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Evidence for the involvement of hydraulic root or shoot adjustments as mechanisms underlying water deficit tolerance in two *Sorghum bicolor* genotypes

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ABSTRACT

Sorghum bicolor (L.) Moench is an ancient drought-tolerant crop with potential to sustain high yields even in those environments where water is limiting. Understanding the performance of this species in early phenological stages could be a useful tool for future yield improvement programs. The aim of this work was to study the response of *Sorghum* seedlings under water deficit conditions in two genotypes (RedLandB2 and IS9530) that are currently employed in Argentina. Morphological and physiological traits were studied to present an integrated analysis of the shoot and root responses. Although both genotypes initially developed a conserved and indistinguishable response in terms of drought tolerance parameters (growth rate, biomass reallocation, etc.), water regulation displayed different underlying strategies. To avoid water loss, both genotypes adjusted their plant hydraulic resistance at different levels: RedLandB2 regulated shoot resistance through stomata (isohydric strategy), while IS9530 controlled root resistance (anisohydric strategy). Moreover, only in IS9530 was root hydraulic conductance restricted in the presence of $HgCl_2$, in agreement with water movement through cell-to-cell pathways and aquaporins activity. The different responses between genotypes suggest a distinct strategy at the seedling stage and add new information that should be considered when evaluating *Sorghum* phenotypic plasticity in changing environments.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench.] is an ancient drought-tolerant crop grown around the world. This species is remarkable for its ability to grow in semiarid and arid regions, even under low or erratic precipitation conditions where other cereal crops are not capable of sustaining high yields. *Sorghum* is grown for food, feed, fiber and fuel (Undersander et al., 2003; Clark, 2007; FAO, 2012). The versatility of its final destination together with its capacity to grow also in environments where water becomes scarce as a consequence of climate change (Morris et al., 2013) are renewing the interest in this species and its use as a next-generation biofuel (Carpita and McCann, 2008; Saballos et al., 2009) is demanding studies to be performed in new environmental conditions. However, variations in climatic conditions, as an increase of air temperature and humidity, could promote the pre-harvest sprouting while grain maturation is taking place (Steinbach et al., 1997). In countries that are highly considered as *Sorghum* producers (*i.e.* Argentina in Latin America, ca. 60% of total production; FAO, 2012), pre-harvest sprouting phenomena has been observed in some inbred lines (Rodríguez et al., 2009). This phenomenon is related to the interruption of dormancy during seed development in plant and, as a consequence, the induction of germination process while still on the parent plant. To avoid this effect, the currently sowed genotypes are obtained from parental lines with a different sprouting behavior. Although *Sorghum* is a drought-tolerant species (Gholipoor et al., 2012) those genotypes that had been selected for their performance to deal with pre-harvest sprouting are not completely dissected in terms of their ability to adjust physiological traits when they are exposed to water deficit

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scenarios. Therefore, our aim is to explore the early plant responses to low water availability in two *Sorghum* parental lines employed in Argentina -RedLandB2 and IS9530- with opposite sprouting behavior, being RedLandB2 susceptible to pre-harvest sprouting and IS9530 resistant to such phenomena (Rodriguez, pers. comm.).

Crop tolerance in response to drought has been addressed in terms of modifications on morphology, anatomy and physiology traits (Shanker et al., 2014; Tardieu et al., 2014). There are several studies related to the intraspecific variability in crop species, but most of them are focused on a particular physiological trait (Tardieu and Tuberosa, 2010). For instance, while several works are mainly focused on the stress impact on the aerial part of the plant (*e.g.* transpiration rate, leaf area, carbon allocation, photosynthetic efficiency; Djanaguiraman et al., 2014; Ogbaga et al., 2014), others are focused on the impact of drought on the root system (*e.g.* root biomass, root elongation rate, root architecture; Passioura, 1988; Sharp et al., 1988; Rogers and Benfey, 2015). In the present work our purpose is to study the whole plant physiological response to water deficit, combining plant hydraulic properties and integrating different aspects of the root system.

One of the main challenges of current drought research is to elucidate the dynamics of plant hydraulic regulation. At the whole plant level, the capacity for moving water is represented by hydraulic resistance. At shoot level, drought-tolerant plants can modify transpiration rate adjusting the number and/or stomatal aperture as well as leaf area. At root level, water uptake is a highly regulated process as it is strongly affected by low water availability in the soil. The anatomy (suberization in exo and endodermis, number and diameter of xylem vessels) and surface area of roots affect water uptake capacity by modifying root hydraulic resistances (Steudle, 2000; Ranathunge and Schreiber, 2011; Lynch et al., 2014). In addition, numerous studies have demonstrated the importance of the cell-to-cell pathway, in particular the role of water channels (*i.e.* aquaporins) in root water permeability (Javot et al., 2003; Li et al., 2014). It has been reported that water channel gene expression can be regulated by drought, salinity and osmotic stress in Arabidopsis (Seki et al., 2002), rice (Kawasaki et al., 2001), wheat (Ayadi et al., 2011), barley (Katsuhara et al., 2003), maize (Zhu et al., 2005) and *Sorghum* (Liu et al., 2014; Reddy et al., 2015). The aquaporins demand rethinking water relations as a whole, considering the adjustment capacity of the cell-to-cell pathway and its contribution to the strategy developed by each genotype in water deficit conditions. Understanding the physiological mechanisms associated with drought tolerance in this species at early phenological stages could be a useful tool for future yield improvement programs (Richards, 2006). Thus, the aim of this work is to study the response of *Sorghum* seedlings under water deficit conditions in the two genotypes RedLandB2 and IS9530. We characterize not only anatomical and physiological parameters at the whole plant level but also analyze the water movement regulationat root and shoot level.

2. Materials and methods

2.1. Plant material and culture conditions

Experiments were performed using two Sorghum [*Sorghum bicolor* (L.) Moench] inbred lines, RedLandB2 and IS9530, which are parental genotypes that originated the regularly sown lines in Argentina (Rodriguez, pers. comm.). Plants were grown under controlled environmental conditions with a 16/8 h light/dark cycle (light intensity of $205 \pm 10\ \mu mol\ m^{-2}\ s^{-1}$), a $60 \pm 2.5\%$ relative humidity in a 21 °C growth chamber. Seeds were sown in plastic containers (330 mL) filled with sterilized sand and moistened with Hoagland solution: 1.25 mM KNO_3, 0.75 mM $MgSO_4$, 1.5 mM$Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 50 μMFeEDTA, 50 μM H_3BO_3, 12 μM $MnSO_4$, 0.70 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.24 μM Na_2MoO_4, and 100 μM Na_2SiO_3 (Javot et al., 2003). Ten days after germination seedlings were separated in two groups, one of them was irrigated periodically and in the other one the irrigation was withdrawn during 15 days. Except for plant growth rate experiments (Fig. S2), seedlings in the three-four leaf stage were used at day 15th for all the measurements and finally harvested for biomass determinations.

2.2. Plant growth rate and leaf area

Whole seedlings of both *Sorghum* genotypes were harvested every three days (3 seedlings per day per treatment) during 13 days. Immediately after each harvesting, plants were divided into root and aerial parts and photographed to measure the increase in root and shoot length (cm) as a function of time (days). Root length is represented by the measurement of the longest root of each seedling. Digital images were analyzed using ImageJ 1.48 v software (http://rsb.info.nih.gov/ij/). To determine fresh weight (FW) of plants, shoots and roots were weighed immediately after cut, and then samples were dried at 60 °C during 48 h (constant weight) to obtain the dry weight (DW). At the end of treatments, leaf blades were photographed with a digital camera to determine leaf area. Images were processed with the software mentioned above.

2.3. Relative water content (RWC) and soil water content (SWC)

Relative water content (RWC) was determined according to Turner (1981). Briefly, leaves of both treated or control seedlings were cut and immediately weighted (fresh weight, FW), followed by immersing them during 24 h in distilled water to determine turgid weight (TW) and finally dried at 60 °C during 48 h to obtain dry weight (DW). Relative water content was calculated as: RWC = (FW-DW)/(TW-DW)*100. Samples of soil were weighted to obtain fresh weight (SFW) and then dried at 60 °C during 48 h to obtain dry weight (SDW). Soil water content was calculated as: SWC = [(SFW-SDW)/(SDW)]*100.

2.4. Stomatal conductance (g_s)

Stomatal conductance was measured using a portable steady state diffusion leaf porometer (model SC-1, Decagon devices, Pullman, WA, USA). Measurement was done at center of the last fully expanded leaf (abaxial face). All the measurements were made between 10:30 and 11:30 AM. Data were analyzed from 3–7 plants from five independent experiments per treatment.

2.5. Predawn water potential (Ψ_h)

Prior to measurement, whole aerial part (stem, leaf blade and leaf sheath) were placed in a plastic box covered with Parafilm® and introduced in a Scholander pressure chamber (Biocontrol Model 4, Argentina) to determine the water potential (Schölander et al., 1965). Measurements were done predawn in 17 seedlings obtained from six independents experiments.

2.6. Osmotic potential (Ψ_{osm})

Leaf blade osmotic potential was measured as previously described by Mahdieh et al. (2008). Briefly, leaf blades were placed in small column with holes at the bottom and immediately frozen in liquid nitrogen. After thawing, the column was placed inside in 1.5 ml centrifuge tube and centrifuged at 4000 × *g* for 4 min at 4 °C using a microcentrifuge. Sap osmolarity of each sample was measured in a vapor pressure osmometer (Vapro 5520, Wescor, USA)

and used to calculate osmotic pressure according to Van't Hoff's equation.

2.7. Measurement of root hydraulic conductance (L_o)

Root hydraulic conductance measurements were carried out as described by Miyamoto et al. (2001), with some modifications. Before starting the measurements, shoots were cut off using razor blades and roots were washed with tap water. The root system of freshly detopped *Sorghum* seedlings was immersed in a 50 mL container filled with hydroponic culture medium and inserted into a pressure chamber (Biocontrol Model 4, Argentina). Root-shoot junction was carefully threaded through the metal lid of the chamber and sealed using low-viscosity dental paste (A + Silicone, Densell). The flux of exudates root (J_v) induced by pressure (P) at 0. 2, 0.3 and 0.4 MPa was determined as described by Matsuo et al. (2009) with some modifications. At each pressure, exuded sap was collected at the surface of the cut root-shoot junction for 3 min on a small piece of tissue paper that was previously pre-weighed. The tissue paper was then weighed on an analytical balance with a sensitivity of 0.1 mg. This process was repeated three times for each pressure. Root hydraulic conductance was obtained from the linear J_v vs. (P) relationship. To measure the effect of water channel inhibitors on the L_o, plants were incubated for 10 min with 50 μM $HgCl_2$ (Carvajal et al., 1996; Sutka et al., 2011; Zhang and Tyerman, 1999) before determination of the exudate flow at the three different pressures.

2.8. Stomatal density and open/close ratio

Stomatal density was determined following techniques described by Foster (1950). Completely expanded leaf blades were separated and the middle portion of the blade was diaphanized with 96% ethanol until all chlorophyll was extracted. Then, blades were treated with 5% (w/v) NaOH, bleached with 50% (v/v) sodium hypochlorite and samples were incubated overnight with chloral hydrate (Foster, 1950). After that, blades were mounted in gelatin in two positions: abaxial and adaxial. Two replicates per treatment and genotype were made. The slides were observed under a light microscope (Zeiss Axioskop 2, Japan) and a digital camera (Nikon E8700, Japan) was used to photograph the samples (between 19 and 28 photographs per treatment). The number and state (open-close) of stomata were measured at 400X in each microphotograph by means of free available software (ImageJ 1.48 v software; http://rsb.info.nih.gov/ij/).

2.9. Xylem vessel number and diameter

In order to characterize the anatomy along the roots, xylem vessels were quantified and measured. For this, roots of control and treated plants (three plants per condition and one root per plant) were finely cut at different positions behind the root apex (0.5, 1, 2 and 4 cm) using a razor blade, mounted and observed under a light microscope (Zeiss Axioskop 2, Japan). A digital camera (Nikon E8700, Japan) was used to photograph the samples. Numbers of xylem vessels were counted from images and xylem diameter was measured using the software above mentioned.

2.10. Statistical analyses

Two-way analysis of variance (ANOVA) was used to analyze the effects of genotype and water deficit treatment on morphological and physiological seedling responses. Post-hoc Tukey's tests were employed for mean comparisons ($P \leq 0.05$). Variable normality and homogeneity of variances were previously verified in order to satisfy ANOVA's assumptions. All statistical analyses were performed with the InfoStat 2014 package (Di Rienzo et al., 2013). Results are presented as Mean ± S.E. (standard error of the mean). Each sample size (n) is indicated in the figure legends. Different upper-case letters indicate significant differences ($P \leq 0.05$) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

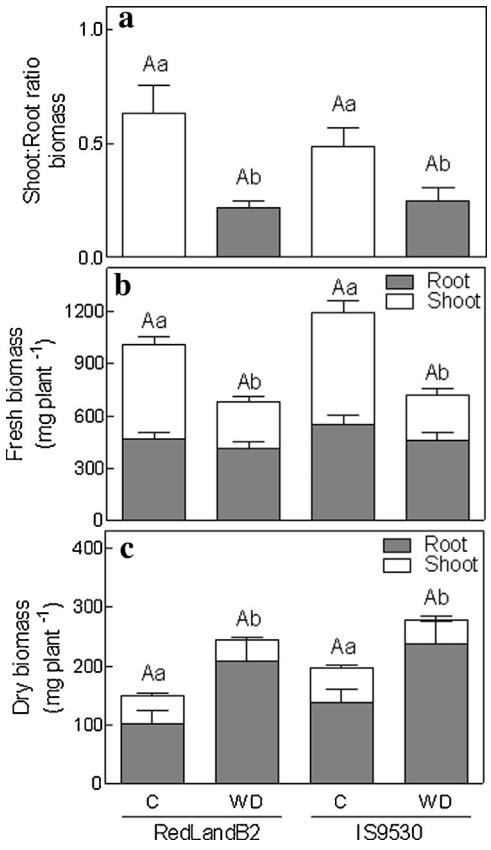

Fig. 1. Shoot:Root biomass ratio (a) fresh (b) and dry (c) biomass of shoot (white bars) and root (grey bars) for seedlings of two genotypes of *Sorghum*: RedLandB2 and IS9530 grown for 15 days under two experimental conditions: control (C) and water deficit (WD). Each bar represents Means ± S.E. of 18 plants ($N = 7$ independent experiments). Different upper-case letters indicate significant differences ($P < 0.05$) between genotypes; lower-case letters indicate significant differences between treatments within each genotype, only for fresh shoot and dry root biomass.

3. Results

3.1. The effect of water deficit on seedling growth is strictly conserved in both genotypes

Seedlings of the two studied genotypes increased root biomass and reduced shoot biomass after 15 days of water deficit as shown by the shoot:root biomass ratio (Fig. 1a). During the 15 days of treatment, soil water content diminished between 80 and 87% compared to the well watered condition (control) for both genotypes (Fig. S1). The soil water content was ca. 13% for control condition that is consistent with field capacity values in sandy soil conditions (Brady, 1990). In accordance with *Sorghum* tolerance to water limiting environments, *Sorghum* seedlings submitted to such condition showed no significant differences in any of the parameters measured during the first seven days when compared to well watered seedlings (Fig. S2). After this period, both genotypes -RedLandB2 and IS9530- started to reduce fresh weight and shoot length (Figs. S2a and e.) and significant differences from well watered seedlings were observed. The reduction profile of those parameters in roots was indistinguishable in the two genotypes (Figs. S2b and f). Both of

Table 1
Different traits for two *Sorghum* genotypes: RedLandB2 and IS9530 grown under control or water deficit condition.

Variable		RedLandB2		IS9530	
		Control	Water deficit	Control	Water deficit
Fresh weight (mg)	Shoot	540.9 ± 47.6**Aa**	267.0 ± 33.5**Ab**	642.5 ± 68.7**Aa**	263.2 ± 37.1**Ab**
	Root	469.7 ± 38.6**Aa**	410.8 ± 39.3**Aa**	551.2 ± 53.3**Aa**	458.5 ± 50.0**Aa**
DW (mg)	Shoot	480.3 ± 3.7**Aa**	380.0 ± 3.5**Ab**	580.2 ± 4.6**Aa**	420.1 ± 4.6**Ab**
	Root	1020 ± 22.4**Aa**	2070.2 ± 36.5**Ab**	1380.7 ± 20.9**Aa**	2370.3 ± 39.4**Ab**
Length (cm)	Shoot	24.4 ± 1.4**Aa**	20.1 ± 0.7**Ab**	26.9 ± 1.0**Aa**	21.9 ± 0.7**Ab**
	Root	9.6 ± 0.8**Aa**	10.5 ± 0.7**Aa**	9.5 ± 0.5**Aa**	13.3 ± 1.9**Aa**
Total biomass (DW, mg)		150.3 ± 25.0**Aa**	245.2 ± 39.4**Ab**	197.0 ± 21.2**Aa**	279.3 ± 43.7**Ab**
Shoot–Root ratio biomass		0.63 ± 0.1**Aa**	0.22 ± 0.03**Ab**	0.49 ± 0.1**Aa**	0.25 ± 0.1**Ab**
Leaf area (cm^2)		21. 98 ± 1.1**Aa**	11.19 ± 1.2**Ab**	24.38 ± 1.96**Aa**	11.01 ± 1.5**Ab**
Water content (mg)		90.9 ± 0.4**Aa**	84.78 ± 1.9**Ab**	90.73 ± 0.5**Aa**	82.07 ± 2.6**Ab**
Relative water content		96.9 ± 1.3**Aa**	81.74 ± 7.4**Aa**	95.82 ± 1.3**Aa**	70.16 ± 7.5**Ab**
Osmotic potential (MPa)		−0.79 ± 0.03**Aa**	−0.8 ± 0.05**Aa**	−0.79 ± 0.03**Aa**	−0.95 ± 0.2**Aa**
g_s ($mmol\ m^{-2} s^{-1}$)		7.14 ± 1.7**Aa**	2.55 ± 0.7**Aa**	10.24 ± 1.96**Ba**	86.87 ± 1.8**Ba**
Waterpotential (MPa)		−0.13 ± 0.02**Aa**	−0.58 ± 0.1 **Ab**	−0.12 ± 0.02**Aa**	−1.23 ± 0.2**Bb**
L_o ($\mu l\ s^{-1}\ MPa^{-1}$)		3.6E-4 ± 4.3E-5**Aa**	1.1E-4 ± 3.1E-5**Ab**	6.3E-4 ± 3.3E-5**Ba**	1.3E-4 ± 4.9E-5**Ab**

DW: dry weight, g_s: stomatal conductance, L_o: hydraulic conductance. Values are Mean ± S.E. of five replicates. Different bold upper-case letters indicate significant differences ($P < 0.05$) between genotype; bold lower-case letters indicate significant differences between treatments within each genotype.

them also increased their root dry weight under water deficit (WD) (Fig. S2d). Therefore, the analysis of the responses of *Sorghum* to WD was explored after 15 days of water shortage. In both *Sorghum* genotypes, total fresh biomass diminished under WD conditions due to a reduction of the aerial fresh weight of the plant while root fresh weight remained without changes (Fig. 1b). While the shoot dry biomass decreased in both genotypes (Fig. 1c, Table 1), total dry biomass showed an increase as a consequence of a rise in dry root biomass. These results demonstrate that both *Sorghum* seedlings were able to cut back water supply to the aerial part and sustain the water status in roots. Seedlings of both *Sorghum* genotypes showed a marked decrease only on shoot length ($P < 0.05$, Fig. 2, upper panels) while this effect was not significant on root length of either genotype (Fig. 2, bottom panels).

3.2. Genotypes showed contrasting hydraulics response to water deficit in shoots

Relative water content (RWC) was studied in both genotypes of *Sorghum* as a meaningful index of plant water status. RedLandB2 and IS9530 showed similar RWC under well watered

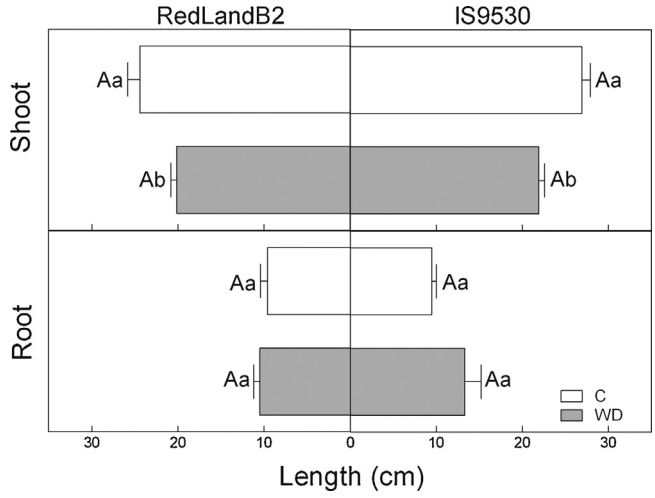

Fig. 2. Shoot and root length from seedlings of two genotypes of *Sorghum*: RedLandB2 and IS9530, grown for 15 days under two conditions: control (C) and water deficit (WD). Each bar represents Mean ± S.E. of 16 plants ($N = 6$ independent experiments). Different upper-case letters indicate significant differences ($P < 0.05$) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

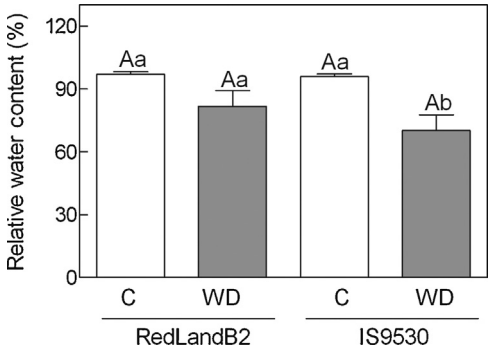

Fig. 3. Relative water content of seedlings of two genotypes of *Sorghum*: RedLandB2 and IS9530, grown for 15 days under two conditions: control (C) and water deficit (WD). Each bar represents Mean ± S.E. of 18 plants ($N = 7$ independent experiments). Different upper-case letters indicate significant differences ($P < 0.05$) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

condition (Fig. 3). However, after 15 days of WD, IS9530 reduced RWC (30%) while RedLandB2 remained unchanged, indicating that RedLandB2 is able to maintain its water status even in WD conditions. Water (Ψ_h) and osmotic potential (Ψ_{osm}) were also measured in both *Sorghum* genotypes. As expected, WD reduced significantly the water potential in RedLandB2 and IS9530. RedLandB2 showed a four-fold reduction compared to control condition while IS9530 showed a ten-fold reduction compared to control condition (Fig. 4a). Interestingly, neither of the genotypes showed differences in osmotic potential values, for both control and WD conditions (Fig. 4b), indicating that solute accumulation is not responsible for the observed reduction in water potential.

In order to study how water deficit affects plant transpiration, we measured two related variables in the aerial part: leaf area and stomatal conductance. Both RedLandB2 and IS9530 had similar values of leaf area and stomatal conductance under control condition (Fig. 5a and b). However, both genotypes reduced their leaf area under WD conditions, only RedLandB2 decreased the stomatal conductance, while IS9530 kept conductance values similar to the control condition (Fig. 5b), which is consistent with the mentioned reduction in RWC and the higher water potential decreased observed (Figs. 3 and 4a). To further explore the stomata status under WD condition, density and open:close stomatal ratio were also analyzed. Under water deficit conditions the genotypes showed differences in terms of stomatal density per leaf blade face. RedLanB2 showed a higher density at the adaxial face while IS9530

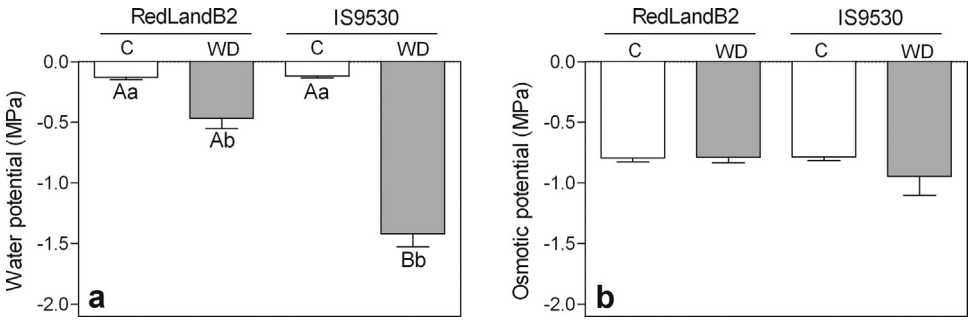

Fig. 4. Water potential (a) and osmotic potential (b) of seedlings of two *Sorghum* genotypes, RedLandB2 and IS9530, grown for 15 days under two conditions: control (C) and water deficit (WD). Each bar represents Mean ± S.E. of 13–24 plants (N = 5–7 independent experiments). Different upper-case letters indicate significant differences (P < 0.05) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

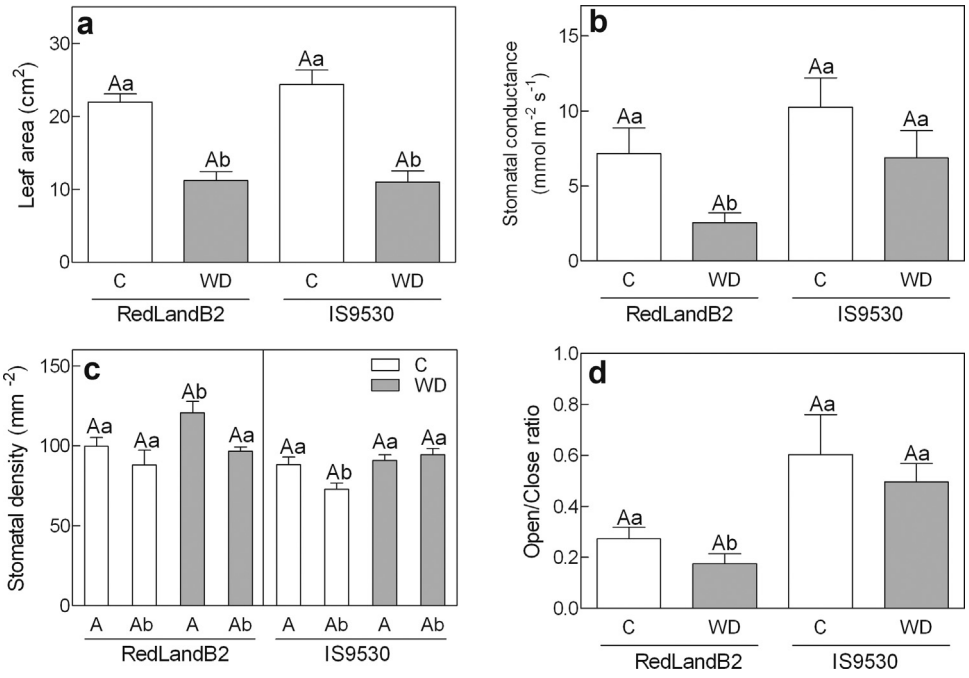

Fig. 5. Leaf area (a), stomatal conductance (b), stomatal density (c) and open/close ratio (d) of seedlings from two *Sorghum* genotypes: RedLandB2 and IS9530, grown for 15 days under two conditions: control (C) and water deficit (WD). Each bar represents Mean ± S.E. of 13–24 plants (N = 5–7 independent experiments). Different upper-case letters indicate significant differences (P < 0.05) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

remained unchanged (Fig. 5c). It was observed that RedLandB2 was the only genotype that *slightly* reduced the open:close stomatal ratio (Fig. 5d). This last result is consistent with the stomatal conductance measurements (Fig. 5b).

3.3. Genotypes showed different participation of root water radial pathway under water deficit

Both genotypes equally presented a high L_o in control conditions and equally reduced it under WD condition (Fig. 6). However, when we measured L_o in the presence of an aquaporin inhibitor ($HgCl_2$) in well watered seedlings, only IS9530 showed a significant reduction of L_o (42%; P < 0.05) while, in RedLandB2 L_o remained indistinguishable from control conditions. Mercury chloride is the most common aquaporin inhibitor in plants, although it must been used with care due to its possible toxicity. In a complementary set of experiments performed with seedlings grown in hydroponic conditions, we assayed the effect of propionic acid, as another aquaporin inhibitor, in the root hydraulic conductance. Only IS9530 showed a reduction of 48% compare to the control condition, similar to 42% obtained in the experiments with $HgCl_2$ (n = 5–16 plants from 3 independent experiments, data not shown). The L_o reduction in IS9530 might reflect the contribution of aquaporins -sensitive to mercurial compounds- to water movement and therefore the contribution of the root cell-to-cell pathway to water management.

It was also important to verify if the root anatomy (in terms of vascular system) was affected. We analyzed the number and diameter of root xylem vessels in both *Sorghum* genotypes along the length of the root (from 0.5 to 4.0 cm). The number of vessels increased from the root apex to the base following a similar pattern in both genotypes and treatments (Fig. S3a; P > 0.05). Only the diameter of xylem vessels was affected in RedLandB2 but not in IS9530. Throughout, RedLandB2 showed a reduction of its vessel diameters under WD conditions, much more markedly next to root tip (0.5 cm; Fig. S3b).

4. Discussion

The low shoot:root biomass ratio observed in well-watered conditions (Fig. 1a) may be due to the ontogenetic stage of plants, since during the early growth more assimilates are allocated to roots in annual plants and, as development continues, the dry matter

allocation changes to aerial parts (Gregory et al., 1997). The initial plant response to drought is the inhibition of shoot growth and the maintenance of root growth, as an adaptive response to maintain the water uptake and reduce water loss by transpiration (Wu and Cosgrove, 2000; Sinclair et al., 2005). For some annual crops, dry biomass accumulation follows a sigmoid pattern in root systems and also it is reported in shoots, although the both phases of growth may not match exactly (Gregory, 2006). In general, a shortage of resources in the root environment -as water deficit- causes changes in biomass assimilation patterns, favoring the root system growth (Brouwer, 1963). Thus, it can be expected that there is a displacement in the biomass reallocation pattern between root and shoot under water deficit conditions.

As expected for a tolerant crop, seedlings of both *Sorghum* genotypes showed a conserved response under limiting water conditions (Muller et al., 2011). Ours experiments were done at an early stage (25 days old, 3–4 expanded leaves seedlings), so it is not expected that the final state of the biomass accumulation pattern is achieved (Fig. S2). At that stage, there was a marked increase in root biomass with a significant impact on the total plant biomass, as root:total biomass ratio indicated (0.68–0.85 and 0.70–0.85 for RedLandB2 and IS9530, respectively). The root growth maintenance in water deficit conditions is a clear benefit to provide plant water supply as it has been previously reported (Sharp and Davies, 1979; Malik et al., 1979; Meyer and Boyer, 1981; van der Weele et al., 2000). Interestingly, both genotypes maintain their biomass allocation response to water deficit, although they developed different strategies to water movement regulation, as discussed below. These results are in accordance with those published by Fracasso et al. (2016) where four genotypes of *Sorghum*, with different tolerance, were screened under drought stress conditions.

4.1. *Both genotypes of* Sorghum *showed different strategies to deal with water deficit*

Under water deficit conditions, RedLandB2 maintained the relative water content of leaves by means of reducing the water potential, stomatal conductance and the open:close stomata ratio (Figs. 3, 4 and 5). By contrast, IS9530 reduced its relative water content (Fig. 3) and water potential (*ca.* 10 times more than Control) but maintained its stomatal conductance and a high open:close stomata ratio (Figs. 4 and 5). A reduction in transpiration rate is considered a typical response to drought stress (Gholipoor et al., 2010). In particular for *Sorghum*, it was reported that the maintenance of low stomatal conductance -under high VPD and low soil water potential conditions- could be advantageous under water limited environments (Choudhary et al., 2013). However, in our approach, the low VPD (data not shown) and the soil water content (Fig. S1) was the same for the two tested genotypes. Transpiration rate depends on both stomatal conductance and root water absorption, both processes involved in maintenance of water homeostasis (Pittermann, 2010). Then, plants have to minimize water loss when the environmental conditions are compromising water uptake (Hommel et al., 2014). Here, one of the differences found between genotypes lies in the physiological strategy to adjust shoot-water demand, suggesting a refined mechanism in water movement regulation. Although, RedLandB2 was able to increase the stomatal density in the leaf abaxial face (Fig. 5c), the strategy is to reduce the number of open stomata, and thus decrease the stomatal conductance under water deficit (Fig. 5b and d). There is an agreement that the different strategies developed for plants to deal with drought can be merged into two contrasting behaviors: anisohydric and isohydric (Klein, 2014; Martínez-Vilalta et al., 2014). Anisohydric plants sustain transpiration rate even in stressful conditions, decreasing water potential (Ψ_h) and relative water content (RWC). By contrast, under the same scenario, isohydric plants reduce transpiration, maintaining constant Ψ_h and RWC (Moshelion et al., 2015). In this framework, the employed *Sorghum* genotypes could be associated with such distinct strategies, considering IS9530 as anisohydric and RedLandB2 as isohydric.

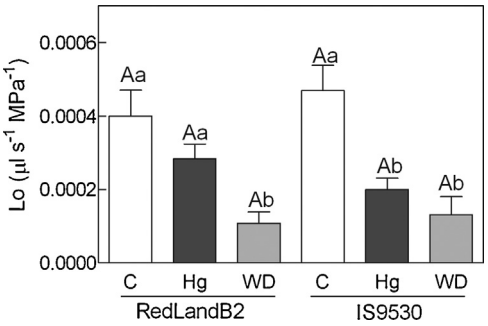

Fig. 6. Properties of root water dynamics: the effect of water deficit and $HgCl_2$ on root hydraulic conductance (L_o) of seedlings from two *Sorghum* genotypes, RedLandB2 and IS9530. Seedlings were grown under control (C) and water deficit (WD) conditions, and incubated with $HgCl_2$ (Hg). In those experiments performed in the presence of 50 μM $HgCl_2$, seedlings were pre-incubated ten minutes before L_o measurements. Each bar represents Means ± S.E. of 9–12 plants ($N = 5$ independent experiments). Different upper-case letters indicate significant differences ($P < 0.05$) between genotypes; lower-case letters indicate significant differences between treatments within each genotype.

Focusing on water uptake by roots, its capacity to manage water under deficit conditions could be defined by different cues that might determine the main pathway for water transport. It is well known that water transport across roots occur through three different pathways: apoplastic, symplastic and transcellular (Steudle et al., 1993; Steudle, 2000). The latter two pathways are normally referred as cell-to-cell pathway, due to the fact that it is impossible to experimentally dissect them. Water transport along the root can be characterized by measuring its hydraulic conductance (Tyree, 2003), which can be modified under stress. Our results demonstrate that both *Sorghum* genotypes studied here reduced their root hydraulic conductance when soil water availability was diminished (Fig. 6). Dissecting water pathways allowed us to analyze its main components as discussed below.

First, mercurial compounds are normally used to evaluate the contribution of cell-to-cell pathway to root water transport, *i.e.* aquaporin activity (Carvajal et al., 1996; Sutka et al., 2011; Zhang and Tyerman, 1999). In *Sorghum* genotypes, only IS9530 showed a hydraulic conductance that was significantly inhibited by $HgCl_2$ (Fig. 6) indicating that the cell-to-cell pathway might contribute in adjusting the root water transport capacity in this genotype. In contrast, RedLandB2 maintained L_o unchanged even when roots were incubated in $HgCl_2$ (Fig. 6). These results are consistent with the idea that RedLandB2 seedling deals with water deficit modulating the water status of the aerial part. Recently, the use of mercurial compounds revealed the participation of aquaporins in the root hydraulic conductance in *Sorghum* plants (Choudhary et al., 2013; Liu et al., 2014). Two *Sorghum* lines (SC15 and SC1205), with dissimilar leaf and root conductance (K_{leaf}, L_o), showed different transpiration rate when they were exposed to $HgCl_2$. The authors interpreted this response as a consequence of the impact of mercurial compound on roots, and it is consistent with the fact that SC15 has a larger number of water channels in its roots than the other line (Choudhary et al., 2013). Liu et al. (2014) studied the effect of silicon application in the amelioration of water loss during osmotic stress. In their case, application of $HgCl_2$ decreased the transpiration rate of seedlings and by silicon application increased the transcription levels of several root aquaporin genes. Thus, the use of mercurial compounds to reveal the aquaporin participation in the root water movement in this specie is also effective. Moreover, *Sorghum* aqua-

porins have been identified, and their expression under several abiotic stresses was also reported (Reddy et al., 2015). Thus, the drop in L_o observed in IS9530, in the presence of mercurial compounds, could be strongly related to the role of aquaporins in the cellular pathway.

Second, xylem anatomy could provide us some clue of the contribution of each pathway when comparing genotypes (for both control and water deficit conditions). The number of xylem vessels showed the same pattern in both genotypes and conditions (Fig. S3a). However, the diameter of xylem vessels was reduced in RedLandB2 when it was exposed to water limiting conditions (Fig. S3b), while IS9530 showed smaller xylem vessels than RedLandB2 in all conditions. These results are in accordance with Cruz et al. (1992) where the diameter of root xylem vessels diminished in water deficit conditions. These could explain the L_o drop (increase of root hydraulic resistance) observed in RedLandB2 and it could be probably associated with an extra suberin deposition in the root exodermis (Passioura, 1982), as reported for other species with similar anatomical properties (Schreiber et al., 2005). However, in IS9530 the response is different, since there are no changes in the number and diameter of the xylem vessels. This could indicate that the cellular pathway could make a difference in terms of water balance.

4.2. Differential contribution of root to plant hydraulic resistance

The plant hydraulic adjustments can be explored analyzing the contribution of root and aerial components to hydraulic resistance. In order to discuss this water management, different parameters have been considered: soil water content (SWC), stomatal conductance (g_s) and root hydraulic conductance (L_o). Plants of both genotypes cope with the same intensity of water deficit (*i.e.* the same pattern of soil water loss, Fig. S1, same VPD) regulating their water management at different points. If the stomatal conductance (g_s) or root hydraulic conductance (L_o) are reduced, then there is an increase in the total hydraulic resistance (Lambers et al., 2008). In RedLandB2, total hydraulic resistance is given at aerial level, as stomatal conductance indicates, but not in IS9530 (Fig. 5), where the root seems to be more important in water movement. In water deficit conditions, both genotypes reduced root hydraulic conductance (Fig. 6) increasing the resistance to water movement. RedLandB2 also showed a reduction in stomatal conductance (reducing the number of open stomata, Fig. 5b and d) having a resistance larger than IS9530. The response of RedLandB2 could be associated with the capacity of this genotype to anticipate the imminent reduction in water availability. However, it could be useful to know the related genes associated to the early response to drought stress, as those described by Pasini et al. (2014). Therefore, RedLandB2 could use water in a conservative way, delaying the water deficit effect (Siqueira et al., 2008), while IS9530 would be regulating at root level by modulating aquaporins activity. It seems likely that plants of IS9530 could have a decoupling between roots and aerial part, which could be reflected on the interaction between absorption and transpiration.

5. Conclusions

The differential response found between *Sorghum* genotypes implicates a distinctive plasticity on water management when they grow under water limiting conditions at seedling stage. On one hand, RedLandB2 is able to respond to soil water restriction earlier in stomatal conductance than IS9530, which do not close its stomata during the 15 days of experiments. On the other hand, IS9530 modulates root water uptake by slightly increasing total hydraulic resistance. We also discuss the importance of the cellular pathway for IS9530, associated with water channel activity.

In summary, this work adds evidence in relation to water management of *Sorghum* species, highlighting the whole plant responses to soil water deficit. We integrated the shoot and root morphological and physiological responses, including the cellular pathway of water movement when seedlings were exposed to water deficit conditions. The different strategies developed by both *Sorghum* genotypes could be an interesting attribute of this crop species, since they can adjust the water management at different levels. This performed approach adds new information for *Sorghum* which can be used to evaluate phenotypic plasticity for changing environments. Our result provides also new insight in terms of anisohydric and isohydric strategies that can be developed by plants in relation to water management. However, an exhaustive study to address the physiological/molecular mechanisms triggered in response to water deficit is still necessary, including in this scenario the role of aquaporins. Besides, our approach make available new evidences related to the performance of Sorghum seedlings of two genotypes with contrast sprouting behavior grown in water limited conditions. This information could be use to improve crop yield in those environments where the soil water content is low and the risk of sprouting phenomena is low too.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2016.01.002.

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