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Changes in root characteristics, gas exchange and water use efficiency following water stress and rehydration of Alfalfa and Sorghum

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Abstract

Soil water stress has paramount effects on water uptake by roots and its use by shoots. In this study, we determined changes in root hydraulic conductivity (Lp_r) and morphology under drought stress and how they regulate shoot gas exchange and water use efficiency in alfalfa and sorghum, two crops with contrasting adaptations to drought, the former exhibiting strong drought avoidance and the later strong drought tolerance. Seedlings of two alfalfa cultivars differing in drought avoidance and one highly drought tolerant sorghum cultivar were subjected to PEG-6000-induced water stress and then rehydrated. The Lp_r declined rapidly after the PEG treatment but largely recovered within 48 hours of rehydration; however, inter-specific and cultivar differences were significant. The rapid change in Lp_r also led to equally rapid changes in leaf water potential, gas exchange and consequently, the instantaneous water use efficiency (WUE_i) in both species. A reasonable correlation was found between Lp_r and WUE_i. Treatment with Hg^{2+} indicated that the water stress-induced changes in Lp_r were due to the involvement of aquaporins. One year old alfalfa and sorghum cultivars subjected to moderate and severe drought stress at the field level had altered root morphology and reduced biomass production and water dissipation but increased water use efficiency of biomass production (WUE_b). These changes were dependent on stress level, species and cultivars. However, not all the root morphological changes contributed to improved water use. For example, the decrease in taproot length negatively affected the WUE_b of alfalfa whereas the increase in root surface area was positively related only to the WUE_b of sorghum. The difference in drought tolerance between species or cultivars was related to their ability to recover the lost Lp_r and CO_2 assimilation after rehydration, as well as the ability to effectively regulate root morphological changes to increase WUE.

Keywords: Root hydraulic conductivity, drought tolerance (DT), gas exchange, water use efficiency(WUE), alfalfa(A) and sorghum (S).

Abbreviations: Lp_r-root hydraulic conductivity; WUE_i-instantaneous water use efficiency; WUE_b-water use efficiency of biomass production; R/S-ratio of root and shoot; MC-mercury chloride; β-ME-β-mercaptoethanol; RVR-the relative variation rate; Ψ_{leaf}-leaf water potential; Pn-including net CO_2 assimilation rate; Gs-stomatal conductance; Tr-transpiration rate; C_i-intercellular CO_2-concentration; WDT-water dissipation by transpiration; DSI_{above} or DSI_{below}-the above- or belowground dry substance stress index; RGR-the relative growth rate root length, SR-root surface area; NR-number of roots (Diameter≥ 1mm); WR-width of tap/seminal roots; LTR-the length of taproot; TLR-total length of roots; MS-Moderate stress; SS-Severe stress

Introduction

Water shortage is a worldwide challenge and its adverse effects on water uptake by roots and uses by shoots results to low-production and even death of food or forage crops. Generally, some behaviors in plants related to water absorption and use, such as xylem water conductance, changes in root components and stomatal movement can reflect different responses to drought and help explain the strength of a plant's drought-tolerance or drought-avoidance. Water flow along the soil-plant continuum, described with a direct physiological parameter, i.e. root hydraulic conductivity (Lp_r), varied with species and conditions (Steudle , 2000; Miyamoto et al. 2001; Matsuo et al. 2009). For example, the attenuation of Lp_r was observed in wheat, maize, lupin, olive, etc., under water stress (Steudle and Peterson 1998；Zhang et al. 2002; Siemens and Zwiazek 2004; Bacelar et al. 2007). The analysis of soil-plant hydraulics can be used to explain and predict the large differences among species or cultivars (Hubbard et al. 2001). Species or cultivars better-adapted to drought stress had higher Lp_r as the soil dried than those less-adapted (Zhao et al. 2004; Mu et al. 2006; Bacelar et al. 2007). However, there is uncertainty about the effect of water uptake by roots on water use by shoots, especially under drought because of scarcity of regulated mechanisms. Additional response to drought by roots occurs through their morphological components. Anatomy, including cortex thickness, xylem vessel diameter and root suberization play a major role in root hydraulics (Bramley et al. 2009). For root components, it is more important to transport water to root surface from soil. Deep rooting is a strategy for desiccation avoidance in natural vegetation and in crops like rice and sorghum (Hund et al. 2009). But enormous roots are not always beneficial to water use by shoots even though a larger ratio of root and shoot (R/S) under drought contributes to better WUE to some extent (Morison et al. 2008). A large root system would consume more photosynthetic end products for their growth and indirectly negate shoot growth during water deficit (Bramley et al. 2009). The dilemma for water-stressed plants is how to share their carbon assimilations for root formation and canopy growth. A common response of plants to water deficiency is an increase in R/S (Sharp and Davies 1979; Morison et al. 2008). However, different components of roots, such as root length and root number, might change differently under drought according to species responses and adaptation to water stress. These diversities resulting from species and eco-physiological properties should be studied to determine and define relationships between different components of roots and WUE.

The main effect of drought stress on shoot (water use) are the decrease in leaf water potential, photosynthesis and growth, due to stomatal closure and lower CO_2 supply to the carboxylating enzymes (Brodribb and Holbrook 2003; Silva and Arrabac 2004; Volker and Wolfgang 2005). In response, there is an increase in drought-induced chemical-signals- such as ABA (Cochard et al. 2002; Brodribb and Holbrook 2003), or electrical-signals-mediated changes in shoots (Grams et al. 2007). Different stomatal behaviours have also been found in some species in response to changes in hydraulic conductivity induced by xylem cavition (*Betula occidentalis*), root pruning (*Abies amanilis*) and defoliation (*Pinus poderasa*) (Hubbard et al. 2001). Therefore, stomatal control and root hydraulic conductivity or variations in root morphology are considered the key controlling factors for WUE under drought. The increase in WUE under water stress was ascribed to biomass production being reduced less by drought than water use (biomass production WUE, WUE_b) (Bacelar et al. 2007; Bramley et al. 2009) or to net CO_2 assimilation rate being decreased less by water stress than transpiration rate (instantaneous WUE, WUE_i) (Li et al. 2007a; Bacelar et al. 2007). Thus, there is a hypothesis that higher-WUE species have better adaptation to drought. More research information is necessary to understand the stomatal or photosynthetic responses of different species or cultivars with differing drought-resistance perhaps, simultaneously with changes in root hydraulic conductivity and morphology under constant drought. This understanding will be very useful to illustrate the mechanism(s) of resistance of plants to water stress. To address these issues, we examined the hydraulic conductivity and morphological components of roots as well as physiological characteristics of shoots related to water use in two contrasting species: alfalfa (*Medicago sativa* L) and sorghum (*Sorghum bicolor* (L.) *Moench*). Alfalfa has a dominant taproot that can develop lots of lateral roots, whereas sorghum develops a root system of several extensively branched individual roots. Traditionally, alfalfa is considered to have strong drought avoidance because of an enormous and deep rooting system (Bai et al. 2001; Li et al. 2010). But alfalfa has a special characteristic of strong water uptake and severely exhausting soil water (Li et al. 2010). Therefore, it is necessary to clarify drought-tolerance in alfalfa. In contrast, sorghum is considered to be a strong physiological drought-tolerant plant with definite drought tolerance mechanisms (Khizzah et al. 1995; Ali et al. 2009). One of our earlier works on the threshold of soil water potential needed for seedling emergence in alfalfa and sorghum suggested a significantly higher moisture environment for alfalfa (Shan et al. 2008). When we compared the ability for osmotic adjustment in both species, we found that sorghum was better than alfalfa (Shan et al. 2008). In addition, sorghum had higher root osmotic pressure (1.2-1.5MPa), stronger root system and more stable production under water stress or drought (Jose et al. 1990; Ali et al. 2009). However, differences in water absorption and use, productivity level and ability to endure drought under the same water stress are poorly understood. We hypothesized that differences exist and proceeded to determine them by examining changes in root hydraulic conductivity and morphology under soil water stress and their regulative role in shoot physiology of alfalfa and sorghum seedlings. To understand the mechanism of drought tolerance between cultivars, we also examined the root hydraulic and morphological characteristics and shoot properties related to water use in two alfalfa species: Long-Dong and Algonquin , the former being a Chinese native cultivar and the latter having higher production and adaptation to wider cultural practices. Our objective was to gain new insight into the physiological and morphological responses that may contribute to drought tolerance in alfalfa and sorghum.

Results

Root hydraulic conductivity (Lp_r) of alfalfa and sorghum

Marked and progressive decline in root hydraulic conductivity (Lp_r) of alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si) seedlings under water stress was observed (Fig.1). The lowest Lp_r occurred after 48 h of water stress, during which the Lp_r of Algonquin was 10.4%, that of Long-Dong was 17.9% and that of Kang-Si 10.4% of control values. A complete recovery of Lp_r did not occur in Algonquin and Long-Dong, which just attained 61.5 and 71.4% of control values at the end of rehydration. However, the Lp_r of Kang-Si recovered to control level only after 36 h of rehydration. Therefore, water stress had much more significant effect on the Lp_r of alfalfa than of sorghum. There was a slight difference between Algonquin and Long-Dong in the response of Lp_r to PEG treatment.

Changes in gas exchange during water stress and rehydration

For both alfalfa varieties and sorghum, the net CO_2 assimilation rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) showed fast declines within the initial 24 h of water stress, paralleling the changes in Lp_r; no significant further declines occurred in the next 24h of stress. After stress for 48h, the Pn declined by 59.4%, Gs by 66.4% and Tr by 62.6% for Kang-Si; the declines were 82.5, 86.4 and 78.8% for Algonquin and 81.5, 88.3 and 85.0% respectively for Long-Dong (Fig.2 I, II and IV). However, the Pn, Gs and Tr of Kang-Si (sorghum) had lesser

Table 1. Effect of MC and MC+β-ME on Lp_r in Algonquin and Long-Dong

	Lp_r （$\times 10^{-7}$ m·s^{-1}·MPa^{-1}）		
Treatments	Without water stress		Under water stress
	Algonquin	Long-Dong	Long-Dong
CK	9.69±3.77 A	10.56±0.54 a	4.30±0.95 a
500 µM/L β—ME	--	10.43±2.40 a	--
1000 µM/L β—ME	--	10.95±1.56 a	--
50 µM/L MC	3.59±2.08 D	4.61±0.59 d	3.71±0.52 b
50 µM /L MC+ 500 µM /L β-ME	5.03±0.58 C	5.26±1.15 c	3.99±0.50 ab
50 µM /L MC + 1000 µM L β-ME	6.67±2.08 B	7.58±1.13 b	4.03±0.11 ab
100 ųM /L MC	2.33±1.15 E	3.63±0.62 d	3.10±0.35 c
100 µM /L MC+ 500 µM /L β-ME	3.00±1.00 DE	5.05±0.80 c	3.80±0.90 b
100 µM /L MC+ 1000 µM /L β-ME	5.00±1.00 C	7.44±1.23 b	4.19±0.90 a

β-ME did not affect root water uptake (Lp_r) in alfalfa. However, MC (Hg$^+$) decreased Lp_r significantly in Algonquin and Long-Dong in a concentration dependent response. A significant recovery effect induced by β-ME was shown. After 24h water stress, Lp_r was decreased by 40.72% in Long-Dong. Additional treatment with 50µM (or 100µM) MC further reduced Lp_r by 13.7% (or 28.1%). The additional decline in Lp_r due to MC was substantially reversed by β-ME, suggesting that AOPs might be present in alfalfa root cells and was responsible for the decline in Lp_r, especially under water stress. Data are shown as mean ± SD of six independent measurements ($P < 0.05$).

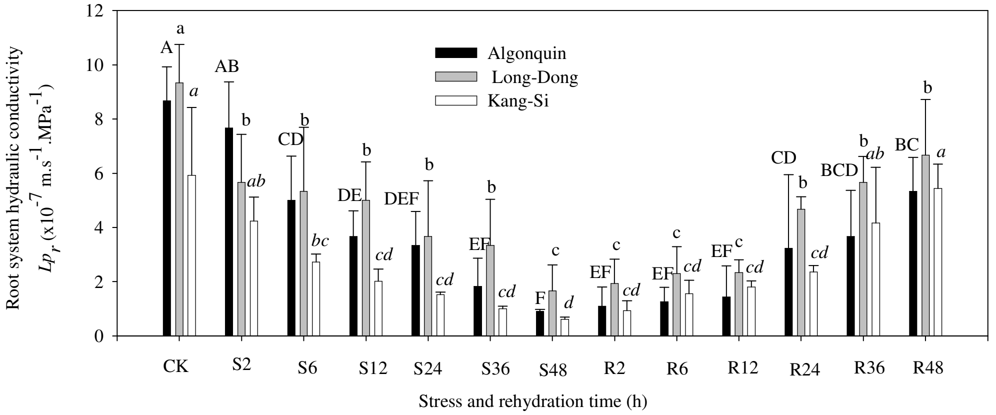

Fig 1. Effect of water stress on root system hydraulic conductivity（Lp_r）of alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si). S and R represent stress and rehydration, respectively. For this and subsequent figures, bars (with standard errors) with the same letters are not significantly different ($P < 0.05$): e capital letters refer to Algonquin, small letters to Long-Dong and italic letters to Kang-Si.

declines and better recovery ($P < 0.05$) than those of alfalfa during water stress and rehydration. The recovery rates for Algonquin at the end of the rehydration were 53.7% for Pn, 38.8% for Gs and 66.2% for Tr; for Long-Dong, the rates were 51.8, 59.7 and 61.0% while for Kang-Si the rates were 93.9, 89.5 and 94.3% respectively. However, the intercellular CO$_2$ concentration (Ci) of only Long-Dong and Kang-Si recovered to the control level by the end of rehydration (Fig.2 III). Therefore, there was a significant difference among gas exchange parameters in response to PEG treatment between alfalfa and sorghum.

Changes in leaf water potential (Ψ_{leaf}) and instantaneous water use efficiency (WUE$_i$) during water stress and rehydration

In response to water stress, the leaf water potential (Ψ_{leaf}) showed typical and time-dependent decline to the lowest values of -0.33 MPa for Algonquin, -0.25 MPa for Long-Dong and -0.52 MPa for Kang-Si, at the 48th h of water stress (Fig.4). During rehydration, the Ψ_{leaf} increased slowly and recovered to 2.14, 1.56 and 1.28 times the respective control in Algonquin, Long-Dong and Kang-Si, respectively. The Ψ_{leaf} of Long-Dong and Kang-Si did recover to their control levels. Water deficit was less influential on the WUE$_i$ of sorghum than alfalfa. In addition, water stress promoted instantaneous water use efficiency (WUE$_i$), defined as the ratio of Pn to Tr (Fig.3), with peak values about 1.8 and 1.7 times higher than respective controls in Algonquin and Long-Dong at 36h of water stress and about 1.2 times higher than control in Kang-Si following 48h of water stress. After rehydration, the WUE$_i$ of sorghum recovered gradually to the control level but that of alfalfa did not, suggesting that the WUE$_i$ of sorghum was affected less than that of alfalfa by water stress. Although the WUE$_i$ changed

Table 2. Effect of drought stress on length of tap/seminal roots, total root length, number of roots (D≥1 mm), width of tap/seminal roots and surface area of roots of alfalfa and sorghum

Root morphology		Long-Dong			Algonquin			Kang-Si		
		CK	MS	SS	CK	MS	SS	CK	MS	SS
Length of tap/ seminal root (cm/pot)	Branching stage	27.48± 4.14 a	22.54± 5.27 b	14.16± 5.29 c	26.85± 3.69 A	21.4± 3.56 B	13.2± 2.72 C	38.58± 2.45	37.77± 2.67	36.6± 1.3
	Harvesting stage	43.55± 3.05 a	37.1± 0.9 b	32.1± 2.8 c	32.92± 1.12 A	29.89± 2.3 AB	27.74± 0.36 B	55.2± 2.2 *a*	59.7± 0.6 *ab*	56.14± 2.34 *b*
	RVR（μm.cm^{-1}.d^{-1}）	*51.16± 9.87*b	*58.75± 10.46*b	*102.5± 6.98*a	*22.72± 8.58*B	*39.13± 15.24*B	*93.29± 11.21A*	*39.86± 5.10*	*53.84± 15.04*	*54.70± 21.2*
Total root length (m/pot)	Branching stage	0.48± 0.04 c	0.73± 0.06 b	1.01± 0.10 a	0.69± 0.16C	0.83± 0.22 B	0.96± 0.21 A	1.12± 0.09 *c*	1.97± 0.23 *a*	1.55± 0.05 *b*
	Harvesting stage	1.11± 0.04 b	1.14± 0.12 b	1.42± 0.11 a	0.91± 0.05 B	1.01± 0.05 AB	1.12± 0.17 A	7.17± 0.57 *b*	8.74± 0.68 *a*	8.95± 0.46 *a*
	RVR（mm.m^{-1}.d^{-1}）	*9.29± 1.05*a	*5.40± 1.69*b	*4.31± 1.11*b	*3.09± 1.49*	*2.20± 1.27*	*2.28± 0.75*	*25.46± 1.56*	*17.57± 0.80*	*21.98± 1.23*
Number of roots (D≥1 mm, piece/pot)	Branching stage	12±1 c	19±7 b	28±5 a	12±5 B	13±4 B	21±4 A	18±3 *c*	30±2 *b*	38±2 *a*
	Harvesting stage	32±13 b	40±9 a	42±10 a	32±4 B	35±2 B	41±4 A	57±4 *c*	78±5 *b*	88±4 *a*
	RVG（×10^{-3} piece. piece^{-1}.d^{-1}）	*10.70± 1.11*a	*8.88± 2.34*a	*5.06± 0.93*b	*10.92± 1.38*A	*11.69± 1.11*A	*8.34± 1.42*B	*12.96± 1.60a*	*11.31± 1.23ab*	*10.51± 1.40b*
Width of taproot or seminal root (cm)	Branching stage	0.30± 0.041 a	0.31± 0.045 a	0.28± 0.034 b	0.30± 0.031	0.29± 0.033	0.29± 0.054	0.35± 0.005 *b*	0.38± 0.008 *a*	0.38± 0.032 *a*
	Harvesting stage	0.62± 0.020 a	0.49± 0.016 b	0.47± 0.007 b	0.63± 0.064 A	0.57± 0.047 B	0.43± 0.013 C	0.41± 0.006 *b*	0.48± 0.008 *a*	0.51± 0.011 *a*
	RVR（μm.cm^{-1}.d^{-1}）	*8.13± 0.33*a	*5.59± 0.30*b	*6.31± 1.07*b	*8.12± 0.68*A	*8.08± 0.59*A	*5.02± 0.66*B	*1.69± 0.43b*	*2.63± 0.73ab*	*4.16± 1.76a*
Surface area of roots (cm^2/pot)	Branching stage	13.47± 2.94 c	19.01± 2.32 a	16.50± 1.63 b	10.73± 1.96 B	16.45± 2.33 A	15.33± 2.45 A	35.72± 3.45 *b*	48.84± 1.17 *a*	36.10± 1.36 *b*
	Harvesting stage	24.97± 2.69 b	34.98± 1.46 a	26.97± 0.10 b	32.40± 2.71 C	42.11± 7.29 A	37.34± 6.67 B	136.25± 10.81 *c*	162.4± 11.08 *a*	150.53± 6.5 *b*
	RVR（×10^{-3} cm^2. cm-^{2}.d^{-1}）	*6.86± 1.72* ab	*8.85± 0.74* a	*4.46± 2.41* b	*12.37± 2.37*A	*11.89± 0.93* A	*9.12± 1.09* B	*14.92± 1.43b*	*14.15± 0.97b*	*17.95± 1.85a*

Drought induced inconsistent changes in root morphological components of soil-cultured alfalfa and sorghum. Significant effects of genotypes and moisture levels were observed on some morphological parameters. Data are shown as mean ± SD of four independent measurements ($P < 0.05$).

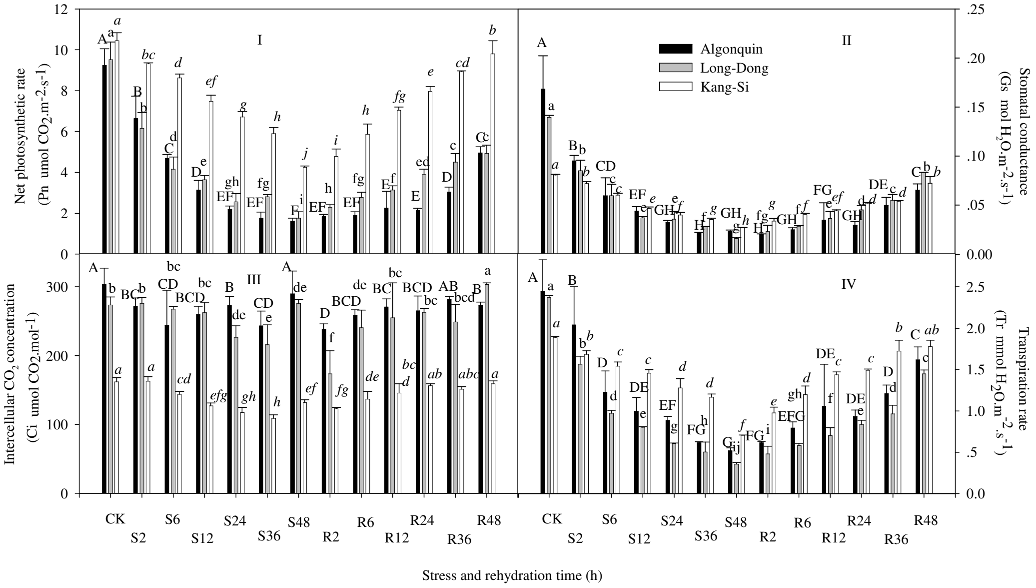

Fig 2. Effect of water stress and rehydration on photosynthetic parameters in alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si). Data are shown as mean ± SD of six independent measurements ($P < 0.05$).

in similar fold in both alfalfa cultivars, the absolute value for Long-Dong was higher than Algonquin.

Inhibition or reversal of water flow in alfalfa seedlings through AQPs with ($HgCl_2$, MC) or/and β-mercaptoethanol (CH_3CH_2-SH, β-ME)

No change was found in the Lp_r of Long-Dong after treating with *β*-ME solution (Table 1). This showed that *β*-ME had no effects on root water uptake in alfalfa. However, at a higher concentration (100 μM), MC resulted in greater reduction of Lp_r than controls (under 100 μM, it declined 76.0 and 65.6% for Algonquin and Long-Dong but under 50 μM MC, it decreased only 63.0% and 56.3%, respectively). It can also be seen that the recovery effects of *β*-ME were significant. Addition of *β*-ME to nutrient solution at 500μM (or 1000 μM) resulted in Lp_r recovery of 47.9% (or 71.8%) in Long-Dong and 31.0% (or 68.8%) in Algonquin. There was a difference in the response of Lp_r to MC and/or *β*-ME treatments in the two cultivars. After 24h of water stress, the Lp_r was decreased by 40.72% in Long-Dong. Additional treatment with 50μM (or 100μM) MC further reduced the Lp_r by 13.7% (or 28.1%). Strikingly, the further decline in Lp_r caused by MC was substantially arrested by treatment with *β*-ME. For example, an addition of 500μM *β*-ME increased the Lp_r to 92.79% of the control value (after 24h water stress; see Table 1), while the addition of 1000μM *β*-ME completely restored the Lp_r. This finding was different from that noted for plants not affected by water stress, in which the 1000μM *β*-ME treatment could only recover Lp_r by 71.8% at the maximum.

Drought induced changes in root morphological components of soil-cultured alfalfa and sorghum

Significant effects of genotypes and water availabilities were observed on some morphological parameters of roots, including root length, root surface area (SR), number of roots (Diameter≥ 1mm, NR) and width of tap/seminal roots (WR) in alfalfa and sorghum (Table 2). Not surprisingly, the length of taproot (LTR) of drought-stressed alfalfa (MS and SS) was significantly less than control ($P<0.05$), but its total length of roots (TLR) was greater (Table 2). Unlike alfalfa, there was no significant difference in the LTR and TLR between drought-stressed and well-watered sorghum at the jointing stage, but the seminal and total root lengths of drought-stressed sorghum were longer at harvesting stage ($P<0.05$, Table 2). These changes were dependent on stress level in alfalfa but not in sorghum. However, the relative variation rate (RVR) of LTR and TLR indicated an improvement in growth of seminal root and an inhibition of total roots in both species by drought stress, though sorghum had bigger ($P<0.05$) RVR of TLR than alfalfa at the same water level. Thus, drought inhibited the elongation of roots much more in alfalfa than in sorghum and much more in Algonquin than in Long-Dong ($P < 0.05$). Also, drought-stressed alfalfa and sorghum had bigger NR and SR, whereas drought-stressed alfalfa had less WR and water-stressed sorghum higher WR, depending on stress levels, genotypes and growth stages. On the other hand, the RVR of NR, WR and SR decreased to the lowest rate in severe drought-stressed alfalfa; values in severe drought-stressed sorghum were much higher than well-watered sorghum. Combined with changes in root length, this indicated that morphological changes in sorghum roots were less affected by water stress than those in alfalfa.

Changes in above- and belowground biomass production in drought-stressed alfalfa and sorghum

Drought stress, especially if severe, significantly ($P< 0.05$) reduced (except at the jointing stage of sorghum) the above- and underground biomass in alfalfa and sorghum (Table 3). This was also confirmed from changes in above- and belowground dry substance stress index (DSI_{above} and DSI_{below}). For drought-stressed sorghum, the decreases in above- and underground biomass production were less pronounced ($P<0.05$) than for alfalfa but the difference between Long-Dong and Algonquin was not statistically significant. For instance at harvest, the aboveground biomass of moderate-stressed alfalfa decreased by 22.47, 54.89, 16.32 and that for severe-stressed alfalfa by 55.03, 10.47 and 25.42% compared with respective control values for Long-Dong, Algonquin and Kang-Si. The belowground biomass of moderate- and severe-stressed Kang-Si decreased by 2.41 and 14.88%, followed by that of Long-Dong (10.51 and 24.05%) and Algonquin (9.72% and 37.77%). Only severe drought-stressed sorghum and alfalfa had an obvious increase in the ratio of roots to shoots (R/S), especially at harvest (Fig.5 I); the R/S of alfalfa increased more than that of sorghum ($P< 0.05$), which was consistent with changes in their biomass production. Of special note was that only the relative growth rate (RGR) of the aboveground biomass in drought-stressed sorghum and alfalfa declined significantly, implying that the effect of water shortage on shoots was higher than on roots and also confirming that the decline in shoots biomass was mainly responsible for variations in R/S.

Water use efficiency of biomass production (WUE_b)

The declining effect of drought stress on water dissipation by transpiration (WDT) varied among species ($P< 0.05$, Table 3). Sorghum had greater WDT but fewer declines in WDT under drought stress than alfalfa. The WDT in moderate-stressed Long-Dong, Algonquin and Kang-Si decreased by 44.20 and 71.43, and 30.26%; that in severe-stressed decreased by 69.65, 29.43 and 45.43% respectively, suggesting differences in drought tolerance. These trends plus the variation in aboveground biomass production under drought stress suggested significant differences ($P< 0.05$) in water use efficiency of biomass production (WUE_b) among species (Fig.5 II). Sorghum had higher WUE_b than alfalfa. For moderate- and severe drought-stressed Long-Dong, the WUE_b were 1.20 and 1.27 times greater; for Algonquin, values were 1.10 and 1.20 times while for Kang-Si values were 1.27 and 1.36 times greater. This is another confirmation of differences in drought tolerance among species.

Discussions

As observed in other plants (Zhang et al. 2002; Siemens and Zwiazek 2004; Bacelar et al. 2007), when the availability of water was limited by PEG-6000, the Lp_r firstly decreased and then the stomata closed rapidly; subsequently, photosynthetic assimilation was affected through declines in Pn, Tr and Ci. As a result, the Ψ_{leaf} started to fall gradually and WUE_i increased slightly, especially at the initial period of water stress in alfalfa and sorghum seedlings. These changes were time-and species-dependent response to water stress. Subsequent rehydration treatment for 48h induced incomplete recovery of these parameters in alfalfa but complete recovery in sorghum. It is inevitable that changes in hydraulic conductivity will indirectly drive changes in stomatal conductance and transpiration (Hubbard et al., 2001). The decline in Lp_r could be due to

Table 3. Effect of drought stress on above- and below-ground biomass, drought stress index (DSI) and water dissipation by transpiration (WDT) in alfalfa and sorghum

		Long-Dong			Algonquin			Kang-Si		
		CK	MS	SS	CK	MS	SS	CK	MS	SS
Above-ground biomass (g/pot)	Branching stage	4.25± 0.43 a	3.57± 0.10 b	2.73± 0.15 c	3.34± 0.47 A	3.28± 0.28 A	2.29± 0.29 B	19.16± 2.32	20.67± 2.95	17.65± 1.87
	Harvesting stage	18.66± 0.60 a	14.46± 0.34 b	8.42± 0.61 c	19.41± 0.50 A	16.24± 0.99 B	8.73± 0.34 C	96.60± 2.44 *a*	86.49± 3.08 *b*	72.04± 0.70 *c*
	RGR（mg $g^{-1}.d^{-1}$)	*16.46± 0.92*a	*14.70± 0.62*b	*11.26± 0.37*c	*19.64± 0.87*A	*17.82± 1.20*B	*14.15± 0.94*C	*18.24± 1.07a*	*16.91± 1.28a*	*13.95± 1.02b*
Below-ground biomass (g/pot)	Branching stage	0.57± 0.014a	0.49± 0.079b	0.46± 0.064b	0.57± 0.135A	0.55± 0.056A	0.35± 0.048B	8.68± 0.29 *a*	7.59± 0.54 *b*	7.54± 0.30 *b*
	Harvesting stage	5.45± 0.33 a	4.88± 0.87 b	4.14± 0.53 c	6.38± 0.30 A	5.76± 0.24 B	3.97± 0.55 B	21.66± 1.20 *a*	21.14± 1.15 *a*	18.44± 1.17 *b*
	RGR（mg $g^{-1}.d^{-1}$)	*25.11± 0.14*	*27.18± 1.98*	*27.61± 1.60*	*27.03± 3.03*	*27.67± 0.86*	*30.36± 3.07*	*10.17± 0.31*	*11.45± 0.58*	*11.16± 0.89*
DSI_{above}		1 --	0.67± 0.02	0.36± 0.05	1 --	0.77± 0.05	0.36± 0.02	1 --	0.90± 0.03	0.75± 0.01
DSI_{below}		1 --	0.90± 0.04	0.76± 0.01	1 --	0.90± 0.04	0.62± 0.09	1 --	0.98± 0.04	0.85± 0.05
WDT (Kg/pot)		19.02± 0.24 a	10.61± 2.00 b	5.43± 0.37 c	16.90± 0.23 A	11.78± 3.61 B	5.13± 0.76 C	25.74± 0.61 *a*	18.42± 2.56 *b*	14.05± 1.76 *c*

Similar decreases were observed for above- and below-ground biomass production and water dissipation by transpiration (WDT) in drought-stressed alfalfa and sorghum. This was also confirmed from changes in above- and below-ground dry substance stress index (DSI_{above} and DSI_{below}). For water-stressed sorghum, the decrease in above- and below-ground biomass was less but that of WDT was higher than alfalfa ($P< 0.05$); the difference between Long-Dong and Algonquin was no statistically significant.

Data are shown as mean ± SD of four independent measurements ($P<0.05$).

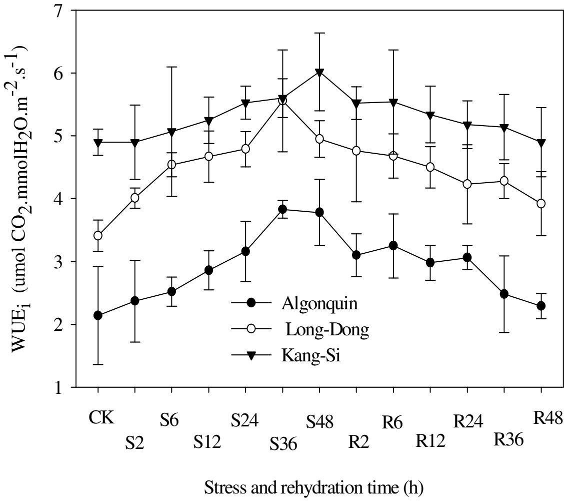

Fig 3. Effect of water stress and rehydration on WUE_i of alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si). Data are shown as mean ± SD of six independent measurements ($P<0.05$).

physiological changes in the root system induced by water shortage, such as the closure of water channel or the decline in AQPs expression, which was confirmed by MC and /or *β*-ME test (Table 1). The $HgCl_2$ reduced Lp_r by 24.1-34.4% in well-watered and 13.7-28.1% in water-stressed alfalfa, which compares reasonably with values found in literature (Kaldenhoff et al. 2008). The 1000μM *β*-ME could nearly restore the Lp_r of water-stressed alfalfa to 71.8% of that in well-watered alfalfa. Therefore, AQPs might be present in alfalfa root cells and was responsible for the decline in Lp_r, especially under water stress, although there was no agreement as to participation of AQPs activities (Carvajal et al. 1996; Bramley et al. 2009). In addition to the decline in osmotic adjustment we reported earlier (Li et al. 2007b), an increase in cortex thickness and decline in root diameter were observed (these changes were not significant, and are thus not shown in this paper). Bramley et al. (2009) indicated that anatomy played a major role in root hydraulics, influencing axial conductance and the distribution of water uptake along the root, with a more localized role for AQPs in wheat and lupines. Influence on photosynthesis and gas exchange characteristics by water stress was another important reason inducing Lp_r decline and incomplete recovery in rehydrated alfalfa. Significantly, all these changes may be the main reasons why Lp_r in water-stressed sorghum also decreased. The obviously positive and non-linear relationship between Lp_r and WUE_i identified

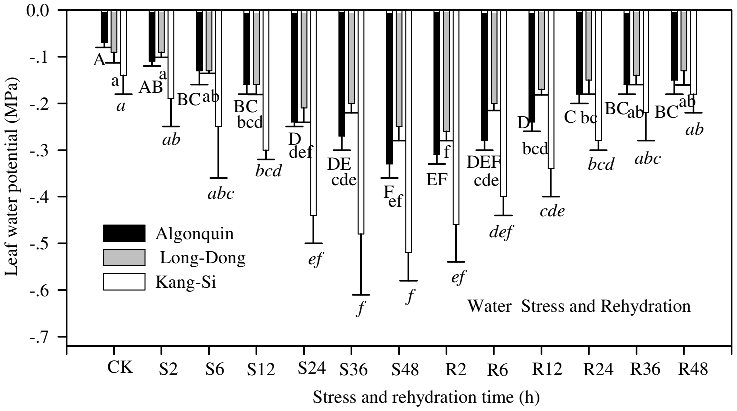

Fig 4. Changes in leaf water potential (Ψ_{leaf}) of alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si). Data are shown as mean ± SD of six independent measurements ($P < 0.05$).

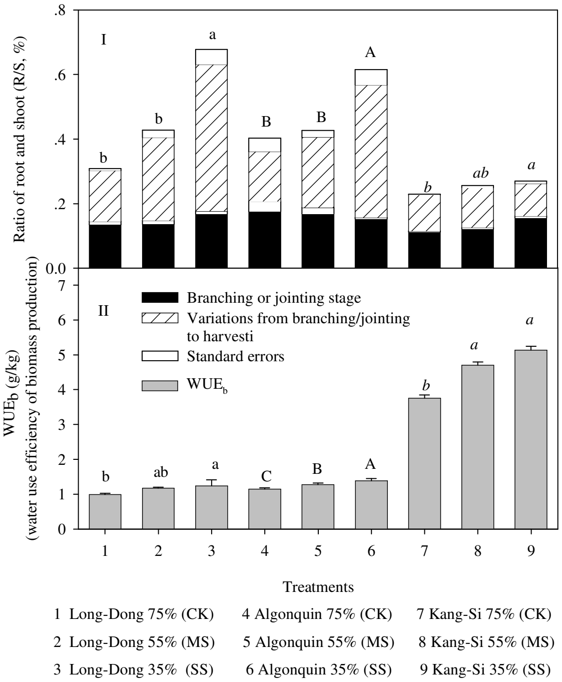

Fig 5. Effect of drought stress on ratio of roots and shoots (R/S, I) and water use efficiency of biomass production (WUE_b, II) of alfalfa (Algonquin and Long-Dong) and sorghum (Kang-Si). Data are shown as mean ± SD of four independent measurements ($P < 0.05$).

hydraulic limits as the cause of partial photosynthetic damage in response to drought , which in turn caused the feed-back inhibitions in recovery of Lp_r in alfalfa and sorghum. We have found that stomatal and non-stomatal limitations were responsible for the decline in photosynthesis of alfalfa at the initial period of water stress (Li et al. 2007a). In addition, the WUE_i showed a gentle change based on the changes in Pn and Tr under water stress and recovered to control levels after rehydration. Therefore, the decline in water absorption induced a slight photosynthetic damage. It was possible the photosynthetic functions of CO_2 fixation, RUBP carboxylation and inorganic phosphorus transformation were inhibited by water stress to some extent (Parry et al. 2002; Bota et al. 2004), resulting to incomplete recovery of Pn, Gs and Tr, then Lp_r after 48h rehydration. Considering the response of Ci to variation in water stress, this effect on Lp_r was more important at the beginning of water stress and rehydration and the inhibition of root water uptake was due mostly to metabolic inhibition, especially at later stages of water stress. In terms of water absorption, water stress affected the Lp_r of alfalfa more severely than that of sorghum and water uptake ability was weaker in alfalfa than in sorghum. Gullo et al. (1998) reported that the Lp_r of olive roots experiencing gentle and medium water stress recovered completely to control level at 24 and 48 h after rehydration. Large differences in water use between species can be attributed in part to differences in their 'hydraulic equipment' (Hubbard et al. 2001). Therefore, water stress had much more effect on gas exchange and photosynthesis in alfalfa than in sorghum. Furthermore, there was a better correlation between Lp_r and WUE_i in sorghum than in alfalfa (Fig.6), suggesting a stronger relationship between root water uptake and "saving water use" in sorghum when water availability diminished and alfalfa was more sensitive to water stress than sorghum. The considerable decrease in biomass of drought-stressed alfalfa and sorghum was attributed to reduced net CO_2 assimilation and subsequently, weakened growth of leaves as reported also for many plants such as olive, wheat, maize, soybean, western larch, tomato and so on (Xiong et al. 2006; Huang et al. 2007; Bacelar et al. 2007). As a consequence, water consumption was reduced, while the ratio of roots to shoots (R/S) and WUE_b increased substantially in drought-stressed alfalfa and sorghum compared with well-watered plants (Tab.3). Studies on the relationship between biomass and WUE_b have shown that changes in root and shoot weights were closely related to those in WUE_b (Huang et al. 2007). Moderate drought stress could improve plant WUE_b through decline in the cost of production (Grimes et al. 1992; Shan et al. 2008); to some extent, the increase in R/S under soil drought reflects the adaptive growth balance of root system and canopy. Combined with the greater decrease in RGR in aboveground biomass than belowground biomass under drought stress, there was an indication that more competitive carbohydrates were allocated to roots, resulting in a greater decline in alfalfa and sorghum shoots yields than roots, in order to maintain root function, such as promoting the absorption capacity to use deep soil moisture, meet the needs of evaporation and plant growth and then reduce drought losses (Bai et al. 2001; Li et al. 2010). Therefore, a decrease in canopy growth played a critical role in increasing the R/S, which was responsible much more than individual root extension and growth, to enduring drought stress in alfalfa and sorghum as reported for soybean (Grimes et al. 1992; Li et al. 2010). The higher decrease in below- and aboveground biomass (RGV, Table 2) and increases in R/S (RVR, Fig.5) suggested a substantially stronger adaptation to drought for sorghum than alfalfa. In addition to the increase in R/S, the other morphological components of root varied in such a way as to cope with prolonged drought in soil-cultured alfalfa and sorghum, suggesting their close relationship with drought resistance of the species. It was traditionally understood that plants with strong and well-developed root system have excellent ability to resist drought stress (Hund et al. 2009). Under prolonged water deficiency, apart from an increase in total length of roots, alfalfa tended to increase its taproot and lateral root length (Li et al. 2010) and NR while sorghum tended to vary its WR, SR and NR, suggesting differences in root morphological adaptation to drought between sorghum and alfalfa. Under drought conditions, decreased root width and increased root length, surface area and number of fine roots were beneficial to the normal growth of alfalfa and sorghum. These changes could improve soil moisture, expand the scope for water uptake, decrease distance from roots to soil and especially improve the effective use of deep soil water, as reported in Bai et al. (2001). More importantly, sorghum root morphological RVR were less affected by water stress than those of alfalfa and much more in Algonquin than in Long-Dong. This might be attributed to differences in drought resistance in sorghum and alfalfa as well as Algonquin and Long-Dong. Matthias and Smith (1997) found smaller stems and leaves of alfalfa after the cessation of irrigation in summer but alfalfa root growth increased at the same time, which was inconsistent with our results. However, we determined that the changes in root morphology and shoot water use and gas exchange were closely related in plants such as alfalfa and sorghum. According to Passioura's (1982) theory on WUE, there are two types of water-use behavior in plants (Bacelar et al. 2007). Sorghum with higher WUE_b appeared to use a conservative water-use strategy, whereas a prodigal water-use strategy was used by alfalfa, whether under well-watered or drought-stressed condition. Similar result was reported in Jefferson and Cutforth (2005) for natural alfalfa field. The relationship between different root morphological components and WUE_b also addressed different water use and drought tolerance mechanisms. For instance from Fig.7, the taproot length was positively related only to alfalfa WUE_b while root surface was only positively correlated with sorghum WUE_b. In addition, the width of taproot and WUE_b were positively correlated in sorghum but negatively correlated in alfalfa. Total length of roots, number of roots ($D \geqslant 1$mm) and R/S were negatively correlated with WUE_b in both species. These relationships were closer, i.e. higher correlation coefficient, in sorghum than in alfalfa. This might be attributed to the different root morphology, in which alfalfa has a dominant taproot with lots of lateral roots, whereas sorghum develops a root system of several extensively branched individual roots. Although soil water deficit promoted the distribution of carbohydrates to roots, resulting to decreases in production of alfalfa and sorghum and consumption of water, it enhanced adaptive changes in root morphological components and a coherent relationship between root and shoot, which ultimately increased the use of deep soil moisture and $WUE_{b.}$ Under soil drought, physiological changes in sorghum did differ from those in alfalfa due to their different biological characteristics, which explained their differences in drought endurance. Variations in biomass, WUE_b and root morphology were less obvious in sorghum than alfalfa when media water potential continued to decline, confirming better adaptation to drought-stress for sorghum. Comparative analysis of the tested alfalfa cultivars showed that under soil drought, the taproot and total root lengths were greater and root surface area and width of taproot declined more in Long-Dong under stress. Therefore, Long-Dong and Algonquin differ in root morphological development, which might lead to different resistance to drought even though their biomass and water use showed no obvious differences. From the above results, it can be concluded that alfalfa and sorghum responded to water shortage by developing different enduring mechanisms though these were based on stomatal closing, reducing transpiration, decreasing water absorption and use and altered root morphology, which inhibited growth of canopy and roots (reduction in photosynthetic assimilation). Our data also showed that the changes in hydraulic conductivity of roots at the initial period of variable water stress were critical to the regulation of water use and gas exchange in alfalfa and sorghum seedlings. In addition, sorghum has stronger drought resistance than alfalfa because of better root hydraulic conductivity and R/S, less affected biomass and gas exchange, higher WUE_b and superior adaptation of root morphological components to prolonged drought stress. However, based on our data, we consider alfalfa to be very promising for cultivation in semi-arid and semi-wet or irrigated areas. To clarify the capacity for drought tolerance in alfalfa, it is necessary to relate the results obtained in this study to extended (two or several year's old alfalfas) cultured in field conditions.

Materials and methods

Plant material and growth conditions

Alfalfa (cultivars: Long-Dong and Algonquin) and sorghum

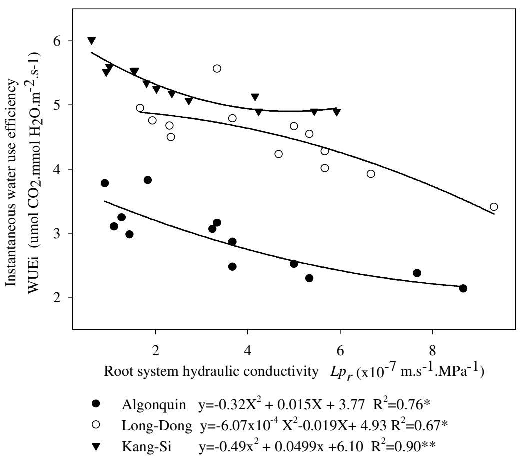

Fig 6. Relationship between Lp_r and WUE_i in alfalfa and sorghum.

(cultivar: Kang-Si) seeds germinated in a climatic chamber at 25℃ after being sterilized with 75% (v/v) ethanol solution for 2 min. Seedlings were transferred to the growth chamber (Model: PGV-36, Canada) and cultured with 1/2-strength Hoagland nutrient solution (pH：5.5-6.0) when the cotyledon (alfalfa) or first euphylla (sorghum) was fully expanded with the following conditions: day/night temperature 23/18 ℃ (alfalfa) or 30/25 ℃ (sorghum), relative humidity 65%, photoperiod 12 h with a photosynthetic photon flux density of 260 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{S}^{-1}$. Culture medium was completely renewed every 3 d and an oxygen pump was used to aerate the solution twice a day at 4 h each time. Potted sorghum (two plants per pot) and alfalfa (five plants per pot) were cultured on 11.3 Kg soil per pot mixed with urea (4.4 g), potassium sulfate (4.4 g), triple phosphate (7.2g) and organic fertilizer (50g) for alfalfa or by 2.2, 4.4, 2.8 and 170g, respectively for sorghum. Each of the pots measured $0.29\times0.29\times0.40\ \text{m}^3$. Seeds were sown on 1 May 2006 (sorghum) and 30 April 2006 (alfalfa). The potted plants were grown in a rain-proof shed during the experiment and irrigated to 75%±5% of field capacity by using tap water until early June 2006. Eight non-planted- pots were used to quantify the evaporation from soil surface.

Experimental treatments

The water stress treatments (water potential: -0.2 MPa; stress time: 48 h) were established by adding PEG-6000 to the nutrient solution when each hydroponic seedling had grown for 40 days (at least 10 mature leaves in alfalfa or 4 mature leaves in sorghum); stressed seedlings were then rewatered for 48 hours by putting them back into 1/2-strength non-PEG Hoagland solution. Control seedlings grew continuously in nutrient solution without PEG-6000. The treatments were replicated four times. Roots of Long-Dong seedlings were immersed for 20 min in mercury chloride ($HgCl_2$; MC) or β-mercaptoethanol (CH_3CH_2-SH; β-ME) solutions as follows: ① 500 μmol/L β-ME solution; ② 1000μmol/L β-ME solution; ③ 50μmol/L MC solution; ④ 100μmol/L MC solution; ⑤ 50μmol/L MC solution, then in 500μmol/L or 1000μmol/L β-ME solution; ⑥ 100μmol/L MC solution, then in 500μmol/L or 1000μmol/L β-ME solution; The roots were also immersed in PEG-6000 solution for 24 h, and then treated as ③, ④, ⑤ and ⑥ above. In the control group, seedlings were cultivated in the unaltered (non-PEG) Hoagland nutrient solution. From 10 June 2006, when soil-cultured alfalfa had 7-8 fully-expanded leaves and sorghum had 3 fully-expanded leaves, three levels of soil moisture with 75%±5% (Control, CK), 55%±5% (Moderate stress, MS) and 35%±5% (Severe stress, SS) of field capacity were imposed on the potted plants. These moisture levels were maintained by periodic weighing of pots and correcting for soil moisture until the plants were harvested. The daily water consumption per pot was noted. The above- and below-ground samples were taken at branching (25 June to 4 July 2006) and anthesis (50% flowering, 18 September to 3 October 2006) of alfalfa or at jointing (2 July to 10 July 2006) and harvesting stages (13 September to 1 October 2006) of sorghum.

Root hydraulic conductivity (Lp_r)

Eight hydroponic seedlings of each species or cultures were selected to measure root hydraulic conductivity (Lp_r) by using a pressure chamber (Model: 3005, Soil Moisture Equipment Co. U.S.A) according to Miyamoto et al. (2001) with minor modification. The whole root system of hydroponicseedlings was immersed in 1/2-strength Hoagland nutrient solution within the pressure chamber when the stems were cut under cotyledons (approximately 2.5 cm above emerging roots) through the pressure chamber lid. Then, the pressure in the chamber was raised in steps of 0.05 MPa up to 0.40 MPa above atmospheric level. Root flow rate was allowed to stabilize (in 1-2 min per pressure) and the flow rate was measured for 1 min at each pressure (t). For a given gas pressure (P_{gas} in MPa), the volume exuded from the root system (V in cm^3) was plotted against time. The slopes of these relationships were calculated and used as unit surface area (m^2) which was measured by the CI-400 root image analysis system (CID Inc. U.S.A). This yielded the volume flow, Jv_r in $\text{m}^3\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Root hydraulic conductivity, Lp_r ($\text{m}^3\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$) was calculated as the slope of the regression line of Jv_r plotted against hydrostatic pressures of 0.05-0.40 MPa according to the equation: $Jv_r = V/(S\times T)$; $Lp_r = Jv_r / P_{gas}$.

Root morphological parameters

The potted plant roots were washed clean with a root washing device after harvesting. Every root was expanded on a piece of transparent paper and scanned with scanner. Root surface area and total root length were measured with a CI-400 root image analysis system (CID Inc. U.S.A). Ruler and vernier caliper were used to measure taproot (or seminal root of sorghum) length and diameter. At the same time, the number of roots (diameter ≥1mm) was recorded. Root volume was determined

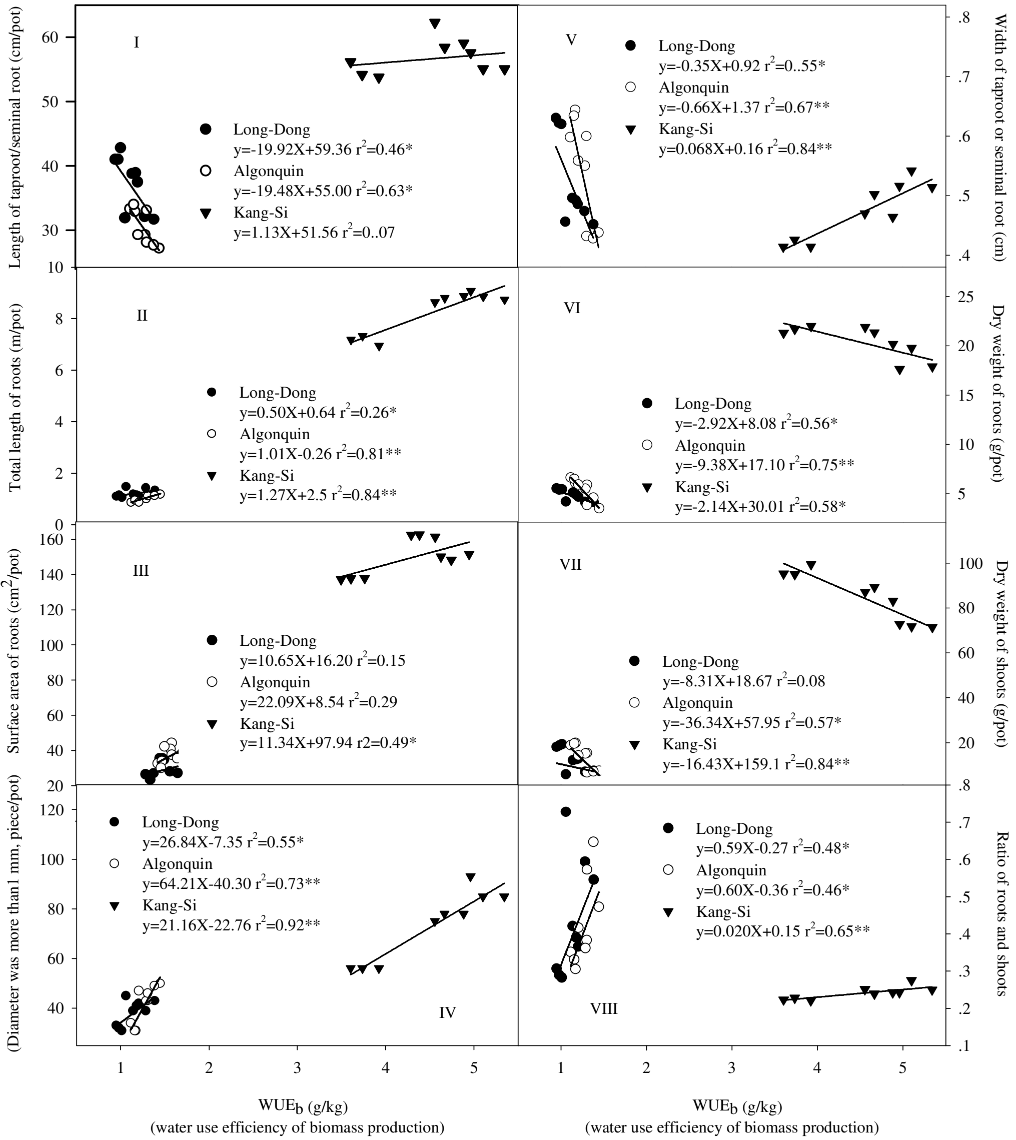

Fig 7. Relationship between WUE_b and root morphological components, including taproot length (I), total length of roots (II), surface area of roots (III), number of roots (D≥1mm, IV), width of roots (V), dry weight of roots (VI), dry weights of shoots (VII) and R/S (VIII) in alfalfa and sorghum.

by drainage method. The root morphology was assessed by calculating the variations in root length, root surface area, number of roots (diameter≥ 1mm) and width of tap/seminal root from branching (jointing stage) to harvesting stages of alfalfa (or sorghum). The relative variation rate (RVR) of each component was calculated as: RVR = [ln (V_2/V_1)] / *d*, where V_1 and V_2 are the values at branching/ jointing and harvesting stages and *d* is number of days from branching/ jointing to harvesting.

Leaf water potential, gas exchange and instantaneous water use efficiency (WUE_i)

The measurement of fully expended leaf water potential (Ψ_{leaf}) was carried out with a pressure chamber (Model: 3005, Soil Moisture Equipment Co. U.S.A) as described in Bacelar et al. (2007). The second and third leaves from the top were measured for alfalfa while the top two fully expanded leaves were measured for sorghum. Leaf gas exchange parameters, including net CO_2 assimilation rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO_2 concentration (Ci) in the top two fully expended leaves of alfalfa or sorghum were measured with a Li-6400 portable photosynthetic system (Li-cor Co. U.S.A) at 9:30- 11:00 am. Measurements of leaf gas exchange parameters of hydroponic seedlings were performed in the growth chamber. Instantaneous water use efficiency (WUE_i) was calculated from the ratio of Pn to Tr. This measurement was repeated 10 times.

Biomass production, water dissipation by transpiration (WDT) and water use efficiency of biomass production (WUE_b)

The soil-cultured plants were harvested at branching and anthesis stages of alfalfa and jointing and harvesting stages of sorghum to assess biomass. The harvested above- and below-ground biomass were dried first at 105°C for 30 min and then at 80 °C to a constant weight. The above- or below-ground dry substance stress index was determined as the ratio of dry substance of drought-stressesed plants to that of well-watered plants. The relative growth rate (RGR) was calculated according to Bacelar et al. (2007) and Boughalleb et al. (2009) with slight modification: $RGR = [\ln (DW_2/DW_1)] / d$, where DW_1 and DW_2 are the biomass at branching/ jointing and harvesting stages and d is number of days from branching/ jointing to harvesting. Water dissipation by transpiration (WDT) per pot was determined as the difference between water consumption per pot for maintaining 75%±5%, 55%±5% and 35%±5% of field capacity and the evaporation from soil surface. At the beginning of this measurement, plant weight per pot was determined to avoid prejudicing the amount of water use per pot. The cumulative amount of WDT per pot d^{-1} during the whole growing season was the total WDT per potted plants. Water use efficiency of biomass production (WUE_b) was determined for each potted plant by dividing total biomass production (above- and belowground biomass) by cumulative water use throughout the growing period (i.e. the total WDT).

Statistical analysis

All data obtained from the measurements were subjected to analysis of variance (ANOVA) using the SigmaPlot 8.0 Dome statistical package and the statistical analysis system (SAS) software. Means were compared using Duncan's multiple range tests at the 5% level of probability. Model analysis was used to determine relationships between variables and differences between parameters of fitted models were evaluated with the *t*-test or F-test.

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