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Chemical signals and their regulations on the plant growth and water use efficiency of cotton seedlings under partial root-zone drying and different nitrogen applications



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Abstract Partial root-zone drying during irrigation (PRD) has been shown effective in enhancing plant water use efficiency (WUE), however, the roles of chemical signals from root and shoot that are involved and the possible interactions affected by nitrogen nutrition are not clear. Pot-grown cotton (*Gossypium* spp.) seedlings were treated with three levels of N fertilization and PRD. The concentrations of nitrate (NO_3^-), absciscic acid (ABA) and the pH value of leaf and root xylem saps, biomass and WUE were measured. Results showed that PRD plants produced larger biomass and higher WUE than non-PRD plants, with significant changes in leaf xylem ABA, leaf and root xylem NO_3^- concentrations and pH values, under heterogeneous soil moisture conditions. Simultaneously, high-N treated plants displayed larger changes in leaf xylem ABA and higher root xylem NO_3^- concentrations, than in the medium- or low-N treated plants. However, the WUE of plants in the low-N treatment was higher than that of those in the high- and medium-N treatments. PRD and nitrogen levels respectively induced signaling responses of ABA/ NO_3^- and pH in leaf or root xylem to affect WUE and biomass under different watering levels, although significant interactions of PRD and nitrogen levels were found when these signal molecules responded to soil drying. We conclude that these signaling chemicals are regulated by interaction of PRD and nitrogen status to regulate stomatal behavior, either directly or indirectly, and thus increase PRD plant WUE under less irrigation.

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1. Introduction

Soil water availability is one of the major environmental factors limiting crop growth and yield formation (Wu et al., 2008; Gonzalez-Dugo et al., 2010). The plant hormone abscisic

acid (ABA) has been suggested many physiological roles in the plant when the soil water potential dropped below optimum levels, including control of stomatal behavior and limiting ineffective water use (Davies and Zhang, 1991; Tardieu and Davies, 1993; Dodd, 2003; Verslues et al., 2006; Pinheiro and Chaves, 2011).

The increase of ABA biosynthesis in both root and shoot and the enhancement of ABA transportation as a root-to-shoot or shoot-to-root signal, accompanied with the subsequent weakening of gas exchange capacity and decrease of growth rate, have been observed in many studies when water availability decreased from optimal level (Davies and Zhang, 1991; Schurr et al., 1992; Tardieu and Davies, 1993; Comstock, 2002; Chaves and Oliveira, 2004; Verslues et al., 2006; Jia and Davies, 2007; Qin et al., 2011; Wang et al., 2012). However, except the effect of ABA on plant shoot phenotype, the elongation of root length and increase of the root surface area were improved by ABA (indicating an indirect role) to ensure the growth-based water and (nitrogen) nutrients uptake (Wittenmayer and Merbach, 2005; Dodd et al., 2009). ABA-deficient mutants (e.g. *flacca*, *aba-1* and *aba-2* etc.) and/or grafted technique have been used to explain the physiological role of shoot-synthesized and root-sourced ABA in plants under suboptimal environmental conditions (Dodd et al., 2008). These studies suggested that the movement of ABA between symplast and apoplast can be inhibited by the alkalization of the xylem sap and the decrease of pH gradients over the cells membrane in stem, leaf or root tissues when the soil dries (Wilkinson et al., 2007; Xue et al., 2014; Dodd et al., 2008), eventually leading to ABA accumulation in mesophyll cells, the guard cells dehydration and then stomatal closure (Jia and Davies, 2007; Netting et al., 2012).

As a signaling molecule, ABA regulates plant physiological behaviors under suboptimal environmental conditions, for example the increased ABA levels weakens gas exchanges capability, declines stomatal conductance and transpiration rates as well as maintains leaf water potential and root water-uptake ability by increasing root: shoot ratio and decreasing leaf surface etc. under drought stress (Wittenmayer and Merbach, 2005; Dodd et al., 2009). However, due to convenience of measurement, many researches focused on shoot growth and regulation of shoot-synthesized ABA.

Additionally, stomatal closure regulated by ABA can be caused by soil nitrogen (N) deficiency, a same mechanism induced by drought (Wilkinson et al., 2007). It has been suggested that N deficiency increases tissue ABA concentrations (Rahayu et al., 2005). It is also well known that water fluxes in the xylem sap are generally reduced by limited water supply. Mineral N fluxes are also declined because N uptake by plants is limited under these conditions; this alters plant N nutrition and can even lead to N deficiency (Wilkinson et al., 2007; Haefele et al., 2008; Wu et al., 2008; Gonzalez-Dugo et al., 2010). NO_3^- is one of the main forms of nitrogen (N) uptake and utilization by plants, and its changes in the xylem sap can alter the pH of xylem sap (Dodd, 2003; Wilkinson et al., 2007). Therefore, NO_3^- concentration in the xylem sap can be reduced under water stress situation (Dodd, 2003; Wilkinson et al., 2007).

Studies indicated that the concentration of NO_3^- in the xylem sap affected stomatal sensitivity to both ABA and soil dryness (Jia and Davies, 2007; Wilkinson et al., 2007), leading

to stomatal opening/closure and even changes in WUE, as the apoplastic pH changed (Davies and Zhang, 1991; Haefele et al., 2008; Qin et al., 2011). It has been proved that the effects of NO_3^- on plant growth were mediated by pH-based ABA redistribution (Wilkinson et al., 2007). It is recently reported that the nitrate transporter 1/peptide transporter (NRT1/PRT) family comprises two ABA transporters (Yann et al., 2013), which suggests the close relationship of ABA and NO_3^- . Therefore, much work should be focused on the available interaction between ABA and supplied N/NO_3^- . Furthermore, numerous studies have been focused on ABA not only because of its key regulation to leaf stomatal conductance especially during the early stages of plant response to water deficit, but also its role in increasing water use efficiency (WUE) in agriculture (Schachtman and Goodger, 2008). An innovative partial root-zone drying model (PRD) has been used to remarkably increase the WUE of various crops including grape, fruit trees, and maize, in the regions of limited water supply (Wang et al., 2009; Jovanovic et al., 2010; Wang et al., 2010, 2012). In the field-grown grapevines study, the use of PRD or deficit irrigation increased WUE by about 40% with a yield decreasing by only 15% (Schachtman and Goodger, 2008).

It has been confirmed that PRD can promote the increase of xylem sap ABA concentrations as compared with full irrigation, which can be explained by the changes of root water potential (Dodd et al., 2010; Puértolas et al., 2014) and other physiological parameters (Dodd, 2009; Sepaskhah and Ahmadi, 2010). Therefore, the increase of WUE and reductions in stomatal conductance are correlated with increased ABA concentrations under PRD treatment (Schurr et al., 1992; Gonzalez-Dugo et al., 2010; Dodd et al., 2010). On the other hand, studies on N fertilization effect under water stress have indicated that N supply can reduce the negative effects of drought stress on plant growth (Wu et al., 2008; Haefele et al., 2008; Cramer et al., 2009; Gonzalez-Dugo et al., 2010). A typical example is that *Sophora davidii* seedlings subjected to severe water deficit under low nitrogen conditions had a higher WUE than well-watered seedlings under high nitrogen conditions (Wu et al., 2008). Most experiments showed positive effect of WUE after supplying of N fertilizer, while few results reported about N effect to WUE (Brueck, 2008). Therefore, although PRD or N supply plays a role in enhancing plant WUE, the involved root-sourced chemical signals (ABA, pH and NO_3^-) and the possible interaction between N nutrition and PRD remain unclear.

The aim of this study is to examine how cotton (*Gossypium* spp.) seedling growth and WUE are affected by the interaction of PRD treatment and different nitrogen levels via cooperatively regulating root-sourced ABA signal and NO_3^- under soil water change. The cotton seedlings supplied with different N levels were subjected to a drought-rewatering treatment, with PRD or full water supplying before drought and after rewatering. The xylem sap in both shoot and root was collected for the analysis of ABA, NO_3^- , and pH, and for the measurement of the biomass, leaf water potential and whole plant water consumption. These results can illustrate the mechanism(s) by which plants adapt to changes in water availability. It is also important to explore how to improve WUE with appropriate N supply, especially for crops cultivated in drought-prone areas or in alternating wet-dry growth conditions.

2. Materials and methods

2.1. Plant materials and culture

Cotton (*Gossypium* spp., HX-25) was selected for in this study. Seeds of HX-25 were sowed in a substrate with distilled water. After emergence, half of HX-25 seedlings were transplanted into $0.06 \times 0.06 \times 0.25$ m pots (A) (one seedling per pot) filled with 0.35 kg soil (total N 0.78 g kg^{-1} , nitrate N 67.66 mg kg^{-1} , total K 22.11 g kg^{-1} , solution K 17.63 mg kg^{-1} , total P 0.81 g kg^{-1} , solution P 31.50 mg kg^{-1} , Organic matter 13.15 g kg^{-1} , respectively, and pH = 7) and grown in a controlled environment with following conditions: day/night temperature $30/23^\circ\text{C}$, relative humidity 65%, photoperiod 12 h with a photosynthetic photon flux density of $330 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The remaining HX-25 seedlings were transplanted into separated pots (B). Pot (B) had the same total volume and shape as the above pot (A). However, each pot (B) was separated equally and vertically in two in the medium by a watertight light plastic barrier before seedlings were transplanted. Soil (0.35 kg) was placed in both sides of each separated pot (B) equally and an emergent seed was transplanted into the medium of this pot (B), to allow equal opportunity for the growth of roots on both sides of the pot (B). These seedlings were grown under the conditions as described above. The water status of soil and seedlings during experiment is shown in Tables 1 and 2.

2.2. Treatments

HX-25 seedlings were supplied with three levels of nitrogen fertilizer (urea, N 45.16%) mixed with soil as base fertilizer: 25 kg N/667m^2 (high N), 17.5 kg N/667m^2 (medium N) and 10 kg N/667m^2 (low N), respectively. The actual amount of nitrogen fertilizer applied was adjusted according to the amount of nitrogen in the original soil. The same amount of nitrogen fertilizer was applied to the entire rootzone of pot (A) while to the left side of the split-rooted pot (B). The right side of pot (B) received no extra nitrogen supply. Water was supplied to pots (A) and pots (B) (entire root-zone) for saturating soil after transplanting. Subsequently, Pots (A) was supplied with water once every 3 days. It was defined as non partial root-zone irrigation/drying (non-PRD) in this paper. Pots (B) were supplied with appropriate water on alternate sides of the pot once every 3 days. It was defined as partial root-zone fertilization with alternate partial root-zone irrigation/drying (PRD) in this paper. Three weeks after transplanting of HX-25 seedlings, water control occurred and all pots were stopped water supply. After 12 days of soil drying, all plants were rewatered to the same level as before drought treating. The daily water consumption per pot was recorded. Parameters were measured every 2–3 days during the periods of drought and rewatering. Each treatment was replicated four times.

Table 1 Water contents of soil during water stress and subsequent rewatering. S and R represent drought stress and rewatering, respectively. Capital letters refer to treatments of high nitrogen (HN), small letters to treatments of medium nitrogen (MN) and italic letters to treatments of low nitrogen (LN). Data are showed as mean \pm SD of six independent measurements ($p < 0.05$). (1) and (2) represent the first water supply side and the second water supply side, respectively.

		Without water supply				With water per 3 days R9	
(Mv)		S0	S3	S6	S12		
Non-PRD	HN	686 ± 11A	583 ± 13B	452 ± 7C	292 ± 17D	566 ± 10B	
	MN	697 ± 18a	528 ± 32b	427 ± 33c	241 ± 29d	536 ± 9b	
	LN	671 ± 23A	548 ± 11B	404 ± 37C	204 ± 7D	519 ± 23B	
PRD	HN	(1)	691 ± 22A	598 ± 18B	492 ± 45C	338 ± 29D	542 ± 19BC
		(2)	576 ± 20B	465 ± 33C	389 ± 39D	272 ± 11E	385 ± 9D
	MN	(1)	676 ± 15a	571 ± 20b	465 ± 16c	314 ± 11d	537 ± 16bc
		(2)	567 ± 25a	432 ± 10b	374 ± 18c	288 ± 70d	371 ± 6c
	LN	(1)	679 ± 21A	555 ± 17B	460 ± 10C	328 ± 6D	536 ± 14B
		(2)	536 ± 21B	447 ± 27C	368 ± 12D	287 ± 9AE	378 ± 13D

Table 2 Leaf water potential of cotton seedlings during drought and subsequent rewatering. S and R represent days of drought stress and rewatering, respectively. Capital letters refer to treatments of high nitrogen, small letters to treatments of medium nitrogen and italic letters to treatments of low nitrogen. Data are shown as mean \pm SD of four independent measurements ($p < 0.05$).

		Without water supply				With water per 3 days
	(MPa)	S0	S3	S6	S12	R9
Non-PRD	High nitrogen	−0.37 ± 0.033D	−0.53 ± 0.010C	−0.89 ± 0.073B	−1.10 ± 0.127A	−0.43 ± 0.010CD
	Medium nitrogen	−0.39 ± 0.009e	−0.60 ± 0.108d	−0.92 ± 0.049b	−1.15 ± 0.029a	−0.45 ± 0.029e
	Low nitrogen	−0.44 ± 0.071D	−0.58 ± 0.015D	−0.87 ± 0.007BC	−1.12 ± 0.052A	−0.43 ± 0.118D
PRD	High nitrogen	−0.40 ± 0.028D	−0.74 ± 0.038C	−0.91 ± 0.049B	−1.22 ± 0.049A	−0.43 ± 0.090D
	Medium nitrogen	−0.43 ± 0.075d	−0.65 ± 0.110c	−0.89 ± 0.062b	−1.25 ± 0.270a	−0.41 ± 0.056d
	Low nitrogen	−0.47 ± 0.051D	−0.68 ± 0.072C	−0.87 ± 0.093B	−1.22 ± 0.057A	−0.38 ± 0.043D

2.3. Collection of leaf blade and de-topped root xylem sap

Xylem sap from the de-topped root and leaf blade of the same seedling was collected by exudation according to Jia and Davies (2007), Liang and Zhang (1997) and Netting et al. (2012) with minor modification. In brief, the base of the third fully expanded leaf from top was cut off in petiole with a sharp razor blade, leaving at most 0.25 mm on the plant. The leaf was immediately placed in pressure chamber (Model: 3005, Soil Moisture Equipment Co., USA) with sealed by a silicone rubber bung so that only the petiole of the blade through the pressure chamber lid and was left outside. After placing the leaf in a whole pressure chamber, the cut petiole surface was cleaned 2–3 times with distilled water to remove all contaminating cell debris. Then, the pressure in the chamber was raised very slowly (0.05 MPa increments). An overpressure (nearly more than 0.2 MPa than leaf water potential) was applied to the leaves (following measurement of leaf water potential) so that about 0.05 ml of sap pre plant could be collected within less than 10 min.

Individual whole plant from the full-soil pots was sealed in the pressure chamber, after which the shoot was cut off from the stem base. The cut surface was rinsed just like described above. Root xylem sap was collected into pre-weighted eppendorf tubes for calculating sap flow rate with a series of overpressure. To collect about 0.05 ml root xylem sap per plant sap flow rate was closely matched with transpiration rate. The collected root xylem sap was immediately used for pH measurement or stored at -80°C for ABA and NO_3^- analyses.

2.4. Absciscic acid (ABA) assay

Analysis of ABA concentrations in leaf and root xylem sap from HX-25 plants was carried out using the radioimmunoassay (RIA) method as described by Zhang et al. (1997) and Netting et al. (2012) with minor modification. Briefly, 50 μl of xylem sap was mixed with 200 μl PBS buffer (phosphate-buffered saline, 50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 6.0, with 100 mM NaCl), 100 μl ^3H -(+)-ABA diluted solution (about 20,000 dpm, 2.5 g/L γ -globulin in PBS buffer, 1:100) and 100 μl diluted ABA antibody solution (about 1:100–300, MAC252, 5 g/l BSA + 4.5 g/l PVP in PBS buffer). The reaction mixture was incubated at 4°C for 60 min and aqueous extracts were obtained as supernatants after centrifuging the extraction mixtures. After that saturated $(\text{NH}_4)_2\text{SO}_4$ and 50% saturated $(\text{NH}_4)_2\text{SO}_4$ were used to precipitate protein sequentially. The bound radioactivity was measured in 50% saturated $(\text{NH}_4)_2\text{SO}_4$ -precipitated pellets with 1.2 ml of a liquid scintillation cocktail using an Ls6500 multi-purpose scintillation counter. The assay sensitivity was calculated based on a standard curve (via measuring different ^3H -(+)-ABA concentrations) according to Zhang et al. (1997).

2.5. Measurement of nitrate concentrations in the xylem sap

Xylem nitrate concentrations were determined using a Low Range Lab Nitrate Test kit (Nitrate Elimination, catalog No. L-NTK-202) with minor modification. Firstly, 50 μl xylem sap was mixed with 100 μl reaction buffer (25 mM KH_2PO_4 ,

0.025 mM EDTA, pH 7.5), 50 μl 2 mM NADH and 20 μl (1 unit) nitrate reductase. After incubation for 30 min at room temperature, 100 μl 1% sulfanilamide in 3 mol L^{-1} HCl and 100 μl 0.02% N-naphthylethylenediamine were sequentially added to the reaction mixture. After incubation for a further 20 min, the absorbance at 540 nm was read in a spectrophotometer for the sample and nitrate standards. The nitrate content was calculated according to a standard curve.

2.6. Measurement of pH value, stomatal resistance, biomass, water dissipation by transpiration, whole plant water use efficiency (WUE) and leaf water potential and soil water content

The pH value of leaf xylem or root xylem sap was determined immediately after collection using digital pH meter (Model 60, JENCO, USA). Stomatal resistance of the top fully expanded leaves was measured at 10:00–12:00 am using a steady state porometer (Li-1600, LiCor Inc, NE, USA).

Daily water dissipation by transpiration and the evaporation from soil surface was determined by weighing every day. At the beginning of this measurement, the plant weight per pot was determined to avoid prejudicing the amount of water use per pot. The cumulative amount of water dissipation by transpiration per pot d^{-1} during the whole experiment was the total water dissipation by transpiration of the potted plants. The water use efficiency of biomass production (WUE) was determined for each potted plant by dividing the total biomass production (above- plus belowground biomass) by cumulative water use throughout the test period (i.e. the total water dissipation by transpiration).

The water potential of top third fully expanded leaf were measured by pressure chamber (Model: 3005, Soil Moisture Equipment Co. U.S.A) as described above. The soil water content per pot was measured by HH2 Moisture Meter (Cambridge, England).

2.7. Statistical analysis

All data obtained from the experiment were subjected to ANOVA (analysis of variance) using the SigmaPlot 8.0, the Dome statistical package and the SAS (statistical analysis system software). Means were compared using Duncan's multiple range tests at the 5% level of probability. Linear regression was used to determine relationships between variables and differences between parameters of fitted models were evaluated with an *F*-test.

3. Results

3.1. ABA signaling in response to the interaction between PRD and nitrogen under drought stress

In all treated plants, the ABA concentration in the xylem sap increased soon along with the decrease of soil water content (Fig. 1, Tables 1 and 2, $p < 0.05$), reached to the top point after 6–12 days drought and then rapidly declined to the pre-drought level after rewetting. ANOVA showed significant effects of PRD and/or N level on ABA (Table 3). During

12 days of drought, the partial root-zone fertilization with alternate partial root-zone irrigation/drying treatment (PRD) induced obvious larger increase (6.66–7.34 folds control levels) but slower reaction of ABA concentration in leaf xylem sap (ABA_{leaf}) compared with non-PRD treatment (4.00–7.12 folds control levels) regardless of N levels. However, ABA concentration of root xylem sap (ABA_{root}) in PRD and non-PRD treatment showed similar changes on ratio and tendency (about 5.00 folds control levels at the 12th day after drought),

irrespective of N level. Moreover, high N improved most for the ABA_{leaf} content (more than 7.00 folds control level), irrespective of irrigation methods, followed by the medium- and low-N treatment (5.38–6.69 folds control levels in PRD-treated plants and 3.70–5.20 folds control levels in non-PRD-treated plants, Fig. 1, $p < 0.05$). Importantly, high or low N levels did semblable changes of ABA_{root} with medium N treatment facing to water deficiency, in both PRD and non-PRD treatments. In addition, there were larger changes

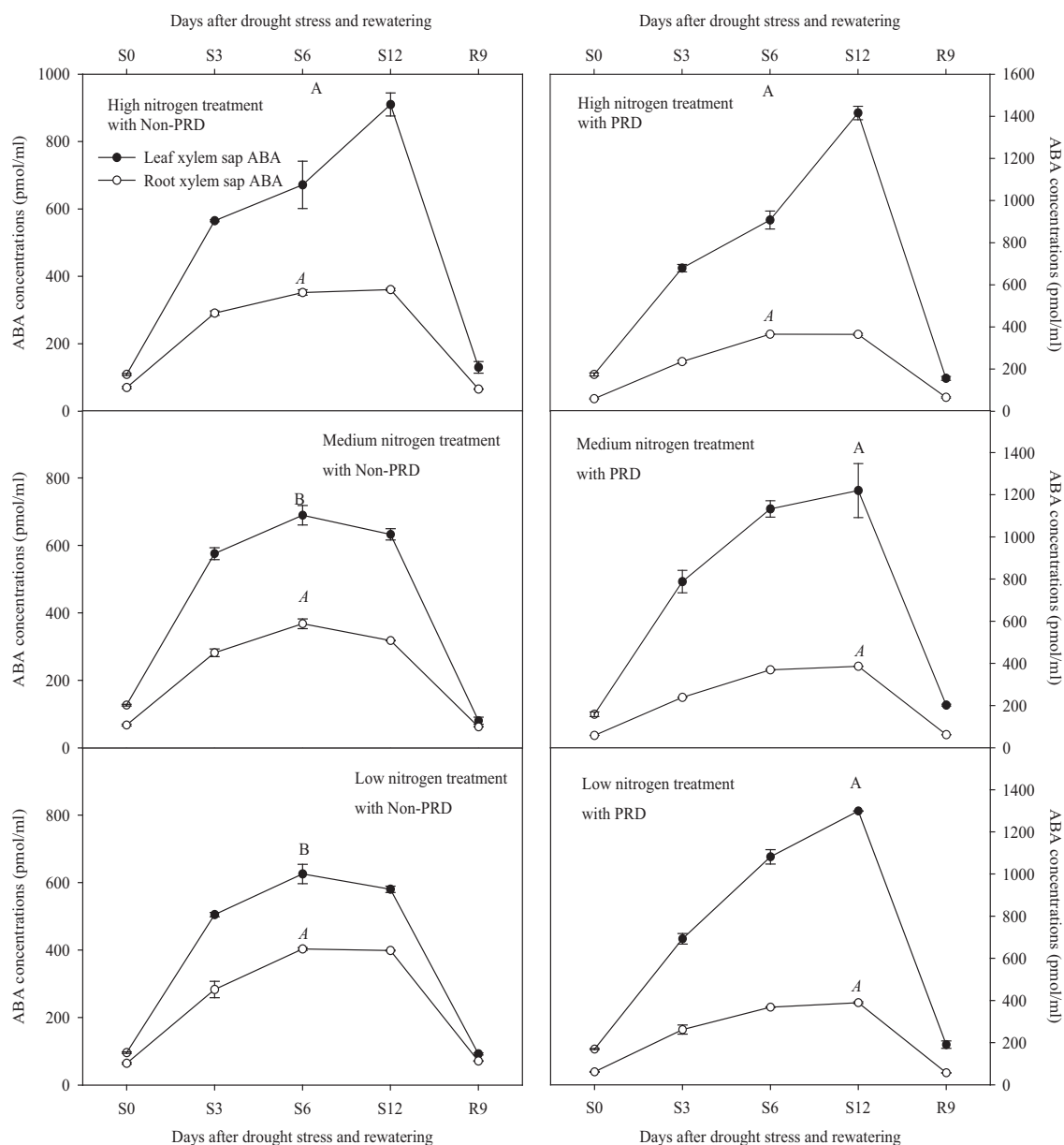


Fig. 1 Change of ABA concentrations in leaf and root xylem sap in PRD and non-PRD cotton seedlings treated with different nitrogen levels during drought stress (S) and subsequent rewatering (R). Two factors (water treatments and nitrogen levels) ANOVA was carried out to the ABA concentrations values but only compared results after drought stress 12 days were showed on this figure. Capital letters refer to ABA concentrations of leaf xylem sap and italic letters to ABA concentrations of root xylem sap. For this and subsequent figures and tables, bars (with standard errors) with the same letters are not significantly different ($p < 0.05$). Data are shown as mean \pm SE of four independent measurements ($p < 0.05$).

Table 3 The effects of PRD, nitrogen supply levels and their interaction on ABA and NO_3^- concentration and pH under drought situation (two factors analysis of variance).

		PRD treatment	Nitrogen levels	PRD \times N levels
Leaf xylem sap	ABA	< 0.0001***	0.0443*	0.0312*
	NO_3^-	0.0482*	0.0187*	0.0304*
	pH	< 0.0001***	0.0472*	0.0092**
Root xylem sap	ABA	0.0422*	0.3890	0.0436*
	NO_3^-	0.0003***	0.0010***	0.0042**
	pH	0.0235*	0.9968	0.9461
Biomass		< 0.0001***	0.0162*	0.0162*
WUE		0.0271*	0.0369*	0.0369*

Note: “*” means that the difference was significant at $p = 0.05$ level; “**” means that the difference was significant at $p = 0.01$ level; “***” means that the difference was significant at $p = 0.001$ level.

in ABA_{leaf} than ABA_{root} during drought stress, irrespective of irrigation methods or N levels.

3.2. NO_3^- concentrations of xylem sap changes facing to PRD and nitrogen level under drought stress

ANOVA also showed significant effects of PRD and/or N level on NO_3^- concentrations (Table 3). PRD induced a obvious slower but larger NO_3^- concentrations increase in leaf xylem sap (4.19–5.49 folds control levels, $\text{NO}_3^-_{\text{leaf}}$) compared with non-PRD treatment (3.52–3.74 folds control levels) during water stress and rewatering in cotton seedlings (Fig. 2, Tables 1–2, $p < 0.05$). However, the increase of NO_3^- concentrations in root xylem sap ($\text{NO}_3^-_{\text{root}}$) of non-PRD-treated plants was larger (2.06–3.39 folds control levels) than in PRD-treated plants (0.45–0.82 folds control levels). At the same time, over N supply induced more increase of $\text{NO}_3^-_{\text{leaf}}$, while low N supply did not change $\text{NO}_3^-_{\text{leaf}}$ obviously, compared with medium-N treatment after 12 days drought. Only low-N improved $\text{NO}_3^-_{\text{root}}$ in PRD but decreased $\text{NO}_3^-_{\text{root}}$ in non-PRD. High- and medium- N showed similar value in response to soil drought 12 days. The data also indicated that there were larger changes in $\text{NO}_3^-_{\text{leaf}}$ than in $\text{NO}_3^-_{\text{root}}$.

3.3. Alkalization in leaf and root xylem sap induced by PRD and different N levels

As drought stress became more severe, pH was alkalized in leaf and root xylem sap in all treatments except for the leaf xylem sap in non-PRD plants, in which pH showed relative constant during the drought treatment (Fig. 3). Similarly, ANOVA showed significant effects of PRD and/or N level on pH value (Table 3). To be more specific, PRD-treated plants showed similar pH increase (only 1.11–1.20 folds of control) with the decrease of soil water content both in leaf and root xylem sap ($p < 0.05$). High nitrogen level induced the increase in pH of both leaf xylem sap and root xylem sap. Whereas, in medium N, pH in leaf xylem sap was the highest, regardless of irrigation methods; while in root xylem sap, different N levels did not change pH with different ratio during 12-day drought stress. Additionally, the pH of leaf xylem sap was higher than that of root xylem sap. Xylem sap pH returned to pre-drought values after rewatering.

3.4. Water consumption, biomass accumulation and plant growth affected by interaction of PRD and nitrogen levels under drought stress

As shown in Fig. 4, although PRD did not change whole plant water consumption in high- and medium-N levels subjected to 12-day drought stress, it improved biomass accumulation more (1.32-fold in high N, 1.41 folds in medium N and 1.54 folds in low N treatment, respectively) compared with non-PRD treatment, irrespective of N levels. Therefore, the WUE of PRD-treated plants were higher than those of non-PRD-treated plants (1.39, 1.49 and 1.08 folds, respectively, in high, medium and low N treatment). However, single leaf water potential in PRD plants were slightly lower than that of non-PRD plants subjected to 12-day water stress (Table 2). Plants in the high- and medium-N treatments showed obviously more water consumption and biomass accumulation than did plants in the low-N treatment, which was observed in both PRD-treated and non-PRD-treated plants. Plants in the low-N treatment showed a higher WUE than those plants in the high- and medium-N treatments. However, single leaf water potential did not show obvious difference among N levels, irrespective of irrigation methods. ANOVA also confirmed that the significant effects of PRD, N level and their interaction on biomass and WUE (Table 3).

In PRD-treated plants, the decrease of growth rate started at the later period of drought and recovered immediately after rewatering; whereas in non-PRD-treated plants, it started at the early period of drought and the recovery was delayed (Table 4, $p < 0.05$). Additionally, PRD-treated plants had higher root to shoot ratio (R/S) than non-PRD-treated plants, even in low-N level. High-N treated seedlings showed the highest growth rate, followed by medium-N and low-N treatment during soil water change. The R/S of medium-N treatment plants displayed smaller changes in response to the drought treatment than those in the high- and low-N treatments.

4. Discussion

ABA regulates stomatal opening and closure when soil water content and N availability change, which makes plants maintain water balance in cell, tissue and individual level through regulating growth and development as well as water use

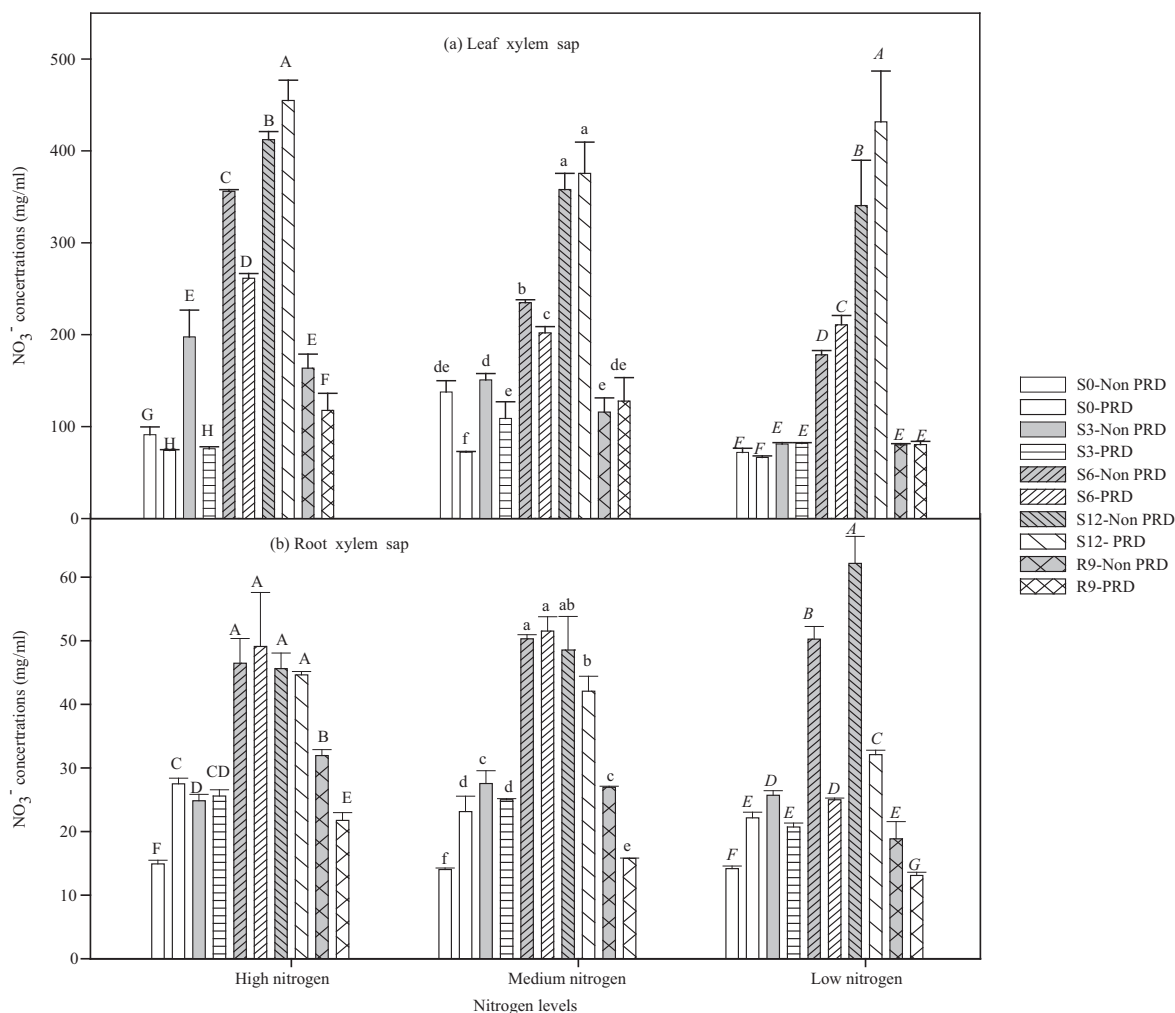


Fig. 2 Change of NO_3^- concentrations in leaf and root xylem sap in PRD and non-PRD cotton seedlings treated with different nitrogen levels during drought stress and subsequent rewatering. Capital letters, small letters and italic letters above error bars represent ANOVA results of high nitrogen, medium nitrogen and low nitrogen treatments, respectively. Data are shown as mean \pm SE of four independent measurements ($p < 0.05$).

(Zhang et al., 1997; Comstock, 2002; Chaves and Oliveira, 2004; Haefele et al., 2008; Jia and Davies, 2007; Li et al., 2011; Poorter et al., 2012). We confirmed that in cotton seedlings, the interaction of N levels and PRD induced obvious changes in plant water status, plant growth and WUE (Table 3) via cooperative regulating of leaf-sourced and root-sourced ABA, NO_3^- and pH (Figs. 1 and 3) to stomatal behaviors in different levels of soil water.

The growth and WUE of cotton seedlings were concerned in this study. The PRD-treated plants exhibit larger biomass and R/S, higher growth rate and WUE, rapid recovery rates from drought stress etc. (Table 4), compared with non-PRD-treated ones during drought and subsequent rewatering. This was consistent with other studies (Li et al., 2010; Poorter et al., 2012; Sampathkumar et al., 2013; Nouna et al., 2016), in which alternate partial root-zone drying/irrigated maize, potato or cotton had the higher WUE than traditional-irrigated plants. On the other hand, 1.5 folds N fertilization did not increase biomass and WUE obviously but raise water consumption of per cotton seedlings; While 0.6-fold N

fertilizer significantly declined biomass and water consumption but increased WUE, irrespective of irrigation methods, compared with medium N supplied cotton seedlings under drought stress and subsequent rewatering. It can be attributed that the appropriate N fertilizer is useful to promote vegetative growth of plants. Nitrogen deficiency is not beneficial for growth maintaining, while N overuse is not helpful to WUE improving. However, Wang et al. (2012) reported that N fertilization did not affect WUE in maize grown in the PRD system. In this experiment, the increase of WUE in low-N level is due to that the decrease of drought-induced biomass was less than water consumption (Table 4 and Fig. 4). It also suggested that even less signal substances accumulations (ABA and NO_3^-) or change (pH) could decrease inefficient water consumption, increased WUE at low N supplied plants under drought finally.

Stomatal opening/closure regulates water consumption and water use via controlling transpiration (Zhang and Davies, 1987; Li et al., 2010). Therefore, the phenotypic changes of cotton seedlings during soil water varied as mentioned above

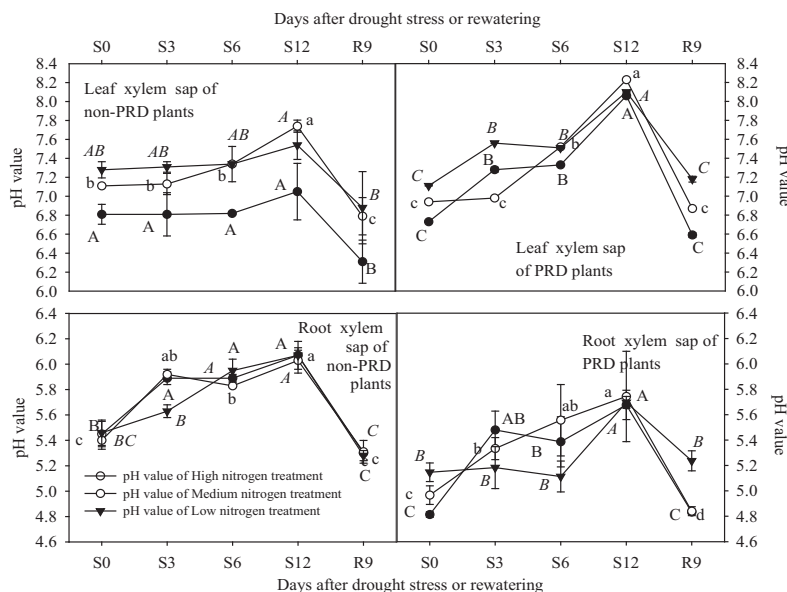


Fig. 3 Change of pH value in leaf and root xylem sap in PRD and non-PRD cotton seedlings treated with different nitrogen levels under drought stress and subsequent rewatering. S and R on the horizontal axis represent days of drought stress and rewatering, respectively. Capital letters, small letters and italic letters above error bars represent ANOVA results of high nitrogen, medium nitrogen and low nitrogen treatments, respectively. Data are shown as mean \pm SE of four independent measurements ($p < 0.05$).

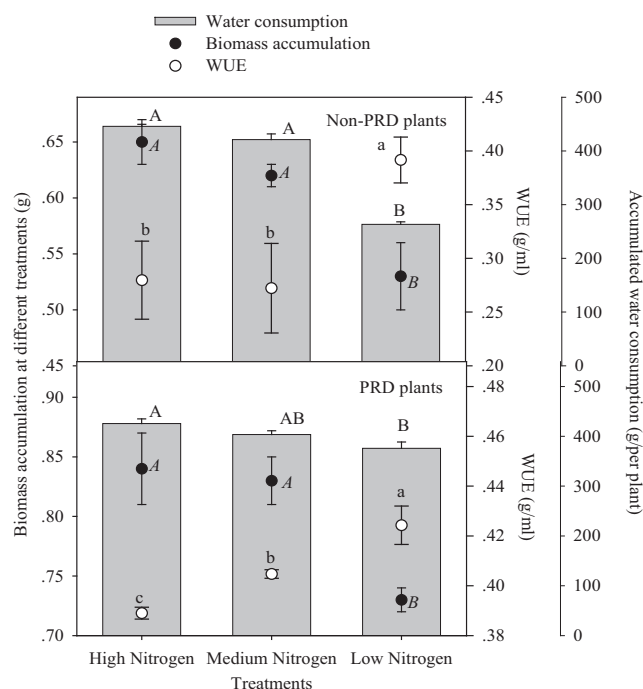


Fig. 4 Accumulated water consumption (square columns), water use efficiency of biomass production (WUE, 'o') and biomass accumulations (●) of cotton seedlings treated with different nitrogen level under drought stress and subsequent rewatering. Capital letters refer to accumulated water consumptions, small letters to WUE and italic letters to biomass accumulations, respectively. Data are shown as mean \pm SE of three independent measurements ($p < 0.05$).

(biomass, leaf water potential, stomatal behavior, R/S etc), are close related to the cooperative regulation of xylem sap ABA, NO_3^- and pH to stomatal opening and closing in PRD and different N levels (Fig. 1–3). ANOVA also confirmed this (Table 3). The decline of soil water availability promotes the increase of ABA and NO_3^- as well as alkalization both in leaf and root xylem sap, irrespective of treatments. Afterwards, the rising of soil water availability recovered them to the control levels (Figs. 1–3). The original role of ABA in controlling stomatal opening and closing induced by root environments water shortage has been clarified on many species plants (Zhang and Davies, 1987; Davies and Zhang, 1991; Chaves and Oliveira, 2004; Jia and Davies, 2007; Wilkinson et al., 2007; Dodd et al., 2008; Kaldenhoff et al., 2008). Most of them thought that the pH fluctuation in apoplast or symplast of leaf changed ABA concentrations near guard cell's apoplas, regulated the stomatal opening and closing finally (Jia and Davies, 2007; Dodd et al., 2008). In PRD, the dried zone of root system improved ABA-dependent pH signal and/or hydraulic signal in root xylem sap, induces stomatal closure and transpiration decrease, while the wet zone of root system maintains water uptake to protect plants avoiding severe water deficit (Dodd, 2003; Dodd et al., 2008). Therefore, the “adapting” to alternative water supply before the onset of long period drought stress promotes the synthesis of root-sourced chemical signals and their transfer from xylem sap to shoot, which stimulates leaf-sourced chemical increase or re-synthesis. This process is responsible for the better growth, higher WUE and rapider recovery from “long-time” drought stress in PRD-treated cotton seedlings.

Xylem sap flow alkalization and the changes of ion concentrations (Ca^{2+} , K^+ and Cl^- etc, which caused turgor pressure reduce and then stomatal closure) might be the original

Table 4 Effect of drought stress and subsequent rewatering on biomass and ratio of root/shoot in cotton seedlings treated with different nitrogen level. S and R represent drought stress and rewatering, respectively. Data are shown as mean \pm SD of four independent measurements ($p < 0.05$).

		(g. dry weight)	S0	S3	S6	S12	R9
Non-PRD	High nitrogen	Biomass of per plant	0.61 \pm 0.01D	0.68 \pm 0.01D	0.87 \pm 0.03C	0.92 \pm 0.00C	1.26 \pm 0.01A
		Increased biomass	–	0.07	0.19	0.05	0.14
		Ratio of root/shoot	0.54 \pm 0.01C	0.56 \pm 0.01C	0.63 \pm 0.01 AB	0.67 \pm 0.01 A	0.53 \pm 0.02C
	Medium nitrogen	Biomass of per plant	0.53 \pm 0.03f	0.58 \pm 0.00ef	0.65 \pm 0.02de	0.73 \pm 0.02d	1.16 \pm 0.04a
		Increased biomass	–	0.04	0.07	0.08	0.21
		Ratio of root/shoot	0.55 \pm 0.02a	0.56 \pm 0.01a	0.58 \pm 0.02a	0.57 \pm 0.03a	0.47 \pm 0.02b
	Low nitrogen	Biomass of per plant	0.50 \pm 0.03E	0.55 \pm 0.05DE	0.66 \pm 0.01 CD	0.71 \pm 0.02C	1.04 \pm 0.07A
		Increased biomass	–	0.05	0.10	0.05	0.14
		Ratio of root/shoot	0.45 \pm 0.01C	0.47 \pm 0.02BC	0.51 \pm 0.02AB	0.54 \pm 0.02A	0.47 \pm 0.02BC
PRD	High nitrogen	Biomass of per plant	0.83 \pm 0.02E	0.97 \pm 0.03DE	1.11 \pm 0.09 CD	1.14 \pm 0.02 CD	1.67 \pm 0.09 A
		Increased biomass	–	0.14	0.13	0.03	0.31
		Ratio of root/shoot	0.54 \pm 0.01B	0.59 \pm 0.01AB	0.63 \pm 0.03A	0.65 \pm 0.04A	0.60 \pm 0.00AB
	Medium nitrogen	Biomass of per plant	0.80 \pm 0.05f	0.94 \pm 0.01ef	1.07 \pm 0.02de	1.17 \pm 0.02 cd	1.63 \pm 0.06a
		Increased biomass	–	0.14	0.13	0.10	0.16
		Ratio of root/shoot	0.61 \pm 0.01cd	0.63 \pm 0.02bcd	0.66 \pm 0.02abc	0.68 \pm 0.01a	0.62 \pm 0.03bcd
	Low nitrogen	Biomass of per plant	0.85 \pm 0.01F	0.98 \pm 0.02E	1.10 \pm 0.03D	1.16 \pm 0.03 CD	1.58 \pm 0.05A
		Increased biomass	–	0.13	0.12	0.06	0.18
		Ratio of root/shoot	0.65 \pm 0.02BC	0.69 \pm 0.01AB	0.74 \pm 0.03A	0.71 \pm 0.01AB	0.62 \pm 0.02C

root-sourced drought signals that induce stomatal closure (Dodd et al., 2008). Accompanied these processes, ABA synthesis was stimulated and combined ABA (such as ABA-GE) in the root xylem sap could be transformed to the free-ABA under a weak acid condition, catalyzed by β -glucosidase and more free-ABA components might promote higher ABA accumulation (Netting et al., 2012). Dodd et al. (2008) confirmed that the ABA concentration in root xylem sap increased as the soil water potential decreased, regardless of whether sap was collected from the wet or dry root system in PRD-treated plants, or from a drought-stressed plant. Alkalinization of the leaf apoplast resulted from drought conditions is attributed to the altering of biochemical functions for maintaining a charge balance in cell (Jia and Davies, 2007; Poorter et al., 2012) and inducing of root-sourced chemical or hydraulic signals. The increased pH delays decomposition metabolism of ABA, blocks ABA diffusion to outside of stomatal cell, then accumulates ABA in apoplast connected with guard cells or re-synthesized ABA in leaves (Wilkinson et al., 2007; Dodd et al., 2008).

Although the interaction of PRD and N levels is obvious but independent effects of PRD and N levels on signal substances are significant while soil water content changes (Figs. 1–3, Table 3). Compared with non-PRD, irrespective of N levels, PRD induces obvious the larger increase of ABA, NO_3^- and pH in leaf xylem sap, but with significant smaller NO_3^- and pH in root xylem sap while drought stress occurred. Leaf-sourced ABA can stay the site or be loaded into the phloem and then transported to the roots. Some of ABA arrived in roots might be deposited in the tissue or metabolized, or with root-synthesized ABA be loaded to the xylem vessels and recirculated to the shoots (Davies et al., 2005; Dodd et al., 2009). The alternation of drying and wetting of

soil water status in PRD might promote sensitivity of stomata to ABA by accelerating synthesis of leaf-sourced ABA or improving recirculation of ABA from root to shoot (Davies et al., 2005; Dodd et al., 2009). Moreover, root-sourced ABA can be regulated by lower root xylem sap pH. We observed nearly unchanged root-sourced ABA and lower root xylem sap pH during long period drought in PRD in the experiment. This process follows by transporting more NO_3^- to shoot with transpiration flow (meant decline of NO_3^- in root xylem sap) and alkalizing leaf xylem sap (more shoot biomass and leaf area means stronger transpiration power). Additionally, leaf xylem sap NO_3^- increase also might be attributed to the N metabolism location change between root and shoot. The reduced N contents increase in xylem sap induced by drought. It might be contributed to the enhancement of pH in apoplast of leaves and the accumulation of ABA in apoplast nearby guard cells (Jia and Davies, 2007; Dodd et al., 2008). It suggested that PRD was beneficial for NO_3^- absorption via root system because of relative stronger transpiration power. Absorbed NO_3^- by roots transfers to the leaf with the transpiration flow, participating in the regulation of ABA synthesis in leaf as reported by Gonzalez-Dugo et al. (2010) and Qin et al. (2011).

Simultaneously, in leaf xylem sap, high N stimulates obvious larger increase of ABA and NO_3^- but lower pH in comparison with medium N both in PRD and non-PRD at drought stress; N deficiency has similar ABA and NO_3^- but lower pH compared with medium N. In root xylem sap, there are no obvious difference on ABA and pH among N levels, but low N improves NO_3^- accumulation in non-PRD and inhibits NO_3^- accumulation in PRD. It suggested that xylem sap alkalization is promoted by both N overdose and deficiency, but it is weaker than medium N under drought stress in this

experiment, especially in leaf xylem sap. Higher nitrogen supply increased NO_3^- resource (Fig. 2), produced larger biomass (Table 4) and faster nitrogen use for growth. Together with alkalization of the xylem sap, NO_3^- plays an important signaling role in regulating ABA concentrations and triggering ABA distribution (Jia and Davies, 2007). Wilkinson et al. (2007) reported that a small increase in N supply could increase the pH of sap, thereby indirectly affecting the ABA accumulation. This process of NO_3^- transferring to the leaf tissues might be easier in PRD or high-N supplied plants because of larger leaf areas and transpiration power (more biomass, Table 4), which also explains why pH values did larger changes in leaf xylem sap than in root xylem sap, both in PRD-treated and non-PRD-treated plants. The NO_3^- concentrations in the xylem sap are a pH-dependent root and shoot signals (Dodd, 2003).

Declined root-sourced NO_3^- in low N level in PRD might be due to changed composition of organic acids, such as the increase of malic acid, which was used to neutralize the hydroxide produced during N transformation and then induced alkalization of xylem sap (Wilkinson et al., 2007; Wang et al., 2012). Thus, the decrease in pH and the decline in ABA and NO_3^- concentrations after rewatering might also be related to a decrease in the malate level, or contributed to maintaining the charge balance of cations and anions in the xylem sap. In the study on maize of PRD, Hu et al. (2009) showed that increased root N absorption in the irrigated zone had a significant compensatory effect on N uptake of whole plant. Gonzalez-Dugo et al. (2010) also reported that although mineral N fluxes in the xylem sap generally decreased under water deficiency, N supplied in one part of the root-zone could complement and provide resources to other parts with no N supply for plant growth in a split-root irrigation treatment. That is why NO_3^- concentrations in root xylem sap in low-N showed larger changes in PRD-treated plant in this study. Earlier research on cotton by Radin and Ackerson (1981) reported that ABA accumulation (in leaves) occurred at a higher water potential in N-deficient cotton plants compared with normal plants. This tendency was more significant in PRD plants than in non-PRD seedlings (see above) in this experiment (before 12-day drought stress, Tables 1 and 2), illustrating an improvement of N deficiency to ABA accumulation at earlier drought stress, which suggests a positive role of PRD treatment and/or N levels.

5. Conclusion

Better growth, larger biomass and higher WUE were shown in PRD plants subjected to soil water deficiency, compared with non-PRD, irrespective of N levels. Higher leaf xylem ABA, NO_3^- and pH, the lower root xylem NO_3^- and pH as well as similar root xylem ABA, in comparison with non-PRD, are responsible for maintaining of phenotype characteristics in PRD. These phenotypic characteristics are controlled by different leaf and root xylem chemical signals, by regulating stomatal behavior under drought stress condition. Obvious significant interaction of PRD and nitrogen levels on chemical signals was exhibited. Whereas high N is benefit to PRD plants growth and biomass increase through stimulating only leaf xylem ABA and NO_3^- increase under relatively low pH, although their WUE decreases. Nitrogen deficiency fails to promote both leaf and root xylem chemical signals to regulate

better biomass and growth, however, WUE increases via inhibiting root xylem NO_3^- accumulation in PRD. Thus, leaf-sourced chemical signal perhaps is sufficient to confer PRD phenotype, especially under high N condition. We conclude that these signaling chemicals are regulated independently and interacted by soil drying conditions, PRD and nitrogen status to regulate stomatal behavior, either directly or indirectly, and thus increase plant WUE in PRD under less irrigation.

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