Hydraulic anatomy affects genotypic variation in plant water use and shows differential organ specific plasticity to drought in *Sorghum bicolor*☆

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ABSTRACT

Genotypic variability and plasticity in hydraulic anatomy are not well-understood in herbaceous monocots. In this study, we used *Sorghum bicolor*, a monocotyledonous, tropical grass model, to understand whether differential plant water use is associated with xylem anatomy and if whole-plant xylem anatomy responds to water stress, justifying differential genotypic sensitivity to drought. In a greenhouse environment, we studied four sorghum genotypes that are known to genetically differ in growth and exhibit differential sensitivity to drought. Under well-watered scenario, transpiration variability and plant growth traits correlated with xylem anatomical traits at both the leaf and stem level, including xylem area and predicted xylem-specific hydraulic conductivity. High water use genotypes had inherently higher hydraulic capacity, but under drought, their transpiration declined at higher fractions of transpirable soil water (*FTSW*) and they showed greater plasticity in hydraulic anatomy. However, lower *FTSW* thresholds and modest anatomical changes were identified in the low water use genotypes with inherently lower hydraulic conductivity. Drought, induced modular phenotypic plasticity in hydraulic anatomy, whereby plasticity in leaf xylem traits was remarkably higher than stem xylem, while root xylem showed a reverse nature of vascular modification. Xylem traits were in agreement with phloem anatomy, irrespective of water regime. Our study indicates that hydraulic anatomy can be critical for herbaceous monocots in determining limits to plant water use and genotypic response to drought with implications on whole-plant functions and habitat ecology.

1. Introduction

A huge body of information is available on plant hydraulic efficiency defined by xylem hydraulic conductivity and hydraulic safety, a set of properties that allow xylem to maintain its integrity and function under negative xylem pressure. Most of this knowledge, however, comes from woody angiosperms, particularly trees, whereas the hydraulic architecture of herbaceous monocots remains largely enigmatic (Choat et al., 2012; Tixier et al., 2013; Lens et al., 2016). Unlike trees, studies on monocot xylem have developed with little reference to their ecology and therefore, the available information on hydraulic architecture in the context of a monocots' habit and habitat are less intensive (Carlquist, 2012a). Yet, herbaceous monocots play important ecological roles in biomes likes grasslands, which are one of the most

important agro-ecosystems representing 26% of the world land area (Foley et al., 2011). Since various valuable crops are grasses, herbaceous monocot species deserve more attention from a hydraulic point of view to understand their responses to shifts in precipitation and temperature (Holloway-Phillips and Brodribb, 2011; Brodribb et al., 2015).

In pasture grasses, drought tolerance is often associated with conservative water uptake behaviour, where the least absolute amount of water is extracted from soil during vegetative growth, but continuity in water uptake is maintained until physiological maturity and reproduction (Zaman-Allah et al., 2011; Vadez et al., 2014; but see Blum, 2009). Traits such as low maximal stomatal conductance, rate of leaf area development, maximum leaf area at anthesis, stomatal frequency, and transpiration sensitivity to VPD were associated to conservative plant water uptake (Vadez et al., 2014). A consensus has been reached that plant water uptake either constitutive (inherent characteristics of a genotype) (Zaman-Allah et al., 2011) or stress-adaptive (Devi et al., 2009), could largely be regulated by hydraulic properties such as stomatal conductance and leaf area expansion (Sperry, 2000; Sperry et al., 2003; Ehlert et al., 2009; Zaman-Allah et al., 2011; Pantin et al., 2012; Vadez et al., 2013, 2014). Unlike trees (but see Gleason et al., 2016), only a few comparative experimental data exist on the structure-function behind xylem vulnerability to embolism in various monocots.

Here, we used the monocotyledonous, tropical C4 grass species *Sorghum bicolor* to investigate how whole-plant xylem anatomy relates to plant water uptake to explain intra-specific differences in growth and productivity under well-watered and water-limited conditions. Differences in xylem anatomy and hydraulic functions are investigated in tandem due to the fact that xylem structure is often designed for dealing with environmental variation and there could be multiple intra-specific hydraulic strategies within a given habitat where each strategy can be intimately cued to xylem anatomy (Carlquist, 2012a). Although intra-specific water use patterns were reported in different commercially important grass species (Kholova et al., 2010; Gholipoor et al., 2010; Gowda et al., 2012; Borrell et al., 2014; Choudhary and Sinclair, 2014), we have limited understanding of the phenotypic variability in xylem traits (e.g. size, number and distribution of xylem vessels) in relation to plant water uptake behaviour (e.g. high vs. low transpiration rates and whole-plant transpiration). It is generally accepted that hydraulic properties determine whole-plant functioning, indicating a developmental coordination between photosynthetic and hydraulic traits (Brodribb, 2009). However, there is little information from grasses that might support such a general coordination. A few studies have attempted to investigate synchronous variation of xylem anatomy, water transport, and photosynthetic traits in grass models, but most anatomical investigations are conventionally organ specific focusing predominantly either leaves (Bresta et al., 2011; Ocheltree et al., 2014; Tabassum et al., 2016) or roots (Salih et al., 1999; Kadam et al., 2015), with little or no attention to the stem. A likely reason could be that the classical soil-plant-atmosphere continuum model considers that axial hydraulic resistance of the stem is smallest relative to that of the leaf and root, especially when short stature grasses (e.g. shortgrass prairie species) are considered, where there is little stem to work with. However, in tall stature grasses with long upright stems, the whole-plant water transport and water use could largely be influenced by stem hydraulic properties (Li et al., 2009).

Plants exposed to water deficit can adjust hydraulic properties and are generally assumed to minimize the risk of embolism by modifying xylem traits like vessel diameter and vessel density (Tognetti et al., 1997; Holste et al., 2006), vessel grouping (Carlquist, 2012b) and pit structure (Plavcová et al., 2013). A central role in hydraulic safety and efficiency is played by xylem vessel size and vessel size distribution (Tyree et al., 1994) and therefore we primarily focused on these traits in the present study. While many reports demonstrated that decreased water availability causes narrower xylem and higher xylem densities, the literature contains many perplexing, contradictory reports on xylem anatomical plasticity perhaps due to reasons like genotypic and organ-

specific responses and anomalies in the duration and intensity of water stress which plants are exposed to. Since anatomical changes are usually the consequences of long-term acclimation to drought, it is necessary that the duration of drought continues for a certain period and is adequate to achieve noticeable changes in xylem structure (Holste et al., 2006; Shao et al., 2008). Drought-acclimated plants may construct safer xylem with small diameter, thick-walled vessels that are resistant to cavitation (Gleason et al., 2012; Hacke et al., 2001). Such structural modification was evident in drought-acclimated sunflower (Nardini and Salleo, 2005) and poplar hybrid (Awad et al., 2010). Drought sensitive species or genotypes may be more vulnerable to embolism (Savi et al., 2016) and may survive prolonged water stress by developing smaller leaves and stronger reduction in plant size unlike the tolerant ones (Regier et al., 2009). Higher vulnerability to embolism and diminished shoot growth may co-operate in formation of narrow xylem vessels and decreased vessel transectional area under drought as suggested in *Vitis vinifera* (Lovisolo and Schubert, 1998). Little attention has been paid to understand how xylem anatomy in grasses may respond to water stress, possibly due to their shorter life-span and lack of secondary growth (an evolutionary constraint) limiting the scope to study them over a longer period of time. Furthermore, the dynamic hydraulic flux between xylem and phloem and lignification of stem tissue (Lens et al., 2016) are some of the emerging intriguing facets of hydraulic plasticity in plants, but such reports on grasses are limited and more anatomical and physiological data are needed.

Unlike many short-statured pasture grasses, the cultivated sorghum genotypes are mostly taller and upright with prominent leaf, stem and root system and therefore considered as an ideal model system for the present study. Further, significant genotypic variation in water uptake behaviour (Vadez et al., 2011), stomatal conductance, and leaf area expansion rate (Shekoofa et al., 2014) have been reported in this semi-arid grass model in relation to its drought tolerance. In this study, all selected sorghum genotypes belonging to one common species (*S. bicolor*) are phenologically similar, but differ in drought sensitivity and productivity, and showed indications to potentially differ in water use behaviour (Quazi et al., 2014). We imposed a long-term (early vegetative stage to early reproductive stage) water stress to investigate water use dynamics during vegetative growth and to examine changes in hydraulic anatomy. We hypothesized that: (i) the whole-plant xylem anatomy would vary among the genotypes differing in plant water use where transpiration rates and whole-plant transpiration would relate to vascular bundle and xylem vessel size and xylem-specific hydraulic conductivity; (ii) xylem anatomical traits would be influenced by productivity traits (e.g. growth and biomass) both within a genotype (under a well-watered and drought scenario) and among the genotypes, and (iii) the whole-plant xylem anatomy would respond to water limitation, but genotypic variation in anatomical plasticity could be expected and differences in drought adaptation may occur between different plant organs. More drought sensitive genotypes would undergo stronger modification in xylem traits (e.g. vessel size, number and distribution) along with stronger reduction in plant size and leaf area reduction than less drought sensitive genotypes.

2. Materials and methods

2.1. Plant materials, growth conditions and establishment

Four post-rainy genotypes including two grain sorghum (M35-1 and SPV1411) and two sweet sorghum (ICSV25280 and ICSSH58) genotypes were selected (Table S1). Sweet sorghum genotypes known for their low water requirement and tolerance to drought (Zegada-Lizarazu and Monti, 2013) are getting preference over grain sorghum for post-rainy farming (Vasilakoglou et al., 2011; Mathur et al., 2017). Sorghum seeds were obtained from the ICRISAT (Patancheru, India) and the experiment was conducted at the Indian Institute of Science Education and Research Pune (Pune, India) between Jul to Sep 2014.

Plastic pots (10 L) were filled with ~9 kg of *vertisol* supplemented with farm yard manure (1:50 v/v) and all pots were placed on bench top in a semi-controlled greenhouse environment (Table S2). Four seeds were sown per pot, irrigated with 500 ml of water immediately after sowing. All pots received 250 ml water on alternate days until the seedlings emerged uniformly. Pots were first thinned to two individuals per pot on 10th day after sowing (DAS) and then to a single plant per pot on 21 DAS. Urea was applied to each pot at the rate of 0.05 g kg^{-1} soil on 22 DAS. All plants were maintained at well-watered conditions by periodic watering until the onset of treatments.

2.2. Drought imposition and assessment of plant water use characteristics

On 26 DAS, pots were saturated with water and allowed to drain overnight. Following morning, each pot was enclosed by a white plastic sheet (< 2 mm thickness) wrapped around the stem to prevent soil evaporation. A slit was cut at the top of the sheet to permit seedling growth and was further sealed with a piece of clear adhesive tape to minimize water loss through the slit. The pots (control and drought) were randomized on the bench top (n = 5) and subsequently weighed between 0830 h to 0900 h using a 30-kg digital balance with 10 g precision. The first weighing at 27 DAS gave the pot water holding capacity weight of each pot and subsequently the treatments were imposed.

Plants were exposed to a dry-down treatment (Vadez and Sinclair, 2001; Devi et al., 2009; Kholova et al., 2010; Choudhary et al., 2013) where they were allowed to lose no more than 100 g of water per day. The difference in re-watering was primarily related to plant stature and allowed the imposition of relatively similar kinetics of stress imposition in these plants. Any excessive transpiration above this maximum daily water loss limit was added back to the pots, as previously described by Vadez and Sinclair (2001). This method prolonged the duration of stress allowing plants to undergo possible developmental changes and revealed temporal transpiration dynamics. The control plants were maintained close to 80% pot water holding capacity by daily pot weighing and bringing the pot weight every day to that level (i.e. 200 g below the saturated weight for the 9 kg pot). The dry-down was terminated on 56 DAS, when strong visible signs of drought-induced morphological changes including leaf wilting and curling etc. appeared. Further continuation of stress would have aggravated the signs leading to damaged plant tissues inappropriate for anatomical examination. For a given periodic interval of 5 days, whole-plant transpiration (T_{WP}) was calculated by adding up the daily T_{WP} values (plus any water added to pots). Total transpiration (T_{total}) for a plant was calculated by adding up the daily T_{WP} data of 29 days. All physiological, growth and anatomical measurements were carried on the plants used in this dry-down experiment.

2.3. Normalized transpiration ratio (NTR) and fraction of transpirable soil water (FTSW)

A separate dry-down experiment was run (n = 5) to determine *NTR* and *FTSW* and to investigate if the *FTSW* threshold has significant genotypic differences. Plants were grown, and treatments were imposed as aforementioned. However, instead of terminating drought on 56 DAS, the dry-down continued longer till the stress was severe. For each drying pot on each day, the transpiration ratio (*TR*) was calculated as: $TR = T_{dry}/T_{ww}$, where T_{dry} is transpiration for each drying pot and T_{ww} is the mean transpiration of well-watered pots. To account for variations due to plant size, second normalization was done by dividing each *TR* value over time by the average *TR* (TR_{ave}) for the first 3 or 4 d of the experiment when the drying pot was still in the well-watered range as (Choudhary et al., 2013): $NTR = TR/TR_{ave}$. This resulted in the normalized transpiration ratio (*NTR*) values which were centered on 1.0 while the plants were well-watered and started decreasing with progression in stress. The dry-down was terminated for a given genotype

when the transpiration of drought stressed plants fell equal to or below 10% (*NTR* < 0.1) of their control counterparts. The *FTSW* was calculated (Kholova et al., 2010; Choudhary et al., 2013), which represents the amount of volumetric soil water residue in the pot available for transpiration. After the experiment, the final pot weight was taken and *FTSW* on each day *n* was calculated as: (pot weight on day *n* − final pot weight)/(initial pot weight − final pot weight).

2.4. Leaf water status, photosynthesis and growth

Leaf relative water content (*LRWC*) was measured on 36, 46 and 56 DAS as: $LRWC = 100 \times [(fw-dw)/(tw-dw)]$ where, *fw* is the fresh weight of leaf discs, *tw* is the turgid weight after re-hydrating the discs for 24 h, and *dw* is the oven-dried weight of discs. Leaf CO_2 assimilation responses including net photosynthesis (P_n), stomatal conductance (G_s), and transpiration (*E*) rates were measured (between 1030 and 1130 h) on top, first fully expanded leaf at the middle of leaf lamina using a portable infra-red gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) with a standard LI-6400 broad-leaf cuvette (6 cm^2). See Appendix A.1.1 in Supplementary material for detailed methodologies on *LRWC* and leaf gas exchange, Appendix A.1.2 in Supplementary material for stomatal traits and foliar pigment levels, and Appendix A.1.3 in Supplementary material for plant growth and biomass yield characteristics.

2.5. Tissue sampling, processing and imaging for anatomy

Tissues were sampled for anatomy on 56 DAS. Leaf samples (n = 5) were harvested from the 10th leaf (numbered acropetally) from the center of leaf (excluding midvein and leaf margin) after normalizing by distance from the leaf tip. The vein counts (including major and minor veins) were made from images taken in 6 × 2 cm^2 wide areas of the sampled leaf specimens (abaxial leaf surface) by a Greenough stereo-microscope (Leica S8 APO, Leica Microsystems, Switzerland) at 4× magnification. For anatomy, the same leaf samples were cut into smaller size (1 × 1 cm), fixed in FAA (5% formalin, 5% acetic acid and 90% ethanol) and stored at 4 °C. The fixed samples were processed, and thin sections were cut, stained, mounted (detailed methodology described in Appendix A.1.4 in Supplementary material) and were finally observed and imaged at 10 and 20× using a Zeiss AxioImager.Z1 microscope (Carl Zeiss, Germany) equipped with a AxioCam HR camera (Carl Zeiss, Germany).

Root segments were sampled (during final harvest) from the secondary and tertiary nodal roots as they were younger than the primary nodal roots and seminal roots (Artschwager, 1948) and developed between 27–56 DAS. At 20 cm distance from the root tip, 5 cm long root segments (n = 4) were collected using sharp razor blades and were fixed in FAA for 2 weeks and later preserved in 70% ethanol at 4 °C. Tissues were processed and thin cross-sections were developed following the similar methods of leaf anatomy (Appendix A.1.4 in Supplementary material). Stem segments (n = 4) were collected at a height of 25–30 cm above soil surface, always at internodes. Since the difference in plant height within genotypes and between treatments (Tables 1 and S5) were not large (though statistically significant), tissue sampling was done without precise normalization by plant height. Stem segments were hand sectioned to ~50 μm thickness with a sharp razor blade, stained, mounted and were finally observed and imaged as described in Appendix A.1.4 in Supplementary material.

2.6. Image analyses and measurements of anatomical traits

Leaf anatomical features (Fig. S1) including leaf vein density (*LVD*), leaf thickness, interveinal distance (*IVD*), and cross-sectional area of vascular bundle (VB), xylem vessel, and phloem elements (Ph) were measured using the open-source ImageJ.exe v1.50i. The VBs of leaf major vein have two large meta xylem vessels (protoxylem was not

Table 1

Shoot and root growth variables, biomass and water use efficiency in four sorghum genotypes under control and drought stress conditions (on 57 DAS). Values are means ± SE (n = 5). Values with different letters in a single row indicate significant difference * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, no significant difference. LMA, leaf mass per area; NR1, number of primary nodal root; NR2, number of secondary nodal root; NR3, number of tertiary nodal root; WUE_{shoot}, water use efficiency based on shoot dry weight; WUE_{total}, water use efficiency based on total plant dry weight.

|	M35-1 (High WU)		SPV1411 (High WU)		ICSV25280 (Low WU)		ICSSH58 (Low WU)		
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	
Plant height (cm)	186.4 ± 9.5	133.7 ± 2.8	164.2 ± 9.5	134.5 ± 4	175.2 ± 7.4	135.2 ± 3.8	148.86 ± 3.5	117.2 ± 2.8	G^{***}, T^{***}, $G \times T^{ns}$
Stem diameter (mm)	13.79 ± 0.41	10.40 ± 0.29	13.16 ± 0.96	10.81 ± 0.24	11.68 ± 0.32	9.45 ± 0.17	11.39 ± 0.55	10.16 ± 0.45	G^{**}, T^{***}, $G \times T^{ns}$
Total leaf area (cm² plant⁻¹)	3806.7 ± 41.5	2105.5 ± 107.4	4042.5 ± 129.6	2234.8 ± 60.5	3056.8 ± 141.7	2285.6 ± 90	2833.4 ± 283.2	2329.8 ± 77.15	G^{***}, T^{***}, $G \times T^{ns}$
LMA (mg cm⁻²)	1.84 ± 0.16	1.88 ± 0.19	2.06 ± 0.13	2.12 ± 0.11	2.19 ± 0.14	2.08 ± 0.07	2.12 ± 0.06	2.36 ± 0.06	G^{*}, T^{ns}, $G \times T^{*}$
Shoot biomass (g dw plant⁻¹)	30.48 ± 1.3	17.85 ± 0.48	27.49 ± 3.6	19.01 ± 0.57	22.58 ± 1.7	18.02 ± 1.1	21.34 ± 2.7	17.56 ± 0.75	G^{*}, T^{***}, $G \times T^{ns}$
Root biomass (g dw plant⁻¹)	5.17 ± 0.34	3.51 ± 0.11	4.55 ± 0.71	3.48 ± 0.18	3.42 ± 0.28	3.34 ± 0.16	3.22 ± 0.59	3.25 ± 0.27	G^{*}, T^{*}, $G \times T^{ns}$
Root:shoot	0.17 ± 0.01	0.19 ± .01	0.18 ± 0.05	0.18 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.17 ± 0.04	0.19 ± 0.02	G^{ns}, T^{ns}, $G \times T^{ns}$
Root volume (cm³ plant⁻¹)	21.72 ± 1.2	8.79 ± 0.51	17.69 ± 3.2	8.49 ± 0.55	11.06 ± 0.75	7.15 ± 0.33	10.70 ± 2.3	7.59 ± 0.61	G^{***}, T^{***}, $G \times T^{*}$
Root surface area (cm² plant⁻¹)	1421.1 ± 74.9	675.2 ± 41	1158.5 ± 200	694.7 ± 60.4	992 ± 79	600.9 ± 40.3	873.3 ± 192.8	610.45 ± 26.14	G^{*}, T^{***}, $G \times T^{ns}$
NR1 (no. plant⁻¹)	9.60 ± 0.98	9.80 ± 0.37	10.20 ± 1.28	10.20 ± 0.97	10.40 ± 0.81	10.20 ± 0.37	10 ± 1.10	8.60 ± 0.40	G^{ns}, T^{ns}, $G \times T^{ns}$
NR2 (no. plant⁻¹)	8.8 ± 0.80	7.0 ± 0.54	5.8 ± 0.58	6.2 ± 0.80	5.6 ± 0.4	4.6 ± 0.51	4.6 ± 0.68	4.4 ± 0.24	G^{***}, T^{ns}, $G \times T^{ns}$
NR3 (no. plant⁻¹)	13.2 ± 0.58	10 ± 1.14	11.2 ± 0.49	8.8 ± 0.58	7.8 ± 0.73	6.2 ± 0.58	7.2 ± 0.37	6.6 ± 0.51	G^{***}, T^{***}, $G \times T^{ns}$
Total plant biomass (g dw plant⁻¹)	35.64 ± 1.3	21.36 ± 0.46	32.04 ± 3.6	22.49 ± 0.64	25.99 ± 1.9	21.35 ± 1.1	24.56 ± 2.4	20.81 ± 0.68	G^{**}, T^{***}, $G \times T^{*}$
WUE_{shoot} (g dw kg⁻¹)	8.49 ± 0.12	8.82 ± 0.39	8.71 ± 0.31	9.66 ± 0.23	8.71 ± 0.3	8.94 ± 0.48	8.67 ± 0.32	9.25 ± 0.3	G^{ns}, T^{*}, $G \times T^{ns}$
WUE_{total} (g dw kg⁻¹)	10.04 ± 0.2	10.76 ± 0.43	10.4 ± 0.36	11.57 ± 0.31	10.09 ± 0.36	10.70 ± 0.5	10.17 ± 0.56	11.06 ± 0.35	G^{ns}, T^{***}, $G \times T^{ns}$

considered) and their cross-sectional areas were combined to express the total cross-sectional area of one metaxylem pair. The equivalent circle diameter (D) was calculated from the total cross-sectional area (A) of a metaxylem pair as (Scholz et al., 2013): $D = \sqrt{4 \times A/\pi}$. The Hagen-Poiseuille equation was applied to calculate the predicted xylem-specific hydraulic conductivity of the major vein (Kh_{major}) according to Scholz et al. (2013) as: $Kh_{major} = \pi D^4/128\eta$, where η is the viscosity of water (10^{-9} MPa). For a minor vein, D was calculated for the total cross-sectional area of a group of short-articulated xylem cells (Fig. S1A) and then the Hagen-Poiseuille equation was applied to calculate the predicted xylem-specific hydraulic conductivity (Kh_{minor}).

For a stem cross-section, multiple overlapping stereomicroscopy images were taken, and the images were assembled and stitched together using Microsoft image composite editor 1.3.4. Radial strips were observed at $10\times$ magnification to characterize and categorize stem VBs into six types (modified from Artschwager, 1948) based on VB and xylem vessel size and structure (Fig. S2) and the number for each VB type was counted per cross-section. The high-resolution images (20 and $40\times$) were used to randomly select five VBs from each type across a radial strip (from pith to rind) and measurements were made for different traits including the cross-sectional area of VB, late metaxylem (MX) and Ph element (protoxylem and lacuna were not considered). These primary measurements were used to derive total late MX and Ph area per stem cross-section and the xylem-specific hydraulic conductivity of stem (Kh_{stem}). The equivalent circle diameter (D) and xylem-specific hydraulic conductivity was calculated for each VB type (taking mean of five different MX pair for each VB type) and then multiplied by the number of the VB to achieve cumulative xylem-specific hydraulic conductivity for each VB type. Finally, all cumulative (for each VB type) hydraulic conductivity values were added up to express Kh_{stem} per stem cross-sectional area.

Images of root cross-sections were analysed to measure xylem traits including the late MX number and cross-sectional area (Fig. S3). These traits were measured for both secondary and tertiary nodal roots. Since none of the root xylem traits showed strong genotype effects, and the treatment effects were modest, the Hagen-Poiseuille conductivity of root xylem was not calculated.

2.7. Statistical analysis

Statistical analysis was performed using STATISTICA v7.0 (StatSoft. Inc., USA). Periodic transpiration data was analysed using repeated measure ANOVA with weighing dates as repeated measures. Two-way ANOVA was used for comparing genotype (G), treatment (T) and genotype $\times$ treatment ($G \times T$) interactions and when a significant $G \times T$ interaction was found, a one-way ANOVA was performed to assess T effect within each single genotype. Multi-factorial ANOVA was performed for stomata and stem anatomical traits. For FTSW threshold analysis, the user defined function of OriginPro (9.0) was used. The change of NTR was plotted against the FTSW using a sigmoidal non-linear model (Muchow and Sinclair, 1991): $Y = 1/\{1 + a*exp(-b*X)\}$, where Y is the dependant variable (NTR), X is FTSW and 'a' and 'b' are empirical coefficients estimated by the non-linear model. The FTSW threshold was calculated (using find X from Y function of non-linear regression) as the fraction of total transpirable soil water for which the NTR equals to 0.95 (Sadras and Milroy, 1996). Relationships between traits were analysed using a general linear model and parametric correlations (Pearson's coefficients, r). Correlations were calculated separately for control and drought conditions to understand the effects of water stress on the relationships. We performed linear regression (using concatenate fit mode of OriginPro 9.0) between log_{10}-transformed values of stem xylem vessel diameter and basipetal stem sampling height to test if plant height had a significant affect on stem xylem size.
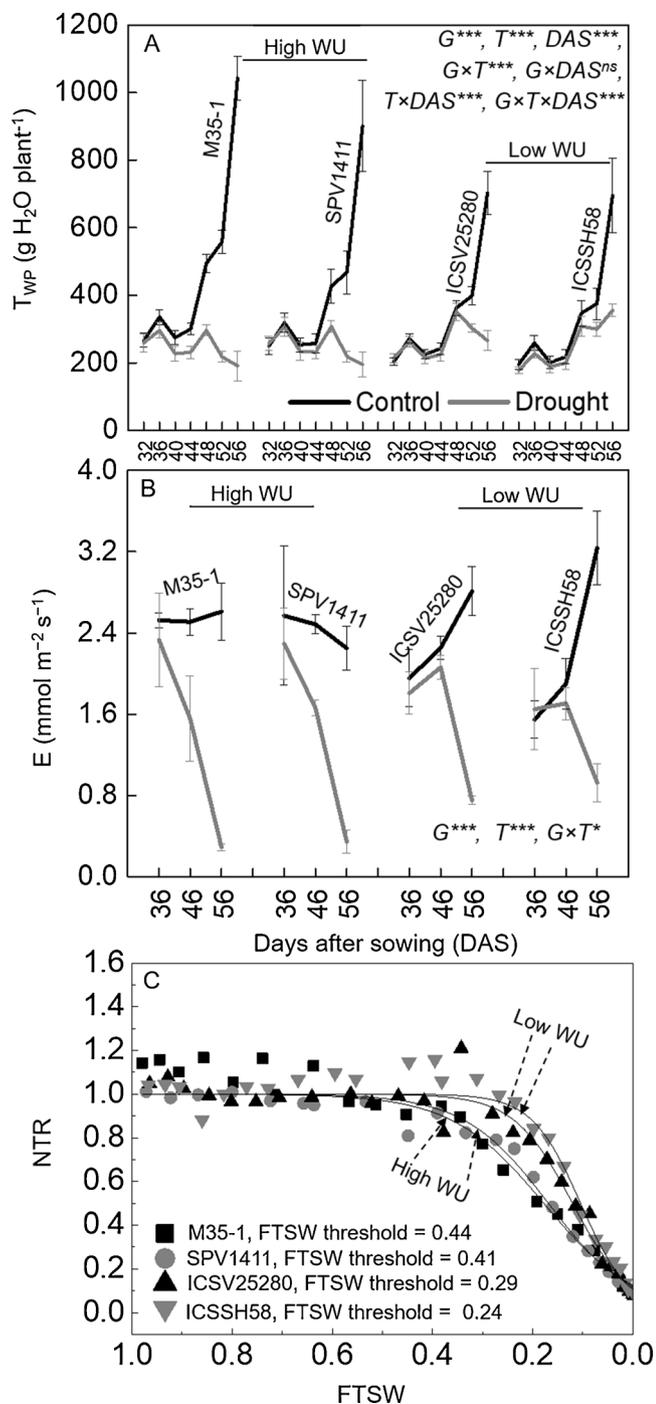
Fig. 1. Transpiration profiles in four sorghum genotypes under control and drought stressed conditions. (A) Periodic whole-plant transpiration (T_{WP}) for the period finishing at the date when the data are plotted. For instance, water transpired at 32 DAS corresponds to the period between 27 and 32 DAS. (B) Rates of transpiration measured on three periodic intervals (36, 46 and 56 DAS). (C) Relationships between normalized transpiration ratio (NTR) and fraction of transpirable soil water (FTSW). The FTSW breakpoints (X_0) where NTR initiated its decline were calculated using a sigmoidal non-linear model (Muchow and Sinclair, 1991). The regression lines were drawn by fitting NTR to FTSW data above and below the respective threshold for transpiration decline in each genotype. Means ± SE (n = 5). The AVOVA results are given for genotype (G), treatment (T), days after sowing (DAS), genotype × treatment (G × T), genotype×DAS (G×DAS), treatment × DAS (T×DAS), and genotype × treatment × DAS (G × T × DAS) interaction. Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and ns, non- significant.

3. Results

3.1. Plant water use characteristics

The well-watered plants of grain sorghum genotypes (M35-1 and SPV1411) consistently exhibited higher T_{WP} compared to sweet sorghum genotypes (ICSV25280 and ICSSH58) for any given periodic interval (Fig. 1A). Progression in time (DAS) had significant effects on T_{WP} showing almost 3.6-fold increase in T_{WP} from 32 to 56 DAS, on an average in all genotypes under well-watered conditions. For a considerably longer duration (as evident on 36 and 46 DAS), transpiration rates (E) were significantly higher in M35-1 and SPV1411 and showed modest change over time (Fig. 1B). In contrary, well-watered sweet sorghum genotypes had significantly lower E, though the rates later increased significantly (Fig. 1B). Cumulative or total transpiration (T_{total}) significantly varied among the well-watered genotypes, whereby the grain sorghum genotypes exhibited a higher T_{total} than the sweet sorghum genotypes (Fig. S4). It is plausible to henceforth refer to them as high (M35-1 and SPV1411) and low (ICSV25280 and ICSSH58) water use ('high WU' and 'low WU') genotypes or groups.

Despite limited soil water, the low WU genotypes maintained T_{WP} values close to their well-watered counterparts till 48 DAS, whereas a large draw-down in T_{WP} was evident in the high WU group (Fig. 1A). Decrease in E was also larger in the high WU genotypes, whereby transpiration rates dropped by ~86.2% from 32 to 56 DAS compared to the ~51.1% decrease of the low WU genotypes (Fig. 1B). Transpiration response to soil drying shows that normalized transpiration ratio (NTR) started declining at fraction of transpirable soil water (FTSW) values ranging between 0.24 and 0.44 (Fig. 1C), and the FTSW threshold values were significantly higher for the high WU genotypes than for the low WU counterparts.

3.2. Leaf water status and photosynthesis

A significant treatment (T) effect was evident on leaf water status showing loss in LRWC with progressive soil water stress, which was more pronounced in high WU genotypes than the low WU counterparts (Fig. 2A). On 56 DAS, LRWC in high and low WU groups dropped to ~48.8 and ~68.7%, respectively compared to their well-watered counterparts. When well-watered, net photosynthetic rates (P_n) were significantly higher in the high WU group for a considerably longer duration (as evident on 36 and 46 DAS; Fig. 2B). However, the rates later dropped sharply in the high WU group unlike the low WU counterparts that showed modest changes in P_n over the treatment (Fig. 2B). When water-stressed, loss in photosynthesis was more pronounced in high WU genotypes than the low WU group. On 56 DAS, P_n decreased to ~93.7 and ~63.9% in the high and low WU groups, respectively (Fig. 2B). The genotypes did not vary in the stomatal traits and no T effect was evident. Though the pigment (chl a, chl b and total chl)) levels decreased on 56 DAS (Table S3), the low WU genotypes maintained relatively higher pigments under drought than the high WU group.

3.3. Plant growth, biomass and water use efficiency

When well-watered, the high WU group exhibited larger plant stature and exceeded the low WU group in plant height, stem diameter, total leaf area, and shoot dry biomass (Table 1). However, water depletion significantly affected growth characteristics in all genotypes, but the effect was stronger in the high WU group (Table 1). Drought decreased the total leaf area by ~44.7 and ~21.4% in the high and low WU groups, respectively. Both shoot and root dry biomass decreased significantly under drought, causing a strong reduction in total plant dry biomass especially in the high WU group (40.06% and 29.8% for M35-1 and SPV1411, respectively) compared to the low WU counterpart (~16.5%). The number of secondary nodal root (NR2) was higher in the high WU group, but no T effect was evident. The number of
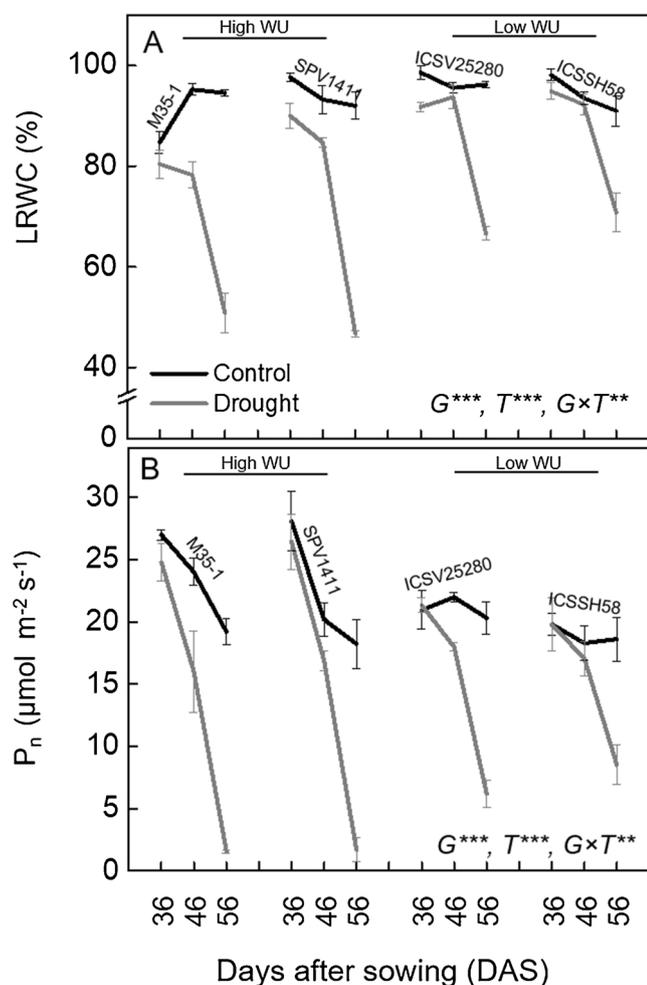
Fig. 2. Periodic changes in leaf water status and net photosynthesis in four sorghum genotypes under control and drought stressed conditions. (A) Leaf relative water content (*LRWC*) and (B) net photosynthetic rate (P_n). Means ± SE (n = 3). The AVOVA results are given for genotype (*G*), treatment (*T*), and genotype×treatment (*G×T*) interaction. Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and *ns*, non- significant.

tertiary nodal root (NR3) was also significantly higher in the high WU group, irrespective of water regimes (Table 1). Though root volume was comparatively higher (~44.7%) in the well-watered high WU group, they showed a stronger reduction (~55.7%) under drought than the low WU group (~32.2%), and the trend was similar also for root surface area. The WUE (*WUE*_shoot and *WUE*_total) values marginally increased under drought but did not differ among the genotypes (Table 1).

3.4. Leaf anatomy

Interveinal distance (*IVD*) increased in all genotypes under drought and leaf thickness was significantly greater in the high WU group under both control and water-stressed conditions (Table S4). Vascular bundle (VB) and xylem vessel cross-sectional area in both minor and major veins showed significant *G* and *T* effects, whereby the area of both VB and xylem vessels significantly increased under drought in all genotypes (Fig. 3). The high WU group had distinctively larger VB (Table S4) and xylem cross-sectional area (Fig. 3A–D) under both control and water-limited conditions. The xylem-specific hydraulic conductivity calculated for both major and minor veins, significantly increased under drought, whereby the high WU group had higher Kh_{major} and Kh_{minor} than the low WU genotypes (Fig. 4B, C). The phloem (Ph) cross-

sectional area (calculated for major veins only) also significantly increased under water stress in all genotypes (Fig. 3F) leading to a ca. 21% higher mean Ph area in the high WU group relative to the low WU counterpart (Fig. 3E). No drought effect was found on leaf xylem to phloem area ratio (*X:Ph*_major), but a marginal *G* effect was evident (Fig. 4A).

3.5. Stem anatomy

The genotypes differed significantly in the size and number of stem VB (described in Appendix A. 2.1 in Supplementary material) and meta xylem (MX) vessel. The size of MX vessel (across different VB types) showed significant genotypic variation, whereby the vessels were distinctively larger in well-watered individuals of the high WU group than the low WU counterparts (Fig. 5). Drought imposition decreased the size of MX vessels, only in large and medium sized VBs (T1–T3), and the effect was more pronounced in the high WU group than the low WU genotypes (Fig. 5). The basipetal stem sampling height had an influence and explains 19.6% of the difference in stem MX size between control and drought treatments (Fig. S7). The high WU group showed significantly larger total MX area (Fig. 6A) and equivalent circle diameter (Fig. S6) relative to the low WU counterparts when well-watered. However, the high WU genotypes showed greater reduction in MX area under drought than the low WU group (Fig. 6A). A similar trend was also recorded for stem xylem-specific hydraulic conductivity (Kh_{stem}) (Fig. 6D). The high WU group showed greater Ph cross-sectional area for each VB type (Fig. 5E–H) and even greater total Ph cross-sectional area (Fig. 6B) when compared to the low WU counterpart. The size of phloem elements did not significantly change under drought (Fig. 6B) and the ratio of total MX to Ph cross-sectional area did not show any genotypic difference and treatment effects (Fig. 6C). Drought stress intensified lignification of stem VBs, which was more pronounced at the rind region (Fig. S8). Lignification was observed in the walls of sclerenchymatic fibre cells surrounding the VB in all four genotypes but was more conspicuous in genotype M35-1 and SPV1411 (Fig. S8).

3.6. Xylem anatomy of secondary and tertiary nodal roots

Root xylem traits did not show significant genotypic variation and were not strongly modified by drought (Fig. 7A, B). The number of MX vessels in NR2 showed a modest decrease under drought (Fig. 7C), but in NR3, the number either moderately decreased (in high WU group) or increased (ICSV25280) or remained unchanged (ICSSH58) (Fig. 7E). In NR2, total MX cross-sectional area showed a modest drought effect, whereby a decrease in MX area was evident in all genotypes (Fig. 7D). Total MX area in NR3 was slightly higher in the high WU group compared to the low WU group when well-watered. Under drought, the total MX area of NR3 decreased only in M35-1 but increased in all other genotypes (Fig. 7F).

3.7. Mutual interrelations between traits

The interrelations between plant water use and different hydraulic traits were examined under both well-watered and water stressed scenario (Table 2). Different variables of transpiration including *E* and T_{WP} positively correlated with leaf (minor vein) and stem xylem area, Kh_{minor}, Kh_{stem}, and total MX area of NR3 under well-watered scenario. Under drought stress, leaf-level traits including intervenial distance (*IVD*), leaf xylem area (minor), Kh_{major}, and Kh_{minor} positively correlated with early T_{WP} and *E* (36 DAS). These relationships were either lost or turned negative (for traits like early *E*, Kh_{major}, and Kh_{minor}) when related to late T_{WP} and *E* (56 DAS). Intervenial distance (*IVD*) correlated positively with early T_{WP} and *E* under drought, but not with late T_{WP}. Late transpiration rates negatively correlated with initial T_{WP}. Early (on 36 DAS) transpiration variables and net photosynthesis (P_n) correlated positively with total leaf area, total plant biomass, root
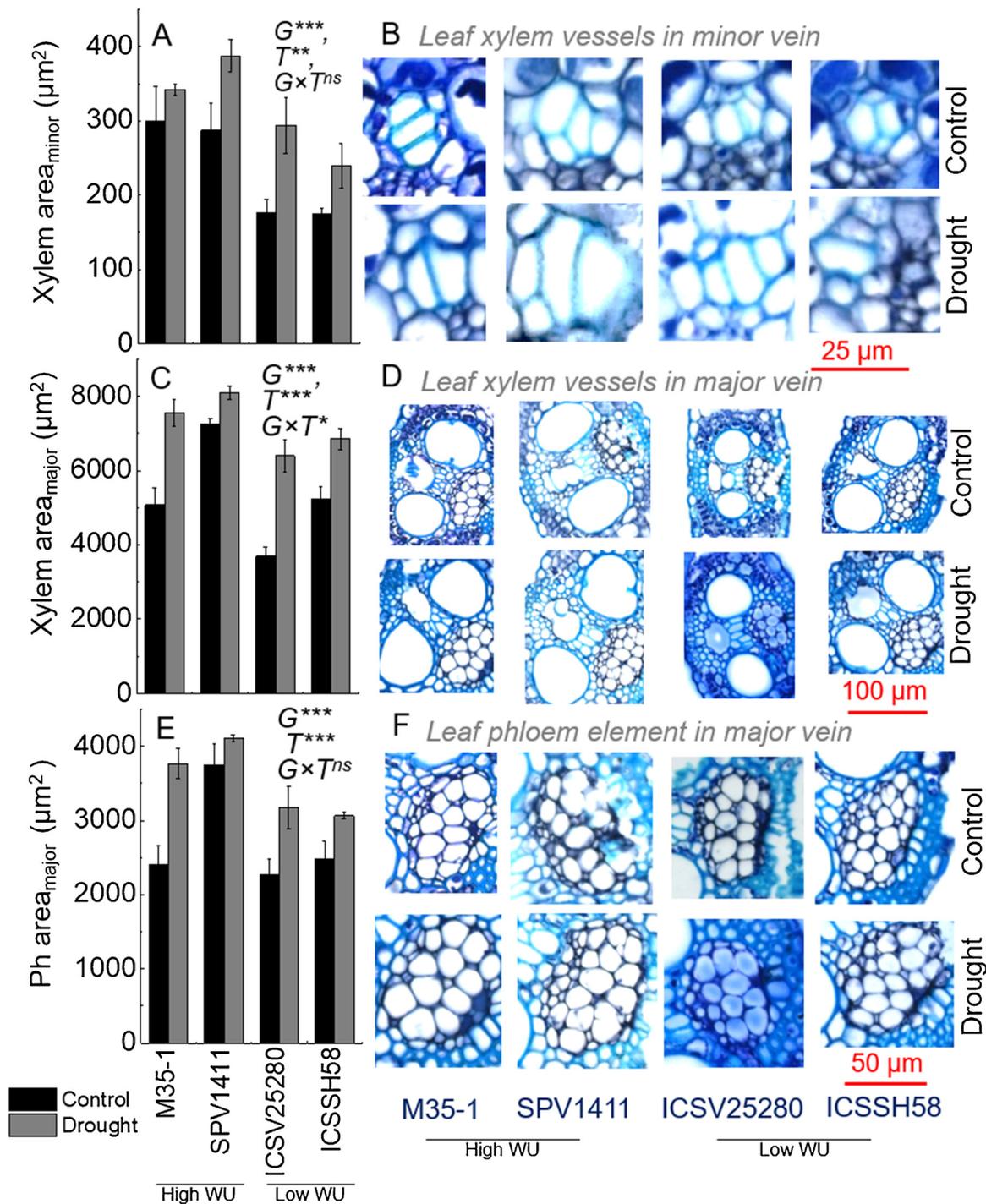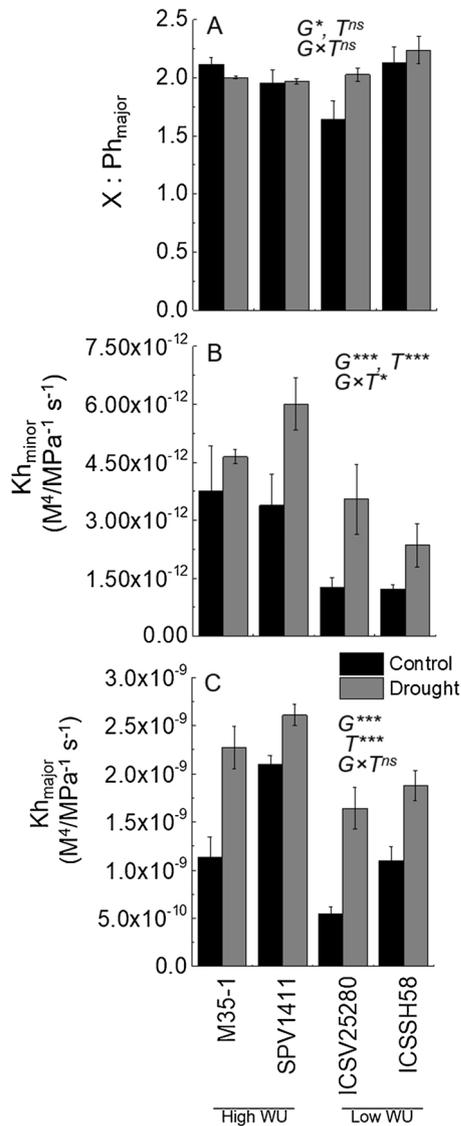
Fig. 3. Leaf anatomical traits in four sorghum genotypes under control and drought stressed conditions (on 56 DAS). (A, C) Xylem vessel cross-sectional area in minor and major veins. (B, D) Microscope images of xylem vessels (stained light greenish blue) in minor and major veins. (E) Phloem (Ph) cross-sectional area and (F) microscope images of Ph in major veins (see Fig. S1 for a comprehensive view of major and minor veins and vessel anatomy). Means ± SE (n = 3–4). The AVOVA results are given for genotype (G), treatment (T), and genotype × treatment (G×T) interaction. Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and *ns*, non- significant (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

volume and numbers of NR2 and NR3 under well-watered conditions. However, the biomass traits were not correlated to either T_{WP} or E when water was limited. Root surface area was negatively correlated to late T_{WP} under water deficit conditions.

Relationships between plant growth and anatomical traits were examined for both water regimes (Table 2). Leaf and stem xylem area strongly correlated with total leaf area and plant biomass, root surface area and NR3 number under well-watered conditions. Although stem and leaf xylem traits did not correlate to biomass under drought, the

nodal root (NR2 and NR3) numbers positively correlated to stem and leaf xylem and Ph area. Significant positive correlations were found between the anatomical traits of leaf and stem under well-watered conditions (Table 2). Leaf xylem area varied positively with stem MX area and Kh_{stem} and both Kh_{major} and Kh_{minor} correlated with Kh_{stem}. The xylem and phloem area positively correlated for both leaf and stem tissue. The MX area of NR3 positively correlated with leaf xylem area (minor vein) and Kh_{minor}, but not with stem. The MX area of NR2 did not correlate either with leaf or stem traits. Under drought,

Fig. 4. Leaf anatomical traits in four sorghum genotypes under control and drought stressed conditions (on 56 DAS). (A) Ratio of leaf xylem to phloem cross-sectional area ($X:Ph_{major}$) in major veins. (B) Predicted xylem-specific hydraulic conductivity of minor (Kh_{minor}) and (C) major (Kh_{major}) veins. Means ± SE (n = 3–4). The AVOVA results are given for genotype (G), treatment (T), and genotype × treatment interaction ($G \times T$). Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and ns, non- significant.

relationships within leaf-level anatomical traits turned stronger compared to the well-watered scenario and the same was true for the stem-level anatomical traits (Table 2). However, the inter-relationships between leaf and stem anatomical traits turned weaker under drought, unlike well-watered scenario.

4. Discussion

### 4.1. Plant water use behaviour affects transpiration response to soil drying

Higher whole-plant transpiration recorded in high WU genotypes was not only driven by a higher plant stature (plant height, leaf area etc.) but also by higher rates of transpiration over a prolonged vegetative growth period. The opposite scenario was evident for low WU genotypes, which can explain their low water use over a long period of vegetative growth. Although E increased in the low WU group by the end of vegetative growth, the existing gap in the magnitude of T_{WP}

between the two groups persisted, indicating that plant stature might have more influence on T_{WP} with progression in vegetative maturity. The high WU group exhibited a greater magnitude of transpiration when well-watered but showed distinctively greater stomatal sensitivity when exposed to drought. The early transpiration profile indicates that the high WU group kept higher E and T_{WP} for a considerable time under drought, followed by a highly sensitive stomatal response to $FTSW$, while the low WU counterparts initially displayed lower E and T_{WP} under drought. Transpiration dropped relatively early in the high WU genotypes upon progressive soil drying, probably to avoid considerable water loss (Kholova et al., 2010; Zaman-Allah et al., 2011) and xylem embolism beside the biochemical cues known to cause stomatal closure (Brodribb, 2009; Lens et al., 2016).

4.2. Hydraulic anatomy varies with genotypic ability to use water and grow

Transpiration rates have been related to the hydraulic conductance of leaf (Brodribb and Jordan, 2008), stem (Martorell et al., 2014) or roots (Rodríguez-Gamir et al., 2016) in woody angiosperms, but such framework has not been widely tested in herbaceous monocots (Gleason et al., 2017). This study demonstrated that the low WU group had lower xylem cross-sectional area and predicted xylem-specific hydraulic conductivity at the leaf (Kh_{major} and Kh_{minor}) and stem (Kh_{stem}) level, indicating hydraulic restrictions to aboveground water flow was sufficiently large to result in lower transpiration rates, despite ample soil water availability. In contrary, we identified larger VBs and MX cross-sectional area (indicating higher transport efficiency) in the high WU group in both leaves and stems, which might have facilitated greater E and T_{WP}. Relationships between leaf and stem hydraulic anatomy of well-watered individuals were strong, indicating a fine coordination and a possible equilibrium between these two components of plant hydraulic continuum (Hubbard et al., 2001; Meinzer et al., 2008; Rodríguez-Gamir et al., 2016), whereas root traits fitted well within this continuum at large morphological scale, but not at a level of anatomy. Hydraulic anatomy even induced limitation on transpiration, when water was limited. The low WU genotypes displaying low hydraulic conductance capacity, transpired less compared to the high WU group, and saved soil moisture for later usage. Consequently, final T_{WP} values negatively correlated to xylem-specific hydraulic conductivity, indicating that genotypes with lower conductance (due to hydraulic limitations) might sustain transpiration for longer periods. Besides, a less efficient but safe conductive pathway has significant broader implications under drought with respect to embolism resistance (Urli et al., 2013).

In this study, xylem size and predicted xylem-specific hydraulic conductivity (for both leaf and stem) correlated positively with leaf area and biomass, which is in accordance with other reports showing xylem hydraulics as important determinants of growth performance (Kondoh et al., 2006; Fichot et al., 2009). An efficient water supply to leaves may allow higher gas exchange rates, which is in agreement with a high correlation between maximum photosynthetic rates and maximum hydraulic conductance across a wide range of growth forms (Brodribb et al., 2007). Similar correlations were observed in our study between net photosynthesis, growth, biomass and xylem traits under well-watered conditions. We considered the initial (36 DAS) P_n values for correlation analysis, because the age effects in fully mature leaves are known to cause a photosynthetic reduction in sorghum (Prasad et al., 2009). We found no genotypic difference in WUE and unlike well-watered situation, the growth and biomass traits did not correlate to xylem size and xylem-specific hydraulic conductivity when plants were water stressed. This indicates little evidence for a superior hydraulic architecture and water use strategy for the given scenario of water stress level and the time-frame within which these observations were made.
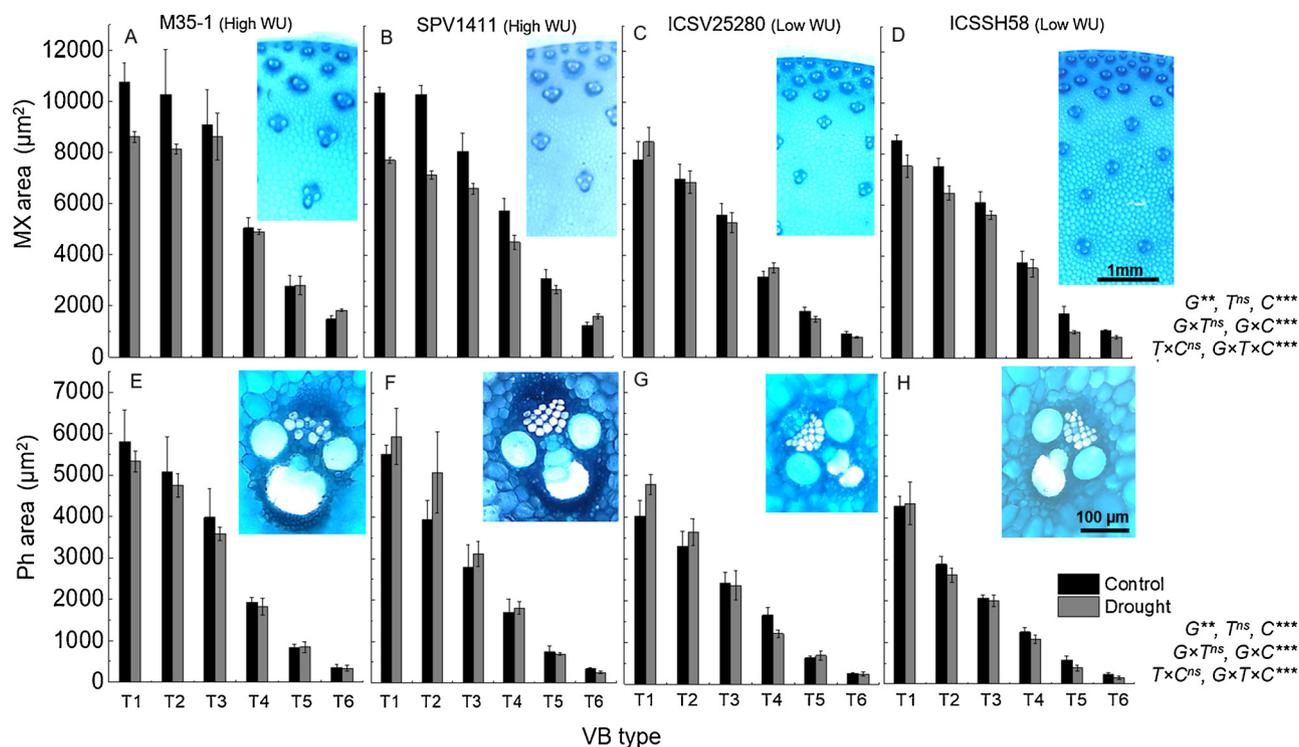
Fig. 5. Stem anatomical traits in four sorghum genotypes under control and drought stressed conditions (on 56 DAS). (A–D) Metaxylem (MX) and (E–H) phloem (Ph) cross-sectional area in different vascular bundle (VB) type. Means ± SE (n = 4). The AVOVA results are given for genotype (G), treatment (T), VB category (C), genotype × treatment (G×T), genotype × VB category (G×C), treatment × VB category (T×C) and genotype × treatment × VB category (G×T×C) interaction. Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and ns, non- significant. Microscope images in inset show representative (A–D) stem cross-section (partial overview) and (E–H) magnified VB-Type 1 (T1) of the respective genotype under well-watered conditions (see Fig. S2 for details on stem VB anatomy and size classification).
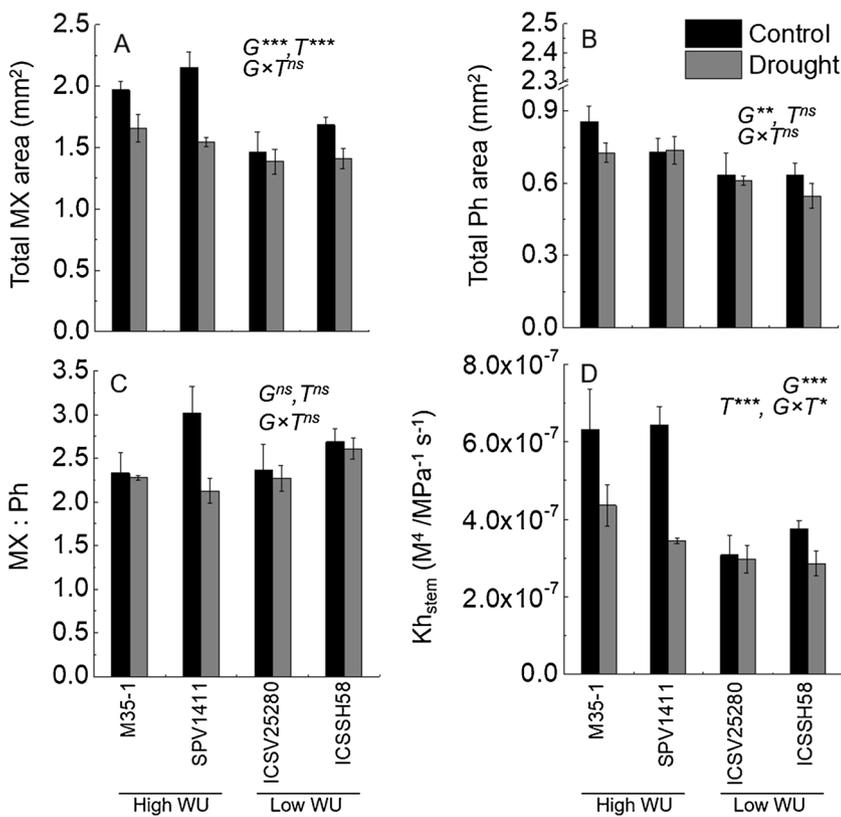
Fig. 6. Stem anatomical traits in four sorghum genotypes under control and drought stressed conditions (on 56 DAS). (A) Total metaxylem (MX) and (B) total phloem element (Ph) cross-sectional area per stem cross-section. (C) Ratio of MX to Ph cross-sectional area and (D) predicted xylem-specific hydraulic conductivity of stem (Kh_{stem}). Means ± SE (n = 4). The AVOVA results are given for genotype (G), treatment (T) and genotype × treatment interaction (G×T). Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and ns, non- significant.
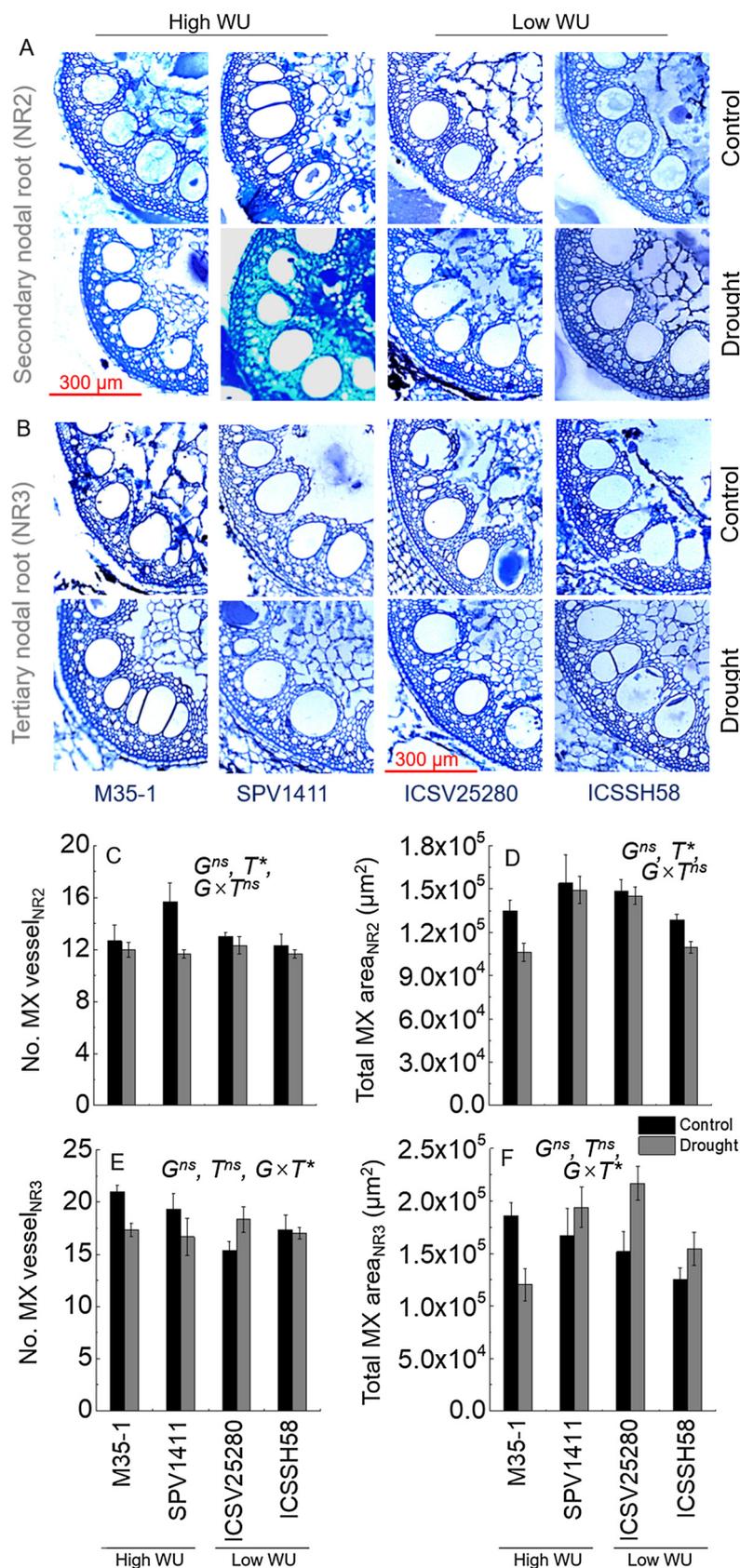
Fig. 7. Root anatomical traits in four sorghum genotypes under control and drought stressed conditions (on 56 DAS). (A) Partial microscopic overview of secondary (NR2) and (B) tertiary (NR3) nodal root cross-sections (at 20 cm distance from the root tip). (C) Number of late metaxylem (MX) vessels in NR2 and (E) NR3. (D) Total MX cross-sectional area in NR2 and (F) NR3. Means ± SE (n = 4). The ANOVA results are given for genotype (G), treatment (T), and genotype × treatment ($G \times T$) interaction. Levels of significance are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and ns, non- significant.

Table 2

Pearson correlation coefficients for linear relationships ($P < 0.05$) between 23 plant traits measured across four sorghum genotypes under control and drought (coloured panels) conditions. Non-significant correlations are indicated as '*ns*'. See the text for trait description. T_{WP}, whole-plant transpiration; P_n, net photosynthetic rate; E transpiration rate; LA, total leaf area; BM, total plant dry biomass; RV, root volume; RSA, root surface area; NR2 no., number of secondary nodal root; NR3 no., number of tertiary nodal root; LVD, leaf vein density; IVD, interveinal distance; X_{major}, xylem cross-sectional area in major vein; X_{minor}, xylem cross-sectional area in minor vein; Kh_{major}, xylem-specific hydraulic conductivity of major vein; Kh_{minor}, xylem-specific hydraulic conductivity conductance of minor vein; Ph_{major}, phloem element cross-sectional area in major vein; $X{:}Ph_{major}$, ratio of xylem to phloem area in major vein; MX_{stem}, total metaxylem area in stem cross-section; Kh_{stem}, xylem-specific hydraulic conductivity of stem; Ph_{stem}, total phloem element area in stem cross-section; MX_{NR2}, total metaxylem cross-sectional area in NR2; MX_{NR3}, total metaxylem cross-sectional area in NR3.

	T_{WP} (36 DAS)	T_{WP} (56 DAS)	P_n (36 DAS)	E (36 DAS)	LA	BM	RV	RSA	NR2 no.	NR3 no.	LVD	IVD	X_{major}	X_{minor}	Kh_{major}	Kh_{minor}	Ph_{major}	$X{:}Ph_{major}$	MX_{stem}	Kh_{stem}	Ph_{stem}	MX_{NR2}	MX_{NR3}
T_{WP} (36 DAS)		0.83	0.88	0.88	0.82	0.64	0.54	ns	0.77	0.86	ns	ns	ns	0.59	ns	0.58	ns	ns	ns	0.69	ns	ns	0.73
T_{WP} (56 DAS)	-0.59		ns	0.73	0.67	0.87	0.58	ns	0.61	0.78	ns	ns	ns	0.53	ns	0.53	ns	ns	0.54	0.63	ns	ns	0.52
P_n (36 DAS)	0.58	-0.71		0.96	0.77	0.52	0.56	ns	0.62	0.80	ns	ns	ns	0.68	0.63	0.76	ns	ns	0.57	0.71	ns	ns	0.68
E (36 DAS)	ns	-0.74	0.73		0.79	0.54	0.53	ns	0.56	0.73	-0.55	ns	ns	0.67	0.61	0.70	0.51	ns	0.50	0.65	ns	ns	0.69
LA	ns	ns	ns	ns		0.68	ns	0.52	ns	0.55	0.79	ns	ns	0.50	0.64	0.64	0.60	0.59	ns	0.65	0.73	ns	ns
BM	ns	ns	ns	ns	0.53		ns	0.64	0.56	ns	0.64	ns	ns	ns	0.69	ns	0.60	ns	ns	0.54	0.69	ns	0.51
RV	ns	ns	0.52	0.64	ns	ns		0.96	0.60	0.74	ns	ns	ns	0.65	ns	0.61	ns	ns	0.69	0.73	ns	ns	ns
RSA	ns	-0.52	ns	0.70	ns	ns	0.70		0.53	0.61	ns	ns	ns	0.60	ns	0.53	ns	ns	0.54	0.61	ns	ns	ns
NR2 no.	0.59	ns	0.73	ns	ns	ns	0.62	ns		0.84	ns	ns	ns	ns	ns	ns	ns	ns	0.54	0.60	ns	ns	0.58
NR3 no.	ns	ns	0.51	ns	ns	ns	ns	ns	0.86		ns	ns	ns	0.65	ns	0.63	ns	ns	0.74	0.85	0.70	ns	0.56
LVD	ns	0.52	-0.66	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	-0.55	0.55	ns	ns	ns	ns	-0.54	ns
IVD	0.56	ns	0.64	ns	ns	ns	ns	ns	0.59	0.55	-0.74		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
X_{major}	0.62	ns	ns	ns	ns	ns	ns	ns	0.60	ns	0.61	ns		ns	0.97	ns	0.90	ns	0.62	0.50	ns	ns	ns
X_{minor}	ns	ns	0.57	0.58	ns	ns	0.67	0.54	0.58	0.61	ns	0.58	0.59		ns	0.87	ns	ns	0.53	0.71	0.54	ns	0.70
Kh_{major}	0.70	-0.59	0.59	0.52	ns	ns	ns	ns	ns	ns	ns	ns	0.86	0.59		0.58	0.92	ns	0.71	0.61	ns	ns	ns
Kh_{minor}	0.60	-0.58	0.69	0.64	ns	ns	ns	ns	0.50	ns	-0.64	0.77	0.72	0.75	0.69		ns	ns	0.56	0.52	ns	ns	0.60
Ph_{major}	0.73	-0.68	0.77	0.70	ns	ns	ns	ns	0.54	ns	-0.50	0.65	0.80	0.66	0.92	0.87		ns	0.67	0.51	ns	ns	ns
$X{:}Ph_{major}$	ns	0.55	-0.73	-0.69	ns	ns	ns	-0.51	-0.50	ns	0.64	-0.59	ns	ns	ns	-0.78	-0.65		ns	ns	ns	ns	ns
MX_{stem}	ns	ns	ns	ns	ns	ns	ns	ns	0.52	ns	0.58	ns	ns	ns	ns	ns	ns	ns		0.89	0.55	ns	ns
Kh_{stem}	ns	ns	ns	ns	-0.54	ns	0.54	ns	0.53	0.58	ns	0.61	ns	ns	ns	ns	ns	ns	0.95		0.57	ns	ns
Ph_{stem}	0.63	ns	ns	ns	ns	ns	0.51	ns	ns	0.52	ns	0.76	0.69	ns	0.59	0.56	0.56	ns	0.73	0.69		ns	ns
MX_{NR2}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.69	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		ns
MX_{NR3}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.78	

4.3. Xylem and phloem anatomy are finely correlated

Our study shows positive correlations between xylem and phloem cross-sectional area in both leaf and stem tissue irrespective of watering regimes indicating structural investment of both vascular tissues varied proportionately. This finding is likely related to the matching requirements of water supply and carbon transport (Savage et al., 2016). The correlation was relatively stronger for leaves than stems, which might be due to minor difference in functional aspects of both organs. Our results contrast with the 'balanced structural-investment' concept, which predicts that wider xylem conduits may function with less phloem (Hölttä et al., 2009; Savage et al., 2016). However, more empirical evidence on xylem-phloem anatomy and physiology is needed to understand their interactions in herbaceous monocots. Interestingly, drought-induced qualitative changes in phloem area were similar to xylem in both leaves and stems, which led to no change in the xylem to phloem ratio under drought. This reinforces the tight mutualistic existence of these two vascular bundle components as emphasised by Savage et al. (2016).

4.4. Drought induces differential organ-specific anatomical plasticity across plant hydraulic continuum

Our results provide interesting evidence of anatomical plasticity in sorghum in response to drought, where the extent and nature of plasticity was strongly organ specific, irrespective of genotype. This provide evidence of modular phenotypic plasticity in herbaceous monocots, which is likely to serve differential physiological needs of structural and functional subunits of the plant hydraulic continuum (de Kroon et al., 2005; Poorter and Ryser, 2015). For instance, the hydraulic anatomy of leaves was remarkably more plastic than either stems and roots. Drought stressed plants, irrespective of genotype, developed larger VB and xylem vessels in both major and minor veins. Larger xylem area and increased interveinal distance caused concomitant decrease in leaf vein density and these all point towards reorchestration of leaf hydraulic architecture in sorghum exposed to water-limited conditions.

Literature dominates with reports showing a common trend of decreased leaf vein size with decrease in soil moisture level (Bresta et al., 2011), which indicates the plastic nature of veins to environmental cues (Sobrado, 2007; Frole, 2008; Olsen et al., 2013). The most likely explanation for this relationship is that drought reduces leaf size, which scales to major vein diameter, but reduces vein length per leaf area (Sack et al., 2012). However, the observed reverse pattern in our study is comparable to reports on *Andropogon gerardii* (Barnes, 1986; Olsen et al., 2013) and the C$_3$ grass *Leymus chinensis* (Wang et al., 2011), for which VBs and xylem vessels were larger at drier sites. As far as we know, there is no functional and developmental explanation for the wider VB and vessels developed under drought. While xylem embolism might be more likely for larger veins with large conduits (Scoffoni et al., 2017a), low vein density and larger xylem vessels in *Leymus chinensis* at drier sites were suggested to minimize investment in xylem construction, while maintaining leaf hydraulic conductance (Wang et al., 2011). Moreover, some reports point towards hydraulic decoupling of leaf and stem, in agreement with the vulnerability-segmentation hypothesis. This hypothesis states that distal organs show a higher vulnerability to xylem embolism, which may contribute to the hydraulic safety of the proximal, more permanent, high-investment tissues of stems in woody plants (Tyree and Ewers, 1991), but it is unknown to what extent this hypothesis applies to herbaceous monocots. Also, it is unclear if the xylem in monocot leaves is equally resistant to embolism as the stem xylem in monocots (Neufeld et al., 1992; Holloway-Phillips and Brodribb, 2011; Bouche et al., 2016; Scoffoni et al., 2017b). The modularity observed raises also interesting questions about compartmentalisation as a potential survival strategy of herbaceous monocots during drought (Guadagno et al., 2017).

Unlike leaves, plasticity in stem hydraulic anatomy was modest and directionally opposite, whereby reduction in VB and xylem size was evident in the high WU genotypes, especially for the larger VB types. Such structural modifications in response to water deficit are consistent with reports on woody angiosperms (Hacke et al., 2006; Fichot et al., 2009) and may indicate a strategy to resist embolism under low water availability or may reflect developmental differences in xylem formation processes and the associated kinetics of cell enlargement, cell-wall deposition, and lignification. The high WU group with bigger MX vessels could be prone to embolism and therefore, had to undergo larger structural modification under drought to develop smaller vessels showing greater stem-level plasticity than the low WU group. Stem xylem embolism resistance capacity of corn genotypes has been shown

to correspond to their relative drought resistance (Li et al., 2009). Reduction in xylem vessel size could also be a strategy to reduce carbon cost or to maintain non-structural carbohydrate levels (Petit et al., 2016).

As the actual stem conductivity was not measured, we don't know the degree of native embolism or to what extent the stem anatomical plasticity resisted xylem embolism. However, visual observations of greater wilting of top leaves, higher leaf desiccation (*LRWC*, Fig. 2A), and stem shrinkage in drought stressed individuals of the high WU group could possibly indicate that these genotypes were more vulnerable to xylem embolism than the low WU group (Barigah et al., 2013; Urli et al., 2013; Bartlett et al., 2016). Contrary to the high WU group, modification in the stem hydraulic anatomy was not observed and probably was not necessary for the low WU genotypes, as they inherently possesed smaller sized stem VBs and MX vessels, and higher density of smaller VB types, which altogether point towards greater inbuilt embolism resistant stem xylem. Without exact quantification, we also observed increased lignification of stem VBs under drought in all genotypes, which could be a general, developmental response of sorghum stem when exposed to drought. Lignification was mainly confined to the peripheral parts of the stem along the entire axis and could be a strategy to increase mechanical stability, to avoid xylem embolism formation, or to avoid collapse of conduits under negative xylem water potentials (Lens et al., 2016; Pereira et al., 2018).

5. Conclusions

Knowledge of xylem hydraulics and extra-xylary water flow in herbaceous monocots is essential to examine how minor anatomical differences may influence water transport and photosynthetic performance under varying water availability. This study clearly illustrates the importance of xylem anatomy as a key aspect of water transport at various levels of the plant water continuum, with consequences for the whole-plant physiology. Genotypes with higher rates of transpiration displayed inherently greater hydraulic capacity but were more sensitive to soil drying, indicating a safety-efficiency trade-off. Xylem anatomy was finely correlated and showed a tight association with the phloem, irrespective of the genotype and water regimes. Leaf and stem xylem traits were highly correlated under ample water conditions but displayed differential plasticity under drought to potentially suit organ-specific functional demands and safety needs. Although this finding requires further empirical support, differential, organ-specific plasticity may shed light on the complex modular phenotypic plasticity within monocots.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors contributions

AG designed the study and wrote the manuscript. AG and SSC carried out the study. SC supervised the dry-down experiment and critically analysed the plant water use data. RS carried out the microtomy and imaging of leaf and root tissues. DB contributed in leaf gas exchange and stomatal trait measurements. SG provided several suggestions on anatomical trait measurements and critically reviewed the manuscript. All authors read and approved the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2018.08.025.

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