesis developed by von Caemmerer and Furbank (1999) was used to simulate the photosynthetic CO$_2$–response curve for maize leaves (Equation 4, Fig. 4). The initial slope of the curve indicates the CE, whereas the plateau denotes the $A_{sat}$. It was found that both CE and $A_{sat}$ were significantly affected by the irrigation regimes; PRI leaves had significantly higher CE and $A_{sat}$ values than the DI leaves when analysed across the N-fertilization rate; whilst N-fertilization rates did not affect the two parameters (Table 2, Fig. 4).

### Photosynthetic light–response curve

The light–response curves of maize leaves under different N-fertilization rates and irrigation regimes are shown in Fig. 5. The light–response curves show important photosynthetic characteristics including $\alpha$, $A_{max}$, $\kappa$, and $R_d$. N-fertilization increased the $A_{max}$ of maize, but not significantly; whereas the irrigation regimes hardly affected $A_{max}$ in maize leaves. $\alpha$, the efficiency of light utilization in photosynthesis, was enhanced with the increase of N levels, although not significantly. Similarly, there were no significant differences for $\kappa$ among irrigation regimes. Interestingly, across the N-fertilization rate, PRI leaves had significantly higher $\kappa$ that DI leaves ($P = 0.046$).

### Discussion

Based on literature surveys and meta-analyses, Sadras (2009) and Dodd (2009) concluded that, given a similar degree of water saving, PRI is superior to DI in terms of improving WUE in several crop species. Consistent with this, it was found here that the intrinsic WUE of maize leaves was significantly higher in PRI than in DI plants (Fig. 1). A higher intrinsic WUE could be achieved by either an increase in $A_{sat}$ or a decrease in $g_s$. In the present study, both possibilities were probably involved, and the trend of changes in $A_{sat}$ and $g_s$ could have been modulated by either the irrigation regime or the N-fertilization treatments, or both. Under N1, while keeping a similar value of $A_{sat}$, PRI was seemingly more efficient in reducing $g_s$ as compared with DI; whereas under N2, $g_s$ ranged from 0.3 mol m$^{-2}$ s$^{-1}$ to 0.4 mol m$^{-2}$ s$^{-1}$ for both PRI and DI plants, and the $A_{sat}$ values were generally higher in PRI than in DI. Under N3, the $g_s$ and $A_{sat}$ remained lower in both PRI and DI plants compared with N1 and N2 treatments. Nevertheless, under both N1 and N2 conditions, an improvement in the $A_{sat}/g_s$ ratio, namely the intrinsic WUE, could be achieved. It was confirmed by comparing the slopes of the two regression lines in Fig. 1 that the intrinsic WUE was significantly higher in PRI than in DI plants. The lowered $g_s$ in the PRI plants under N1 might have been a result of fine-tuning of stomatal control by the ABA signalling under PRI, as has been suggested in many earlier studies (e.g. Liu et al., 2006, 2009). While most of those studies have exclusively highlighted the significance of a lowered $g_s$ induced by the xylem-borne ABA signalling in contributing to the improvement of WUE, the potential role of an increase in the photosynthetic capacity in enhancing crop WUE under PRI irrigation has received less attention. The present study was, therefore, designed to illustrate whether the photosynthetic capacity of maize leaves is improved by PRI in relation to its counterpart DI.

In $C_4$ plants, photosynthetic efficiency can be evaluated by several parameters relating to the efficiencies of CO$_2$ transportation, concentration, and fixation. It is widely accepted that measurement of the carbon isotope discrimination ($\Delta^{13}C$) of leaf samples can reflect the environmental influences on the efficiency of photosynthesis in plants (Farquhar et al., 1989). For instance, measurements of $\Delta^{13}C$ in $C_4$ plants have been shown to vary in response to soil water availability (Buchmann et al., 1996; Salindra et al., 1996). Models relating $C_4$ photosynthesis to $\Delta^{13}C$ suggest that changes in $\Delta^{13}C$ are largely the result of increases in $\Phi$ (Farquhar et al., 1989). Here, the result in maize leaves showed that PRI decreased $\Delta^{13}C$ compared with DI and this decrease might have been a result of lowered $\Phi$ as exemplified by the significant correlation.
between the two variables (Table 1, Fig. 3b). In the present experiment, \( \Phi \) estimated from \( \Delta^{13}C \) and \( C_i/C_a \) (Equation 3) was similar to those reported for other C4 plants (Bowman et al., 1989; Saliendra et al., 1996; Meinzer et al., 1998). Most interestingly, \( \Phi \) was significantly higher in the DI (0.38) than in PRI plants (0.31); and these values of \( \Phi \) implied that close to 40% of the CO2 fixed by PEPC and transported to bundle-sheath cells was subsequently leaked back to the mesophyll under DI, while for PRI this values was ~30%. Bowman et al. (1989) have shown that \( \Phi \) values could reach 0.55 in water-stressed maize leaves. However, the significantly lowered \( \Phi \) in the PRI leaves as compared with the DI leaves observed here was not due to a better water status of the plants as both SWC and \( W_l \) were identical for the two irrigation regimes (Table 1). Earlier studies have indicated that the magnitude of \( \Phi \) in C4 plants was determined by both the physical conductance of bundle-sheath cell walls and the balance between Rubisco and PEPC activity (Farquhar et al., 1989). Particularly under drought stress the increased \( \Phi \) may be caused by a reduced activity of C3, relative to C4, cycle enzymes; namely a lowered Rubisco to PEPC activity ratio (von Caemmerer and Furbank, 1999). In addition, Ranjith et al. (1995) found that the Rubisco to PEPC activity ratio decreases linearly with declining leaf N content. Therefore, a lowered leaf N content could be associated with an increase in \( \Phi \). In the present study, although the leaf N content was not determined, based on the fact that a significant portion of leaf N is present in the chlorophyll, the significantly higher CCI value for PRI than for DI

**Fig. 3.** Relationships between carbon isotope discrimination (\( \Delta^{13}C \)) and the ratio of intercellular to ambient CO2 concentration (\( C_i/C_a \)) and the bundle-sheath leakage to CO2 (\( \Phi \)) of maize leaves exposed to different irrigation and N-fertilization treatments. Values are the means±SE (n=4).

**Fig. 4.** Photosynthetic CO2–response curves of maize leaves exposed to different irrigation and N-fertilization treatments (the measurements were made at a PPFD of 1500 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)). The two regression curves (based on Equation 4) are made for the DI and PRI plants, respectively, across the three N-fertilization rates. For DI leaves, the carboxylation efficiency (CE) and the CO2-saturated photosynthetic rate (\( A_{sat} \)) were 0.69±0.03 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) and 23.9±1.20 \( \mu \)mol m\(^{-2}\) s\(^{-1}\), respectively; both were significantly less than those for PRI leaves (i.e. 0.98±0.09 and 31.7±2.55, respectively). Statistical comparisons (two-way ANOVA) of the parameters between the irrigation and N-fertilization treatments and their interactions are shown in Table 2.

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**Table 2.** Output of the two-way analysis of variance (ANOVA) for PEPC carboxylation efficiency (CE, \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) and the CO2-saturated photosynthetic rate (\( A_{sat} \), \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) derived from the photosynthetic CO2–response curve (Equation 4, Fig. 4) and for the maximum apparent quantum yield of CO2 (\( \alpha \), \( \mu \)mol CO2 mol\(^{-1}\) photons), the irradiance-saturated rate of gross photosynthesis (\( A_{max} \), \( \mu \)mol m\(^{-2}\) s\(^{-1}\)), the dark respiration rate (\( R_d \), \( \mu \)mol m\(^{-2}\) s\(^{-1}\)), and the dimensionless convexity term (\( \kappa \)) derived from the photosynthetic light–response curve (Equation 5, Fig. 5) of maize leaves as affected by the irrigation (DI and PRI) and N-fertilization (N) treatments

| Factor | CE | \( A_{sat} \) | \( \alpha \) | \( A_{max} \) | \( R_d \) | \( \kappa \) |
|--------|----|---------------|-------------|-------------|---------|----------|
| Irrigation * | * | NS | NS | NS | NS | * |
| \( N \) | NS | NS | NS | NS | NS | NS |
| Irrigation \( N \) | NS | NS | NS | NS | NS | NS |

* indicates the significance level at \( P < 0.05 \); NS denotes non-significance.
treatments and their interactions are shown in Table 2. ANOVA) of the parameters between the irrigation and N-fertilization treatments (the measurements were made at a CO2 concentration of 400 μl−1).

The two regression curves (based on Equation 5) are made for the DI and PRI plants, separately, across the three N-fertilization rates. For DI leaves, the maximum apparent quantum yield of CO2 (α, mol CO2 mol−1 photons), the irradiance-saturated rate of gross photosynthesis (Amax, μmol m−2 s−1), the dark respiration rate (Rd, μmol m−2 s−1), and the dimensionless convexity term (κ) were 0.037±0.001, 43.1±1.05, 1.35±0.11, and 0.73±0.04, respectively; while for the PRI leaves, the values were 0.035±0.001, 40.2±1.20, 1.87±0.22, and 0.90±0.03, respectively. Here, only the convexity (κ) of the curve for PRI leaves was significantly greater than for the DI leaves. Statistical comparisons (two-way ANOVA) of the parameters between the irrigation and N-fertilization treatments and their interactions are shown in Table 2.

Fig. 5. Photosynthetic light–response curves of maize leaves exposed to different irrigation and N-fertilization treatments (the measurements were made at a CO2 concentration of 400 μl−1).

plants may indirectly confirm that the former had higher N contents in the leaves, and this might have led to a higher Rubisco to PEPC activity ratio and thereby a lowered Φ in the PRI plants. However, when analysed across all the combinations of the irrigation and N-fertilization treatments, there was no clear link between CCI and Φ of maize leaves (Fig. 2b, f), and Φ did not respond to N-fertilization rates (Table 1). The reasons for the disassociation between the N-fertilization rates/or leaf N status (CCI) and photosynthetic capacity parameters of maize leaves observed here remain unknown. It is speculated that most of the plants used here might have had been grown under luxury N conditions as the N requirement for achieving Amax is much less in C4 than in C3 plants (Sinclair and Horie, 1989). This was unlike the case in the study by Ranjith et al. (1995) where the plants were exposed to N stress. Therefore, rather than leaf N content, other unknown factors might have exerted a role in maintaining a small Φ in the PRI maize leaves.

It is believed that a small Φ will be beneficial for achieving a high level of photosynthesis in C4 plants (Farquhar et al., 1989). von Caemmerer and Furbank (1999) found that increasing Φ could cause a decline in both CE and Asat derived from the CO2–response curve. In good agreement with this, here it was found that, across the three N-fertilization rates, both CE and Asat were significantly less in DI than in PRI plants (Fig. 4, Table 2). Similarly finding has been observed by Tahi et al. (2007) in tomato plants where the Rubisco carboxylation efficiency was less affected by PRI than by DI treatments in relation to the fully irrigated controls. In addition, increased Φ may result in a decline in the light use efficiency of C4 plants, because CO2 that leaks from the bundle-sheath cells is either lost or re-fixed by PEPC in the mesophyll, thus increasing the energy costs for CO2 fixation (Farquhar, 1983; Hatch et al., 1995; Watling et al., 2000). However, analysis of the photosynthetic light–response curve of maize leaves indicated that there was no difference in the quantum yield (α) between the PRI and DI plants (Table 2, Fig. 5). von Caemmerer et al. (1997) suggested that the relationship between Φ and α is non-linear, so that a relatively large increase in Φ actually has a rather small impact on α. In addition, the irrigation regimes and N-fertilization rates had no significant effects on most of the photosynthetic parameters derived from the light–response curve of maize leaves, except for the convexity (κ) of the curve, which was significantly higher in the PRI than in DI plants (Table 2). Earlier work by Ogren (1993) had pointed out that κ determines the photosynthetic efficiency in the intermediate light range under natural growing conditions. A greater κ means that Asat increases much more rapidly to Amax with increasing PPFD. Consistent with this, in the present study, it was observed that at a PPFD of 1500 μmol m−2 s−1, the photosynthetic efficiency, as exemplified by the higher CE and Asat derived from the CO2–response curve, was significantly enhanced under the PRI treatment. As has been discussed previously, the lower Φ could have contributed to the higher CE and Asat in the PRI leaves; however, more investigations are necessary to explore the biochemical and physiological mechanisms underlying the increase in κ under PRI treatment.

In conclusion, the results suggest that, in comparison with the DI treatment, the PRI irrigation enhances WUE of maize leaves, which was associated with an improved photosynthesis capacity as indicated by a lowered Φ and consequently a higher CE and Asat under normal light conditions.

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