Transpiration efficiency: Further insights from C4 cereals species comparison

Vincent Vadez¹,²,*, Sunita Choudhary², Jana Kholova², C Tom Hash², Rakesh Srivastava², A Ashok Kumar², Anand Prandavada³, M Anjaiah²,§

¹ Institut de Recherche pour le Développement (IRD), UMR DIADE, University of Montpellier, 911 Av Agropolis BP65401, 34394, Montpellier, France
² International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Telangana, India
³ Multi-Crop Research Centre, Corteva Agriscience, Agriculture Division of Dow-DuPont, Tunki-kalsa, Wargal Mandal, Siddipet 502336, Telangana State, India
§ Deceased

* correspondence: vincent.vadez@ird.fr; v.vadez@cgiar.org

© The Author(s) 2021. Published by Oxford University Press on behalf of the Society for Experimental Biology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Highlights

C4 species vary for TE. Soils and source/sink balance alter TE, in a specie-dependent manner. Soil/rhizosphere together with root/plant conductance may explain part of the TE differences under high evaporative demand.
Abstract

An earlier review on transpiration efficiency (TE) reported a tight link between high TE and transpiration restriction under high vapor pressure deficit (VPD). This paper builds on it and addresses other factors altering TE (shoot biomass/water transpired), namely species difference among major C4 cereals, soil texture, and source/sink relationships. Maize genotypes (n=10) had overall higher TE than pearl millet (n=10), and somewhat higher than sorghum (n=16). TE was higher in high clay- than in sandy soil, and that effect was crop-dependent. Maize showed large TE and yield variations among soils, while pearl millet TE showed no soil variation. This also suggested specific soil-species fitness. Removal of cobs drastically decreased TE in maize, but not in pearl millet, suggesting that source/sink balance also drove TE variations. The species differences in TE are interpreted to account for differences in transpiration restriction capacity under high VPD but also to breeding history having possibly favored maize’s source/sink balance, suggesting a breeding scope to increase TE in pearl millet and sorghum. The soil results open a new avenue to better understand stomatal regulation and transpiration restriction under high VPD leading to TE differences, where considering soil hydraulic characteristics and root system together appears to be critical. Finally, the results on sink alteration highlight the importance of sink strength in regulating transpiration / photosynthesis and consequently in influencing TE.

Key words: Climate change, root hydraulics, soil hydraulics, rhizosphere, drought, aquaporin, breeding.
Introduction

In an earlier review on transpiration efficiency (TE) (Transpiration efficiency: New insights into an old story, Vadez et al., 2014), the authors reviewed considerable evidence for the existence of a genetic term to the denominator portion of a TE formula (TE = 0.6Ca *(1-Ci/Ca)/(Wi-Wa) proposed earlier (Condon et al., 2002), where Ci and Ca are the stomatal chamber and ambient CO₂ concentrations, respectively, and Wi and Wa are the stomatal chamber and ambient vapour pressures, respectively). A more detailed framework describes TE at different scales (Sinclair et al., 1984). The denominator portion of the TE formula is akin to a vapor pressure deficit (VPD) factor prevailing at the leaf level, which had long thought to be out of reach for genetics, being considered an environmental term. However, this factor was hypothesized to be potentially affected by the capacity of certain genotypes in now many species to restrict transpiration under high VPD (Vadez et al., 2013a), therefore leading to a lower average VPD value over the course of the day in such genotypes, and then a higher TE (shown in modelling outputs in Sinclair et al., 2005). In other words, this review provided new insights on potential causes of variation for TE, moving away from the debate on the Ci/Ca ratio on which much work had concentrated until then.

In this paper, we propose to add further insights into this “rejuvenated” TE outlook, bringing experimental evidence for other drivers of TE variation, building on but going beyond the transpiration restriction to increasing VPD. These aspects are related to: (i) differences among C4 cereal species; (ii) soil effects; (iii) source/sink effects. We propose to do that in part from the standpoint of the comparison among three C4 species: maize, sorghum and pearl millet. These are the main C4 cereals, accounting for 197, 42, 33 Million ha worldwide respectively, and a productivity of 5.71, 1.42 and 0.94 ton ha⁻¹ (FAO stat, 2018). Maize has received a lot of breeding efforts over the past century to improve its productivity and expand its area of geographical adaptation. However, maize is also considered as relatively sensitive to several abiotic stresses such as drought, low soil fertility or water logging. By contrast, pearl millet and sorghum are considered very hardy crops, tolerant to harsh growing conditions like poor soil fertility, high air temperature or water limitation. Although they stand as the only choice of staple crops for farming communities of the semi-arid tropics, they are also considered as orphan crops and have received a lot less breeding improvement efforts than maize.

The species difference is motivated by the fact that there is very limited work having tried to compare TE across species. Comparing TE across conditions is indeed difficult, because TE
depends on VPD conditions in the environment, and requires normalization of the biomass/transpiration ratio (i.e. TE) for VPD (proposed initially by de Wit et al., 1958 and used for instance in Seversike et al., 2014). In relation to drought adaptation, although maize is considered less adapted than pearl millet and sorghum, there is actually very limited quantified evidence for this assertion (except Muchow et al., 1989 a, b). Specifically, for this paper, there is very limited data comparing TE among these crops. Bierhuizen and Slatyer (1965) define TE = k/(e*-e), where the term e*-e represents the gradient of vapor pressure between the leaf and the air, k is a constant that a priori only distinguishes C3 from C4 species (Tanner and Sinclair 1983; Sinclair et al., 1984), and encompasses the Ci/Ca from the definition above in Condon and colleagues (2002, 2004). Therefore, the TE definition from Bierhuizen and Slatyer (1965) implies that TE should be similar in maize, sorghum and pearl millet. Here TE is measured in these species under different soil water stress intensities and VPD conditions, and an assessment of possible inter-specific differences is made.

The soil aspects are motivated by the fact that water flows through a continuum from the soil to the root to the shoot and to the atmosphere. Earlier reports and reviews on TE hypothesized that a restriction in the movement of water in the plant, possibly in the roots (Sadok and Sinclair, 2010; Schoppach et al., 2014; Choudhary and Sinclair, 2014; Vadez 2014), could explain the transpiration restriction under high evaporative demand. However, there was no consideration of a possible role of the soil in this response of transpiration to increasing VPD conditions. Soils are known to have different hydraulics capacity (e.g. Sinclair 2005; Roy et al., 2018). The rhizosphere, i.e. that minute layer surrounding roots and “connecting” the roots to the soil, has also received recent attention, especially in its role to sustain the soil-plant continuum under progressive soil drying (Carminati et al., 2016, 2017; Ahmed et al., 2018; Carminati et al; 2020; Carminati and Javaux 2020). However, there is no work attempting/addressing a possible connection between the soil hydraulic conductivity and transpiration restriction under increasing VPD. This is addressed here indirectly by looking at TE in different soils with putative difference in hydraulic characteristics and different VPD conditions.

The reproductive sink aspects are motivated by the earlier consideration over the main driver for differences in TE (TE = 0.6Ca *(1-Ci/Ca)/(Wi-Wa), Condon et al., 2002). From this definition, TE is maximized when the ratio Ci/Ca is minimized, and since the ambient carbon is essentially constant in the lifetime of a crop, then Ci has to be kept low. This is possible either by reducing the stomatal conductance of the leaf, or by increasing its carboxylation efficiency. Here we hypothesized that any differences in the sink capacity for carbon
compounds could simply lead to differences in transpiration efficiency. Despite the close relationship between photosynthetic capacity and stomatal conductance (Wong et al., 1979; Ainsworth and Bush 2011; Kelly et al., 2014), the coupling between photosynthesis and stomata opening is far from being tight (Lawson and Blatt; 2014; Lawson and Vialet-Chabrand, 2018). This could explain in part putative TE differences among genotypes but also species. We present TE data where source/sink ratios have been altered. Therefore, the objectives of this paper were threefold: (i) test C4 species differences in TE; (ii) test soil effects on TE and discuss a possible role for soil/rhizosphere hydraulic conductivity in the transpiration restriction phenotype; (iii) test sink effects on TE and discuss a possible link between source/sink relationships, leaf photosynthetic rates, and TE.

**Material and Method**

**Genetic material**

Thirty-six genotypes were used, i.e. 10, 16, and 10 of maize, sorghum, and pearl millet (see brief description in Table 1). The maize material were hybrids and included seven breeding material from the Pioneer breeding pipeline for South Asia, plus a released Pioneer hybrid and two checks (Monsanto 900MG and a public check). The sorghum material were lines; some were released as cultivars by ICRISAT or State Agricultural Universities in India. The pearl millet material were elite single cross hybrids with 843A, a downy mildew resistant male sterile line, as female parent. The pollinators were either parents of genetic populations or of popular hybrids in India. These materials were chosen with no *a priori* knowledge of their genetic distance and while they did not represent the range of variation available in each of the crop species, they provided a representation of elite material well adapted to the geographical zone where the tests were carried out.

**Plant growth conditions and experimental description**

Plants were grown individually in lysimeters, which consisted of PVC tubes of 25-cm diameter and 2.0 m length. These are described in earlier reports (Vadez et al., 2011a; Vadez et al., 2013b). Five experiments were carried out, two for the species comparison in different water regimes and VPD in one soil (Alfisol) (Exp 1 and 2), two for the soil effect comparison in four different soils and different VPD and in fully irrigated conditions (Exp 3 and 4), and one (Exp 5) for the source/sink effect comparison in one soil (Alfisol) and fully irrigated conditions. Exp1 and 5 were sown on 2nd November 2012 and harvested on 2nd Feb 2013. Exp 2 was sown on 13th June 2013 and harvested on 4th October 2013. Exp 3 was sown on
11<sup>th</sup> Nov 2014 and harvested on 5<sup>th</sup> Feb 2015. Exp 4 was sown on 16<sup>th</sup> June 2015 and harvested on 16<sup>th</sup> Sept 2015. Exp 1, 3 and 5 were postrainy season experiments, characterized by a typical medium to high evaporative demand with a mean maximum daily vapor pressure deficit (VPD) of 2.30 - 2.40 kPa. Exp 2 and 4 were rainy season experiments, characterized by a typical low to medium mean maximum daily VPD of 1.40 kPa (Exp 2) and 2.00kPa (Exp 4) (Fig. 1).

**Comparison across different species, water regimes, and VPDs (Exp 1 and 2)** - In both experiments a terminal water stress was imposed by stopping irrigation at different points in the crop cycle. In Exp1, one sole water stress treatment was imposed by stopping irrigation at 60 DAS in maize, 57 DAS in sorghum, and 44 DAS in pearl millet, corresponding to tassel emergence in maize, and flowering in sorghum and pearl millet. This last irrigation watered back the lysimeters to about 90% field capacity. In Exp2, three water stress treatments were imposed (WS1, WS2, and WS3), by stopping irrigation at the time of pearl millet flowering (WS1), sorghum flowering (WS2) and maize male flowering (WS3). These were imposed at 48, 61, and 68 DAS. In this experiment, the last irrigation was a 2L water addition to each tube. In both experiments, a fully irrigated control was used (well-watered, WW), which was watered back to 90% field capacity after each weighing and given an additional 2-4 L of water between weighings when two consecutive weighings were separated by more than two weeks. Exp 1 experienced higher VPD conditions than Exp 2 (Fig. 1).

**Comparison in different soils and VPDs under fully irrigated conditions (Exp 3 and 4)** - Four soils were used. The Alfisol (same as in Exp 1, 2, 5) and the Vertisol were collected from the ICRISAT farm and were described recently (Bhattacharyya et al., 2020). These had sand:silt:clay percentages of 64:9:27 for the Alfisol and of 31:19:50 for the Vertisol. One was a loamy sand soil and consisted of sediment collected from the bottom of an empty water tank at the ICRISAT campus. The last soil was a sandy soil generated from the mixing of construction sand and Alfisol in a 3:1 ratio (v/v). Exp 3 experienced higher VPD conditions than rainy season Exp 4 (Fig. 1).

**Comparison in different source/sink balance in fully irrigated conditions (Exp 5)** - Different source/sink balances were obtained by severing the panicles as they were coming out. Maize cobs were removed before silking and one severing was sufficient. Sorghum and pearl millet panicles were removed at the time of booting. However, several passes were necessary to
remove new panicles from tillers or even nodal tillers in most pearl millet genotypes and some sorghum. The reproductive organs were dried in the oven and their weight taken at final harvest.

Transpiration follow-up and transpiration efficiency measurement

The weighing of the lysimeters started at 27, 27, 18, 26, and 27 DAS in Exp 1 to 5, respectively. A total of six, (Exp 1 and 5), seven (Exp 2), and nine (Exp 3 and 4) weighings of the lysimeters were carried out during the crop cycle, providing five, six and eight measurements of transpiration. The weighings were carried out with a S-type load cell (Mettler-Toledo, Geneva, Switzerland), with a 200 kg capacity and precision of 20 g. Soil evaporation was prevented by covering the soil surface with a plastic sheet on top of which a 2-cm layer of plastic beads was applied. Plastic sheets and beads were applied before the first weighing. The sum of the transpiration values gave the total plant transpiration, also referred to as ‘water used’ in the figures, which was used to compute transpiration efficiency (TE) as the ratio of the total plant biomass (panicle plus vegetative aboveground biomass) divided by the total transpiration. The difference between the first weighing, when the tubes were at field capacity, and the last weighing gave the total water extracted from the lysimeters. Water extracted represented a measure of the plant capacity to extract water from the soil profile under water stress. At harvest, plants were separated into leaf, stem, panicle or cob. For maize, the tassel was included in the stem fraction. The panicles or cobs were threshed to obtain grain weight. In Exp 5, the weight of the severed panicles and cobs was added to the vegetative biomass to obtain a total biomass. The experimental designs were complete randomized split-split block design with either water regime, soil, or panicle removal as the main factor, crop as the main sub-factor, and genotypes randomized within each sub-factor five times. One- and two-way ANOVA were carried out to analyze genotypic and genotype-by-treatment interactions effects within each specie. LSD values were used to compare the means among treatments and crops.

Results

Species differences in TE, yield, and water use, in different VPD conditions

There were large differences in TE among the three crops. Under WW conditions, maize had higher TE than sorghum, itself having higher TE than pearl millet in both seasons (Fig. 2A & 2B). Under WS conditions, it was only in the high VPD season that maize had lower TE than sorghum and TE similar to pearl millet (Fig. 2A), whereas in the three WS treatments of the
low VPD experiment maize had higher TE than sorghum, itself again having higher TE than pearl millet (Fig. 2B). Yield results were in close agreement with the TE results in these lysimetric experiments with a plant population similar to the field (Fig. 3A & 3B). Maize had a higher yield than sorghum and pearl millet both in WW and WS conditions, regardless of the VPD conditions. Sorghum yield was similar to pearl millet in the high VPD season (Fig. 3A), but higher than pearl millet in the low VPD season, regardless of the water treatment (Fig. 3B). Water use results were somewhat different from the TE and yield data. In the high VPD season maize used more water than sorghum and pearl millet, although not in proportion to its biomass increase (Fig. 3C). Interestingly, in the low VPD season, water use was similar in all three crops, and was even slightly higher in pearl millet, despite the higher maize yield (Fig. 3D). Maize also extracted more water from the soil profile under WS condition in the high VPD season, and water extraction was in the same order in the three crops in the low VPD season (Suppl. Fig. 1A & 1B).

Soil effects on TE, yield, and water use, in different VPD conditions
During the high VPD season, there were important TE differences among soils, in the order of 2 g biomass L$^{-1}$ water transpired (Fig. 4A). By contrast, during the low VPD season, there was no TE variation among the different soils, all having TE within a narrow and non-significant range of 3.0-3.3 g biomass L$^{-1}$ water transpired (Fig. 4B). During the high VPD season, TE was the highest of all soils in the Vertisol, and lowest of all soils in the sandy soil, being intermediate and at similar level in the Alfisol and the loamy sand soil (Fig. 4A). Interestingly, when looking at TE differences among soil for each species, TE did not vary among soils in pearl millet in the high VPD season (Fig. 5A). In contrast, TE varied mostly among soils in maize, with the highest values in the Vertisol and Alfisol and lowest in the sandy soil. For sorghum, TE variations were limited to the Vertisol where TE was the highest (Fig. 5A). During the low VPD season, there was no soil effect on the TE of each individual crop, except for a higher TE in the Vertisol for pearl millet (Fig. 5B).

Maize grain yield was again much higher than in sorghum and pearl millet during the high VPD season (Fig. 6A), whereas maize biomass was only superior to sorghum in the low VPD season (Fig. 6B). Grain yield variations across soils during the high VPD season were very consistent with the variations found in TE, with maize showing large yield variations across soils – lowest in the sandy soil and highest in the Vertisol and Alfisol. By contrast, the grain yield hardly varied across soils in sorghum and did not vary in pearl millet (Fig. 6A). During the low VPD season, there were hardly any significant biomass variations across soils in
maize and sorghum (both crops had a slightly lower biomass in the sandy soil) and only a lower biomass in pearl millet in the sandy soil (Fig. 6B). Water use also followed the trend of TE variations (Fig. 6C & 6D). During the high VPD season, maize had lower water use in the sandy soil, and this was also the case for sorghum and pearl millet (Fig. 6C). During the low VPD season, the water use variations were limited and were somewhat lower in the sandy soil for all three crops (Fig. 6D).

**Source-sink effects on TE and water use**

Removal of panicles had dramatic but contrasting effects on transpiration efficiency among crop species. Under well-watered conditions, removal of maize cobs decreased TE by more than two units, i.e. about 35% of its value under control conditions. In sorghum, the drop in TE was also significant upon removal of the panicles, although lower in magnitude than in maize, i.e. about one unit or about 20% of its value under control conditions. By contrast, in pearl millet there was even a modest 0.5-unit increase in TE upon removal of the panicles (Fig. 7A). Under water stress conditions, results were similar for sorghum and pearl millet, whereas in the case of maize, TE was unaffected by the removal of the cobs (Fig. 7B).

Removal of the reproductive organ had a dramatic effect in maize, where biomass decreased by more than 50% under well-watered conditions (Fig. 8A). By contrast, the decrease in biomass of sorghum was only about 25% and there was no decrease in pearl millet (Fig. 8A). Under water stressed conditions, the trend was similar in maize, and there was no biomass difference in sorghum and pearl millet (suppl. Fig. 2A). Despite this dramatic decrease in biomass in maize, water use of maize under well-watered conditions was only decreased by about 25%, whereas it was fairly similar between the two treatments in sorghum and pearl millet (Fig. 8B). In other words, the water usage of maize did not decrease as much as its biomass did, then explaining the important decrease in TE upon removal of the reproductive organs (Fig. 8B). The trend was similar under water stress (suppl. Fig. 2B).

**Discussion**

**Species differences in TE**

Overall, maize had higher TE than sorghum and pearl millet, and more so under fully irrigated conditions. Here, importantly, the TE difference between maize and sorghum was only seen in the Alfisol, where the large set of genotypes from Experiments 1 and 2 were tested, and somewhat in the sandy soil. The lysimetric system used in this study was earlier used to deliver robust assessments of plant water use throughout the cropping cycle and of
TE in sorghum (Vadez et al., 2011b), pearl millet (Vadez et al., 2013b), groundnut (Vadez and Ratnakumar 2016), along with a very relevant agronomic evaluation of yield (Vadez et al., 2014). Therefore, the breadth of results presented here are not an artefact. Despite the importance of TE as a yield enhancement factor in water limited environments, the literature is scant on C4 species comparison in TE, with only five studies comparing maize, sorghum, and pearl millet or finger millet side by side (Singh and Singh, 1995) (Muchow, 1989a, b) (Schittenhelm and Schrooter, 2014), (Rurinda et al., 2014), van Oosterom et al., 2021) three of them assessing WUE. Overall, the results reported tend to conclude the superiority of maize in terms of yield performance (e.g. Farre and Faci, 2006), even under fairly limited water availability (Rurinda et al., 2014), in agreement with our data. With regards to WUE values, Singh and Singh (1995) and Farre and Faci (2006) reported a similar range of WUE values for maize, sorghum and pearl millet, and a slight advantage for sorghum under water stress. Zegada-Lizarazu and colleagues (2012) found that sweet sorghum had higher WUE than maize under water deficit, and so did Bhattarai et al (2020) and Roby et al. (2017). However, few genotypes were used in these studies compared to the 36 we used. Some of our sorghum genotypes were at par with maize genotypes (data not shown), suggesting species comparison needs to include a wide range of genotypes to represent the species. Only one recent study used a large range of genotypes, i.e. eight maize hybrids and twenty-one 2dwarf and 3dwarf sorghum genotypes, and showed no TE difference between the two species (van Oosterom et al., 2021). Unfortunately, the soil type used in this study was not mentioned in the paper. We speculate that the soil type could have been similar to the Vertisol we used, their experiment being set in Gatton, Queensland, Australia, where this soil type predominates. Hence, the absence of TE difference between maize and sorghum in a putative Vertisol (van Oosterom et al., 2021) would agree with the absence of TE difference between maize and sorghum that we also found in Vertisol (Fig. 4). In conclusion, the TE difference among species distinguished notably maize and pearl millet, while the TE advantage of maize over sorghum appeared to be linked to the soil type, Alfisol and sandy soil eliciting a TE difference between maize and sorghum. Additional data would also be required to fully take into account possible root:shoot ratio difference among species and its consequences on the TE calculations.

Our interpretations for these species differences are several, besides the possible soil effect in the sorghum vs maize differences. A first explanation could be species differences in the capacity to restrict transpiration under high VPD that would lead to TE differences (see Vadez et al 2014 for a review). A recent report investigating water saving traits in these same
genotypes of maize, sorghum and pearl millet indeed showed that all three crops recurred to this strategy, although to a much larger extent in maize than in sorghum and pearl millet (Choudhary et al., 2020). Since most pearl millet and sorghum material used in this study lacked transpiration sensitivity to high VPD, compared to maize genotypes, they would have lower TE as hypothesized earlier (Sinclair et al., 2005). In fact, many of the elite varieties of sorghum and pearl millet used in these experiments were bred for the rainy season environment, i.e. targeting seasons with predominantly low VPD conditions, and hence did not require transpiration restriction as an adaptive trait. This could explain the large TE differences found in this work, at least in the high VPD season, and the difference with the results from van Oosterom et al., (2021) that likely used sorghum genotypes bred for the terminal stress conditions of Australia. A second explanation is the breeding history. Maize has been bred for about a century, whereas breeding for sorghum and pearl millet is a lot more recent (the 70’s) and has not received the financial and research support that maize did (Bänziger et al., 2006). Plant growth rate increase over time is one of the factors explaining the maize yield increases, which could be related to sustained photosynthetic rate during grain filling (Hammer et al., 2009), and which could also have driven up TE. There, we hypothesize that an increased sink strength of modern maize varieties like those used in this study could have boosted photosynthesis and consequently driven the Ci/Ca ratio down in the leaves, thereby increasing TE according to Condon et al’s TE definition (2002). This could have explained why TE difference among species were found in both seasons, especially in the low VPD season when no transpiration restriction would have been expected. A third explanation could be a difference in the biomass allocation as suggested recently (Velázquez et al., 2017), whereby sorghum and pearl millet could have larger biomass allocation to roots compared to maize. A possible caveat in our data is indeed that roots were not extracted and were not accounted in the biomass. Root:shoot ratio appear to vary in maize and sorghum, from values reaching 0.10 in maize (Ordonez et al., 2020) to values around 0.30 in sorghum (van Oosterom et al., 2011). Applying these values to correct biomass results of Experiments 1 and 2 removed the differences between maize and sorghum under WW conditions but not under water stress, where maize kept a TE advantage. Therefore, our experiments showed the TE superiority of maize over pearl millet, and over sorghum at least in Alfisol and sandy soils especially under water stress, having direct effects on yields, and with a wider range of TE values in sorghum and pearl millet than in maize. This knowledge along with the large intra-species variation for TE in sorghum and pearl millet opens a scope to breed for higher TE in these two crops and fill the TE gap that their C4 physiology theoretically allows.
Transpiration restriction would be an avenue toward this. Learning from the breeding history of maize, with regards to source/sink relationships (see section below), is another one.

**Soil effects on TE**

Soil had an effect on TE, and this effect was species-dependent. Very few studies have looked at the effect of soil on water productivity. So far, Razzaghi et al (2012) found no differences in water use efficiency (WUE) among soils and Bello et al (2019) found very small WUE differences between two sandy soils. However, a simulation study showed that maize productivity was sensitive to soil hydraulic properties, and water productivity would be differently affected by climate change in different soils (Pinheiro et al., 2019). Our interpretations for the TE differences among soils are two-fold, and build upon the hypothesis of a link between the capacity to restrict transpiration under high VPD and high TE (Sinclair et al., 2005; Vadez et al., 2014).

On the one hand, differences in soil matric potential between the different soils could have altered the transpiration response to the high VPD conditions. Soil texture, where the fraction in clay, silt and sand particles are important factors, is considered to be the main driver of the water retention properties of soils (Zhuang et al., 2001; Scharwies and Dinneny 2019). The sandy soil and the vertisol might then have opposite properties. Higher soil matric potential in the sandy soil, and to a certain extent in the Alfisol, would have let plants uptake soil water more quickly from the soil profile to support the high transpiration demand in the high VPD season, then leading to lower TE, whereas a higher soil water retention in a soil with lower matric potential, like in the case of the high clay content of the Vertisol (50%) could have induced a transpiration restriction leading to higher TE. This interpretation would agree with the results of a study using soil super absorbent polymers, aiming at increasing soil water retention in a sandy soil, and where WUE increased in maize with the treatment, whereas root density also increased to allow better access of roots to water (Yang et al., 2019). An additional argument in favor of this interpretation is that there was no soil effect on TE in the low VPD season. The location of water retention in the soil remains unclear. Newman (1969) states that pararhizal resistance, i.e. the resistance of the soil to water movement, could be a factor limiting water movement towards the roots, even at water content values close to field capacity. Other viewpoints (Sperry et al., 1998; Carminati et al., 2017; Ahmed et al., 2018), in opposition to a report stating that rhizosphere resistance would likely not be an issue (Newman 1969), show that the connection of the root to the soil through the rhizosphere is a critical factor. The rhizosphere, i.e. that thin layer between the root and the soil, could indeed
disrupt the connection between the soil and the plant and create a situation in which the water movement toward the roots is reduced and trigger stomatal closure to maintain plant hydraulic integrity (Carminati et al., 2016). A recent report (Carminati and Javaux, 2020) argues that better understanding the plant-soil hydraulic connection is indeed essential to fully understand plant responses to drought, especially under progressive drying. As stated by these authors, the water potential decrease between the bulk soil and the soil-root interface would increase dramatically at different saturation levels for different soil types (Carminati and Javaux, 2020). This could have happened in our lysimeters receiving weekly irrigation only and creating cycles of drying and re-wetting without reaching a water stress status. The production of mucilage to maintain hydraulic connection between the root and the soil in the case of drought could be an important aspect (Ahmadi et al., 2017; Ahmed et al., 2018). Root hairs also appear to be an active part of this connection between the root and the soil for water. For instance, in barley mutants without root hairs, plant response to drought was more abrupt in the mutants than in the wild type under water stress, although there was no difference under fully irrigated conditions (Carminati et al., 2017). The hypothesis of mucilage or root hairs having an effect on the transpiration response to high VPD has not been tested and represents an exciting avenue to explore.

But these putative differences in soil hydraulics are not enough to explain observed species differences in TE, since the soil interacted with crop species. Our second interpretation then involves differences in root hydraulic conductance of the three species, in interaction with the soil, which could have altered the way plants responded to the increased evaporative demand. Root density, distribution in the soil volume, conductivity, anatomy, are possible root traits that could determine the overall root hydraulic conductance (Ahmed et al., 2018), with possible species differences, and with possible soil differences. The root to shoot area ratio could also be an important factor in the putative species differences because rhizosphere conductance becomes limiting for water absorption below certain threshold ratios, and these threshold values depend on the soil (Sperry et al., 1998). The fact that TE was the same in pearl millet in the sandy soil as in the vertisol, and was at the level of maize TE in the sandy soil, suggests root hydraulic conductance differences between maize and pearl millet in the vertisol, or between maize and sorghum in the Alfisol, i.e. a higher hydraulic conductance in pearl millet or sorghum allowing them to support transpiration under high evaporative demand in these types of soil. Whether the pearl millet root occupied the soil volume in a different way than maize in the Vertisol, in a way that the hydraulic connection between the root and the soil could be maintained, is unknown. Recent results suggest that indeed pearl
millet has a dense and profuse root system allowing a small gradient of soil water potential even as the soil dried (Cai et al., 2020). These results also imply that crop species grown in a high VPD environment would have specific soil type fitness, for instance maize did not fit well the sandy soil under such conditions, whereas pearl millet fitted well all types of soil. In summary, there is a lot of published work on soil hydraulics and on root hydraulics, but these two research domains have not often been abridged except recently. This interconnection offers a few exciting hypotheses to explain crop species differences in TE.

First, additional work would be needed to test whether soils of different matric potentials are able to trigger different Tr-VPD response curves, whether and how much these response curves are species-dependent, and how these curves depend on the soil water content.

Second, on the root side, there is a crucial lack of data on root traits contributing to hydraulic conductance in these crops species, on their interaction with soil texture, on the root to shoot area ratios, and how these differences affect Tr-VPD response curves. This work tackling both soil and root variations and their interactions is needed to have a more complete picture of transpiration restriction under high VPD that duly takes into account soil differences and crop-soil interactions.

**Source-sink effects on TE**

There was a strong effect of removing the reproductive organs on TE in maize, but not in pearl millet and sorghum. A possible caveat in these TE data of Exp 5, is the fact that roots were not taken into account in the calculation, and roots could have become a sink for carbon in the absence of the maize cobs. For instance, the root:shoot ratio dramatically increased in four sorghum hybrids that were exposed to water stress and had poor seed set from values around 0.30 under control conditions to about 0.70 in plants with no seed set (van Oosterom et al., 2011). However, for maize this is very unlikely because biomass decreased from 263 g plant⁻¹ to 127 g plant⁻¹, and the maintenance of a similar total biomass (root + shoot) would have implied a root mass of about 130g in maize, giving a root/shoot biomass ratio of about 1.0, which is totally outside the more typical range of 0.04-0.13 across a diversity of maize fields (Ordonez et al., 2020), and then even much higher than the root:shoot ratio value in sorghum with no seed set (van Oosterom et al., 2011). Therefore, removal of cobs in maize depressed biomass accumulation in this high VPD season, but did not decrease transpiration to a similar extent. Our interpretation is that transpiration regulation under high VPD, which is an active water saving trait in maize (Choudhary et al., 2020), could have been lost in this crop because of the loss in sink capacity. In a study in apple, the loss in the regulation ability
of stomata was indeed reported following de-fruiteding, when de-fruiteding was done at a late stage (Wibbe and Blanke 1995). Here, WUE increased when de-fruiteding was done during the summer, but decreased when de-fruiteding was done at fruit maturity, putatively because photosynthate then went to other perennial sinks like shoots and roots in the summer. The maintenance of a high transpiration rate in maize is also unclear and could relate in part to the need to dissipate light energy by photorespiration (Busch, 2020; Kozaki and Takeba 1996). Decreasing sink capacity usually leads to an inhibition of photosynthesis (Ainsworth and Bush 2011), which is triggered by stomatal closure following the sensing of the excess glucose level by the hexokinase (Kelly et al., 2014). However, this coordination is not always tight. For instance, a transgenic study found that photosynthetic rate could be depressed by about 75% compared to the wild type in lines having a lower Rubisco content without any change in the stomata opening (von Caemmerer et al., 2004). Similarly, the photosynthetic rate of ageing leaves declined while transpiration remained intact or declined less, resulting in a lower intrinsic WUE at the leaf level in aging leaves (Atkinson et al., 1989). This could have also been the case in our maize results where high transpiration under high VPD would then have led to decreased TE while depressing mass accumulation in the absence of a sink. By contrast, sorghum, and more so pearl millet, kept producing tillers and panicles, including nodal tillers in the case of pearl millet, and may never have had a real sink limitation like in maize.

There is very limited work approaching that question of a relationship between TE or WUE with the sink capacity of crops, and more specifically with the source-sink relationships and their role in the regulation of photosynthesis and/or stomatal conductance. Several studies have shown links between TE and biomass allocation aspects reflecting source/sink relationships. For instance, TE was high and similar to maize in a sweet sorghum line (Zegada-Lizarazu et al., 2012), which could be a consequence of the sucrose sink in the sorghum stem acting on the photosynthetic efficiency. Similarly, TE was high and at par with maize in forage sorghum lines (Bhattarai et al., 2020; Roby et al., 2017), which could have been caused by less leaf area supporting a higher biomass sink, leading to a higher TE (Condon et al., 2002). In other crops like sunflower, TE was positively associated to a decrease in the biomass allocation to leaf area, and to a decrease in leaf area to biomass ratio (Velázquez et al., 2017). Similarly, in wheat and groundnut, TE was positively related to an increased biomass allocation to stem (Masle and Farquhar, 1988; Puangbut et al., 2009).

In summary, the section on species differences for TE and this one suggest that source-sink balance is likely to affect TE values. Stronger sink strength in maize as a consequence of
decades of breeding would have in part given an advantage over less bred crops like pearl
millet and sorghum. Hence, interpreting TE differences from the angle of sink strength and
source/sink balance is possibly an interesting avenue to explore for further TE improvements.
Interestingly, this also involves aspects of biomass allocations between root and shoot, with
possible consequences on and interactions with the soil-plant hydraulic balance.

Concluding remarks
Other factors than the transpiration restriction under high VPD, namely soils and source/sink
balance, can alter TE in a crop-dependent manner. Despite having physiologically similar
photosynthetic apparatus, maize had an advantage over pearl millet for TE, less though under
water stress, and over sorghum in the Alfisol. This relates in part to its higher capacity to
restrict transpiration under high VPD in this set of genotypes, but also to its breeding history
having possibly altered source-sink balance and/or photosynthetic efficiency. Soils - here we
interpret soil hydraulic characteristics - had a strong influence on TE, with higher TE in the
high-clay vertisol, although in a crop-dependent manner since TE varied among soils in
maize but not in pearl millet. Our interpretation goes for root trait differences among species,
specific to soils, which alter the soil-plant hydraulic continuum in ways that plant
transpiration under high VPD is eventually differently restricted across species and lead to
TE differences that are soil-specific. These results call for a lot more data about soil effects
on the transpiration restriction, on species differences in soil-specific root traits taking part in
the root hydraulic conductance, and on root to leaf area ratios that determine the hydraulic
limits of the rhizosphere conductance in a soil-dependent manner. Source-sink relationships
also had a strong influence on TE, exemplified by the removal of cobs in maize dramatically
depressing TE while no effect was noted in pearl millet. This opens a scope to further
improve TE from harnessing the characteristics of the source-sink balance that contribute to
increased TE, looking for instance at leaf to biomass ratio or sink strength.
Data availability
Data are available upon request to the corresponding author.

Supplementary data
Supplementary figure 1 presents water extraction results under water stress conditions from Experiment 1 and Experiment 2 – Supplementary figure 2 presents biomass and water use under water stress conditions from Experiment 5.

Acknowledgements
This paper is dedicated to the memory of M Anjaiah, scientific officer at ICRISAT, who played a key role in the quality of data acquired from the lysimeters for many years, including the data from this paper. Authors are grateful to Corteva AgriScience for making a set of maize hybrid available for this research. Funding for the experimental work comes from the CGIAR Research Program on Dryland Cereals (CRP-DC) and from a research grant between Pioneer and ICRISAT. Authors are grateful to Rekha Baddam for assistance with the statistical analysis. The paper was written and supported under the Make Our Planet Great Again (MOPGA) ICARUS project (Improve Crops in Arid Regions and future climates) funded by the Agence Nationale de la Recherche (ANR, grant ANR-17-MPGA-0011).

Author’s contribution
VV designed the study, undertook the experiments and wrote the paper - SC and JK contributed to data collection – CTH and RK provided genetic material of pearl millet – AAK provided genetic material of sorghum - AP provided genetic material of maize
Reference

Ahmadi K, Zarebanadkouki M, Ahmed MA, Ferrarini A, Kuzyakov Y, Kostka SJ, Carminati A 2017. Rhizosphere engineering: Innovative improvement of root environment Rhizosphere 3, 176-184.

Ahmed MA, Passioura JB, Carminati A 2018. Hydraulic processes in roots and the rhizosphere pertinent to increasing yield of water-limited grain crops: a critical review. Journal of Experimental Botany 69, 3255-3265. doi:10.1093/jxb/ery183

Ainsworth EA, Bush DR. 2011. Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. Plant Physiology 155, 64–69

Atkinson CJ, Davies WJ, Mansfield TA 1989. Changes in stomatal conductance in intact ageing wheat leaves in response to abscissic acid. Journal of Experimental Botany 40, 2021-2028.

Bänziger M, Setimela PS, Hodson D, Vivek B 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agricultural Water Management 80, 212–224.

Bello ZA, Walker S, Tesfahuney W 2019. Water relations and productivity of two lines of pearl millet grown on lysimeters with two different soil types. Agricultural Water Management 221, 528-537.

Bhattacharyya T, Wani SP, Pal DK, Sahrawat KL, Pillai S, Nimje A, Telpande B, Chandran P, Chaudhury S 2020. ICRISAT, India soils: yesterday, today and tomorrow. Current Science 110, 1652-1670.

Bhattarai B, Singh S, West CP, Ritchie GL, Trostle CL 2020. Water Depletion Pattern and Water Use Efficiency of Forage Sorghum, Pearl millet, and Corn Under Water Limiting Condition. Agricultural Water Management. 238, 106206. https://doi.org/10.1016/j.agwat.2020.106206

Bierhuizen JF, Slatyer RO. 1965. Effect of atmospheric concentration of water vapor and CO2 in determining transpiration-photosynthesis relationships of cotton leaves. Agriculture Meteorology 2, 259-270

Busch FA 2020. Photorespiration in the context of Rubisco biochemistry, CO2 diffusion and metabolism. The Plant Journal 101, 919-939.

Cai G, Ahmed MA, Dippold MA, Zarebanadkouki M, Carminati A 2020. Linear relation between leaf xylem water potential and transpiration in pearl millet during soil drying. Plant and Soil https://doi.org/10.1007/s11104-019-04408-z

Carminati A, Javaux M 2020. Soil rather than xylem vulnerability controls stomatal response to drought. Trends in Plant Science 25, 868-880.

Carminati A, Ahmed MA, Zarebanadkouki M, Cai G, Lovric G, Javaux M 2020. Stomatal closure prevents the drop in soil water potential around roots. New Phytologist 226, 1541-1543.
Carminati, A., Zarebanadkouki, M., Kroener, E., Ahmed, M.A., Holz, M. 2016. Biophysical rhizosphere processes affecting root water uptake. Annals of Botany 118, 561-571.

Carminati, A., Passioura, J.B., Zarebanadkouki, M., Ahmed, M.A., Ryan, P.R., Watt, M., Delhaize, E. 2017. Root hairs enable high transpiration rates in drying soils. New Phytologist 216, 771–781.

Choudhary, S., Guha, A., Kholova, J., Pandravada, A., Messina, C.D., Cooper, M., Vadez, V. 2020. Maize, sorghum, and pearl millet have highly contrasting species strategies to adapt to water stress and climate change-like conditions. Plant Science, 295, 11297. https://doi.org/10.1016/j.plantsci.2019.110297

Choudhary, S., Sinclair, T.R. 2014. Hydraulic conductance differences among sorghum genotypes to explain variation in restricted transpiration rates. Functional Plant Biology 41, 270-275. http://dx.doi.org/10.1071/FP13246

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D. 2004. Breeding for high water-use efficiency. J. Exp. Bot 55, 2447–2460. doi: 10.1093/jxb/erh277

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D. 2002. Improving intrinsic water-use efficiency and crop yield. Crop Science 42, 122-131

de Wit, C.T. 1958 Transpiration and crop yields. Verslandbouwk onderz 64.6. Inst of biol and chem Res on field crops and herbage. Wageningen, The Netherlands

FaoStat 2018. http://www.fao.org/faostat/en/#data/QC/visualize

Farré, I., Faci, J.M. 2006. Comparative response of maize (Zea mays L.) and sorghum (Sorghum bicolor L. Moench) to deficit irrigation in a Mediterranean environment. Agricultural Water Management 83, 135–143

Hammer, G.L., Dong, Z.S., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., Cooper, M. 2009. Can Changes in Canopy and/or Root System Architecture Explain Historical Maize Yield Trends in the U.S. Corn Belt? Crop Science 49, 299-312.

Kelly, G., Sade, N., Attia, Z., Secchi, F., Zwieniecki, M., et al. 2014 Relationship between Hexokinase and the Aquaporin PIP1 in the Regulation of Photosynthesis and Plant Growth. PLoS ONE 9(2): e87888. doi:10.1371/journal.pone.0087888

Kozaki, A., Takeba, G. 1996 Photorespiration protects C3 plants from photooxidation. Nature, 384, 557–560.

Lawson, T., Blatt, M.R. 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology 164: 1556–1570

Lawson, T., Vialet-Chabrand, S. 2018. Speedy stomata, photosynthesis and plant water use efficiency. New Phytologist 221, 93-98.
Masle J, Farquhar GD 1988. Effects of soil strength on the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. Plant Physiology 86, 32–38.

Muchow RC 1989a. Comparative productivity of maize, sorghum and pearl millet in a semi-arid tropical environment I. Yield potential. Field Crops Research 20, 191–205.

Muchow RC 1989b. Comparative productivity of maize, sorghum and pearl millet in a semi-arid tropical environment II. Effect of water deficits. Field Crops Research 20, 207–219.

Newman EI 1969. Resistance to water flow in soil and plant: Soil resistance in relation to amounts of roots: Theoretical estimates. Journal of Applied Ecology, 6, 1-12.

Ordóñez RA, Archontoulis SV, Martínez-Feria R, Hatfield JL, Wright EE, Castellano MJ 2020. Root to shoot and carbon to nitrogen ratios of maize and soybean crops in the US Midwest. European Journal of Agronomy 120, 126-130. https://doi.org/10.1016/j.eja.2020.126130

Pinheiro EAR, van Lier QdJ, Šimůnek J 2019. The role of soil hydraulic properties in crop water use efficiency: A process based analysis for some Brazilian scenarios. Agriculture Systems 173, 364-377.

Puangbut D, Jogloy S, Vorasoot N, Akkasaeng C, Kesmala T, Rachaputi R 2009. Association of root dry weight and transpiration efficiency of peanut genotypes under early season drought. Agricultural Water Management 96, 1460–1466. doi: 10.1016/j.agwat.2009.04.018

Razzaghi F, Plauborg F, Jacobsen SE, Jensen CR, Andersen MN 2012. Effect of nitrogen and water availability of three soil types on yield, radiation use efficiency and evapotranspiration in field-grown quinoa. Agricultural Water Management 109, 20-29.

Roby MC, Salas Fernandez MG, Heaton EA, Miguez FE, VanLoocke A 2017. Biomass sorghum and maize have similar water-use efficiency under nondrought conditions in the rain-fed Midwest U.S. Agricultural and Forest Meteorology 247, 434-444

Roy D, Jia XH, Steele DD, Lin DQ 2018. Development and Comparison of Soil Water Release Curves for Three Soils in the Red River Valley Soil Science Society of America Journal 82:568–577 doi:10.2136/ssaj2017.09.0324

Rurinda J, Mapfumo P, van Wijk MT, Mtambanengwe F, Rufino MC, Chikowo R, and Giller KE 2014. Comparative assessment of maize, finger millet and sorghum for household food security in the face of increasing climatic risk. European Journal of Agronomy 55, 29-41.

Sadok W, Sinclair TR 2010. Transpiration response of ‘slow-wilting’ and commercial soybean (Glycine max [L.] Merr.) genotypes to three aquaporin inhibitors under high evaporative demand. Journal of Experimental Botany, 61: 821–829.
Scharwies JD, Dinneny JR 2019. Water transport, perception, and response in plants. Journal of Plant Research.  
https://doi.org/10.1007/s10265-019-01089-8

Schittenhelm S, Schroetter S 2014. Comparison of drought tolerance of maize, sweet sorghum and sorghum-sudangrass hybrids. Journal of Agronomy and Crop Science 200, 46-53.

Schoppach R, Wauthelet D, Jeanguenin L, Sadok W. 2014. Conservative water use under high evaporative demand associated with smaller root metaxylem and limited trans-membrane water transport in wheat. Functional Plant Biology, 41: 257–269.

Seversike TM, Sermons SM, Sinclair TR, Carter TE Jr., Rufty TW 2014. Physiological properties of a drought-resistant wild soybean genotype: Transpiration control with soil drying and expression of root morphology Plant and Soil 374, 359-370.

Sinclair TR, Tanner CB, Bennett JM 1984. Water use efficiency in crop production. BioScience 34, 36-40.  
https://www.jstor.org/stable/1309424

Sinclair TR 2005. Theoretical Analysis of Soil and Plant Traits Influencing Daily Plant Water Flux on Drying Soils. Agronomy Journal 197, 1148-1152.

Sinclair TR, Hammer GL, van Oosterom EJ. 2005. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Functional Plant Biology 32, 945-952

Singh BR and Singh DP 1995. Agronomic and physiological responses of sorghum, maize and pearl millet to irrigation. Field Crops Research 42, 57-67.

Sperry JS, Adler FR, Campbell GS, Comstock JP 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. Plant Cell and Environment 21, 347-359.

Tanner CB, Sinclair TR 1983. Efficient water use in crop production: research or re-search? In: HM Taylor et al. (eds), Limitations to Efficient Water Use in Crop Production. Madison. WI: ASA, CSSA and SSSA, pp 1-27

Vadez V, Deshpande SP, Kholova J, Hammer GL, Borrell AK, Talwar HS, Hash CT 2011a. Staygreen QTL effects on water extraction and transpiration efficiency in a lysimetric system: Influence of genetic background. Functional Plant Biology 38, 553-566

Vadez V, Krishnamurthy L, Hash CT, Upadhyaya HD, Borrell AK 2011b. Yield, transpiration efficiency, and water use variations and their relationships in the sorghum reference collection. Crop and Pasture Science, 62 (8) 1-11. DOI: 10.1071/CP11007.
Vadez V, Kholova J, Zaman-Allah M, Belko N 2013a. Water: the most important ‘molecular’ component of water stress tolerance research. Functional Plant Biology 40, 1310-1322  
http://dx.doi.org/10.1071/FP13149

Vadez V, Kholova J, Yadav RS, Hash CT 2013b. Small temporal differences in water uptake among varieties of pearl millet (Pennisetum glaucum (L.) R. Br.) are critical for grain yield under terminal drought Plant Soil 371 (1), 447-462 DOI 10.1007/s11104-013-1706-0

Vadez V 2014. Root hydraulics: the forgotten side of root in drought adaptation. Field Crops Research 165, 15-24 DOI: 10.1016/j.fcr.2014.03.017

Vadez V, Kholova J, Medina S, Aparna K, Anderberg H 2014. Transpiration efficiency: New insights into an old story. Journal of Experimental Botany 65, 6141-6153 doi:10.1093/jxb/eru040

Vadez V, Ratnakumar P 2016 High transpiration efficiency increases pod yield under intermittent drought in dry and hot atmospheric conditions but less so under wetter and cooler conditions in groundnut (Arachis hypogaea (L.)). Field Crops Research 193, 16-23, http://dx.doi.org/10.1016/j.fcr.2016.03.001

van Oosterom EJ, Borrell AK, Deifel KS, and Hammer GL 2011. Does Increased Leaf Appearance Rate Enhance Adaptation to Post anthesis Drought Stress in Sorghum? Crop Science 51, 2728-2740. doi: 10.2135/cropsci2011.01.0031

van Oosterom EJ, Kulathunga MRDL, Deifel KS, McLean GB, Barrasso C, Wu A, Messina C, Hammer GL 2021. Dissecting and modelling the comparative adaptation to water limitation of sorghum and maize: role of transpiration efficiency, transpiration rate and height. In silico Plants 3, 1-12

Velázquez L, Alberdi I, Paz C, Aguirrezábal L and Pereyra Irujo G 2017 Biomass Allocation Patterns Are Linked to Genotypic Differences in Whole-Plant Transpiration Efficiency in Sunflower. Frontiers in Plant Science 8:1976. doi: 10.3389/fpls.2017.01976

von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ, Raines CA 2004. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco Journal of Experimental Botany 55, 1157-1166

Wibbe ML, Blanke MM 1995. Effect of defruiting on source-sink relationship, carbon budget, leaf carbohydrate content and water use efficiency of apple trees. Physiologia Plantarum 94, 529-534.

Wong SC, Cowan IR, Farquhar GD 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282: 424-426

Yang W, Guo S, Li P, Song R, and Yu J 2019. Foliar antitranspirant and soil superabsorbent hydrogel affect photosynthetic gas exchange and water use efficiency of maize grown under low rainfall conditions. Journal of the Science of Food and Agriculture 99, 350-359 DOI 10.1002/jsfa.9195
Zegada-Lizarazu W, Zatta A, Monti A 2012. Water uptake efficiency and above- and belowground biomass development of sweet sorghum and maize under different water regimes. Plant and Soil 351, 47–60.

Zhuang J, Nakayama K, Yu GR, Mizayaki T 2001. Predicting unsaturated hydraulic conductivity of soil based on some basic soil properties Soil and Tillage Research 59, 143-154
**Figure captions**

**Figure 1.** Minimum and maximum temperature (A, C, E, G) and minimum and maximum relative humidity percentage (B, D, F, H) in Exp 1 and 5 (A, B), Exp 2 (C, D), Exp 3 (E, F), and Exp 4 (G, H).

**Figure 2.** Transpiration efficiency (TE) in maize, sorghum, and pearl millet, measured in lysimeters (Vadez et al., 2014) in Experiment 1 (A) and Experiment 2 (B), in several water regimes, i.e. well-watered (WW), or water stress imposed at the time of flowering of each crop in Experiment 1 (WS), or imposed for all crops at the time of pearl millet flowering (WS3), sorghum flowering (WS2) or maize male flowering (WS1) in Experiment 2. Data are means (+/- SE) of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotypic mean data were the average of five replicated lysimeters in each genotype. Bars in the graph represent LSD (P<0.01) to compare species within water treatment.

**Figure 3.** Grain yield (A, B) and total water use (C, D) in maize, sorghum, and pearl millet, measured in lysimeters (Vadez et al., 2014) in Experiment 1 (A, C) and Experiment 2 (B, D), in several water regimes, i.e. well-watered (WW), or water stress imposed at the time of flowering of each crop in Experiment 1 (WS), or imposed at the time of pearl millet flowering (WS3), sorghum flowering (WS2) or maize male flowering (WS1) in Experiment 2. Data are average (+/- SE) of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotypic mean data were the average of five replicated lysimeters per genotype. Bars in the graph represent LSD (P<0.01) to compare species within water treatment.

**Figure 4.** Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in different soils in Experiment 3 (A) and Experiment 4 (B), under fully irrigated conditions. Data are means (+/- SE) of species means within soils. Species mean data were the average of three genotypes in each species. Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare soil treatments.

**Figure 5.** Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in maize, sorghum and pearl millet and in different soils in Experiment 3 (A) and Experiment 4 (B), under fully irrigated conditions. Data are species means (+/- SE) in different soils. Species mean data were the average TE across three genotypes in each species. Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare each crops across the different soils. In experiment 3 (A), LSD to compare crop differences in TE was 0.37 g biomass L\(^{-1}\) water transpired. In experiment 4 (B), LSD to compare crop differences in TE was 0.25 g biomass L\(^{-1}\) water transpired.

**Figure 6.** Grain yield (A), shoot dry weight (DW) (B), and total water used (C, D) measured in lysimeters (Vadez et al., 2014), in maize, sorghum and pearl millet and in different soils in Experiment 3 (A, C) and Experiment 4 (B, D), both under fully irrigated conditions. Data are species means (+/- SE) in different soils. Species mean data were the average across three
genotypes per species. Genotype means were the average of five replicated lysimeters per genotype. Bar in the graph represents LSD (P<0.01) to compare each crops across the different soils. In experiment 3 (A, C), LSD to compare crop differences for each soil in grain yield was 6.6 g plant$^{-1}$ and in water use was 1.8 L plant$^{-1}$. In experiment 4 (B, D), LSD to compare crop differences for each soil in shoot DW was 9.0 g plant$^{-1}$ and in water use was 2.1 L plant$^{-1}$.

**Figure 7.** Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in maize, sorghum and pearl millet, where reproductive organs (female organ in maize) were either left untouched (with panicle) or removed (no panicle), under either well-watered conditions (A), or water stress conditions (B). Data are means (+/- SE) of genotype means within species and panicle removal treatment (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare panicle removal treatment effect within each crop.

**Figure 8.** Total dry weight (DW) (A) and total water used (B) measured in lysimeters (Vadez et al., 2014), assessed in maize, sorghum and pearl millet, under well-watered conditions, where reproductive organs (female organ in maize) were either left untouched (with panicle) or removed (no panicle). Data are average (+/- SE) of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotype means were the average of five replicated lysimeters per genotype. Bar in the graph represents LSD (P<0.01) to compare panicle removal treatment effect within each crop.
Table 1: Description of the genetic material used in maize, sorghum, and pearl millet. Or pearl millet, materials are male pollinator on top of 843A, except for Pusa 322. All these genotypes were elite materials in their respective breeding programs.

| Crop       | Type               | Characteristics                                                                 | Drought response          |
|------------|--------------------|--------------------------------------------------------------------------------|----------------------------|
| **Maize**  |                    |                                                                                |                            |
| 8315622    | Hybrids            | Tall, high yield                                                              | Moderately drought tolerant|
| 18270413   | Hybrids            | Short, early maturity                                                         | Drought tolerant           |
| 783527     | Hybrids            | Medium tall, Stable, girth ear                                                | Drought tolerant           |
| 4695575    | Hybrids            | broad leaf, high yield, early maturity                                        | Drought sensitive          |
| 22525674   | Hybrids            | medium yield, medium maturity                                                 | Drought tolerant           |
| 9424780    | Hybrids            | High yield, big cob, late maturity                                            | Drought sensitive          |
| 14746185   | Hybrids            | Tall, high consistent yield, late flowering                                   | Drought tolerant           |
| 30V92      | Hybrids            | High yield, response to high plant density, medium maturity                    | Drought tolerant           |
| 900M Gold  | Hybrids            | Medium tall, high yield, late maturity                                        | Drought sensitive          |
| Public Check | Hybrids            | Medium, high yield, late maturity                                             | Drought sensitive          |
| **Sorghum**|                    |                                                                                |                            |
| 296B       | Breeding line      | Rainy season maintainer Line                                                   | Drought sensitive          |
| BTx623     | Breeding line      | Template line for genome wide analysis, Hi-coverage BAC library, Low canopy temp | Drought sensitive          |
| E 36-1     | Breeding line      | Non-stay green check line                                                      | Drought tolerant           |
| ICSR93024  | Breeding line      |                                                                              |                            |
| ICSV1      | Breeding line      |                                                                              |                            |
| ICSV700-P10| Breeding line      |                                                                              |                            |
| ICSV745    | Breeding line      |                                                                              |                            |
| ICSV93046-P1| Breeding line       |                                                                              |                            |
| IS18551    | Breeding line      |                                                                              |                            |
| IS9830     | Breeding line      |                                                                              |                            |
| M 35-1     | Breeding line      |                                                                              |                            |
| N13        | Breeding line      |                                                                              |                            |
| PB15220-1  | Breeding line      |                                                                              |                            |
| PB15881-3  | Breeding line      |                                                                              |                            |
| PVK 801-P23| Breeding line      |                                                                              |                            |
| S35        | Breeding line      |                                                                              |                            |
| **Pearl Millet** |                  |                                                                                |                            |
| 841B       | Single Cross       | Medium tall, medium-early flowering                                          | Drought sensitive          |
| Pusa 322   | Single Cross       | 841B x PPMI 301                                                              | -                          |
| 863B       | Single Cross       | Medium tall, medium-early flowering                                          | Drought tolerant           |
| GB8735     | Single Cross       | Medium height, early flowering                                                | Drought escaper            |
| ICMP 451-P6| Single Cross       | Tall, long panicle bristles                                                   | Drought tolerant           |
| H777/833-2 | Single Cross       | Short, many tillers, photoperiod-sensitive early flowering; seedling heat stress tolerant | Drought sensitive          |
| ICMV-4S 92222 | Single Cross          |                                                                              | -                          |
| PT732B-P2  | Single Cross       | d2 dwarf, photoperiod-sensitive late                                           | -                          |
| PRLT       | Single Cross       | Medium tall, early flowering; seedling heat stress sensitive                   | Terminal drought tolerant  |
| Tift 238D1-P158 | Single Cross     |                                                                              | -                          |
Figure 2. Transpiration efficiency (TE) in maize, sorghum, and pearl millet, measured in lysimeters (Vadez et al., 2014) in Experiment 1 (A) and Experiment 2 (B), in several water regimes, i.e. well-watered (WW), or water stress imposed at the time of flowering of each crop in Experiment 1 (WS), or imposed for all crops at the time of pearl millet flowering (WS3), sorghum flowering (WS2) or maize male flowering (WS1) in Experiment 2. Data are means (± SE) of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotypic mean data were the average of five replicated lysimeters in each genotype. Bars in the graph represent LSD (P<0.01) to compare species within water treatment.
Figure 3. Grain yield (A, B) and total water use (C, D) in maize, sorghum, and pearl millet, measured in lysimeters (Vadez et al., 2014) in Experiment 1 (A, C) and Experiment 2 (B, D), in several water regimes, i.e. well watered (WW), or water stress imposed at the time of flowering of each crop in Experiment 1 (WS), or imposed at the time of pearl millet flowering (WS3), sorghum flowering (WS2) or maize male flowering (WS1) in Experiment 2. Data are average +/- SE of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotypic mean data were the average of five replicated lysimeters per genotype. Bars in the graph represent LSD (P<0.01) to compare species within water treatment.
Figure 4. Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in different soils in Experiment 3 (A) and Experiment 4 (B), under fully irrigated conditions. Data are means (+/- SE) of specie means within soils. Specie mean data were the average of three genotypes in each specie. Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare soil treatments.
Figure 5. Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in maize, sorghum and pearl millet and in different soils in Experiment 3 (A) and Experiment 4 (B), under fully irrigated conditions. Data are species means (+/- SE) in different soils. Species mean data were the average TE across three genotypes in each species. Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare each crops across the different soils. In experiment 3 (A), LSD to compare crop differences for each soil in TE was 0.37 g biomass L−1 water transpired. In experiment 4 (B), LSD to compare crop differences for each soil in TE was 0.25 g biomass L−1 water transpired.
Figure 5. Grain yield (A), shoot dry weight (DW) (B), and total water used (C, D) measured in lysimeters (Vadloc et al., 2014). In maize, sorghum and pearl millet and in different soils in Experiment 3 (A, C) and Experiment 4 (B, D), both under fully irrigated conditions. Data are species means (+/−SE) in different soils. Species mean data were the average across three genotypes per species. Genotype means were the average of five replicated lysimeters per genotype. Bar in the graph represents LSD (P<0.01) to compare each crops across the different soils. In experiment 3 (A, C), LSD to compare crop differences for each soil in grain yield was 6.6 g plant⁻¹ and in water use was 3.8 L plant⁻¹. In experiment 4 (B, D), LSD to compare crop differences for each soil in shoot DW was 9.0 g plant⁻¹ and in water use was 2.3 L plant⁻¹.
**Figure 7.** Transpiration efficiency (TE), measured in lysimeters (Vadez et al., 2014), assessed in maize, sorghum and pearl millet, where reproductive organs (female organ in maize) were either left untouched (with panicle) or removed (no panicle), under either well-watered conditions (A), or water stress conditions (B). Data are means (+/- SE) of genotype means within species and panicle removal treatment (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotype means were the average of five replicated lysimeters in each genotype. Bar in the graph represents LSD (P<0.01) to compare panicle removal treatment effect within each crop.
Figure 8. Total dry weight (DW) (A) and total water used (B) measured in lysimeters (Yadez et al., 2014), assessed in maize, sorghum and pearl millet, under well watered conditions, where reproductive organs (female organ in maize) were either left untouched (with panicle) or removed (no panicle). Data are average (+/- SE) of genotype means within species (n = 10 for maize, n = 16 for sorghum, n = 10 for pearl millet). Genotype means were the average of five replicated lysimeters per genotype. Bar in the graph represents LSD (P<0.01) to compare panicle removal treatment effect within each crop.