

The transpiration rate sensitivity to increasing evaporative demand differs between soil textures, even in wet soil

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ARTICLE INFO

Keywords:

Canopy conductance
Maize
Pearl millet
Plant hydraulic conductance
Restricted/ limited transpiration rate
Soil hydraulic conductivity
Soil texture
Sorghum
Vapor pressure deficit (VPD)

ABSTRACT

Many efforts to improve crop yields in water-limited environments have been directed towards identifying genotypes capable of restricting their transpiration rate (TR) at high vapor pressure deficit (VPD). This has proven challenging due to the dependence of the TR-VPD relationship on environmental conditions. In this context, however, the impact of edaphic properties on the TR response to VPD has largely been overlooked as experiments investigating the TR-VPD relationship are usually performed in wet soil conditions. Hence, the soil is not expected to be limiting the water supply to the canopy at high VPD. Nonetheless, soil (hydraulic) properties are known to shape plant growth and the development of the plant hydraulic system. Thereby, they might indirectly affect plant water use during rising VPD, even in wet soils. To test the soil dependency of the TR-VPD relation, we measured the TR response of genotypes of three important C₄ cereals - maize, sorghum, and pearl millet - to increasing VPD in two soil textural classes (sandy loam vs. clay loam). We show that the TR response to rising VPD differed among soil textures in wet conditions. Plants grown in sandy loam exhibited a higher initial slope in TR during increasing VPD (slope1), a restriction in TR at lower VPD (VPD_{BP}), and a greater difference in TR before and after the VPD_{BP} ($slope_{diff}$), compared to plants grown in clay loam. Additionally, plants grown in more conductive soils (i.e., sandy loam) systematically exhibited higher maximum canopy conductance (i.e., slope1) and restricted their transpiration rate at lower VPD levels (VPD_{BP}), resulting in a greater reduction in transpiration. This aligns with a hydraulic mechanism underpinning TR response to VPD. We advocate that considering soil texture is valuable in breeding for water conservation based on TR restriction under increasing VPD.

Introduction

Due to global climate change, many places on Earth, especially subtropical regions, await a hotter and drier future (IPCC, 2022). The resulting rise in saturated vapor pressure (i.e., the amount of water vapor that air can hold at saturation) and the decrease in actual atmospheric vapor pressure lead to an increase in atmospheric evaporative demand – the vapor pressure deficit (VPD, kPa). The VPD (relative to atmospheric pressure, P_{am} , kPa) drives plant transpiration (TR, mg H₂O s⁻¹) for a given leaf area (LA, cm²) and canopy conductance (gc, mg s⁻¹

cm⁻² e.g., Jarvis and McNaughton, 1986):

$$TR = \frac{VPD}{P_{am}} * LA * gc. \quad (1)$$

Transpiration drives water fluxes across the soil-plant-atmosphere continuum (SPAC), generating a suction within the leaf xylem vessels that drives water movement from the soil to the roots along a gradient in water potentials in dependence on the hydraulic conductivity of the single compartments of the SPAC (Swaef et al., 2022). Rising VPD typically causes a larger TR (Eq. (1)) due to the increasing gradient in the vapor pressure between the relatively dry atmosphere and the moist

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inner space of the leaf. However, many plant species and genotypes within the species have evolved the capacity to limit TR during atmospheric drought by expressing a constitutively low TR or by restricting TR upon a critically high VPD through partial stomatal closure (Vadez et al., 2013, 2014; Sinclair et al., 2017).

In an agronomic context, it has been suggested that restricting TR during increasing VPD can be beneficial for yield formation in water-limited environments like the subtropics. Limiting TR at high VPD may lead to an increase in transpiration efficiency (TE = total biomass/total transpired water, Vadez et al., 2014) and water conservation in the soil during the vegetative stage for phases that are more critical for yield-making, like the reproductive phase (Sinclair et al., 2005; Ahmed et al., 2018). However, it was also shown that limiting TR under rising VPD might have detrimental impacts on yield due to unnecessary limitation in carbon assimilation and productivity in environments where water is not limiting (Messina et al., 2015; Sadok et al., 2019), emphasizing the strong context-dependency of the agronomic benefit of the TR-VPD relationship (Sinclair et al., 2005, 2010; Tardieu, 2012; Tardieu et al., 2018). Hence, understanding which plant traits and environmental conditions impact transpiration regulation during atmospheric drought is an important component in developing environment-specific breeding strategies for improved crop drought adaptation.

Biophysical processes regulating the TR response to increasing VPD

Low TR during high VPD can be related to a consecutively low stomatal conductance (i.e., a low initial slope between TR and VPD) or stomatal closure (i.e., the decline in stomatal conductance as predicted by Eq. (1)) upon a certain threshold VPD (commonly referred to as the VPD breakpoint at which TR increase is restricted, VPD_{BP} , kPa).

Explanations of regulatory principles driving stomatal closure range from stomata passively responding to a decline in plant water status (Brodrribb and McAdam, 2011) to stomatal control being actively mediated through dynamics of phytohormones, like the production and degradation of abscisic acid (ABA, e.g., Sussmilch et al., 2017). Additionally, hydraulic frameworks of stomatal regulation propose transpiration regulation to be an adaptive response to soil-plant hydraulics as a function of the interaction between transpiration water demand and soil water supply (Sperry et al., 1998; Sperry and Love, 2015, 2016; Wang et al., 2020). The hydraulic framework allows us to predict the TR response to various environmental conditions.

Using the hydraulic approach after Carminati and Javaux (2020), as implemented in Wankmüller and Carminati (2022), we will discuss which plant hydraulic variables are expected to impact the TR response to increasing VPD in wet soils.

Plant hydraulic traits shaping the TR response to increasing VPD

The TR response to increasing VPD is typically experimentally investigated in wet soil conditions in order to disentangle reasons for transpiration down-regulation (i.e., soil vs. atmospheric drying, Koehler et al., 2023). In wet soil conditions, the soil hydraulic conductivity (K_s , $m^3 s^{-1} kPa^{-1}$) is large compared to the plant hydraulic conductance (K_{plant} , $m^3 s^{-1} kPa^{-1}$, e.g., Draye et al., 2010) and, therefore, not expected to limit plant water supply. Likewise, the soil water potential (ψ_{soil} , kPa) is negligible compared to typical leaf water potentials (~ -1 MPa) in wet soils. Under these conditions, the leaf water potential is approximated as:

$$\psi_{leaf} = - \frac{TR}{K_{plant}} \quad (2)$$

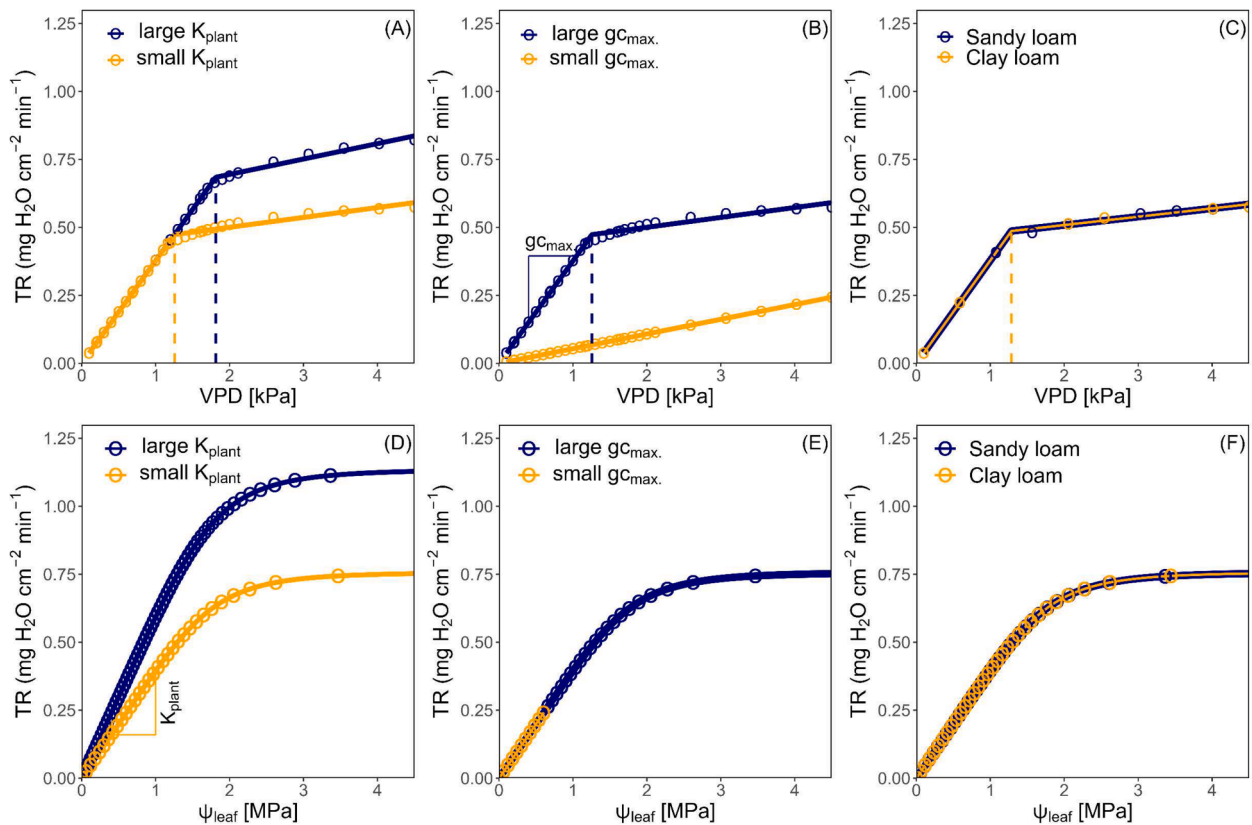


Fig. 1. Simulated relation between the transpiration rate (TR) and the vapor pressure deficit (VPD, upper panel), and between TR and the leaf water potential (ψ_{leaf} , lower panel) for two hypothetical plants differing in plant hydraulic conductance (K_{plant} , A, D), maximum canopy conductance ($g_{c,max}$, i.e., maximum potential plant water demand, B, E), and texturally differing soil substrates (C, F). Predicted by the soil-plant hydraulic model of Carminati and Javaux (2020) as implemented in Wankmüller and Carminati (2022). Varied fitting parameters can be found in Supplementary Material Table S1.

This simplified equation (Eq. (2)) indicates that a low *plant hydraulic conductance* (i.e., the slope between TR and the leaf water potential (ψ_{leaf} , kPa) in wet soil, Fig. 1D) will cause a more pronounced decrease in leaf water potential (ψ_{leaf} becoming more negative) for increasing transpiration, triggering an earlier stomatal closure as denoted as an earlier VPD_{BP} (Fig. 1A, yellow curve). Indeed, experimental results indicate that less hydraulically conductive plants restrict transpiration at relatively lower VPD during atmospheric drying (Sinclair et al., 2008; Choudhary et al., 2014; Jafarikouhini et al., 2020), with some exceptions (e.g., Jafarikouhini et al., 2022; Mandour et al., 2023) that will be discussed below.

Additionally, it was demonstrated that stomatal sensitivity to increasing VPD is higher when plants exhibit a high *canopy conductance* at low VPD (i.e., a high *maximum canopy conductance*, $g_{c_{max}}$, $\text{mg H}_2\text{O cm}^{-2} \text{ min}^{-1}$, Oren et al., 1999; Ocheltree et al., 2014; Ranawana et al., 2021). A plant with a higher maximum canopy conductance (i.e., a higher slope between TR and VPD at low VPD) experiences a larger TR per leaf area and requires larger gradients and, hence, a larger drop in leaf water potential to sustain that transpiration (Fig. 1E, blue curve). Thus, a higher maximum canopy conductance implies stomatal closure and restrictions in transpiration at lower VPD_{BP} (Fig. 1B, blue curve, Eq. (3) as combination of Eqs. (1) and (2):

$$\psi_{leaf} = -\frac{g_{c_{max}} * VPD}{K_{plant}} \quad (3)$$

Plants with a low maximum stomatal conductance are expected to sustain the resulting lower water demand until a relatively higher VPD, which appears as a linear TR over a limited tested range of VPD (Fig. 1B, yellow curve).

The counteracting effect of K_{plant} and $g_{c_{max}}$ on the transpiration rate response to increasing VPD has received insufficient attention in experiments investigating the role of K_{plant} in shaping the TR response to VPD. However, it might be the key to resolving the origin for contrary observations, whether VPD_{BP} is actually lower for less hydraulically conductive plants as described above. Therefore, our primary objective in this study is to concentrate on the role of the maximum potential plant water demand ($g_{c_{max}}$) in shaping the TR response to increasing VPD. Based on Eq. (3) and Fig. 1B and Fig. 1E, we expect plants that exhibit a higher maximum potential plant water demand (i.e., a higher $g_{c_{max}}$) to be more sensitive to atmospheric drying (restricting TR at lower VPD_{BP}).

Do soil physical properties modify the TR response to increasing VPD in wet soil?

Despite restrictions in TR during increasing VPD (as expressed by the VPD_{BP}) being expected to primarily result from constraints in K_{plant} (at a given $g_{c_{max}}$), Vadez et al. (2021) have recently suggested that maize, sorghum, and pearl millet plants grown in contrasting soil textures might have a different TR profile in response to VPD, even in wet soil, which would explain the observation that their transpiration efficiency (TE) differed between soil textures. The idea that the edaphic environment shapes the TR response to increasing VPD in wet soil has not been experimentally investigated to date. From a hydraulic perspective, we would not expect the TR response to increasing VPD to differ between hydraulically differing soil textures (Fig. 1C and Fig. 1F), considering that differences in soil hydraulic conductivity in the wet range are likely negligible compared to the resistance to water flow as opposed by the plant in wet soils (, Draye et al., 2010).

However, variations in soil properties were shown to induce differences in the development of the plant hydraulic system (i.e., K_{plant} , e.g., Koehler et al., 2022) and the maximum potential plant water demand ($g_{c_{max}}$) by its impact on plant growth (anatomically, e.g., maximum stomatal conductance, Katerji and Mastorilli, 2009; Tramontini et al., 2013; Lavoie-Lamoureux et al., 2017, and architecturally, e.g., leaf area, Poeplau and Kätterer, 2017; Cai et al., 2021; Vetterlein et al., 2022).

Thereby, the soil substrate could indirectly modify the TR response to increasing VPD.

Building on this, the second objective of this study is to examine the effect of soil texture on the TR response to increasing VPD in wet soil conditions. We expect the TR sensitivity to increasing VPD to be soil substrate-specific, not necessarily due to soil hydraulic limitations in the first place, but due to differences in plant growth between soils.

Material and methods

We investigated the TR response to increasing VPD in an experiment that was conducted in January-February 2019 at the International Crop Research Institute for the Semi-Arid Tropics in Patancheru (ICRISAT, Lat. 17.511100, Long. 78.275204), using two soils typical for the region: Alfisol (sandy loam) and Vertisol (clay loam). The aim was to determine the effect of soil texture on the TR sensitivity to rising VPD.

Plant material and growth conditions

We selected a total of six genotypes of three species of C_4 cereals: two genotypes of sorghum (*Sorghum bicolor* L., R16, and S35), pearl millet (*Pennisetum glaucum* L., H77/833-2, and PRLTx843A), and maize (*Zea mays* L., 30V92, and 900 M Gold), respectively. Within each species, the selected genotypes were previously reported to express contrasting transpiration responses to increasing VPD. In sorghum, R16 was shown to transpire linearly with increasing VPD, while S35 mostly showed a restricted transpiration response (VPD breakpoint, VPD_{BP}) upon a certain threshold VPD (Kholová et al., 2014; Vadez et al., 2015). In pearl millet, H77 was shown to be VPD-insensitive, while PRLT expressed a VPD-sensitive behavior (Kholová et al., 2010, 2016; Tharanya et al., 2018; Choudhary et al., 2020). Lastly, in maize, 900 M Gold was shown not to express a VPD_{BP} as opposed to 30V92 (Choudhary et al., 2020).

A total of 72 plants (six replicates per treatment) were grown in glasshouse conditions under optimal water supply. The plants experienced natural daylight oscillations with day/ night temperature averages of 27.9/ 22.2 °C and 60.2/ 69.4 % relative humidity. A data logger (Tinytag Ultra 2 TGU-4500 Gemini Datalogger Ltd, Chichester, UK) recording glasshouse temperature and relative humidity (RH) on a half-hourly basis was positioned at the canopy height. Plants were grown in plastic pots (26.7 cm in diameter and 20 cm in height) filled with 8 kg of soil. Maize seeds were sown seven days after sorghum and pearl millet seeds to account for growth rate differences. Di-ammonium phosphate at a rate of 2.5–3 g per pot was applied before sowing. All pots were initially fertilized with 300 ml of nutrient solution [after Tharanya et al. (2018): Macronutrients - MgSO_4 (2.05 mM), K_2SO_4 (1.25 mM), $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ (3.3 mM), KH_2PO_4 (0.5 mM), Fe-EDTA (0.04 mM), Urea (5 mM); and Micronutrients - H_3BO_3 (4 μM), MnSO_4 (6.6 μM), ZnSO_4 (1.55 μM), CuSO_4 (1.55 μM), CoSO_4 (0.12 μM), Na_2MoO_4 (0.12 μM)].

Soil properties

Six replicates per genotype were planted in sandy loam (Alfisol) and clay loam (Vertisol), respectively (Fig. 2).

The soils were collected from the ICRISAT farm and are described in detail by Bhattacharyya et al. (2020). Prior to pot filling, the soils were sieved to a particle size smaller than 1 cm to ensure homogeneous soil packing. The soil hydraulic properties were measured in two (sandy loam) to three (clay loam) replicates using HYPROP2 (METER ENVIRONMENT, München, Germany). The soil water retention and the hydraulic conductivity curves were fitted using the PDI-variant of the bimodal constrained van Genuchten model (after Iden and Durner, 2014 and Peters, 2014) and can be found in Supplementary Material Table S2.

Screening genotypes for transpiration response to increasing VPD

When five to six leaves were fully developed (23 days after sowing

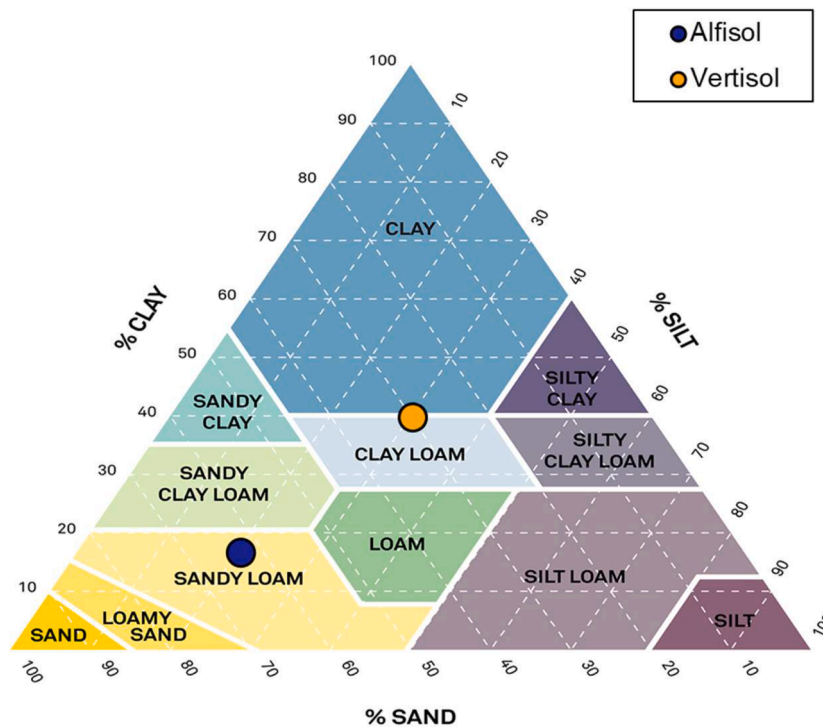


Fig. 2. Soil texture triangle (METER ENVIRONMENT, München, Germany) showing the soil texture of the soils (Alfisol – sandy loam, Vertisol – clay loam) used in the experiments. Based on Vadez et al., 2021.

(DAS) for maize, and 30 DAS for sorghum and millet), the pots were watered to saturation in the evening and allowed to drain overnight to reach the maximum pot water holding capacity. The following morning, the soil surface was covered with a round plastic sheet and a 2–3 cm layer of plastic beads to minimize soil evaporation. Subsequently, plants were transferred to four climate chambers in a randomized manner (Conviron, Model PGW36, Controlled Environment Limited, Winnipeg Manitoba, Canada) with day/ night temperature of 33.5/ 26 °C and relative humidity and 60/ 85 % for the rest of the day for acclimatization. The photosynthetic photon flux density in the climate chambers at canopy height ranged between 491 and 503 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A data logger recorded temperature and relative humidity every five minutes at canopy level. On the day of the experiment, plants were exposed to an ascending series of 5–6 VPD levels (depending on the performance of the climate chamber) ranging from 1.01 to 4.76 kPa by simultaneously varying temperature and relative humidity (Supplementary Material Table S3), in accordance with other studies that characterized the transpiration response to rising VPD (Gholipoor et al., 2013; Schoppach et al., 2017; Sivasakthi et al., 2020). Each VPD level was maintained for one hour. A fifteen-minute transition was allowed between successive VPD levels in which the pots were weighed. Transpiration was measured gravimetrically from changes in pot weight for each new VPD level. Transpiration rate (TR, $\text{mg H}_2\text{O cm}^{-2} \text{min}^{-1}$) was calculated as transpiration (T, mg) per unit of leaf area (LA, cm^2) and per unit of time (min) as:

$$\text{TR} = T * \text{LA}^{-1} * \text{min}^{-1} \quad (4)$$

The leaf area was determined at the end of the experiment using a leaf area meter (LI-3100C AREA METER, LICOR). A subset of the roots (50%) were scanned using WinRHIZO (version 5.0a; Regent Instruments, Quebec, Canada). Lastly, biomass dry weight was recorded for the individual plant parts (leaves, stems, roots).

Data analysis

In accordance with other studies characterizing the TR-VPD relationship (Kholová et al., 2010; Schoppach and Sadok, 2012; Schoppach et al., 2017; Tharanya et al., 2018), a linear or linear segmented regression was fitted per genotype-soil texture-combination (as described by Fletcher et al., 2007) using the R segmented-package version 1.6–4 (Muggeo, 2023). The linear and the linear segmented regression were performed and compared for each genotype-soil texture combination, and the best fitting model was determined based on an extra sum-of-squares F test ($p < 0.05$). The linear model was described as:

$$\text{TR} = y - \text{intercept} + (\text{slope} * \text{VPD}). \quad (5)$$

The linear segmented regression was described as:

$$\text{TR} = (\text{slope}_1 * \text{VPD}_{BP} + y - \text{intercept}_1) + (\text{slope}_2 * (\text{VPD} - \text{VPD}_{BP})), \quad (6)$$

Where slope_1 , VPD_{BP} and $y - \text{intercept}_1$ characterize the first linear segment (i.e., the intercept, slope, and the VPD breakpoint, VPD_{BP}); and slope_2 characterizes the second linear segment. This method searches iteratively for a possible breakpoint and tests whether the slopes between the two linear segments are significantly different. If the slopes differed significantly, the segmented linear regression was retained. Otherwise, a simple linear regression was applied.

From the fits per genotype-soil texture-combination, the coefficient estimates and standard errors for the slope of the first linear segment or the linear regression slope (hereafter referred to as slope_1), the VPD_{BP} , and the slope difference before and after the VPD_{BP} (in case of a segmented linear regression, slope_{diff}) were obtained. The benefit of fitting a linear or segmented linear regression is that the coefficients can be attributed a physical/ physiological meaning. However, comparing two resulting models (i.e., TR-VPD response curves) statistically is not directly possible with fitting either a linear or a segmented linear regression due to the different amounts of fitting parameters. The pro-

cedure of testing for statistical differences in the TR-VPD response curves between groups (e.g., genotypes or soils) will be described below.

Further, we determined the percentage of reduction in transpiration rate ($TR_{reduction}$, after Franks and Farquhar, 1999 and Ryan et al., 2016) which occurred due to the restricted TR response upon the VPD_{BP} (Eyland et al. (2023), Fig. 3). This metric is an integration of regression parameters specific to the segmented regression, namely, the VPD_{BP} and the slope of the second linear segment. The percentage of reduction in TR allows for a direct comparison between segmented and linear regression approaches. The percentage of reduction in TR was obtained by dividing the measured transpiration rate ($TR_{meas.}$) at the highest tested VPD level (4.76 kPa) by the potential maximum transpiration rate that a plant would have reached at the highest tested VPD level ($TR_{pot.}$) if it did not restrict transpiration upon the VPD_{BP} :

$$TR_{reduction} = \left(1 - \frac{TR_{meas.}}{TR_{pot.}}\right) * 100. \quad (7)$$

The potential maximum transpiration rate was obtained by extrapolating the linear regression of the first linear segment (in the case of a segmented linear regression) to a highest tested VPD.

Statistical analyses

Simple hypothesis testing (e.g., ANOVA, ANCOVA etc.) or linear modeling for identifying differences in the TR-VPD relationship between genotypes or between soils is invalid with the present dataset considering the non-independence of TR-observations (e.g., TR at VPD-level 5 will depend on TR at VPD-level 4) given the repeated measures (multiple TR measurements at different VPD levels for the same sample).

To test for statistical differences in the whole TR-VPD profile (i) between genotypes of the same crop species grown in the same soil and (ii) between soils per genotype, we used generalized nonlinear least squares models (gnls and SSlogis functions in R version 4.1.2) in the form of:

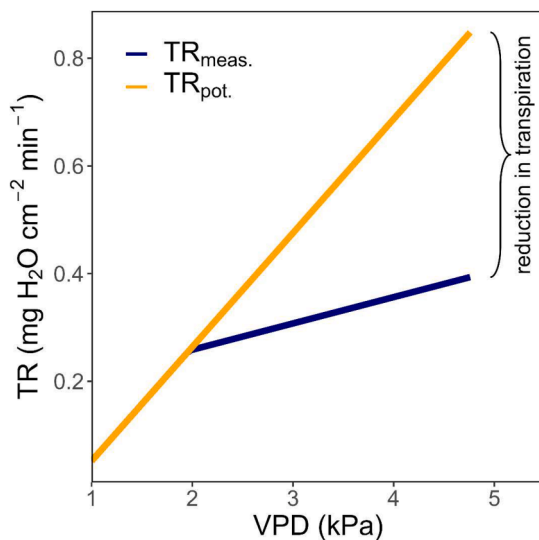


Fig. 3. Example of the calculation of the transpiration rate reduction ($TR_{reduction}$) according to Franks and Farquhar (1999) and Ryan et al. (2016). The slope of the linear regression defining the first segment in case of a segmented regression was extrapolated (orange line) to the maximum measured VPD to get a measure of the maximum potential transpiration rate ($TR_{pot.}$) that a plant would have reached if it did not restrict the transpiration rate upon the critical threshold VPD (VPD breakpoint, VPD_{BP}). Subsequently, the maximum potential transpiration rate was compared to the measured transpiration rate ($TR_{meas.}$) at the highest tested VPD to quantify $TR_{reduction}$ (Eq. (7)).

$$TR = \frac{asym}{\left(1 + e^{\left(\frac{infl - VPD}{slope1}\right)}\right)} \quad (8)$$

with $asym$ representing the asymptotic TR-value that the function approaches as VPD tends to positive infinity, $infl$ being the VPD at which TR is halfway between its maximum and minimum values along the sigmoidal function (inflection point), and $slope1$ determining the steepness of the curve at the inflection point.

We fitted two logistic models to each comparison (soil-wise and genotype-wise). The first model fitted one curve to the combined data from both groups that ought to be compared. The second model fitted two separate curves, i.e., one per group of interest with two sets of parameters: $asym$, $infl$, and $slope1$, and $\Delta asym$, $\Delta infl$, $\Delta slope1$, the latter characterizing the difference between the curves for both groups. The parameters for the logistic regression were defined as:

$$asym + x * \Delta asym$$

$$infl + x * \Delta infl$$

$$slope1 + x * \Delta slope1$$

with x being a dummy covariate ($x = 0$ for group 1 and $x = 1$ for group 2, update.gnls function in R version 4.1.2). In the following, we tested if the Δ -values for each parameter are significantly different from zero. If none of them was different from zero, the two curves were considered as not statistically significantly different. For that, the two models were compared using `anova()` in R version 4.1.2 (Supplementary Material Fig. S1-S2). If a p -value < 0.05 indicated that the two models are significantly different, we concluded that the two groups are significantly different in their TR-VPD response. The benefit of the logistic mathematical form of fitting the data is that it can describe approximately linear data as well as clearly non-linear data (i.e., those that express a VPD_{BP}), making it possible to directly compare them statistically.

Further, we tested if other investigated traits (e.g., root properties, leaf area) differed statistically significantly between genotypes of the same species and between soil textures for the same genotype. For that, the following steps were taken. First, the Bartlett Test of Homogeneity of Variances was conducted (`bartlett.test` function in R version 4.1.2). If the variances in each of the groups were the same (p -value > 0.05), an ANOVA was conducted (`aov` function in R version 4.1.2). Subsequently, the residuals of the ANOVA-outcome were tested for normal distribution using the Shapiro-Wilk Normality Test (`shapiro.test` function in R version 4.1.2). If the residuals were normally distributed (p -value > 0.05), the Tukey Honest Significant Differences – test was applied post-hoc in order to identify the groups that differed significantly (`TukeyHSD` function in R version 4.1.2). If the variances in each of the groups were not the same (Bartlett Test of Homogeneity of Variances p -value < 0.05) or the residuals of the ANOVA-outcome were not normally distributed (Shapiro-Wilk Normality Test p -value < 0.05), a Kruskal-Wallis Rank Sum Test was conducted (`kruskal.test` function in R version 4.1.2), followed by Dunn's Test (post-hoc) in order to identify the groups that differed significantly (`dunn.test` function in R version 1.3.5).

Results

Soil textures differed in hydraulic conductivity in the wet range

As the experiment was conducted in wet soil conditions, we will compare soil hydraulic conductivity between the two textures in the wet range (until -30 hPa). The soil hydraulic conductivity in the wet range was almost twice as high for sandy loam ($44.9 \pm 2.1 \text{ cm day}^{-1}$) compared to clay loam ($25.2 \pm 1.0 \text{ cm day}^{-1}$).

Plant water use sensitivity to increasing VPD differed significantly between soils

Plants differed significantly in their TR response to increasing VPD according to soil texture (Table 1, Fig. 4), while genotypic differences were less consistent, showing significant differences primarily among sorghum genotypes in both soils and pearl millet genotypes in clay loam (Table 1).

For all tested genotypes grown in sandy loam, the estimate of the threshold VPD upon which plants restricted the increase in TR with rising VPD (VPD_{BP}) was relatively low (1.89–2.10 kPa) compared to plants grown in clay loam (2.37–3.23 kPa, Fig. 5A). Pearl millet genotype H77 did not express a VPD_{BP} in clay loam. The first slope of the segmented regression (slope1, i.e., initial TR) was higher (0.18–0.30 mg cm⁻² min⁻¹ kPa⁻¹) for sandy loam grown plants than for clay loam grown plants (0.07–0.15 mg cm⁻² min⁻¹ kPa⁻¹, Fig. 5B). Lastly, plants expressed a comparatively large difference between the first and second slope of the segmented regression ($slope_{diff}$) when grown in sandy loam (|0.094–0.215| mg cm⁻² min⁻¹ kPa⁻¹) compared to when grown in clay loam (|0.000–0.086| mg cm⁻² min⁻¹ kPa⁻¹, Fig. 5C). This resulted in a considerably higher reduction in actual transpiration compared to potential transpiration ($TR_{reduction}$) for the majority of sandy loam grown plants (23–44 %) compared to clay loam grown plants (0–24 %, Fig. 5D).

Plant water demand and supply determining plant architectural traits did not consistently differ between soils

Leaf surface area (indicating plant water demand) did not vary consistently between soil textures (Fig. 6). While leaf area did not significantly differ between soils in maize genotypes and in pearl millet genotype PRLT, it was significantly larger for clay loam grown plants than for sandy loam grown plants in pearl millet genotype H77, and in sorghum genotypes (Fig. 6).

Root:shoot ratio (indicating water demand to water supply ratio) did not significantly differ between soils across genotypes, implying that a greater leaf area (i.e., greater water demand) was compensated for by more roots (i.e., greater water supply, Supplementary Material Fig. S3).

Table 1

Overview of coefficient estimates and their standard errors from the linear and linear segmented regression per genotype-soil texture combination: the slope of the first linear segment in case of a segmented regression or the regression slope in case of a simple linear regression (slope1), the VPD upon which the increase in TR with rising VPD was restricted (VPD_{BP}), and the difference in the two slopes in case of a segmented regression ($slope_{diff}$). In addition, genotypic differences (merged rows) or soil texture differences (same shade of gray) are indicated by p-values < 0.05 from the comparison of the two gnl models (for details, see Supplementary Materials Fig. S1-S2).

Soil	Genotype	Species	slope1 (mg cm ⁻² min ⁻¹ kPa ⁻¹)		VPD_{BP} (kPa)		$slope_{diff}$ (mg cm ⁻² min ⁻¹ kPa ⁻¹)		genotypic difference?	soil texture difference?
			coefficient estimate	standard error	coefficient estimate	standard error	coefficient estimate	standard error	p-value	p-value
Sandy loam	30V92	Maize	0.302	0.105	1.89	0.22	-0.215	0.107	0.52 *10 ⁰	0.49 *10 ⁻¹¹
Sandy loam	900M Gold	Maize	0.239	0.091	1.99	0.30	-0.157	0.093		0.25 *10 ⁻⁰⁵
Sandy loam	H77	Pearl millet	0.208	0.094	1.93	0.35	-0.128	0.096	0.21 *10 ⁰	0.21 *10 ⁻⁰⁶
Sandy loam	PRLT	Pearl millet	0.258	0.064	2.04	0.17	-0.208	0.065		0.7 *10 ⁻¹⁰
Sandy loam	R16	Sorghum	0.246	0.087	1.94	0.24	-0.178	0.089	0.21 *10 ⁻³	0.19 *10 ⁻¹⁰
Sandy loam	S35	Sorghum	0.183	0.067	2.10	0.43	-0.095	0.069		0.35 *10 ⁻¹⁹
Clay loam	30V92	Maize	0.135	0.013	2.75	0.20	-0.086	0.016	0.76 *10 ⁻¹	0.49 *10 ⁻¹¹
Clay loam	900M Gold	Maize	0.152	0.031	2.60	0.51	-0.066	0.037		0.25 *10 ⁻⁰⁵
Clay loam	H77	Pearl millet	0.074	0.008					0.11 *10 ⁻¹	0.21 *10 ⁻⁰⁶
Clay loam	PRLT	Pearl millet	0.110	0.019	2.37	0.57	-0.033	0.021		0.7 *10 ⁻¹⁰
Clay loam	R16	Sorghum	0.099	0.016	3.23	0.42	-0.071	0.035	0.1 *10 ⁻¹	0.19 *10 ⁻¹⁰
Clay loam	S35	Sorghum	0.114	0.017	2.66	0.31	-0.063	0.021		0.35 *10 ⁻¹⁹

The higher the initial TR, the more pronounced the reduction in transpiration

The VPD breakpoint (VPD_{BP}) upon which transpiration became restricted during increasing VPD (Fig. 7A), and consequently the reduction in transpiration (Fig. 7B) were significantly related to the initial TR at low VPD (slope1, Fig. 7). In sandy loam, plants exhibited the higher initial slope in transpiration with increasing VPD and the earlier reduction in stomatal conductance (i.e., lower VPD_{BP}) and, in turn, the highest reduction in transpiration. In clay loam, the slope of the first linear segment was comparatively less steep, and the reduction in transpiration was smaller.

Discussion

We investigated the effect of soil texture on the water use response to increasing VPD in two genotypes of sorghum, pearl millet, and maize, respectively. We hypothesized that (i) the transpiration rate response to VPD is soil-specific, and (ii) plants with high water demand (i.e., a high maximum canopy conductance) are more sensitive to increasing VPD. We find that plant water use regulation in response to increasing VPD differs between soils in a consistent way, even in well-watered soil conditions. The same is not reflected in plant architectural properties that impact plant water demand and supply. However, differences in maximum potential plant water demand scale with soil hydraulic conductivity in the wet range. Further, we show that plants exhibiting high TR at low VPD have an earlier onset of transpiration restriction as VPD increased and, hence, a more pronounced reduction in actual transpiration compared to potential transpiration.

Soil properties affect plant water use during increasing VPD, even in wet soil conditions

Plant water use response to atmospheric drying differs significantly between soils. In sandy loam, plants show a strongly segmented TR response (high initial TR, Fig. 5B; early restriction in TR increase with rising VPD, Fig. 5A; big difference in TR beyond the VPD_{BP} compared to low VPD conditions, Fig. 5C), resulting in a great reduction in actual

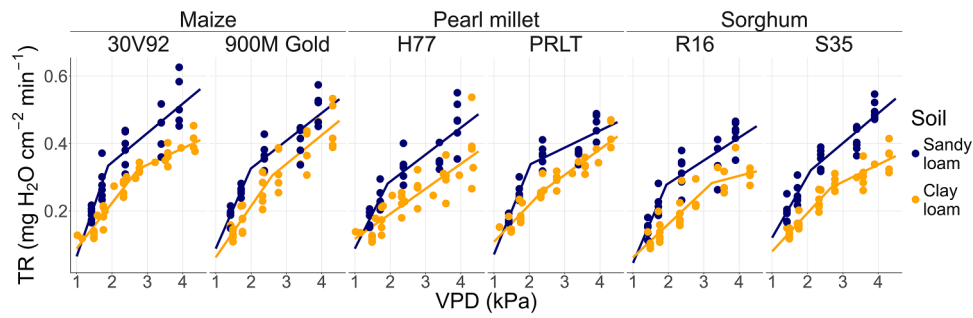


Fig. 4. Overview of the transpiration rate (TR) response to increasing VPD for the individual genotypes (columns: maize genotypes 30V92 and 900 M Gold, pearl millet genotypes H77 and PRLTx843A, sorghum genotypes R16 and S35) grown in different soil textures (colors). The points represent the measured data, and the lines show the result of the linear or segmented linear regressions.

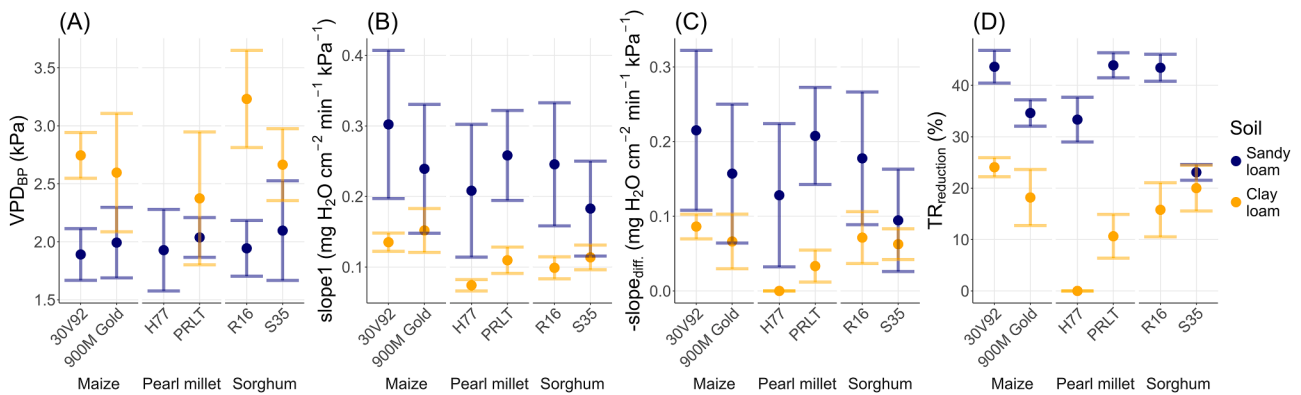


Fig. 5. Overview of the coefficient estimates of the linear and segmented linear regression of the relation between transpiration rate (TR) and VPD between soils per genotype: (A) threshold VPD (VPD breakpoint, VPD_{BP}) upon which plants restrict the TR increase with increasing VPD over the tested range of VPD in case of a segmented regression (nonexistent within the tested range of VPD for linear response), (B) the slope of the linear regression or of the first segment of the segmented regression in the TR-VPD relationship, (C) the difference between the slopes of the first and the second linear segment of the segmented TR-response to increasing VPD (zero for linear response), and (D) the reduction in actual transpiration compared to potential transpiration ($TR_{reduction}$ (%)) at the highest tested VPD (zero for linear response).

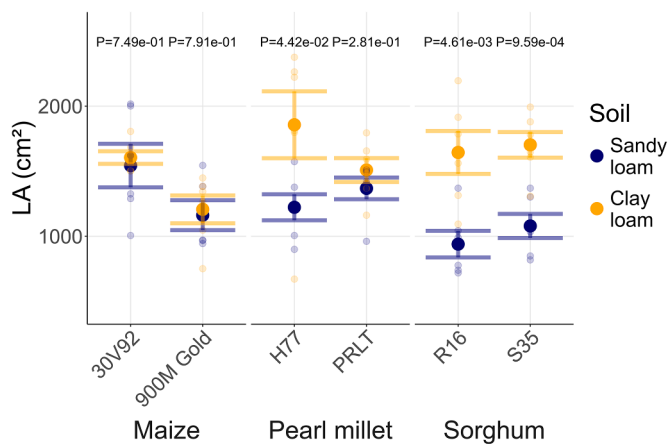


Fig. 6. Comparison of leaf area (LA) between soils per genotype (maize genotypes 30V92 and 900 M Gold, pearl millet genotypes H77 and PRLTx843A, sorghum genotypes R16 and S35). The transparent points represent all measurements. The opaque points represent means per soil and genotype. The vertical lines represent the standard error. P-values < 0.05 indicate statistical differences between soils.

transpiration compared to potential transpiration (Fig. 5D). Contrastingly, plants grown in clay loam show a TR response to increasing VPD that is closer to a linear response (moderate initial TR, Fig. 5B; restriction in TR at higher VPD, Fig. 5A; small difference in TR beyond the

VPD_{BP} , Fig. 5C), resulting in a small to no reduction in actual transpiration compared to potential transpiration (Fig. 5D).

Considering (i) that the experiments were conducted in wet soil conditions, meaning that soil water limitations are not expected to play a role for transpiration limitations, and (ii) that plants grown in different soils did not systematically differ in architectural traits that would impact water demand and supply (LA, root:shoot ratio, Fig. 6, Supplementary Material Fig. S3), the result is not straightforward from a hydraulic point of view (Fig. 1C). However, we found that plants express a higher max. canopy conductance (i.e., maximum potential plant water demand, slope1) when grown in soils that are more conductive under moist conditions (K_s , Supplementary Material Fig. S4). Plants seem to have a way of sensing soil texture (e.g., mechanosensing, Sparke and Wünsche, 2020), thereby adapting their maximum canopy conductance (potentially to the hydraulic conductivity of the soil). Determining the underlying physiological/ molecular mechanisms that may explain how plants sense differences in soil texture and correspondingly respond to them by changes in canopy conductance goes beyond the scope of this study. However, the dependence of canopy (i.e., stomatal) conductance on soil texture has also been demonstrated in other studies. In grapevine, Tramontini et al. (2013) similarly found that stomatal conductance was lower for clay-rich soils (which correspond to Vertisol, clay loam, in our case) than for sandy soil. In a lysimetric experiment with six crops, Katerji and Mastrorilli (2009) concordantly found that stomata conductance was comparatively low in clayey soils. In light of these findings, exploring the transpiration response to increasing VPD across different soil textures in a more realistic setting, such as in lysimeters or under field conditions, becomes particularly intriguing. This is

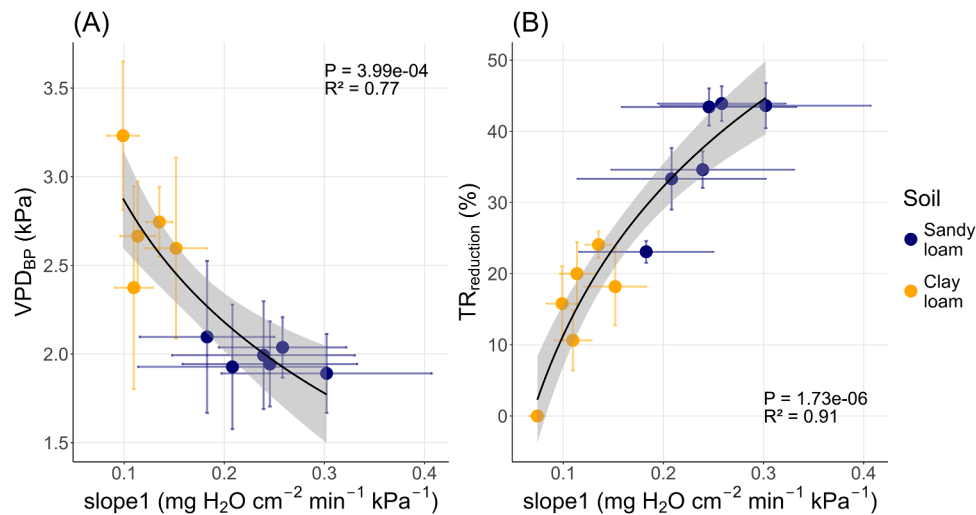


Fig. 7. Relation between the slope of the first segment of the segmented linear regression (slope1), and (A) the threshold VPD (VPD breakpoint, VPD_{BP}) upon which plants restricted the TR increase with increasing VPD over the tested range of VPD, and (B) the hypothetical reduction in transpiration.

especially relevant as uniform root and soil water distribution assumption may no longer hold true under field conditions, compared to in potted conditions like here. Further, soil texture-specific adaptations of plant anatomical features (e.g., endodermal cell dimensions, Kholová et al., 2016) that can impact the development of the plant hydraulic system (Parent et al., 2009; Lynch, 2019) might have affected plant hydraulic conductance in different soil textures. This remains to be explored.

It was proposed that the expression of a segmented transpiration rate response to increasing VPD might be a critical physiological ‘trait’ that contributes to drought tolerance by saving water in water-limited environments (Sinclair et al., 2005). However, in light of these findings, whether plants limit their transpiration rate at high VPD seems also to be a plastic response to environmental conditions. This is supported by mainly finding non-significant differences in the TR-VPD response between contrasting genotypes of the same species grown in the same soil (Table 1). We conclude in this section that whether and when plants express a restricted transpiration response to increasing VPD does not depend only on atmospheric conditions (temperature, VPD, e.g., Severson et al., 2013; Choudhary et al., 2014; Riar et al., 2015) but also on edaphic conditions, even in wet soil. Until now, the latter has been ignored.

Pronounced transpiration rate sensitivity to rising VPD for plants with high initial water use

We found clear evidence that plants must restrict their transpiration rate earlier (i.e., at lower VPD, Fig. 5A) and consequently experience greater reductions in transpiration rate (Fig. 5D) when their maximum canopy conductance (g_{Cmax} , i.e., slope1, Fig. 5B, Jafarikouhini et al., 2022) is high (Fig. 7). In other words, stomatal sensitivity to increasing VPD is higher when the canopy conductance at low VPD is high, which is in line with previous studies and the used hydraulic framework (e.g., Oren et al., 1999; Ocheltree et al., 2014; Ranawana et al., 2021; Jafarikouhini et al., 2022). This is additionally supported by finding the only genotype that expresses a linear response (H77) in Vertisol where plants show a low initial transpiration rate (Fig. 5B) and the VPD_{BP} is expressed late (at high VPD, Fig. 4A), indicating that the absence of a VPD_{BP} could be interpreted as the VPD_{BP} potentially occurring at higher than tested VPD. This remains to be tested systematically.

Theoretically, a plant with a higher max. canopy conductance (and hence, also higher transpiration rates per leaf area) requires larger quantities of water to be moved through the plant (assuming equal leaf

area), resulting in a more negative leaf water potential (assuming equal plant hydraulic conductance, blue curve, Fig. 1E) to sustain transpiration, compared to a plant with a relatively smaller max. canopy conductance (yellow curve, Fig. 1E). Assuming that stomata close at a critical leaf water potential, a higher maximum canopy conductance causes earlier stomatal closure and restriction of transpiration (blue curve, Fig. 1B) compared to a plant with a lower max. canopy conductance (yellow curve, Fig. 1B). This has recently been simulated theoretically by Cai et al. (2023) in the case of soil drying and it applies also to the case of atmospheric drying and its effect on the decrease in leaf water potential. While this trade-off between the plant’s maximum capacity to transpire (and consequently, to assimilate carbon) and stomatal sensitivity to increasing VPD is well known from a stomatal functioning point of view (Oren et al., 1999), it might still have important implications for crop model prediction of yield benefits that might occur from a restricted TR response to increasing VPD. The opposite of a ‘conservative genotype’ expressing a VPD_{BP} is commonly modelled as an aggressively water using ‘risky genotype’ that transpires at the same initial rate (slope1) but linearly (no VPD breakpoint, Sinclair et al., 2005; Messina et al., 2015; Sadok et al., 2019). However, this scenario applies only for two hypothetical plants with equal canopy conductance that differ primarily in plant hydraulic conductance. In light of our current findings, it might be more realistic to model the opposite as a genotype that restricts TR at high VPD but transpires at a lower initial rate until then. In summary, restrictions in transpiration rate at high VPD should be considered both from the standpoint of the VPD breakpoint (whether it occurs and when) and from the initial TR rate, i.e., slope1, as also emphasized by Vadez et al. (2013).

Our results are useful to resolve the inconsistency observed in previous research concerning the role of plant hydraulic conductance for sustaining transpiration at high VPD. While the prevailing hypothesis is that more hydraulically conductive plants can sustain unlimited transpiration rates until comparatively higher VPD (blue curve, Fig. 1A), Jafarikouhini et al. (2022) have recently found that a low plant hydraulic conductance was associated with restricting transpiration at lower VPD within the group of genotypes that expressed a segmented transpiration response (VPD_{BP}), while genotypes expressing a linear transpiration response had an even lower conductance in maize (*Zea mays* L.). Similarly, Mandour et al. (2023) observed no difference in whole-plant hydraulic conductance between faba bean (*Vicia faba* L.) genotypes expressing differential transpiration rate responses to increasing VPD. We illustrated here that a high maximum canopy conductance has a negative effect on stomatal sensitivity to VPD (Figs. 1B, 7A). In contrast, a high plant hydraulic conductance is

expected to have a positive effect on stomatal sensitivity to VPD (Fig. 1A). Consequently, the transpiration rate response to increasing VPD depends on both, maximum plant water demand and plant hydraulic conductance. When investigating just one of the independent variables (e.g., only the plant hydraulic conductance), the effect on the transpiration rate sensitivity to increasing VPD might not be straightforward. For instance, it is likely that a less conductive plant adapts its maximum potential water demand according to the capabilities of its hydraulic system. During high atmospheric water demand, such a plant will experience less steep gradients in water potential compared to plants with a high canopy conductance. Thereby, the plant with the lower canopy conductance might be able to sustain the resulting lower transpiration rate linearly despite the relatively low internal hydraulic conductance. Future studies should, therefore, be directed towards monitoring maximum canopy conductance *as well as* plant conductance in their effect on the transpiration rate response to rising VPD.

Lastly, the inverse relationship between the VPD breakpoint and the maximum canopy conductance brings a new perspective on the idea that an early expression of the VPD breakpoint could be advantageous for soil water conservation in specific environments (e.g., Sinclair et al., 2005; Vadez et al., 2014). Transpiring larger amounts of water initially but reducing transpiration at lower VPD levels may result in similar quantities of total water transpired by the end of the day, compared to when a plant consistently transpires lower quantities of water throughout the day. Hence, the benefit of restricting transpiration at low VPD for soil water conservation is not as obvious when taking its relation to the maximum canopy conductance into account. This emphasizes once more the importance of context-specific temporal dynamics for whether a segmented transpiration rate response to increasing VPD will lead to soil water conservation (besides the supposedly positive impact on transpiration efficiency, Vadez et al., 2014) and can thereby be beneficial for yield (Tardieu, 2016; Tardieu et al., 2018).

Conclusion

This study indicates that variation in soil texture may play a large role in regulating the transpiration rate response to increasing VPD, even in wet soil. We found that plant water use regulation, i.e., transpiration rate, during atmospheric drying is soil-specific, even in wet soil. This has not been systematically demonstrated so far, and it emphasizes the role of the growth environment for the expression of the restricted transpiration response, which is an important implication for crop modeling, breeding, and crop management.

Secondly, we found that the transpiration rate sensitivity to rising VPD increased with increasing plant water demand at low VPD (i.e., maximum canopy conductance). Considering the interplay between the maximum canopy conductance and the VPD breakpoint is essential to resolve the inconsistencies regarding the role of the plant hydraulic conductance for the VPD breakpoint.

CRedit authorship contribution statement

Tina Koehler: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Ákos Botezatu:** Writing – review & editing, Investigation. **Tharanya Murugesan:** Writing – review & editing, Supervision, Investigation. **Sivasakthi Kaliamoorthy:** Writing – review & editing, Supervision, Investigation. **Jana Kholová:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Walid Sadok:** Writing – review & editing, Validation. **Mutez Ali Ahmed:** Resources, Supervision, Writing – review & editing, Funding acquisition. **Andrea Carminati:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jana Kholova reports financial support was provided by German Academic Exchange Service. Tina Koehler reports financial support was provided by The German Federal Ministry of Education and Research. Andrea Carminati reports financial support was provided by The German Federal Ministry of Education and Research. Jana Kholova reports financial support was provided by Ministry of Agriculture of the Czech Republic. Jana Kholova reports financial support was provided by Faculty of Economics and Management, Czech University of Life Sciences Prague. Walid Sadok reports financial support was provided by USDA NIFA - Minnesota Agricultural Experiment Station. Mutez Ahmed reports was provided by German Academic Exchange Service. Akos Botezatu reports was provided by German Academic Exchange Service. Tharanya Murugesan reports was provided by German Academic Exchange Service. Sivasakthi Kaliamoorthy reports was provided by German Academic Exchange Service. - If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

Acknowledgements

We gratefully acknowledge the whole ICRISAT Crop Physiology team for fundamental support in planning and conducting the experiment, especially but not exclusively Rekha Baddam. Moreover, we thank Daniel Sebastian Moser (previously from the Soil Physics group of the University of Bayreuth) for continuing some of the related work. We thank Prof. Dr. Mohsen Zare (Technical University of Munich) and Dr. Andreas Cramer (ETH Zurich) for their support in obtaining the soil hydraulic properties. Lastly, we thank Prof. Dr. Hans-Peter Piepho (University of Hohenheim) for his guidance regarding the statistical analyses.

Funding

The exchange with ICRISAT was funded by DAAD (PPP Project Nr. 57390361). This work was supported by The German Federal Ministry of Education and Research (BMBF) in the context of the RhizoTraits-project (031B0908). The contributions from Dr. Jana Kholová were supported by the Ministry of Agriculture of the Czech Republic, grant number QK23020058 “Precision agriculture and digitisation in the Czech Republic” and the internal grant agency of the Faculty of Economics and Management, Czech University of Life Sciences Prague, grant no. 2023B0005 (Oborově zaměřené datové modely pro podporu iniciativy Open Science a principů FAIR). The contribution of Prof. Dr. Walid Sadok was supported by USDA NIFA - Minnesota Agricultural Experiment Station, Grant/Award Number: MIN-13-124.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.stress.2024.100506](https://doi.org/10.1016/j.stress.2024.100506).

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