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Water-deficit treatment followed by re-watering stimulates seminal root growth associated with hormone balance and photosynthesis in wheat (*Triticum aestivum* L.) seedlings

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Abstract Elucidating the growth responses of roots to water status will reveal physiological mechanisms underlying drought tolerance and water conservation in plants. Hydroponic experiments were conducted using two winter wheat cultivars, Wangshuibai (drought-sensitive) and Luohan 7 (drought-tolerant), and a water deficit was induced using a 20 % (m/v) aqueous solution of polyethylene glycol 6000 (−0.6 MPa). The lack of water significantly reduced the plant dry weight, leaf area, total root length (TRL) and surface area in seminal (SRs) and nodal roots (NRs), but the effects were less pronounced in Luohan 7 than in Wangshuibai. After re-watering, leaf area, TRL and surface area of Luohan 7 increased significantly, as compared to the controls, due to rapid compensatory growth of SRs, while those of Wangshuibai were still significantly reduced. Under water-deficit conditions, the concentrations of indole-3-acetic acid (IAA) and cytokinin (CTK) and their ratio (IAA/CTK) in SRs and NRs of both cultivars were significantly lower than those of controls, but increased after re-watering. However, Luohan 7 showed significantly increases in IAA/CTK of SRs as compared to the control. Net photosynthetic rate was much lower during water deficit in both cultivars, but it was enhanced significantly after re-watering, especially for Luohan 7. Moreover, sucrose content was significantly increased in leaves while reduced in roots under water-deficit conditions. After re-watering, sucrose content in leaves of both cultivars and in roots of Wangshuibai was severely reduced, while the values in roots of Luohan 7 were significantly increased as compared to the control. These results indicate that the drought-tolerant cultivar has a greater ability to maintain plant growth under water deficit and greater compensatory growth in SRs associated with higher IAA/CTK and photosynthetic products supply after re-watering.

Keywords Winter wheat (*Triticum aestivum* L.) · Water deficit · Root morphology · Hormone balance · Photosynthesis

Introduction

Drought is a major constraint to global crop production (Shane et al. 2010; Chaves and Oliveira 2004). As a worldwide planting cereal crop, winter wheat production requires more water than other crops due to its longer growth period. In China, drought occurs more frequently during winter and spring seasons, which severely affects the formation of photosynthetic organs and tiller number during seedling period, hence reduces the accumulation of materials and energy and grain yield. So breeding more tolerant cultivars to drought stress is becoming an urgent target for high yield and water-saving cultivation.

As the main organ for capturing water and nutrients from soil, the plant root system is strongly affected by the water status of the soil (Osmont et al. 2007; Hermans et al. 2006; Malamy 2005). However, it has strong morphologic plasticity (e.g., changes in length and in the number of lateral roots and root hairs) to adapt to heterogeneous and changing environments (Valladares et al. 2007; Gruber et al. 2013). For example, under drought conditions, plants

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develop deep and extensive root systems as an adaptive response (Serraj et al. 2004), including thick roots with an increased length and density (Siopongco et al. 2006). Deeper roots are important for plant growth under drought conditions (Sanguineti et al. 2007; Araus et al. 2008): deep roots may absorb water from deep soil layers to help plants survive under drought stress (Gowda et al. 2011). However, decreased root growth in response to drought has been reported (Vandoorne et al. 2012). These responses differ according to the crop species and the timing and intensity of drought (Kano et al. 2011). Therefore, it is important to explore the mechanisms regulating the morphology of the root system in relation to water status to increase crop yields under drought conditions (Uga et al. 2013).

The morphology of the root system is associated with endogenous hormones. Indole-3-acetic acid (IAA) and cytokinin (CTK) play important roles in regulating and modulating the formation of lateral roots in response to environmental changes (Swarup et al. 2008; Kuderová et al. 2008; Casimiro et al. 2003; Malamy 2005). CTK is mainly produced by the root cap meristem and negatively regulates meristem size and root length (Dello Ioio et al. 2007). IAA is mainly synthesized in the shoot apical meristem and is transported from shoots to roots through the phloem (Woodward and Bartel 2005; Ljung et al. 2001) to promote root branching and the formation of adventitious roots (Casimiro et al. 2001; Woodward and Bartel 2005; Quint et al. 2009). Therefore, CTK and IAA have antagonistic roles during root formation (Aloni et al. 2006). It has been reported that water stress reduced the synthesis of CTK in roots and decreased its upward transportation (Li and Li 2007). Under drought conditions, the endogenous contents of IAA usually decreased (Yang et al. 2001). Nevertheless, there are reports that drought did not change IAA levels in leaf and root tissues (Mahouachi et al. 2007) and that moderate drought stress increased IAA transport in the root tip (Xu et al. 2013). Thus, those changes in distribution and concentrations of IAA and CTK in plants would regulate root morphology under different water status. Unfortunately, the regulatory mechanism remains unclear.

The morphology of roots may also be regulated by the concentration of sucrose. In wheat, increased lateral roots are associated with increased concentrations of glucose and sucrose in the roots (Bingham et al. 1998). In tobacco, there is a relationship between the growth rate and sugar content of roots (Schiefelbein and Benfey 1991). Previous studies have also demonstrated that an increase in sucrose metabolism under osmotic stress may induce the root system to produce more branches (MacGregor et al. 2008). Karthikeyan et al. (2007) found that the exogenous supply of sucrose increased the density of lateral roots in *Arabidopsis*, and this effect was enhanced by the addition of

IAA. Nevertheless, our understanding of these phenomena is mostly based on studies of *Arabidopsis thaliana*. Therefore, it is important to explore the regulatory mechanisms of food crops to improve their drought tolerance. However, our understanding of these regulatory mechanisms remains limited.

In crops, root morphology is closely associated with the growth and development of shoots because of the role of roots in water and nutrient uptake (Samejima et al. 2004; Zhang et al. 2009). Under drought conditions, rice roots produce signals to regulate leaf stomatal conductance, transpiration, and shoot growth (Siopongco et al. 2008). Two root types are distinguished in cereals: seminal roots (SRs, also called primary roots) and nodal roots (NRs, also called secondary or crown roots) (Manske and Vlek 2002). Both SRs and NRs in wheat maintain active function throughout all plant stages (Sanguineti et al. 2007). It is reasonable to speculate that SRs may be equally or even more vital than NRs under conditions of water stress for capturing water and nutrients from the soil and sustaining plant growth because they can penetrate more deeply into the soil (Manske and Vlek 2002). However, most studies of plant responses to water stress have been performed using the whole root system (Serraj et al. 2013); few studies have considered SRs under such conditions (Sahnoune et al. 2004). In addition, many studies have focused on the negative effects of a water deficit on root growth (Rostamza et al. 2013). Information on the effects of water stress and re-watering conditions on the morphologies of SRs and NRs of wheat, and the relationship with shoot growth, is unavailable.

Therefore, the objectives of the present study were to: (i) examine the responses of wheat in terms of SR and NR morphology to water deficit and re-watering conditions and elucidate the regulatory mechanism, and (ii) to clarify the relationship between root morphology and shoot growth. Our results may be used to uncover physiological mechanisms of drought tolerance and to select or breed wheat cultivars that are adapted to water-deficit conditions.

Materials and methods

Plant materials and experimental design

Two wheat cultivars with different responses to drought (Luohan 7, a drought-tolerant cultivar; and Wangshuibai, a drought-sensitive cultivar) were used for hydroponic experiments (Ma et al. 2012). Uniform seeds were surface-sterilized with 20 % hydrogen peroxide for 10 min, rinsed with distilled water, and then germinated in a Petri dish with two pieces of filter paper. The seeds were transferred to vermiculite until the bud length was approximately

1 cm. Uniform wheat seedlings at the one-leaf stage were transplanted to plastic containers (32 cm long, 21 cm wide, and 11 cm tall) filled with modified Hoagland nutrient solution containing 1 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2 mM KNO_3, 0.5 mM $CaCl_2$, 0.5 mM $(NH_4)_2SO_4$, 1 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM NaCl, 5 µM Fe-EDTA, 0.55 µM $MnSO_4 \cdot H_2O$, 0.0385 µM $ZnSO_4 \cdot 7H_2O$, 2.35 µM H_3BO_3, 0.0165 µM $CuSO_4 \cdot 5H_2O$, and 0.0065 µM H_2MoO_4. The seedlings were cultivated in a greenhouse under 16/8 h of light/dark conditions at 18/8.5 °C (day/night). The pH of the nutrient solution was controlled at 5.50 ± 0.05 by adding 0.1 mM HCl or 0.1 mM NaOH every day. The nutrient solutions were changed every 3 days.

The seedlings were divided into two batches at the four-leaf stage. One batch was treated with 20 % (m/v) polyethylene glycol 6000 (−0.6 MPa) (Michel and Kaufmann 1973) to induce a water deficit for 5 days. Another batch served as controls (CK). After inducing water deficit, the seedlings were re-watered until there was no difference in leaf relative water content (LRWC) between the control and deficit-exposed plants of cultivar Luohan 7 (Fig. 1). The experiment had a completely randomized block design with three replicates.

Leaf samples were collected to determine the LRWC on 1, 3, and 5 days after water deficit, and on 1 and 3 days after re-watering treatment, respectively. Gas exchange was examined on the same day. After these experiments, another batch of samples was taken and divided into two groups. One group was further divided into leaves, SRs, and NRs, and all samples were frozen in liquid nitrogen and stored at −80 °C. The other group was used for dry weight measurements. These plants were divided into shoots and roots and dried at 105 °C for 20 min and then at 85 °C to obtain a constant weight.

Measurement of total root length (TRL) and root surface area (RSA)

Uniform roots were chosen after the control and experimental treatments. SRs and NRs were separated and rinsed with distilled water, and then scanned using a scanner (Epson 1680, Suwa, Japan) and finally analyzed using WinRHIZO Pro Vision 5.0 (Elmira, ON, Canada) to obtain the TRL and RSA.

The relative growth rate (RGR) of the TRL was calculated using the equation

$$\text{RGR of TRL} = (L_2' - L_1')/(L_2 - L_1),$$

where L_1' and L_2' are the TRLs of the water deficit-treated plants on day 5 of stress treatment and day 3 of re-watering, respectively, and L_1 and L_2 are the TRLs of control plants measured at the same time as L_1' and L_2', respectively.

The RGR of the RSA was calculated using the equation

$$\text{RGR of RSA} = (A_2' - A_1')/(A_2 - A_1),$$

where A_1' and A_2' are the RSAs of the water deficit-treated plants on day 5 of stress treatment and day 3 of re-watering, respectively, and A_1 and A_2 are the RSAs of control plants measured at the same time as A_1' and A_2', respectively.

Determination of the LRWC and leaf area

The LRWC was determined as described previously (Meng et al. 2013). Leaf area was measured by using a LI-3000 area meter (Li-Cor Inc., Lincoln, NE, USA).

Gas exchange measurements

The net photosynthetic rate (Pn) and stomatal conductance (Gs) were measured for the top leaf between 09:00 and 11:00 a.m. using a LI-6400 portable photosynthesis system (Li-Cor Inc.). The leaf chamber CO_2 content was managed at ca. 370 µmol mol^{-1} and the photosynthetic active radiation was set to 1000 mmol m^{-2} s^{-1}.

Determination of the sucrose content

Dried samples were ground into a powder and analyzed for the sucrose content. Dried samples (0.1 g) were extracted three times with 80 % (v/v) ethanol at 80 °C to extract sugars, and then centrifuged at 3,000 × g. The supernatant
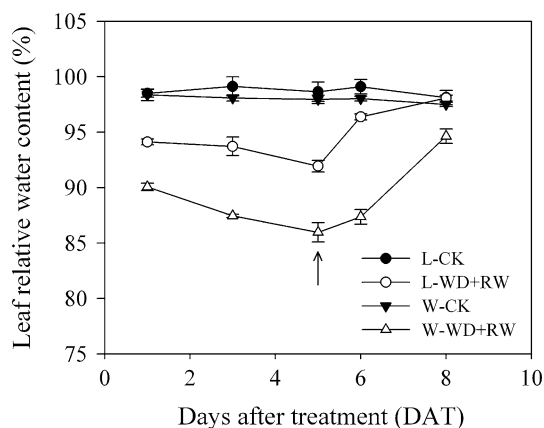
Fig. 1 Changes in relative water content in leaves of Luohan 7 (L) and Wangshuibai (W) after water-deficit treatment (WD) and re-watering (RW). *Uparrow* represents the start of re-watering; L-CK refers to control Luohan 7 plants; L-WD + RW refers to Luohan 7 plants exposed to water-deficit conditions + re-watering; W-CK refers to control Wangshuibai plants; and W-WD + RW refers to Wangshuibai plants exposed to water-deficit conditions + re-watering. The data are given as mean ± standard error (n = 3)

was collected to measure the sucrose content using the resorcinol method (Zhang and Qu 2003).

Measurement of endogenous hormones

The methods used for the extraction and purification of IAA, zeatin (Z) + zeatin riboside (ZR), and isopentenyladenine (iP) + isopentenyladenine riboside (iPR) were essentially as described previously (Yang et al. 2001; Liu et al. 2011). Briefly, approximately 0.5 g of frozen tissue was ground in 5 ml of 80 % (v/v) methanol extraction solution containing 1 mM butylated hydroxytoluene as an antioxidant and put into a centrifuge tube, and then incubated in the dark at 4 °C for 4 h. After incubation, the tubes were centrifuged at 10,000 × g for 15 min at 4 °C. Chromosep C18 columns (C18 Sep-Park Cartridge; Waters Corp., Milford, MA, USA), prewashed with 5 ml of 80 % methanol, were used to filter the supernatants. The filtrates were dried using a freeze dryer (ALPHA 2–4/LSC; Christ Corp., Harz, Germany) and dissolved in 1 ml of phosphate-buffered saline (pH 7.5) containing 0.1 % (v/v) Tween 20 and 0.1 % (w/v) gelatin. The endogenous hormones were analyzed using an enzyme-linked immunosorbent assay (ELISA).

All reagents used in the ELISA, including the monoclonal antigens and antibodies against IAA, Z + ZR, and iP + iPR and immunoglobulin G-horseradish peroxidase were purchased from the Phytohormones Research Institute of China Agricultural University (Beijing, China). The concentrations of IAA, Z + ZR, and iP + iPR were determined by ELISAs as described previously (Yang et al. 2001). The CTK concentration was calculated based on the Z + ZR and iP + iPR concentrations.

Statistical analysis

The results represent the means of three replicates and were subjected to a two-way analysis of variance to identify significant differences between cultivar and water status using SPSS (ver. 13.0; SPSS, Chicago, IL, USA) software.

Results

Effects of water deficit and re-watering on plant dry weight and leaf area

Water-deficit treatment significantly reduced plant dry weight and leaf area in both cultivars. Compared to controls, the magnitude of the effect was lower in Luohan 7 than in Wangshuibai (Table 1). Though the plant dry weight of the two cultivars was markedly lower after re-watering treatment, the decreases in Luohan 7 were from 38 to 35 % after re-watering, while those in Wangshuibai were from 51 to 55 %, indicating that the plant

growth rate was higher in Luohan 7. Moreover, no significant change in leaf area between the stressed and control plants after re-watering in Luohan 7, while significant decreases were observed in Wangshuibai. These data suggest that the drought-tolerant cultivar maintained a greater capacity for plant growth under water-deficit conditions and exhibited a fast compensatory ability during re-watering.

Effects of water deficit and re-watering on TRL and RSA

Under water-deficit conditions, the TRL and RSA of the SRs, NRs, and total roots (TRs) in both cultivars decreased significantly, but the decreases were lighter in Luohan 7 than those in Wangshuibai (Table 2). After re-watering, the TRL and RSA of the TRs showed no significant differences in Luohan 7, as compared with the controls, but they remained lower in Wangshuibai. However, the TRL and RSA of the SRs in Luohan 7 were significantly higher than those in controls after re-watering. On the contrary, the TRL and RSA were significantly lower in the NRs of Luohan 7 as well as in both the SRs and NRs of Wangshuibai after re-watering. Furthermore, for both stressed cultivars, the growth rates of the TRL and RSA in the SRs were much higher than those in controls after re-watering, which were different from those in the NRs (Fig. 2). Moreover, the growth rates of the TRL and RSA in the SRs of Luohan 7 were 3.1 and 2.7 times greater than those of Wangshuibai, respectively, indicating that the compensatory growth speed of the SRs in Luohan 7 was much faster than that in Wangshuibai after re-watering. Thus, the growth rates of the TRL and RSA in the TRs of Luohan 7 were much higher than those of the controls after re-watering, whereas those of Wangshuibai were lower than those of the controls. Due to the compensatory growth of the SRs, the TRL and RSA in the TRs of Luohan 7 exhibited no significant difference compared to controls after re-watering.

Effects of water deficit and re-watering on the concentrations of IAA and CTK

Water-deficit treatment decreased the concentrations of IAA and CTK in the SRs and NRs of the two cultivars (Fig. 3). Furthermore, the IAA concentrations were higher in the SRs of Luohan 7 after re-watering than in controls; however, they were still lower both in the SRs and NRs of Wangshuibai (Fig. 3a, b). In addition, the CTK concentrations were lower in the SRs of Luohan 7 after re-watering, while they were higher in the NRs of Luohan 7 and both in the SRs and NRs of Wangshuibai (Fig. 3c, d).

Under water-deficit conditions, the IAA/CTK decreased significantly in the SRs and NRs of both cultivars (Fig. 4). Furthermore, it decreased less in Luohan 7 than in

Table 1 Effects of water-deficit treatment (WD) and re-watering (RW) on plant dry weight and leaf area in two wheat cultivars

| Cultivar	Treatment	Dry weight (mg plant^{-1})			Leaf area (cm^2 plant^{-1})
		Shoot	Root	Plant	
Day 5 of water-deficit treatment (DAW)					
Luohan 7	CK	235.29a	71.36a	306.65a	41.12b
	WD	136.75c	53.38b	190.13c	36.63c
Wangshuibai	CK	208.55b	68.74a	277.29b	58.01a
	WD	97.77d	37.81c	135.58d	30.55d
Day 3 of re-watering (DAR)					
Luohan 7	CK	378.28a	98.50a	476.78a	45.62b
	RW	224.95c	74.94b	310.88c	45.13b
Wangshuibai	CK	342.04b	98.92a	440.96b	67.51a
	RW	145.39d	54.22c	199.61d	37.56c

Values within measurement time followed by different letters are significantly different ($P < 0.05$)

Table 2 Effects of water-deficit treatment (WD) and re-watering (RW) on total root length (TRL) and root surface area (RSA) in seminal roots (SRs), nodal roots (NRs), and total roots (TRs) of two wheat cultivars

Cultivar	Treatment	TRL (cm plant^{-1})			RSA (cm^2 plant^{-1})		
		SRs	NRs	TRs	SRs	NRs	TRs
Day 5 of water-deficit treatment (DAW)							
Luohan 7	CK	720.42b	136.01b	894.43b	72.24a	23.21b	95.44b
	WD	574.64c	104.36c	679.00c	50.98b	15.08c	66.06c
Wangshuibai	CK	802.62a	277.26a	1079.88a	69.45a	34.43a	103.88a
	WD	311.65d	108.46c	420.11d	30.18c	15.23c	45.42d
Day 3 of re-watering (DAR)							
Luohan 7	CK	768.98c	361.17b	1130.15b	86.98b	53.47a	140.44a
	RW	1008.66a	161.65c	1170.31b	107.98a	24.16b	132.14a
Wangshuibai	CK	866.48b	444.19a	1310.67a	85.01b	55.01a	140.02a
	RW	494.29d	153.54c	647.84c	53.59c	21.14b	74.74b

Values within measurement time followed by different letters are significantly different ($P < 0.05$)

Fig. 2 Relative growth rate of the total root length (**a**) and root surface area (**b**) in the seminal roots (SRs), nodal roots (NRs), and total roots (TRs) of two wheat cultivars. *Different small letters* indicate significant differences at the 0.05 level. The data are given as means + standard error (n = 3)
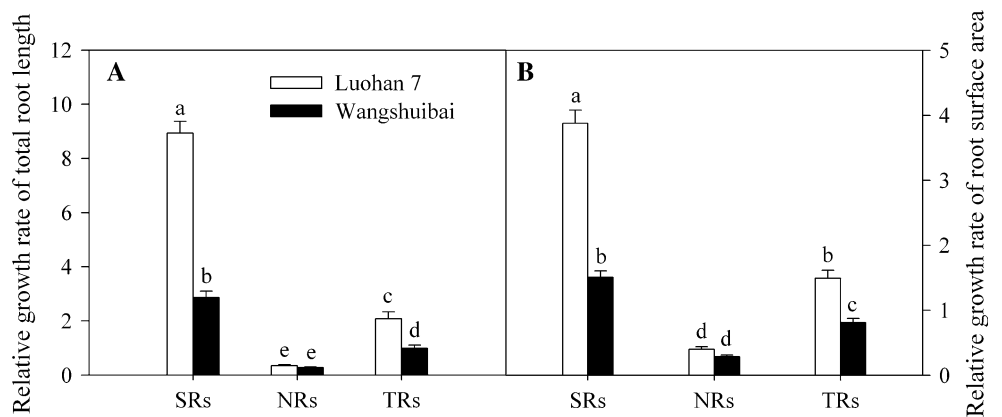
Wangshuibai both in the SRs and NRs. After re-watering, the ratio increased significantly in both cultivars. In addition, the ratio in SRs of Luohan 7 was significantly higher than that in controls after re-watering, but the ratios were lower in the NRs of Luohan 7 and in the SRs and NRs of Wangshuibai.

Effects of water deficit and re-watering on Pn and Gs

For two cultivars, the Pn and Gs were decreased significantly under water-deficit conditions, but the decreases were less in Luohan 7 than that in Wangshuibai (Fig. 5). After re-

Fig. 3 Effects of water-deficit treatment (WD) and re-watering (RW) on the IAA and CTK concentrations in the seminal roots (SRs) and nodal roots (NRs) of two wheat cultivars. DAW refers to days after water-deficit treatment; DAR refers to days after re-watering; IAA refers to Indole-3-acetic acid; and CTK refers to cytokinin. The concentrations of CTK are the total concentrations of zeatin (Z) + zeatin riboside (ZR) and isopentenyladenine (iP) + isopentenyladenine riboside (iPR). *Different small letters* indicate significant differences at the 0.05 level. The data are given as means + standard error (n = 3)
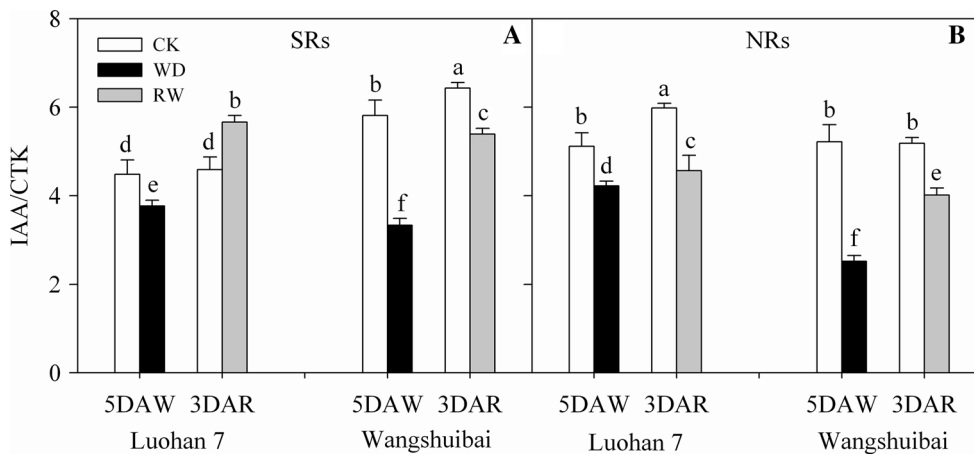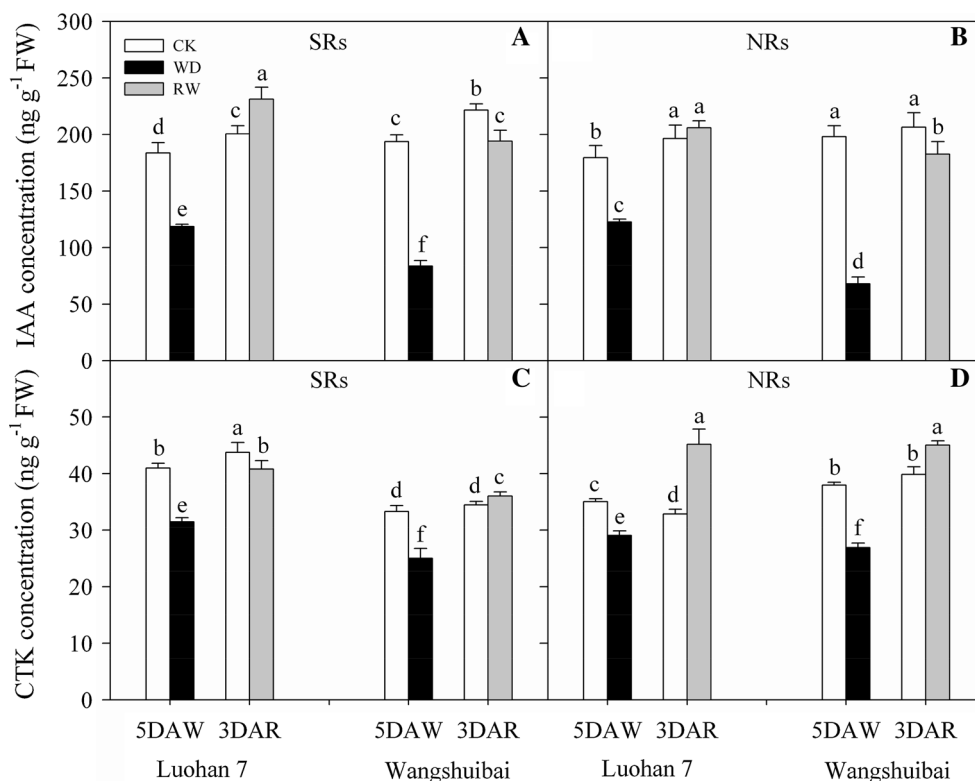
Fig. 4 Effects of water-deficit treatment (WD) and re-watering (RW) on the IAA/CTK in the seminal roots (SRs, **a**) and nodal roots (NRs, **b**) of two wheat cultivars. DAW refers to days after water-deficit treatment; DAR refers to days after re-watering; IAA refers to Indole-3-acetic acid; and CTK refers to cytokinin. The concentrations of CTK are the total concentrations of zeatin (Z) + zeatin riboside (ZR) and isopentenyladenine (iP) + isopentenyladenine riboside (iPR). *Different small letters* indicate significant differences at the 0.05 level. The data are given as means + standard error (n = 3)

watering, the Pn and Gs in the deficit-exposed plants were higher than those in controls of Luohan 7, but the values were lower than those in controls of Wangshuibai.

Effects of water deficit and re-watering on sucrose content

The sucrose content increased significantly in leaves and decreased significantly in roots in both cultivars

under water-deficit conditions (Fig. 6). However, the sucrose content increased less in the shoots and decreased less in the roots of Luohan 7 compared to Wangshuibai. After re-watering, the sucrose content decreased in the leaves of the stressed plants for both cultivars (Fig. 6a). In addition, the sucrose content increased significantly in the roots of the stressed plants for Luohan 7, while it decreased significantly in Wangshuibai (Fig. 6b).
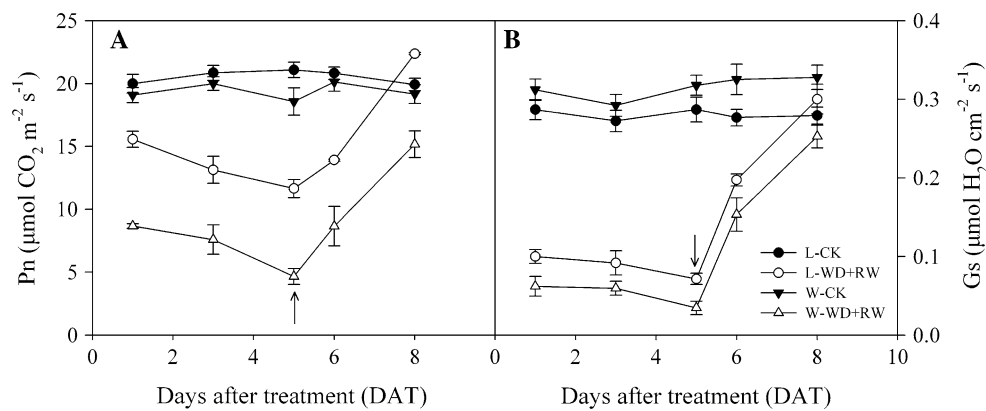
Fig. 5 Changes in the net photosynthetic rate (Pn, **a**) and stomatal conductance (Gs, **b**) of Luohan 7 (L) and Wangshuibai (W) after water-deficit treatment (WD) and re-watering (RW). *Uparrow* represents the start of re-watering; L-CK refers to control Luohan 7 plants; L-WD + RW refers to Luohan 7 plants exposed to water-deficit conditions + re-watering; W-CK refers to control Wang-shuibai plants; and W-WD + RW refers to Wangshuibai plants exposed to water-deficit conditions + re-watering. The data are given as mean ± standard error (n = 3)

Fig. 6 Effects of water-deficit treatment (WD) and re-watering (RW) on the sucrose content in the leaves (**a**) and roots (**b**) of two wheat cultivars. DAW refers to days after water-deficit treatment; DAR refers to days after re-watering. *Different small letters* indicate significant differences at the 0.05 level. The data are given as means + standard error (n = 3)
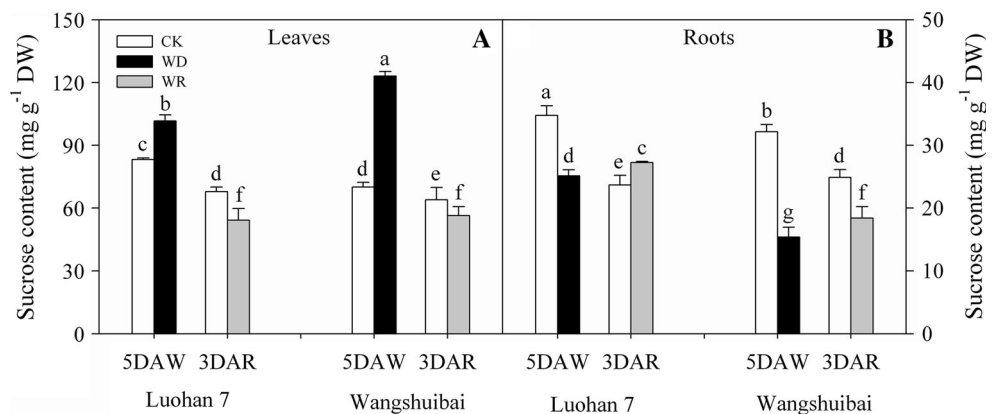
Discussion

Many studies have demonstrated that roots can change their morphology to adapt to changes in the external environment (Valladares et al. 2007; Kano et al. 2011). Chu et al. (2014) proved that the root length and surface area of paddy rice (*Oryza sativa* L.) decreased under alternate wetting and drying irrigation. Here, the TRL and RSA decreased significantly under water-deficit conditions (Table 2). However, prior to this study, little information was available on the morphology of the SRs and NRs in wheat cultivars with different responses to drought under re-watering. Our results showed that the SRs underwent compensatory growth during re-watering. Furthermore, the compensatory growth of the SRs in Luohan 7 occurred more rapidly than in Wangshuibai during re-watering, resulting in a longer TRL and larger RSA in the SRs of Luohan 7, as compared with the controls, after re-watering (Fig. 2; Table 2). There was no pronounced difference in the TRL and RSA of the whole plant between the stressed

plants and controls for Luohan 7 after re-watering due to the extra growth of the SRs (Table 2). These results imply that during re-watering after water-deficit treatment, the extra growth of the SRs promoted overall root growth in the drought-tolerant cultivar.

Blilou et al. (2005) observed that the allocation of IAA, controlled by five *PIN* genes, regulates cell division and elongation in the primary root. In the present study, the IAA concentrations were higher in the SRs of Luohan 7 alone after re-watering than in the controls (Fig. 3a, b), indicating that the higher growth rate of the SRs in Luohan 7 after re-watering was partly due to the increased root IAA concentration. Furthermore, the effects of IAA on root morphology are associated with CTK (Aloni et al. 2006; Blilou et al. 2005; Dello Ioio et al. 2007). CTK is believed to play an important role in promoting shoot growth but inhibiting root growth (Dello Ioio et al. 2007; Rahayu et al. 2005; Howell et al. 2003). It has been shown that the balance between IAA and CTK regulates the formation of roots (Blilou et al. 2005; Dello Ioio et al. 2007; Garay-

Arroyo et al. 2012). Here, we detected a positive relationship between TRL and IAA/CTK in the roots under water-deficit conditions and re-watering. Our results indicate that the growth rate of the TRL was significantly correlated with the root IAA/CTK, and that water status regulated the TRL (especially for SRs) by influencing the concentrations of IAA and CTK and the balance between them. Therefore, the drought-tolerant cultivar exhibited a compensatory growth of the SRs in response to the higher root IAA/CTK during re-watering.

It is thought that cultivars possessing a root system with good soil penetration ability can capture more moisture and, therefore, maintain a high LRWC under water-deficit conditions (Luo 2010; Yue et al. 2006). Moreover, under drought conditions, water shortages in leaves would decrease Pn due to stomatal closure (Medrano et al. 2002). Here, we observed that the LRWC of Luohan 7 reduced to a lesser degree under water-deficit conditions and recovered more quickly during re-watering, and these changes were closely associated with changes in TRL and RSA in SRs especially after re-watering (Fig. 1; Table 2). During re-watering, the higher growth rates of the TRL and RSA in SRs of Luohan 7 contributed to a higher LRWC, leading to a higher Pn in the deficit-treated plants, as compared with the controls (Figs. 1, 2, 5a). Therefore, Pn was closely associated with SR growth of winter wheat seedlings during re-watering. In the present study, we also observed that leaf expansion in the drought-tolerant cultivar was faster during re-watering due to promoted growth of SRs, which would contribute to the improved Pn (Table 1). These results were confirmed using data obtained from the Wangshuibai cultivar. Thus, the extra growth of the SRs in the drought-tolerant cultivar contributed to the increases in photosynthesis and shoot growth.

There is an interdependent relationship between roots and shoots. Active roots can improve shoot characteristics by supplying ample water and nutrients; in turn, the improvement in shoot functions ensures a sufficient supply of carbohydrates to the roots to promote growth (Yang et al. 2004; Zhang et al. 2009). An important carbohydrate, sucrose, accumulates in leaves through photosynthesis, and its metabolism results in energy production, required for plant growth (MacGregor et al. 2008). It has been reported that the formation of roots may also be regulated by the sucrose content in plants. In many plants, a lack of nutrition can change the root structure due to the accumulation of sucrose or starch in leaves, altering the distribution of carbon in the root system (Hermans et al. 2006). Studies of A. thaliana have shown that under osmotic deficit an increase in sucrose metabolism induces the root system to produce more branches (MacGregor et al. 2008). In this study, we observed that water-deficit treatment decreased the metabolism of sucrose, resulting in the accumulation of

sucrose in leaves and inhibiting its transportation to roots (Fig. 6). In addition, a higher IAA concentration in SRs and a more active sucrose metabolism was observed in Luohan 7 after re-watering, increasing the growth rate of SRs (Figs. 3a, 6). One possible reason is that sucrose metabolism in leaves stimulates the transport of IAA to roots; thus, increased IAA in roots could promote sucrose transport from leaves to roots, stimulating root growth further, which is in accordance with the finding by Bhalerao et al. (2002).

Water-deficit treatment limits the growth and decreases the biomass of plants (Shane et al. 2010; Chaves and Oliveira 2004). In agreement with this, our results showed that the plant dry weight of both cultivars decreased significantly ($P < 0.05$) under water deficit (Table 1). However, the decreases were less severe in Luohan 7; this can be explained by the less decreases in the activity of the roots and Pn in the leaves under water-deficit conditions. It has been reported that deeper roots, especially deeper SRs, are important and required for plant growth under drought conditions (Sanguineti et al. 2007; Araus et al. 2008). However, little is known about those effects after water-deficit treatment. Our results indicate that the faster growth rate of SRs in Luohan 7 promoted the recovery of the whole root system during re-watering and, thus, accelerated the absorption of moisture to improve the Pn, all of which contributed to the higher growth rate of Luohan 7.

In conclusion, water-deficit treatment reduced plant growth, but the drought-tolerant cultivar possessed a greater ability to maintain plant growth. Extra growth of the SRs, regulated by the higher root IAA/CTK after re-watering, promoted the recovery of such root traits as TRL and RSA. Moreover, the higher water status promoted by the improved TRL and RSA during re-watering promoted photosynthesis, which in turn contributed to higher shoot and root growth rates in the drought-tolerant cultivar by accelerating the synthesis and transport of sucrose.

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