Comparative effects of deficit irrigation and alternate partial root-zone irrigation on xylem pH, ABA and ionic concentrations in tomatoes

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Abstract

Comparative effects of partial root-zone irrigation (PRI) and deficit irrigation (DI) on xylem pH, ABA, and ionic concentrations of tomato (Lycopersicon esculentum L.) plants were investigated in two split-root pot experiments. Results showed that PRI plants had similar or significantly higher xylem pH, which was increased by 0.2 units relative to DI plants. Nitrate and total ionic concentrations (cations+anions), and the proportion of cations influenced xylem pH such that xylem pH increases as nitrate and total ionic concentrations decrease, and the proportion of cations increases. In most cases, the xylem ABA concentration was similar for PRI and DI plants, and a clear association between increases in xylem pH with increasing xylem ABA concentration was only found when the soil water content was relatively low. The concentrations of anions, cations, and the sum of anions and cations in PRI were higher than in the DI treatment when soil water content was relatively high in the wetted soil compartment. However, when water content in both soil compartments of the PRI pots were very low before the next irrigation, the acquisition of nutrients by roots was reduced, resulting in lower concentrations of anions and cations in the PRI than in the DI treatment. It is therefore essential that the soil water content in the wet zone should be maintained relatively high while that in the drying soil zone should not be very low, both conditions are crucial to maintain high soil and plant water status while sustaining ABA signalling of the plants.

Key words: Abscisic acid, anions, cations, pH, partial root-zone drying, xylem sap.

Introduction

A worldwide shortage of freshwater resources and more frequent and severe drought due to climate change has stimulated research into water-saving irrigation strategies aimed at producing more crops per drop. Enhancing crop water and nutrient use efficiency is essential for increasing crop productivity and sustaining the environment.

Deficit irrigation (DI) and alternate partial root-zone irrigation (PRI) are water-saving irrigation techniques being intensively studied in many regions of the world on a wide range of crops and fruit trees. DI is a method that irrigates the entire root zone with an amount of water less then the potential evapotranspiration during drought-insensitive growth stages and the minor stress that develops has minimal effects on the yield (English and Raja, 1996). PRI is a further refinement of DI and was developed based on the findings from the laboratory split-root experiments (Blackman and Davies, 1985) utilizing the plant root-to-shoot chemical signals to influence shoot physiology in drying soil (Davies and Zhang, 1991; Tardieu and Davies, 1992). The principle behind PRI is alternately to let one part of the root system be exposed to soil drying while the other part is irrigated in order to keep the leaves hydrated. PRI has been demonstrated to result in considerable water savings—in some cases almost a doubling of water use efficiency (WUE) (Kirda et al., 2007). Accumulated evidence has demonstrated that, given the same amount of irrigation water, PRI is superior to DI in terms of yield maintenance and an increase in WUE (Dodd, 2009; Liu et al., 2009; Sadras, 2009; Wang et al., 2011).
2010a, b, c), as well as an increase of carbon (C) and nitrogen (N) concentrations in the plant biomass (Wang et al., 2009, 2010a, b, c).

It is widely believed that the production of abscisic acid (ABA) in the drying roots and its transport to the leaves in the xylem stream play a dominant role in the chemical signalling of soil water status and in the control of stomatal conductance (Loveys, 1984; Zhang and Davies, 1990; Davies and Zhang, 1991; Sauter et al., 2001; Davies et al., 2002; Liu et al., 2005; Dodd, 2007; Schachtman and Goode, 2008). Earlier studies have shown that both PRI and DI can induce the ABA-based root-to-shoot chemical signals regulating stomatal conductance and leaf expansion growth thereby increasing water use efficiency (WUE) (Dodd, 2007; Wang et al., 2010a). However, accumulated evidence indicated that, at a similar degree of soil water deficit in the whole root zone, PRI plants process greater xylem ABA concentrations relative to DI plants and hence lead to a better control of plant water loss thereby causing further improvement to WUE (Dodd, 2007; 2009; Wang et al., 2010a). In addition to ABA acting as the root-to-shoot signal, changes in the pH of the xylem sap commonly observed under drought stress can be another important signalling molecule and may act synergistically with ABA signalling. In many plant species, xylem sap pH becomes more alkaline when plants are drought-stressed, which leads to the accumulation of ABA in the apoplastic resulting in enhanced stomatal closure and even reduced growth (Wilkinson and Davies, 1997, 2002; Hartung et al., 1998; Davies et al., 2002; Schachtman and Goode, 2008). The mechanism of pH change in the xylem sap may involve nitrate availability. A low nitrate concentration in the xylem may lead to a switch of nitrate reductase activity from the shoots to the roots (Lips, 1997), and this gives rise to greater concentrations of inorganic acids such as malate in the xylem hence alkalinizing the xylem sap (Kirkby and Armstrong, 1980; Patonner et al., 1999). It is known that N is the most essential nutrient for plants and is thought to play a role in modifying root-to-shoot signalling under drought (Goode and Schachtman, 2010). Many studies have noticed a reduction in xylem nitrate concentration as soil dries (Gollan et al., 1992; Bahrun et al., 2002), whilst some researches have also observed that xylem nitrate concentrations increase as soil dries (Goode et al., 2005; Jia and Davies, 2007). In addition, soil drying can modify the uptake of other inorganic ions into the xylem, i.e. potassium and calcium, which may influence the strength of the xylem-borne hormonal signalling (Wilkinson et al., 2001).

The xylem conduits are the main pathway for the long-distance transport of water and nutrients from the root to the shoot (Marschner, 1995). Thus, the characterization of changes in xylem sap composition under drought is important to illustrate the mechanisms of crop response to soil water and nutrient dynamics. In addition to the aforementioned drought effects on ABA concentration and pH in the xylem, other parameters such as the xylem ionic constitution may also act as signals in root–shoot communication under conditions of soil water deficits (McDonald and Davies, 1996). Moreover, the xylem sap composition has been used as a diagnostic tool of soil nutrient availability in relation to the nutrient requirements of adult trees (Stark et al., 1989), and the qualification of nutrient status in the xylem sap may be an alternative for assessing crop nutrient uptake. Our recent studies have revealed that PRI can improve plant N nutrition and optimize N distribution in the canopy when compared with DI treatment, which may partly account for the greater WUE in the PRI tomato plants (Wang et al., 2010a, b). The improved plant N nutrition under the PRI treatment could be mainly attributed either to a greater mineral N availability in the soil (Wang et al., 2010b) or to a higher root N acquisition ability.

In the present study, in order to exploit the potential of PRI as a management tool in improving crop water and nutrient use efficiencies further, its effects on xylem sap pH, ABA concentration, and ionic concentrations compared with DI treatment at different soil and plant water status were investigated in tomato plants grown under different soil N availability, namely, achieved by mineral and organic N fertilizations. Our objective was to examine whether the drying and wetting cycles imposed by the PRI treatment could enhance nutrient uptake and intensify xylem-borne ABA signalling via modification of xylem pH and ionic concentrations compared with its counterpart DI treatment. The possible interactions between soil N bioavailability and xylem-borne ABA signalling under different irrigation regimes were also discussed.

Materials and methods

Experimental set-up

Two experiments were conducted in April–June 2009 (Exp. I) and August–October 2010 (Exp. II) in a greenhouse located at the experimental farm of the Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark. The soil was classified as a sandy loam having a pH of 6.7. The soil contained 12.9 and 1.4 g kg⁻¹ total C and N in Exp. I, and 10.3 and 1.0 kg⁻¹ total C and N in Exp. II, respectively. The soil had a volumetric soil water content of 30% at pot water-holding capacity and of 5% at permanent wilting point. The soil was sieved passing through a 2 mm mesh before filling the pots. The pots used were 10 l (17 cm in diameter and 50 cm deep) and were evenly divided into two vertical compartments by plastic sheets which were glued to the walls of the pots by a silicon sealant such that water exchange between the two compartments was prevented. The bottom of the pots was fixed by mesh (1.5 mm) which allows free drainage. At the fifth leaf stage, tomato (Lycopersicon esculentum L., var. Cedrico) seedlings were transplanted into the pots. The average soil water content was monitored by a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment Corp., CA, USA) with 33 cm probes installed in the middle of each soil compartment. The climate conditions in the greenhouse were set as: 20/17±2 °C day/night air temperature, 16 h photoperiod and >500 μmol m⁻² s⁻¹ photosynthetic active radiation (PAR) supplied by sunlight plus metal-halide lamps.

Irrigation and N fertilizer treatments

In Exp. I, the soil was mixed thoroughly and homogeneously with 1.6 g N as NH₄NO₃ together with 25.0 g finely-ground maize straw (particle size <1.5 mm), which had a total N content of 16.8 g kg⁻¹.
and total C content of 391.5 g kg\(^{-1}\). In Exp. II, 4.0 g inorganic N pot\(^{-1}\) as NH\(_4\)NO\(_3\) in the mineral nitrogen (MN) treatment and 4.0 g organic N pot\(^{-1}\) as grounded maize straw (particle size <1.5 mm), which had a total N content of 28.8 g kg\(^{-1}\) and total C content of 411.4 g kg\(^{-1}\) in the organic nitrogen (ON) treatment were thoroughly mixed into the soil. In both experiments, 0.87 g pot\(^{-1}\) and 1.66 g pot\(^{-1}\) of P and K, respectively, were mixed into the soil to meet the macronutrient requirement for plant growth.

In both experiments, the plants were subjected to PRI and DI treatment during the flowering and early fruiting stages. In PRI, one soil compartment was watered to 29–30% while the other was allowed to dry to 7–13%, then the irrigation was shifted between the two soil compartments (denoted as PRI-N and PRI-S); in DI, the same amount of water used for PRI was evenly irrigated into the two soil compartments. The experiment was a completely randomized design with 12 and 6 replicates for each treatment in Exps I and II, respectively. The pots were watered daily at 09.00 h and 16.00 h, respectively, in Exp. I and Exp. II. Plant water use (PWU) between the two successive irrigation events was computed based on the amount of irrigation, TDR soil moisture measurements and the soil volume concerned. The water used for irrigation was tap water with negligible concentrations of nutrients. The irrigation treatments lasted 27 d and 34 d in Exps I and II, respectively, during which period each soil compartment of the PRI plants had experienced three dry/wet cycles (Figs 1, 2).

**Sampling, measurements, and analysis**

Sampling was conducted on the 13th, 20th, and 27th day after the onset of the irrigation treatment (DAT) with four replicates in each treatment in Exp. I, and on the 34th DAT with six replicates in each treatment in Exp. II. Dry biomass of plant samples was determined after oven-drying at 70 °C to constant weight. After grinding in a ball mill, the plant samples were analysed for total N using the Dumas dry combustion method in a system consisting of an ANCA-SL Elemental Analyzer coupled to a 20-20 Mass Spectrometer (Europa Scientific Ltd. Crewe, UK).

On each sampling day, midday leaf water potential was measured with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on fully expanded upper canopy leaves from 10.00 h to 11.00 h. Stomatal conductance was measured after irrigation during the treatment period on upper canopy fully expanded leaves by a leaf porometer (Decagon Devices, Inc., USA). Xylem sap was collected by pressurizing the roots of the potted plants in a Scholander-type pressure chamber. The entire pot was sealed into the pressure chamber and the shoot was detopped at 5–10 cm from the stem base. With the stem stump protruding outside the chamber, pressure was applied slowly until the root water potential was equalized. The cut surface was cleaned with pure water and dried with blotting paper. The pressure was increased gradually until it equalled leaf water potential of the plants in order to obtain a sap flow rate similar to the whole plant transpiration rate to avoid dilution effects (Dodd et al., 2008). However, as the hydraulic resistance of the shoot was removed, the flow rate could be higher than the actual transpiration rate, and thus the ABA and ionic concentration in the sap might have been underestimated. 1.0 ml of xylem sap was collected using a pipette from the cutting surface into an Eppendorf-vial wrapped with aluminium foil. All sap samples were frozen immediately after sampling and stored at –80 °C until analysis.

ABA concentration in the xylem sap was determined by ELISA using the protocol of Asch (2000). Anions and cations in the xylem sap were determined by ion chromatography (Metrohm AG, Herisau, Switzerland). Anions were determined on a Metrosep A Supp 4 analytical column (4×125 mm 1.8 mM Na\(_2\)CO\(_3\)/1.7 mM NaHCO\(_3\) eluent), and cations were determined on a Metrosep C4-100 analytical column (4×125 mm, 1.7 mM nitric acid/0.7 mM dipicolinic acid (DPA) eluent). Xylem pH was determined with a microelectrode (model PHR-146, Lazar Research Laboratories, Inc. CA, USA) interfaced with a pH meter (Model 60, Jenco Instruments Inc. CA, USA) after sap was allowed to thaw for 30 min.

**Statistical analyses**

The data were subjected to analysis of variance using SAS GLM (SAS Institute, Inc., 2004) at a significant level of 5%. Duncan’s multiple range test and Independent t test were applied to assess the differences between treatments at a significant level of 5%. Regression analyses were used to determine the relationships between the measured parameters.

**Results**

**Soil and plant water status**

Volumetric soil water content (% vol.), midday leaf water potential (LWP), and root water potential (RWP) at each
sampling date were measured, and plant water use (PWU) between the two successive irrigation events was calculated (Tables 1, 2, 3). In PRI, soil water contents were dependent on which side of the root system was being irrigated (Fig. 1). The soil water contents in the irrigated soil compartment after each irrigation event was close to pot holding capacity, while in the drying soil compartment the soil water content was below 10% and 14% in Exp. I and Exp. II, respectively. However, the averaged soil water content in the whole pots was similar for PRI and DI in both experiments (Table 1). It was notable that before irrigation the soil water contents in the PRI wetted zone were only slightly higher than those in the PRI drying soil compartment in Exp. I (Table 1).

In Exp. I, DI and PRI plants had similar RWP, LWP, and PWU, except for the significantly lower RWP in the DI relative to PRI on 13 DAT and significantly lower LWP in the PRI compared with the DI treatment on 20 DAT (Table 2). Likewise, similar LWP, RWP, and PWU values were observed between DI and PRI treatment across the N fertilizer treatments in Exp. II (Table 3). Across the irrigation treatments, the plants under the MN treatment had significantly lower LWP, but had significantly higher PWU than those of the ON treatment; whereas the RWP was similar for MN and ON plants. The analysis of variance showed that there were no significant interactions between the irrigation and N fertilizer treatments for the above variables in Exp. II. In addition, the averaged value of stomatal conductance during the treatment period was similar between DI and PRI plants in both experiments (Fig. 3).

**Table 1.** Changes of soil water content (% vol.) in the pots on each sampling date under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments in Exp. I and Exp. II, respectively

| Treatment | Exp. I 13 DAT | Exp. I 20 DAT | Exp. I 27 DAT | Exp. II 34 DAT Mineral N | Exp. II 34 DAT Organic N |
|-----------|--------------|--------------|--------------|---------------------------|--------------------------|
| DI        | 20.8±1.0 ±9.5±0.8 | 18.4±0.2 ±7.4±0.1 | 19.4±0.7 ±8.2±0.2 | 19.1±0.1 ±14.4±0.4 | 19.3±0.8 ±15.9±1.1 |
| PRI-N     | 9.9±0.2 ±9.0±0.3 | 7.7±0.2 ±7.3±0.1 | 7.9±0.2 ±7.2±0.4 | 10.7±0.2 ±10.5±0.2 | 13.6±0.3 ±13.6±0.4 |
| PRI-S     | 30.0±0.0 ±14.6±0.2 | 30.0±0.0 ±7.8±0.3 | 30.0±0.0 ±7.7±0.5 | 29.0±0.0 ±17.9±0.6 | 29.0±0.0 ±21.0±0.3 |
| PRI-average | 19.9±0.1 ±11.8±0.2 | 18.9±0.1 ±7.6±0.2 | 18.9±0.1 ±7.4±0.4 | 19.9±0.1 ±14.2±0.4 | 21.3±0.2 ±17.3±0.3 |

**Table 2.** Leaf water potential (LWP), root water potential (RWP), and plant water use (PWU) of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments in Exp. I

Values are means ±SE (n=4–6). Different letters within a row at each sampling date indicate significant differences between treatments according to independent t test at *P* <0.05.

| Parameter     | 13 DAT       | 20 DAT       | 27 DAT       |
|---------------|--------------|--------------|--------------|
| LWP (MPa)     | DI  -1.2±0.1 a | PRI -1.1±0.1 a | 20:0.1 a -1.4±0.1 a |
| RWP (MPa)     | DI -0.5±0.1 b | PRI -0.1±0.0 a | 20:0.1 a -1.3±0.1 a |
| PWU (mL plant⁻¹) | 730±5±6.8 | 781.3±3.1 a | 1084±4±17.0 a |

**Table 3.** Summary of analysis of variance on the effect of experimental factors and their interaction on leaf water potential (LWP), root water potential (RWP), and plant water use (PWU) of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments on 34 DAT in Exp. II

Values are means ±SE (n=12). Different letters within a row of each experimental factor indicate significant differences at *P* <0.05.

| Parameter     | Irrigation treatment | N fertilizer | Irrigation×N |
|---------------|-----------------------|--------------|--------------|
| LWP (MPa)     | DI -0.6±0.0 a | PRI -0.6±0.0 a | 0.816       |
| RWP (MPa)     | DI -0.2±0.0 a | PRI -0.3±0.0 a | 0.493       |
| PWU (mL plant⁻¹) | 425.0±19.2 a | 445.8±23.8 a | 0.307       |

**Xylem sap constituents: ABA, pH, anions, and cations**

In Exp. I, on 13 DAT, the ABA concentration in the DI plants was the highest, which was significantly greater than in the PRI plants (Table 4). However, on 20 and 27 DAT, xylem ABA concentration was similar in the DI and PRI treatments (Fig. 3).
plants. The xylem pH was similar in the DI and PRI plants on 13 and 20 DAT, whereas on 27 DAT, significantly higher xylem pH was observed in the PRI plants. In Exp. II, xylem ABA concentration and pH was slightly higher in the PRI than in DI plants across the N fertilizer treatments (Table 5). Across the irrigation treatments, the plants under MN treatment had significantly higher xylem ABA concentration and significantly lower xylem pH compared with the ON plants. Analysis of variance showed that there were no significant interactions between the irrigation and N fertilizer treatments for the above variables in Exp. II.

In Exp. I, on 13 DAT, significantly greater concentrations of chloride, phosphate, sulphate, ammonium, calcium, total cations, and the sum of anions and cations were found in the PRI compared with the DI plants (Table 6). However, on 20 and 27 DAT, DI plants had a higher concentration of anions and cations, total anions, total cations, and the sum of anions and cations when compared with the PRI plants.

In Exp. II, across the N fertilizer treatment, significantly greater concentration of chloride, nitrate, calcium, and total cations, total anions, total cations, and the sum of anions and cations were observed in the PRI as compared with the DI plants (Table 7). When analysed across the irrigation treatments, it was found that plants under the MN treatment had significantly higher concentration of nitrate, ammonium, calcium, magnesium, and total anions, total cations, and the sum of anions and cations, compared with those under the ON treatment. By contrast, the concentration of phosphate, sulphate, sodium, and potassium were similar or slightly higher in the ON treatment, and the concentration of chloride was significantly greater compared with the MN treatment. The analysis of variance showed there were no significant interactions between the irrigation and N fertilizer treatments for the above variables except for chloride in Exp. II.

**Relationships between measured parameters**

When analysed on the data across the two experiments, it was found that RWP declined exponentially with decreasing averaged soil water content in the whole pot (Fig. 4A), and was positively correlated with LWP and the sum of anions and cations in the xylem (Fig. 4B, C) while negatively correlated with xylem ABA concentration (Fig. 4D). Significant positive linear relationship between xylem pH and the xylem ABA concentration was only found in Exp. I (Fig. 4E). There was a significant negative linear relationship between nitrate concentration and xylem pH (Fig. 4F). Moreover, a significant negative linear relationship was also observed between the sum of anions and cations and the xylem pH (Fig. 4G); while there was a significant positive linear relationship between the proportion of cations to the total ions in the xylem and the xylem pH (Fig. 4H). In addition, a significant positive linear relationship was found between the concentrations of the total anions and the total cations (Fig. 4I).

**Table 4.** Xylem ABA concentration and pH of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments in Exp. I

| Parameter | 13 DAT | 20 DAT | 27 DAT |
|-----------|-------|-------|-------|
|           | DI    | PRI   | DI    | PRI   | DI    | PRI   |
| ABA (pmol ml⁻¹) | 144.8±15.5 a | 166.9±18.9 a | 1545.8±418.8 a | 140.1±11.5 b | 3488.8±363.3 a | 11.5±1.5 b |
| pH        | 5.1±0.0 a | 5.0±0.0 a | 3488.8±363.3 a | 5.4±0.1 a | 3373.5±274.1 a | 5.6±0.1 a |

**Table 5.** Summary of analysis of variance on the effect of experimental factors and their interaction on xylem ABA concentration and pH of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments on 34 DAT in Exp. II

Values are means ±SE (n=12). Different letters within a row of each experimental factor indicate significant differences at P <0.05.
Table 6. Xylem sap constitutes of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments in Exp. I. Values are means ± SE (n=4). Different letters within a row at each sampling date indicate significant differences between treatments according to Independent t test at P <0.05.

| Constitute (mol m⁻³) | 13 DAT | PRI  | 20 DAT | PRI  | 27 DAT | PRI  |
|----------------------|--------|------|--------|------|--------|------|
| Chloride             | 4.2±0.8 b | 6.1±0.1 a | 0.8±0.1 a | 0.6±0.0 a | 0.9±0.3 a | 0.6±0.1 a |
| Nitrate              | 36.3±5.9 a | 37.8±5.9 a | 1.1±0.3 a | 0.4±0.1 b | 0.6±0.2 a | 0.3±0.0 a |
| Phosphate            | 2.3±0.1 b | 3.2±0.1 a | 0.9±0.2 a | 0.5±0.1 a | 1.6±0.5 a | 0.6±0.1 a |
| Sulphate             | 13.5±2.5 b | 18.5±1.0 a | 3.6±0.8 a | 2.2±0.5 a | 6.4±1.8 a | 3.3±0.3 a |
| ∑anions              | 56.3±8.2 a | 65.5±4.9 a | 6.4±0.9 a | 3.7±0.5 b | 9.5±2.6 a | 4.8±0.4 a |
| Sodium               | 1.0±0.2 a | 1.1±0.2 a | 0.3±0.0 a | 0.1±0.0 b | 0.2±0.0 a | 0.1±0.0 a |
| Ammonium             | 2.0±0.3 b | 3.9±0.5 a | 0.3±0.1 a | 0.2±0.1 a | 0.3±0.0 a | 0.2±0.0 a |
| Potassium            | 25.3±1.1 a | 30.6±5.5 a | 7.2±1.0 a | 4.4±0.8 a | 6.7±1.1 a | 5.0±0.4 a |
| Calcium              | 15.1±1.7 b | 21.6±1.1 a | 2.2±0.8 a | 1.0±0.3 a | 5.6±2.2 a | 3.1±0.2 a |
| Magnesium            | 7.7±1.3 a | 9.3±0.2 a | 1.1±0.4 a | 0.5±0.2 a | 1.8±0.7 a | 1.1±0.1 a |
| ∑cations             | 51.1±3.2 b | 66.6±4.5 a | 11.1±1.9 a | 6.2±1.4 a | 14.6±3.9 a | 9.5±0.3 a |
| ∑(anions+cations)    | 107.4±1.3 b | 132.1±5.1 a | 17.5±2.7 a | 9.9±1.9 a | 24.1±6.5 a | 14.3±0.6 a |

Table 7. Summary of analysis of variance on the effect of experimental factors and their interaction on xylem sap constitutes of tomato plants under deficit irrigation (DI) and partial root-zone irrigation (PRI) treatments on 34 DAT in Exp. II. Values are means ± SE (n=12). Different letters within a row of each experimental factor indicate significant differences at P <0.05.

| Constitute (mol m⁻³) | Irrigation treatment | N fertilizer | Irrigation×N |
|----------------------|----------------------|--------------|--------------|
|                      | Di                  | PRI          | Mineral N    | Organic N   | P value     | P value     |
| Chloride             | 3.0±0.3 b           | 4.1±0.5 a    | 2.4±0.4 b    | 4.7±0.2 a   | <0.001      | 0.016       |
| Nitrate              | 7.9±2.1 b           | 11.0±2.4 a   | 16.0±0.3 a   | 2.9±1.6 b   | <0.001      | 0.092       |
| Phosphate            | 4.0±0.3 a           | 4.6±0.5 a    | 3.9±0.5 a    | 4.7±0.3 a   | 0.168       | 0.773       |
| Sulphate             | 11.1±1.6 a          | 13.4±1.4 a   | 11.9±1.7 a   | 12.5±1.3 a  | 0.780       | 0.869       |
| ∑anions              | 26.0±2.7 b          | 33.1±2.5 a   | 34.2±2.3 a   | 24.9±2.6 b  | 0.009       | 0.552       |
| Sodium               | 0.2±0.0 a           | 0.2±0.0 a    | 0.2±0.0 a    | 0.2±0.0 a   | 0.719       | 0.096       |
| Ammonium             | 0.8±0.2 a           | 1.0±0.2 a    | 1.6±0.2 a    | 0.2±0.0 a   | <0.001      | 0.259       |
| Potassium            | 13.6±1.4 a          | 17.0±1.1 a   | 15.2±1.4 a   | 15.4±1.3 a  | 0.920       | 0.240       |
| Calcium              | 11.5±1.3 b          | 15.7±1.7 a   | 16.3±1.4 a   | 11.0±1.5 b  | 0.013       | 0.746       |
| Magnesium            | 3.9±0.5 a           | 5.2±0.6 a    | 5.6±0.5 a    | 3.5±0.5 b   | 0.008       | 0.912       |
| ∑cations             | 30.1±3.2 b          | 39.0±3.1 a   | 38.9±3.2 a   | 30.2±3.1 b  | 0.049       | 0.750       |
| ∑(anions+cations)    | 56.0±5.6 b          | 72.1±5.2 a   | 73.1±5.3 a   | 55.1±5.2 b  | 0.017       | 0.903       |

Shoot and root dry biomass and shoot N accumulation

PRI and DI plants had similar shoot and root dry biomass in Exps I and II (Fig. 5). Nonetheless, In Exp. I, shoot N accumulation was consistently higher in the PRI than in the DI plants (Fig. 6). In Exp. II, shoot N accumulation was significantly higher in the PRI than in the DI plants under MN treatment (Fig. 6), whereas PRI and DI had similar shoot N accumulation under ON treatment.

Discussion

In order to investigate the effects of different soil water dynamics imposed by PRI in relation to DI on xylem pH, ABA, and ionic concentrations in tomato plants, xylem sap was sampled during the experimental period at different soil and plant water status in the two experiments. The root system communicates changes in soil water availability to the shoot via xylem hydrostatic pressure (root water status) and non-hydraulic (chemical composition of the xylem) signals (Davies et al., 1990, 1994; Tardieu and Davies, 1993). Plant RWP is dependent on the soil water content in both of the soil compartments, as indicated by the significant positive exponential relationship between RWP and the averaged soil water content in the pots (Fig. 4A) (Liu et al., 2006). In addition, LWP had a significant positive linear relationship with RWP (Fig. 4B). Hence, the root and leaf water status is intimately related to soil moisture and changes of soil water dynamics imposed by irrigation treatments. Besides, plant RWP positively correlated with the total cations and anions in the xylem (Fig. 4C), whereas a significant negative linear relationship was found between RWP and the xylem ABA concentration (Fig. 4D), indicating that the soil and root water status in the PRI and DI treatments significantly influenced the xylem ABA level and the concentrations of cations and anions in the xylem.
Numerous studies have shown that, in many plant species, xylem sap pH becomes more alkaline when plants are drought-stressed (Hartung and Radin, 1989; Gollan et al., 1992; Wilkinson and Davies, 1997; Jia and Davies, 2007) or when soils are flooded (Jackson et al., 2003). In the present study, similar or significantly higher xylem pH was found in PRI than in DI treatment (Table 4), where xylem pH in PRI plants was 0.2 units greater than those of DI plants, except on 13 DAT in Exp. I (Tables 4, 5). It has been reported that xylem pH may be influenced by the ionic concentration of the xylem sap and lowered nitrate concentration in particular is often associated with increasing in xylem pH (Gollan et al., 1992; Schurr et al., 1992). Gollan et al. (1992) found that, in drought-stressed sunflower plants, a decrease in xylem nitrate concentration was accompanied by an increase in xylem pH and an excess of cations in the xylem sap. In good agreement with this, it was observed here that xylem pH was negatively correlated with xylem nitrate concentration under PRI and DI treatments (Fig. 4F), indicating that a lowered nitrate concentration in the xylem might have caused the increase in xylem pH. Moreover, it was also noticed that different N-fertilizations had significantly affected xylem pH. When analysed across the irrigation treatments, significantly lower xylem pH was observed in the MN than in the ON treatment; whilst xylem nitrate concentration was significantly higher in the MN compared with the MN. This result again confirms the negative relationship between xylem pH and xylem nitrate concentration. The availability of nitrate to the roots influences the pH of the xylem sap and is probably due to the modified uptake and transport of particular inorganic species (Raven and Smith, 1976). In the present study, a significant negative linear relationship was also found between xylem pH and the total concentration of cations and anions (Fig. 4G), whereas a significant positive linear relationship was observed between xylem pH and the
proportion of cations to the sum of cations and anions (Fig. 4H). These results imply that, in addition to nitrate adjusting xylem pH, the total concentration of cations and anions and the proportion of cations might also partially be responsible for the changes of xylem pH in the PRI and DI plants. Nevertheless, it was also found here that the balance between cations and anions was generally maintained (Fig. 4I), providing ‘charge balance’ in the PRI and DI plants grown under different N fertilizer treatments. Goodger and Schachtman (2010) suggested that, in order to maintain the
charge balance, biochemical functions in maize roots are redirected for adaptation to different nutrient and moisture conditions and this redirection results in changes in the xylem sap pH or proton flux. However, further studies are needed to elucidate the effect of ‘charge balance’ on xylem pH under dramatic soil water dynamics such as PRI.

ABA is a weak acid (pK_a=4.75) and it is distributed within the cellular compartments of the plant according to the anion-trap concept and the Henderson–Hasselbalch equation (Slovik et al., 1995), and apoplastic pH may act synergistically with ABA on both guard cell functioning and cell expansion. In the present study, it was observed that, in most cases, the xylem ABA concentration was similar for PRI and DI plants. It is known that an increased xylem pH can result in significant intensification of ABA signalling and stomatal closure may occur even at a xylem ABA concentration similar to well-watered plants (Hartung, 1983; Hartung and Radin, 1989; Wilkinson and Davies, 1997). However, a clear association between increases of xylem pH with increasing xylem ABA concentration was noted here only when the soil water content was relatively low in the PRI and DI treatments (Fig. 4E). In addition, in the present study, the difference between PRI and DI in regulating stomatal conductance was hardly detected (Fig. 3) (de Souza et al., 2003, 2005; Liu et al., 2008).

Our recent studies in potato and tomato have demonstrated that PRI could improve plant N nutrition, which might have partly contributed to the greater WUE in those plants (Wang et al., 2009, 2010a). Leaves and other parts of the shoots are supplied with mineral nutrients from the xylem. One of the first steps in this process is the secretion/loading of ions into the xylem by the stellar parenchyma (Wegner and Raschke, 1994). Under soil water deficits, the translocation of N and other minerals from bulk soil to the root surface is reduced; under such circumstances the root nutrients acquisition could be maintained only by an increase in the concentration of the ions (Flowers and Yeo, 1986). As discussed previously, both soil and root water status significantly influenced the concentration of cations and anions in the xylem. As the PRI and DI plants transpired a similar amount of water between the irrigation events (Tables 2, 3), the absolute amount of those ions being taken up by the roots should follow the same pattern as their concentrations in the xylem. However, it should be noted that the temporal ionic concentrations in the xylem sap were dependent on the soil and plant water status at the time of sampling and which might not reveal the time-integrated total ion uptake by the roots. For instance, in PRI, when soil water is still easily available in the wet zone of the root system, i.e. 13 DAT in Exp. I and 34 DAT in Exp. II, the concentrations of anions and cations, including nitrate and ammonium, were higher in the xylem of PRI plants (Tables 6, 7), and the sum of anions and cations in PRI were significantly greater than DI treatment in both experiments, even through the average soil water contents in the whole pot was similar for the two irrigation treatments. However, when both soil compartments in the PRI treatment were severely water-stressed just before the irrigation, i.e. 20 DAT and 27 DAT in Exp. I, the acquisition of soil water and nutrients by root was reduced and, consequently, the ions are simply not available to the plant. Under such circumstances the concentrations of anions and cations in the xylem sap of the PRI plants were even slightly lower compared with the DI plants. Nevertheless, in both experiments with the same amount of irrigation water for the PRI and DI plants, although the shoot and root dry biomass was similar for PRI and DI plants (Fig. 5), N accumulation in the shoot was constantly higher in the PRI than in the DI plants (Fig. 6), indicating that the more dynamic changes of soil water content induced by PRI treatment, compared with DI treatment, enhanced the uptake of nutrients in a long term. Several reasons may explain this phenomenon. Firstly, during PRI treatment half of the root system was irrigated close to pot water-holding capacity, the favourable soil water condition in the root zone could benefit nutrient transportation (diffusive and/or mass flow) from the bulk soil to the root surface thereby facilitating root nutrient uptake. Secondly, the drying and wetting cycles in the soil profile under PRI could stimulate soil organic matter mineralization hence increasing the availability of mineral nutrients in the soil leading to enhanced nutrient uptake (Wang et al., 2010a, b). Thirdly, studies have shown that root hydraulic conductivity is enhanced by PRI treatment and

**Fig. 6.** Shoot N accumulation of tomato plants during DI and PRI treatments in Exps I and II. Values are means ±SE (n=4 and 6 for Exps I and II, respectively). Different letters at each sampling date indicate significant differences between treatments according to independent t test at P <0.05.
which may be associated with an increased aquaporin activity (Sade et al., 2009; Hu et al., 2011). It is also known that the uptake of nitrate could be sensed and translated into changes in the aquaporin activity (Clarkson et al., 2000) and the association between increased aquaporin activity and uptake of nitrate may indicate a link between active nitrate transporter and aquaporin activity (Vandeleur et al., 2005). If all of those were true, then PRI might have enhanced the root nutrient-uptake capacity, resulting in higher anions and cations in the xylem. However, further investigations are necessary to examine the above possibilities. Our present study has shown that, in order to optimize the PRI strategy to improve both water and nutrient use efficiencies, it is essential that the soil water content in the wet zone should be maintained relatively high while that in the drying soil zone should not be very low, both conditions are crucial to maintain high soil and plant water status while sustaining the ABA signalling of plants.

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