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Large Variations in the Transpiration of Sorghum Canopies Under High Evaporative Demand Are Positively Related to Water Use Efficiency

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ABSTRACT

Transpiration restriction under high vapour pressure deficit (VPD), measured indoors with individual plants, increases water use efficiency (WUE). However, VPD is not the only factor driving transpiration, individual plants in a field rapidly become a canopy, and reports on the transpiration restriction versus WUE are scant. We analyzed the transpiration response to the evaporative demand (Penman-Monteith reference evapotranspiration, ET_{ref}) and WUE in sorghum canopies outdoors. These responses showed no plateau at high ET_{ref} in 47 genotypes. The slope of the resulting linear relationship over the whole range of ET_{ref} showed a large genetic variability. Unexpectedly, this slope was positively correlated with WUE in experiments with high ET_{ref}. Conversely, a (classical) negative correlation was observed under low ET_{ref}. Genotypes with high WUE and response to ET_{ref} allowed maximum light penetration into the canopy, via more erect leaf orientation. VPD in the canopy was also lower than in open air when the leaf area index reached 2.5-3. We interpret that higher WUE related to a larger proportion of plant photosynthesis being contributed by lower level leaves that received light and faced lower VPD than leaves exposed to air VPD. This study opens new opportunities, agronomic and genetic, to improve WUE.

1 | Introduction

Agriculture needs to ensure food security and to become more water efficient. This is especially true in drought-prone areas in which climate change and limited water resource for irrigation seriously threaten the future of millions of persons (Rosegrant et al. 2009; Kang et al. 2017; Vadez et al. 2023). Among other avenues, this requires to improve the water use efficiency (WUE) of crops (Tardieu 2022; Vadez et al. 2024). However, WUE has different definitions in the literature, thereby causing confusion in the analysis of potential traits underlying high WUE.

Intrinsic WUE (WUE_{int}) is defined as the ratio of photosynthesis to stomatal conductance or transpiration at leaf level (Condon et al. 2002; Medlyn et al. 2011). WUE_{int} is measured

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over short time scale and can be assessed with gas exchange measurements of stomatal conductance and photosynthesis, targeting leaves or leaf spots. The ^{13}C discrimination ($\Delta^{13}C$) in plant tissues, especially in C3 species but also recently in maize, a C4 species (Blankenagel et al. 2022), has been used as a proxy for WUE_{int}. WUE_{int} has a large genetic variability and heritability in several species (Condon 2004; Coupel-Ledru et al. 2016; Avramova et al. 2019; Kenney et al. 2014; Li et al. 2017; Al-Salman et al. 2024).

Another definition of WUE is at plant level, namely as the biomass accumulated per unit transpired water (WUE_{bm}), with time definitions ranging from weeks to months (Vadez et al. 2011; Chenu et al. 2018). WUE_{bm} also has a large genetic variability and heritability in several species (Vadez et al. 2011; Coupel-Ledru et al. 2016; Ryan et al. 2016; Vadez and Ratnakumar 2016), and can be measured in lysimeters (Vadez et al. 2014; Zhi et al. 2022), or in pot-based experiments (Halperin et al. 2017; Fletcher et al. 2018). WUE_{bm} integrates many physiological processes beyond those involved in WUE_{int}, for example night transpiration (Coupel-Ledru et al. 2016), plant nitrogen status and aging (Prieto et al. 2012), spatial variability of transpiration and photosynthesis in the canopy (Perez et al. 2019), maintenance of expansive growth under low water availability (Tardieu et al. 2014) or row spacing and plant density (Barbieri et al. 2012; Echarte et al. 2020; Pilloni et al. 2022; Pilloni et al. 2024). It is WUE_{bm} that we consider in this paper.

A mechanism used by plants to avoid excessive water loss per unit of carbon gain under high VPD is to close stomata at high VPD, resulting in a plateau of transpiration rate (Turner et al. 1984). Genotypes possessing this trait could achieve higher WUE_{int} under high VPD than genotypes that would keep on transpiring. Indeed, WUE_{int} decreases with vapour pressure deficit (VPD) because transpiration, but not photosynthesis, increases with VPD (Medlyn et al. 2011). A modelling study then predicted that restricting transpiration under high VPD achieved higher WUE_{bm} than not restricting transpiration (Sinclair et al. 2005). This seminal study triggered extensive investigation to evaluate the range of genetic variation in the transpiration response to increasing VPD. The latter was indeed observed in several species such as soybean (Fletcher et al. 2008), wheat (Schoppach and Sadok 2012), pearl millet (Kholová et al. 2010a; Choudhary et al. 2020), maize (Gholipoor et al. 2013; Choudhary et al. 2020), sorghum (Gholipoor et al. 2012; Choudhary et al. 2020), chickpea (Zaman-Allah et al. 2011) and rice (Affortit et al. 2022). Genotypes that most close stomata during the highest VPD hours have highest WUE_{int} (Gilbert et al. 2011) and WUE_{bm} (Sinclair et al. 2005). Genetic variability for WUE_{bm} in sorghum (Donatelli et al. 1992, Vadez et al. 2011; Xin et al. 2009), or in maize (Ryan et al. 2016), was therefore interpreted as a consequence of the plant ability to restrict transpiration in response to VPD, resulting in a plateau of transpiration rate at high VPD (Sinclair et al. 2005; Hatfield and Dold 2019). While this mechanistic interpretation is exciting because it opens an opportunity to develop improved cultivars with the capacity to restrict transpiration under high VPD and higher WUE_{bm}, there are several critical issues that need to be addressed, in addition to others raised in a recent viewpoint (Gleason et al. 2025):

i. In the modelling studies showing the benefit of transpiration restriction (Sinclair et al. 2005; Kholová et al. 2014; Messina et al. 2015; Sinclair et al. 2010), all leaves were assumed to be exposed to high light and VPD conditions. Most experiments relating transpiration to VPD indeed reproduce the same condition, most often in growth chambers with plants well separated from one another (Kholová et al. 2010b; Schoppach and Sadok 2012; Ryan et al. 2016). This raises the question whether there is also genotypic variation in the transpiration response to VPD if plants were to be assessed as part of a crop canopy.

ii. Only VPD was used as a driver of transpiration in experiments exploring the genetic diversity for the transpiration response to the evaporative demand, carried out in growth chamber under constant light intensity of typically 500 $\mu mol\ m^{-2}\ s^{-1}$ (Fletcher et al. 2008; Kholová et al. 2010a). Outdoor, light intensity fluctuates between hours and days, typically up to and above 1500 $\mu mol\ m^{-2}\ s^{-1}$ in semi-arid tropical conditions (Adeniji et al. 2020). This raises the question whether there is also genotypic variation in the response of transpiration to the evaporative demand taking light into account, calculated via the Penman-Monteith equation (Allen et al. 1998), rather than to the VPD only (Kholová et al. 2010b; Vadez et al. 2013; Geetika et al. 2019; Blum 2009; Condon 2004).

iii. Experimental results relating transpiration response to the evaporative demand and WUE_{bm} are scant.

The above paragraphs suggest that a direct analysis of the response of the evapotranspiration to the evaporative demand and of its relationship to WUE_{bm} in outdoors experiments is essential, because of differences in environmental conditions sensed by plants indoor or in the field. Our first hypothesis is that the transpiration response would fit better to a metrics of evaporative demand that also includes radiation and not only VPD. Our second hypothesis, supported by recent evidence (Jaikumar et al. 2025), is that genotypes allowing more light to reach leaves from the lower canopy, for example via steeper leaf angles, would have higher plant WUE_{bm}. In this case, leaves from the inner canopy would open their stomata, and contribute to plant photosynthesis while experiencing a milder VPD, thereby resulting in a higher WUE_{int} at plant level. The proportion of incident light that reaches the lowest parts of the canopy, an essential feature under this hypothesis, has a clear genetic variability (Yin and Struik 2015) and is related to plant architecture (Niinemets 2010), plant density (Song et al. 2013; Song et al. 2013) and the vertical distribution of leaf area (Perez et al. 2019).

Our objective was therefore to estimate the genotypic variation in the transpiration response to high evaporative demand (Penman-Monteith) of plants grown in canopy, and to assess if it correlates to WUE_{bm}, in two panels of genotypes grown outdoor in Senegal and India, and in a glasshouse platform in France, with two canopy structures (12 or 24 per m^2). For that, we assessed the transpiration response of studied genotypes to a range of evaporative demand (ET_{ref}). This was carried out in well-watered plants to avoid confusions of effects between evaporative demand and the depletion of soil water reserve. We

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used for that a lysimeter system (Vadez et al. 2014; Fletcher et al. 2018) where both WUE_{bm} and transpiration in response to the evaporative demand could be simultaneously assessed (Geetika et al. 2019). Because results were largely counter intuitive, we also explored the potential cause of these results, in particular those related to canopy architecture and microclimate, in field and glasshouse experiments.

2 | Materials and Methods

2.1 | Genetic Material and Experiments

A panel of 20 elite sorghum hybrids, some provided by seed companies, was used in lysimeter experiments in India (panel A). This panel has been used earlier to test the genotype response to increased sowing density (Pilloni et al. 2024). A panel of 27 lines from the germplasm collection of ICRISAT (International Crop Research Institute for Semi-Arid Tropics) was used in field and lysimeter experiments in Senegal (panel B). This panel derives from a sorghum germplasm panel tested for its response to planting density in the field at ICRISAT-HQ (India), and lines selected in panel B where those most contrasting in this response (unpublished). A lysimeter experiment and a pot experiment in glasshouse and in France used 9 and 2 genotypes of panel B respectively.

2.2 | Transpiration Response to the Evaporative Demand in a Lysimeter Setup Outdoors

Two experiments were carried out in the lysimeter platform (LysiField, Vadez et al. 2014) of ICRISAT (Hyderabad, India, 17°30 N; 78°16 E; elevation 549 m) during the dry (February to May) and rainy (August to October) seasons of 2018, which largely differed for VPD (4.7 vs 2.8 kPa on average) and light intensity (2217 vs 1545 μmol m^{-2} s^{-1}) (Figure 1). The lysimeter setup consisted of PVC tubes (20 cm diameter, 1.20 m length) arranged side by side in a trench and filled with alfisol, weighed at regular time intervals. Plants growing individually in each lysimeter formed a crop canopy. To maximise the range of transpiration values, we also varied the leaf area index (LAI) by setting up two plant density treatments. Four replications were used in both experiments, organised as a complete randomised block design with density as the main factor and genotypes randomised in the main blocks. Each replication consisted of a set of 4 tubes arranged in a square, all tubes carrying the same genotype. In the high density treatment, each tube carried one plant, whereas in the low density treatment there were only 2 plants in four tubes, leading to plant densities of 20 and 10 plants m^{-2}, respectively

Another lysimeter experiment took place in the outdoors lysimetric facility of the ISRA/CNRA station (Bambey, Senegal, 14° 41 N; 16° 27 W, elevation 20 m) during the 2021 dry season (March to June 2021) characterised by high VPD and light (4.9 kPa and 2068 μmol m^{-2} s^{-1} respectively) (Figure 1). The platform had the same characteristic as the LysiField platform with small differences: tubes were 25 cm in diameter and 1.50 m long, they were filled with a sandy soil. The experimental design was the same as above, except that high and low plant densities were 16 and 8 plants m^{-2}, respectively.

Transpiration was calculated from regular weighings of the lysimeters, taking into account the water given after each weighing. Lysimeters were lifted with a block-chain pulley and weighed with a S-type load cell every 7th–10th day. Direct soil evaporation was prevented by a 2-cm layer of plastic beads (India) or a 5-cm layer of gravel (Senegal) located on the soil surface. Plants were fully irrigated during the entire

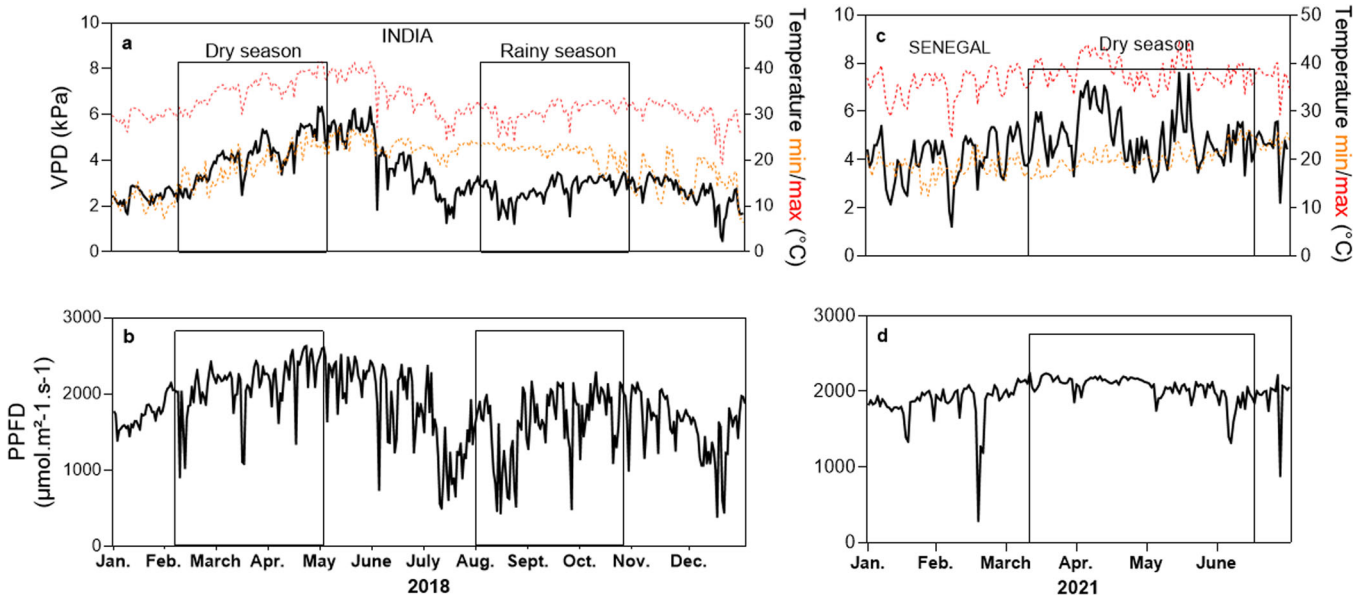

FIGURE 1 | Daily vapour pressure deficit (VPD, black), minimum (orange) and maximum (red) temperature in experiments in India (ICRISAT meteorological station in 2018) (a), and Senegal in 2021 (CNRA Bambey station) (c). Photosynthetically active Photon Flux Density (PPFD) in experiments in India (ICRISAT meteorological station in 2018) (b), and Senegal in 2021 (from nearby Niakhar meteorological station in 2021) (d). Empty frames correspond to the periods of lysimetric measurements.

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experiment. To ensure this, the lysimeters were watered to field capacity before initiating the weighings. Then, after each weighing on day 'n', each tube was individually re-watered on the basis of its initial field capacity weight, so that:

$$\text{Water added (L)} = \text{Field capacity weight (kg)} - \text{Weight day 'n'} - 2$$

The 'minus 2' component was there to avoid the risk of water drainage down the tubes. With this "buffer", lysimeters were re-watered to about 80%–90% field capacity after each weighing, and lysimeters were large enough that plants faced no water stress until the next watering.

In India, lysimeters were weighed 9 and 8 times during the rainy and dry seasons, respectively, resulting in 30 transpiration values for each genotype across the two seasons and the two plant densities. In Senegal, lysimeters were weighed 11 times, giving 20 transpiration values for each genotype across the two plant densities. In both cases, measurements covered a period that could vary from 7 to 10 days intervals between consecutive weighings. In both locations, all the tubes were weighted in 1 day and re-watering done the day after for all lysimeters.

Evaporative demand (ET_{ref}) was calculated during the same periods of time (between two weighings of lysimeters). Because it was one of the first time ET_{ref} was used as a proxy for the evaporative demand, we compared two ways to compute it. The first method was based on the simulation of the transpiration of a reference genotypes by using the APSIM crop simulation model parametrised with a sorghum genotype having a phenology and leaf area similar to those of genotypes of panel A, based on Priestley-Taylor equation (PRIESTLEY and TAYLOR 1972). The transpiration of this reference genotype, simulated with unlimited water supply, is akin to an environmental variable, and was considered as a reference transpiration. The second method involved the Penman-Monteith equation corrected by a crop coefficient (Kc) (Zotarelli et al. 2014) which takes into account the change of LAI with time, ranging from 0.4 to 0.85 according to plant stages (Piccinni et al. 2009). The ET_{ref} calculation, updated by FAO (Allen et al. 1998), is such that:

$$ETref = \frac{0.408\Delta(Rn-G) + \gamma\frac{900}{T+273}\mu(es\text{-}ea)}{\Delta + \gamma(1 + 0.34\mu)}$$

where Δ is the slope of the saturation vapour pressure versus temperature relationship, Rn is the net radiation, G is the soil heat flux, γ is the psychrometric constant, T is temperature, μ is wind speed, and (es - ea) represents the vapour pressure deficit of the air.

For both methods, we used as inputs the daily temperature, hygrometry, wind and solar radiation data collected on site at the ICRISAT and Bambey meteorological stations. Only the second method was used for the Senegal experiment because there was no genotype with existing parameters in APSIM that had phenology and leaf area similar to genotypes of panel B. Daily ET_{ref} values obtained with either method were then averaged between two weighings of lysimeters. Since ET_{ref} was computed in mm m^{-2} day^{-1}, transpiration data, initially measured in kg of water loss per replication (4 tubes), were also converted into mm m^{-2} day^{-1}, by dividing raw transpiration values by the surface area of each replicate (4 tubes) and by the number of days between two weighing dates. Daily ET_{ref} values were then plotted against the average daily transpiration values for the same periods. The transpiration response of each studied genotype to ET_{ref} was examined for India and Senegal, using transpiration and ET_{ref} over periods of 7 to 10 days, combining data from the dry or rainy seasons in India, and combining data from the two plant density treatments in both locations.

2.3 | Transpiration Response to the Evaporative Demand in the Glasshouse

An experiment was performed in Montpellier, France in April-May 2021, at the IRD automated lysimetric platform located in a glasshouse. Light conditions and VPD were recorded and fluctuated in the glasshouse, with VPD values ranging from 1 kPa at sunrise to 3.5 kPa at 2 pm. 16 L pots were filled with a mix of loamy clay agricultural soil and sand, each containing four plants. Three replicates of each genotypes were grown. Plastic beads covered the soil surface (4–5 cm layer) to avoid water loss due to direct soil evaporation. 15 days after sowing, transpiration was measured every week during 5 weeks. After each weighing, pots were watered back to 500 g below field capacity, as described above for the lysimeters. Five days before the end of this period, the pots were transferred on top of automatic load cells and organised in mini canopies of 20 plants m^{-2}. Tables that supported these load cells (10 per table) were 1.6 m^2. The load cells recorded the weight of each pot every 30 min for a 4-day period. In this experiment, the response of transpiration to the evaporative demand was measured during 4 days before final harvest, when plants were the largest and formed a canopy. A data analysis pipeline (adapted from (Kar et al. 2020) generated a smoothed transpiration profile for each of these 4 days. Then the response of transpiration to the evaporative demand was estimated during the 3 h preceding the maximum transpiration, corresponding to the maximum evaporative demand on any particular day.

2.4 | Measurement of WUE_{bm} in Canopy-Grown Plants

WUE_{bm} was calculated as the ratio of above ground biomass to the cumulated transpiration between the beginning and the end of weighing of the lysimeters in the trials described above (India, Senegal, and IRD-France). Plants were harvested at 13 and 14 weeks after sowing in the dry and rainy seasons respectively in India, at 14 weeks in Senegal, and 7 weeks in France. The biomass was dried in an oven during 72 h at 60°C and weighted. At the beginning of the transpiration measurements it was assumed that this initial plant biomass differed little among genotypes and was then unlikely to alter WUE_{bm} differences among genotypes, as discussed earlier (Vadez et al. 2011).

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2.5 | VPD and Light Penetration Assessment Within Canopies

These experiments were meant to measure VPD inside plant canopies and compare it between density treatments and to air VPD. This was done both in a field experiment in Senegal and in a glasshouse experiment at CIRAD, France.

The field experiment was carried out at the CNRA Bambey station, near and simultaneously with the lysimeter experiment. It consisted of 2×4 m micro plots sown with one genotype each, with three repetitions per genotype. The low density plots harboured 11 plant m^{-2} consisting in 4 plant rows, 15 cm between plants within a row, and an inter row spacing of 60 cm. High density (HD) plots harboured 22 plants m^{-2} with an inter row spacing of 30 cm and the same plant distribution otherwise. Before sowing, the field was fertilised with di-ammonium phosphate at a rate of 100 kg/ha and top dressing with 100 kg/ha urea 4 weeks after sowing. Daily temperature, hygrometry were collected on site at the CNRA Bambey meteorological station, whereas the radiation data was recorded in another meteorological station located 25 km south from the field. Temperature and relative humidity sensors were positioned in the low and high density plots of the field experiment in Bambey (TinyTag ultra 2, TGU-4500, Gemini Datalogger Ltd, Chichester, UK). The sensors were placed inside the canopy, positioned between two plant rows within the plot and attached to a stem at mid-canopy height. The sensors were covered with open polystyrene boxes to avoid direct solar radiation. Temperature and relative humidity were recorded every 30 min during 6 weeks in the field trial. Air and canopy VPD were calculated based on relative humidity (RH) and air temperature values measured every 30 min, either 2 metres above the canopy for air VPD (i.e. from the meteorological station located 50 m from the field) or at mid height of the canopy. Every week, the position of sensors was adjusted according the height of the canopy. Recording of data started at 4 weeks after emergence.

The glasshouse experiment was carried out at CIRAD Montpellier in April 2020 with a daytime VPD of 3 kPa maintained over the studied period, with the sole purpose of comparing intra-canopy VPD between two planting densities. Plants were grown in square pots of 4-L capacity filled with horticultural potting soil, which is composed of coarse and fine peat, clay, sand and perlite with a pH of 5.5 and an EC of 0.2. Pots were placed on 1 m^2 tables. The high and low density treatments consisted in 1 m^2 tables with 24 or 12 pots. Each treatment was replicated three times for each genotype in the glasshouse. Similar temperature and relative humidity measurements as those done in the field in Senegal were performed in this glasshouse experiment with two genotypes from panel B in April 2020. The sensors were placed at 15 cm above the soil surface (i.e., few centimeters above the pot brim). Recording of data started at fifteen days in the glasshouse and lasted for 15 days. At 16 and 22 days after sowing, leaf area index (LAI) was measured in both low and high-density canopies using a light sensor (Spectrol LI-80, Li-Cor).

The field experiment was also used to measure putative genotypic differences in light intensities at different heights within the canopy, and in leaf area index (LAI), obtained from Photosynthetic Photon Flux Density (PPFD) measurements in both low and high-density canopies. A light sensor (LI-190R-BNC-2 Quantum Sensor) was placed above the canopy, and a sensor bar (LI-191R-BNC-2 Line Quantum Sensor) placed at the ground level. LAI was calculated indirectly as $LAI = \ln(\frac{I}{Io})/k$ where Io is the incident light above the canopy, I is the light at ground level, and k is the crop extinction coefficient that was set at 0.6 for sorghum (Flénet et al. 1996). The Photosynthetic Photon Flux Density (PPFD) was measured 45 and 60 days after sowing with a light sensor (LI-190R-BNC-2 Quantum Sensor) placed above the canopy, and two sensor bars (LI-191R-BNC-2 Line Quantum Sensor) fixed perpendicularly on a pole placed vertically in the plots and adjusted respectively at mid-canopy level and at ground level. Mid canopy height was assessed for each genotypes thanks to graduations marks on a vertical pole. All sensors were connected to a data logger (LI-1500 Light Sensor Logger), and data were recorded between 12:30 to 1:30 pm. Each data point was the mean of four replicates in each plot. At 44 days after sowing, a light measurement was also done at ground level in a plot of high and low density at four time-point during this particular day, at 7am, 10am, 2 pm and 5 pm.

2.6 | Statistical Analysis

Statistical analysis involved analysis of variance, *t*-test, linear and second order regressions which were performed using GraphPad Prism version 9.2.0 for Windows, (GraphPad Software, San Diego, California USA, www.graphpad.com). Slopes and time courses of transpiration in the IRD glasshouse experiment were smoothed and analyzed using an analysis pipeline under R software version 4.1.2, adapted from (Kar et al. 2020).

The transpiration response to ET_{ref} was first compared to that to VPD (Figure 2), trying different possible functions. Several models were also compared to analyze the transpiration response to ET_{ref}. To test for a putative break in linear regressions, a segmental linear regression model was used as in Schoppach and Sadok (2012). The model involves the fitting of two linear segments joined at a common breakpoint. The equations are defined as follows:

$$Y = b1 + m1 * X, \text{ for } X \leq Xbreak \text{ (first segment), and}$$

$$Y = b2 + m2 * X, \text{ for } X > Xbreak \text{ (second segment).}$$

The software optimises the location of the breakpoint (Xbreak) to minimise the sum of squares residuals across both segments. Optimisation was performed using nonlinear least-squares regression. The software iteratively determined the optimal parameters, including the slopes (m1, m2), intercepts (b1, b2), and the breakpoint (Xbreak). A continuity constraint was enforced to ensure that the Y-values at the breakpoint were equal for both segments. The goodness-of-fit was assessed using the coefficient of determination (R^2) and residual analysis.

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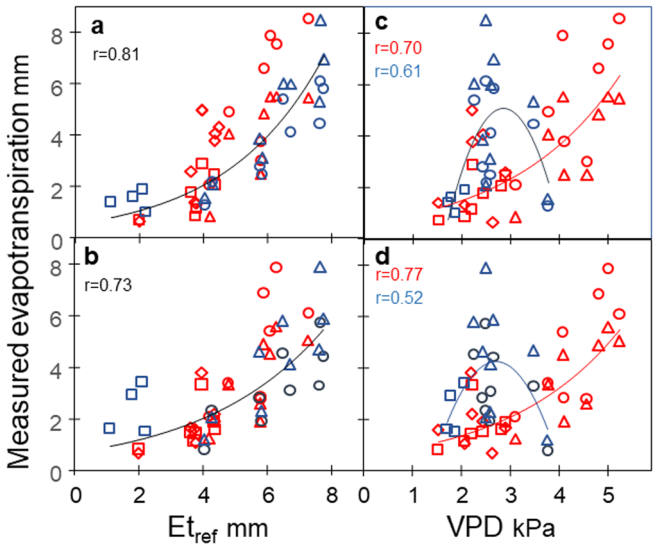

FIGURE 2 | Measured evapotranspiration as a function of Penman Monteith's reference evapotranspiration (Et_{ref}) or air vapour pressure deficit (VPD). (a, c) Two typical genotypes of panel A tested in India (red) and panel B tested in Senegal (blue) with high measured evapotranspiration (ICSV93046 and G12, respectively). (b, d) Two typical genotypes of panel A tested in India (red) and panel B tested in Senegal (blue) with low measured evapotranspiration (ICSB404 and G13 respectively). All experimental situations are represented in each panel. Red circles: India dry season, high plant density (HD). Red triangles: India, dry season, low density (LD). Red squares, India, rainy season HD. Red diamonds India, rainy season LD. Blue circles: Senegal dry season HD. Blue triangles: Senegal dry season, LD. Blue squares: greenhouse in Montpellier. In (a) and (b), data could be fitted to a single exponential function. In (c) and (d), data had to be fitted to different functions for data acquired either in India or in Senegal + Greenhouse. The other genotypes are presented in SI 1 and 2. [Color figure can be viewed at wileyonlinelibrary.com]

A polynomial model was also tested and compared to these linear and segmental linear models. We conducted a comparison of model fit using the Fisher test to evaluate whether quadratic or segmented regression models provided a significantly better fit than a linear model. For each genotype, we tested the models under two conditions: evaporative demand (ET ref) and vapour pressure deficit (VPD). The p-values indicate whether the more complex models significantly improve the fit compared to the linear regression. Based on the results, we determined the preferred model for each case.

Broad sense heritability was calculated as in Falconer et al, 2005, $H^2 = \frac{\sigma g^2}{\sigma p^2}$, where H^2 is the ratio of total genetic variance to total phenotypic variance plus residuals, with σg^2 the squared genotypic variance and σg^2 the squared phenotypic variance obtained through analysis of variance, as recommended by (Schmidt et al. 2019). In the model, the phenotypic variance, or environmental effect, was the variance between values of each of the four replicates within each genotype and treatment and genotypic effect was the part of the variance explained by the genotype within the four replications across genotypes. The same approach was used in the calculation of heritability for the different traits presented in this study.

3 | Results

3.1 | Which Model for Comparing Genotypes? Linear Relationships With ETref Allowed Comparison Across Environments, Relationships With Air VPD Did Not

The first step was to choose the most appropriate driving variable for the transpiration response, either evaporative demand (ET_{ref}) or VPD, across environments (either India or Senegal) and all genotypes of panels A and B. For that, we fitted measured transpirations with either ET_{ref} or VPD via quadratic equations. For example ETr fitted with ET_{ref} (Figure 2a,b) with a common model for two genotypes with high evapotranspiration rates (ETr) (Figure 2a). The same result was observed for two genotypes with low ETr (Figure 2b). By contrast, the fitting of ETr with VPD could not use the same model for data coming from either India or Senegal (Figure 2c,d), indicating that VPD could not be used as a reliable variable to compare genotypes. The whole panels are described in Figures S1 and S2). For panel A tested in India, the fitting with ET_{ref} was always better than with VPD, although fitting with VPD was also very good (Figure S1). For the whole panel B measured in Senegal (Figure S2) the fitting with VPD was clearly not proper and only the fitting with ET_{ref} was relevant.

We then compared different models for each genotype. To highlight putative changes in slopes at high evaporative demand, we fitted the data with linear, segmental linear, and polynomial models. In panel A measured in India, we did not observe any flattening of curves nor significant break point for polynomial and segmental models respectively (Figure S1). Similar results were observed in panel B, measured in Senegal (Figure S2, Table 1). We therefore considered ET_{ref} as the driving variable for transpiration rate in the following analyses, and used linear regressions to compare genotypes for the sensitivity of their transpiration responses to ET_{ref}.

3.2 | The Sensitivity of Transpiration Rate to Evaporative Demand and WUE_{bm} Had Large Genetic Variabilities in Both Datasets

The slopes of linear regressions, called 'sensitivity of transpiration to ET_{ref}' hereafter, significantly differed between genotypes in the data set with panel A in India (Figure 3a, $p = 0.03$), with a high heritability ($H^2 = 0.56$, Tables S1 and S2). Hence, the studied genotypes differed in their ability to transpire at a given evaporative demand with up to three-fold differences, for example, from 2.3. to 6.28 mm d^{-1} for a common ET_{ref} of 5.80 mm d^{-1}. Importantly, the slopes for each genotype were independent of leaf area index (LAI), at both the first ($r = 0.2$, $p = 0.42$) and the second ($r = 0.03$, $p = 0.86$) dates of LAI measurement (Figure S3a,b). Similar results were observed whether ET_{ref} was calculated either via the Penman-Monteith equation corrected for leaf area, or with the APSIM model, with a very high correlation between calculated slopes (Figure S4a). The genetic variability of sensitivities of transpiration to ET_{ref} was even larger in panel B tested in Senegal ($p = 0.02$), with slopes ranging four-fold with a heritability of $H^2 = 0.41$. For example,

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TABLE 1 | Results of the Fisher test comparing the fit of quadratic and segmented regression models against a linear model for different genotypes under two conditions: Evaporative demand (ET ref) and vapour pressure deficit (VPD). The *p* values indicate whether the more complex models provide a significantly better fit than the linear model. Linear regression was the best choice for all cases, as neither the quadratic nor the segmented regression models significantly improved the fit.

	Fisher test for comparison of fit with linear model (*p* values)			
	Evaporative demand (ET_{ref})		VPD	
	Quadratic	Segmental regression	Quadratic	Segmental regression
India global data set				
CSH 16	0.09	0.06	0.09	0.06
ICSB 404	0.26	0.41	**0.03**	0.08
ICSH 14002	0.63	0.84	0.56	0.59
ICSH 28001	0.69	0.87	0.73	0.76
ICSR 101	0.43	0.43	0.37	0.27
ICSR 14001	0.29	0.36	0.46	0.47
ICSR 196	0.34	0.47	0.40	0.45
ICSR 89058	0.26	0.43	0.11	0.28
ICSV 112 (CSV 13)	0.29	0.39	0.30	0.40
ICSV 15013	0.50	0.79	0.67	0.79
ICSV 25302	0.87	0.97	0.73	0.81
ICSV 25308	0.94	0.97	0.85	0.90
ICSV 25316	0.98	0.94	0.87	0.85
ICSV 745	0.35	0.43	0.35	0.38
ICSV 93046	0.35	0.64	0.29	0.47
Isiap Dorado	0.27	0.17	0.27	0.23
MR 750	0.25	0.21	0.24	0.28
NTJ-2	0.60	0.79	0.65	0.76
PVK 801	0.39	0.48	0.39	0.44
S 35	0.52	0.68	0.65	0.71
Preffered model	Linear regression		Linear regression	
Senegal global data set				
G1	0.70	0.88	0.52	0.32
G10	0.92	0.98	0.41	0.36
G11	0.84	0.95	0.50	0.40
G12	0.74	0.80	0.37	0.30
G13	0.95	0.97	0.68	0.42
G14	0.81	0.91	0.49	0.61
G15	0.91	0.97	0.78	0.59
G16	0.89	0.97	0.89	0.60
G17	0.60	0.77	0.38	0.25
G18	0.94	0.99	0.97	0.83
G19	0.75	0.92	0.55	0.46
G2	0.44	0.72	0.46	0.36
G20	0.91	0.98	0.77	0.96
G21	0.98	0.99	0.98	0.85
G22	0.99	0.99	0.94	Unstable
G23	0.53	0.81	0.82	0.76

(Continues)

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TABLE 1 | (Continued)

	Fisher test for comparison of fit with linear model (p values)			
	Evaporative demand (ET_{ref})		VPD	
	Quadratic	Segmental regression	Quadratic	Segmental regression
G24	0.72	0.89	0.61	0.49
G25	0.92	0.99	0.82	0.68
G26	0.85	0.96	0.86	0.77
G27	0.46	0.76	0.33	0.33
G3	0.73	0.89	0.56	0.40
G4	0.80	0.95	0.79	0.72
G5	0.67	0.85	0.62	0.51
G6	0.80	0.94	0.31	0.32
G7	0.82	0.92	0.60	0.42
G8	0.57	0.81	0.71	0.49
G9	0.45	0.70	0.59	0.43
Preffered model	Linear regression		Linear regression	

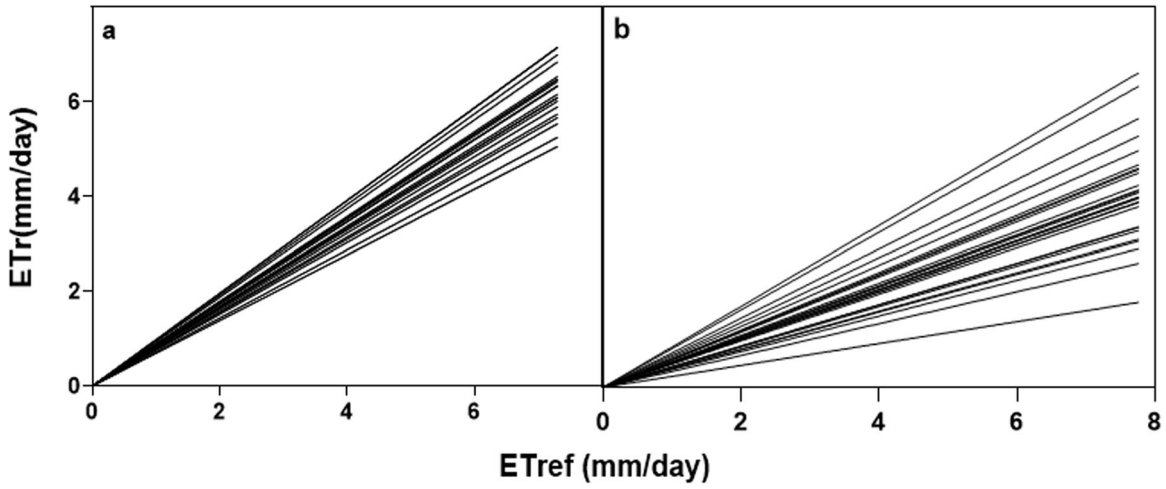

FIGURE 3 | Measured evapotranspiration (ETr) plotted against the reference evapotranspiration (ETref), for the 20 genotypes of the panel A tested in India (a) and the 27 genotypes of the panel B tested in Senegal (b). Regression lines for all studied genotypes (one line per genotype), fitted with 4 replications per genotype and treatment ($p < 0.0001$). The slope of each regression was used to characterise the genotypes for the sensitivity of the transpiration in response to evaporative demand.

transpiration rate ranged from 1.03 to 9.14 mm d^{-1} for a common ET_{ref} of 5.83 mm d^{-1} (Figure 3b). Similar results were also observed in the glasshouse experiment with 9 genotypes from panel B, where the sensitivities of transpiration to ET_{ref} differed between genotypes ($p < 0.01$, $H^2 = 0.39$).

WUE_{bm} showed a high genetic variability in panel A during both the rainy and dry seasons in India (8.4–11.9 and 2.2–3.2 g. kg^{-1}, respectively, $p < 0.0001$ and < 0.01), with a high heritability calculated across seasons ($H^2 = 0.64$) (Figure 4a,b). A significant genotype-by-season interaction for WUE_{bm} was observed ($p = 0.0004$) (Table 2). Similar results were observed in Senegal with panel B, with a high genetic variability for WUE_{bm} (1.4–3.9 g. kg^{-1}, $p < 0.01$) and a high heritability ($H^2 = 0.65$, Figure 4c).

3.3 | Genotypes That Transpired Most at a Given Reference Evapotranspiration Had the Highest WUE_{bm} in the Dry Season, and the Lowest in the Wet Season

Counter intuitively, the genotypes that most transpired at a given ET_{ref} (highest slopes presented above) had the highest WUE_{bm}, with a high correlation between WUE_{bm} and the sensitivity of transpiration to ET_{ref} in the dry season in India

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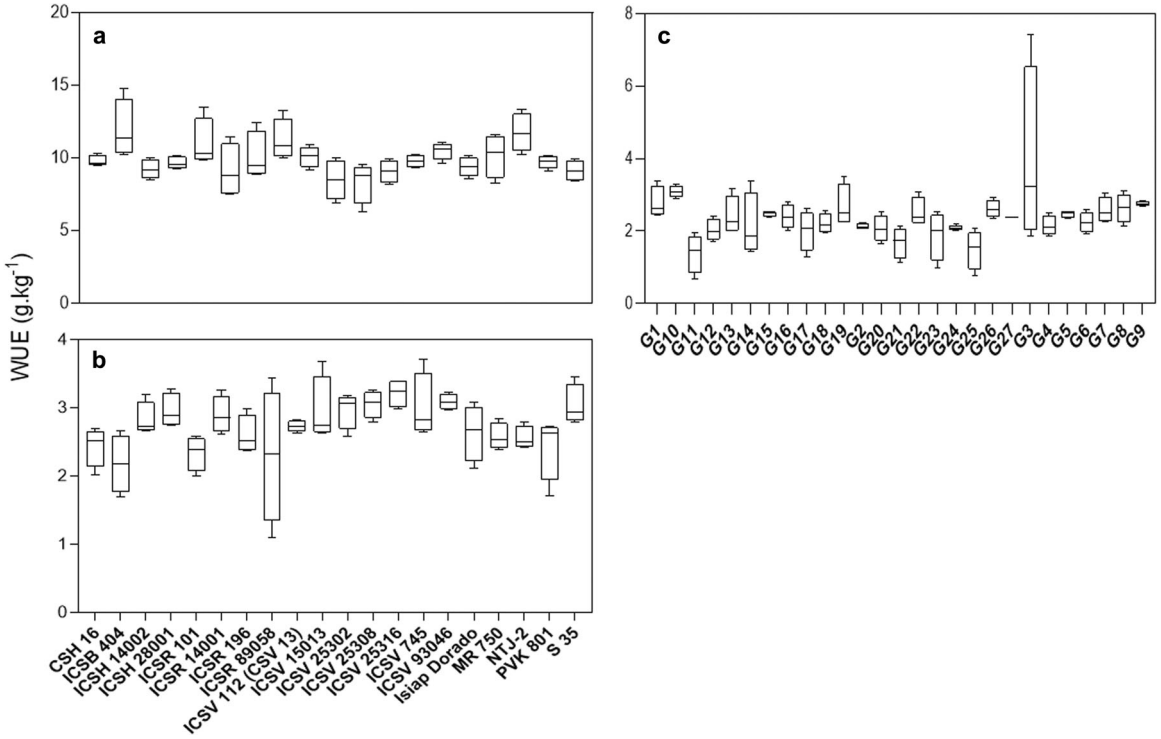

FIGURE 4 | Water use efficiency (WUE, grams of biomass per kilogram of water used) for the 20 genotypes of the panel A grown on the lysimeter platform during (a) the low and (b) the high VPD season 2018 at the ICRISAT station, (c) for the 25 genotypes of the panel b during the 2021 high VPD season in Bambey. Data are the mean of four replications per genotype.

TABLE 2 | ANOVA table showing the genotypic variability for the water use efficiency (WUE) in the dry and rainy season experiment in India and the dry season experiment in Senegal.

Source of variation	Dry season 2018	Rainy season 2018	Dry season 2021
Location	India	India	Senegal
Genotypes panel	A	A	B
Two-way ANOVA			
Genotype	*	****	*
Genotype × Season	***	NA	NA

**$p < 0.01$; *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$.

($r = 0.64$, $p < 0.0001$,) (Figure 5a) and lower correlation but also significant in Senegal ($r = 0.54$, $p < 0.01$) (Figure 5b). The same pattern was observed in the glasshouse experiment with 9 genotypes of panel B and carried out under high VPD, where the sensitivity of transpiration to ET_{ref}, measured during the 3 h of highest evaporative demand, was positively correlated with WUE_{bm} ($r = 0.78$, $p < 0.05$) (Figure S5).

Conversely, the relationship between WUE_{bm} and the sensitivity of transpiration to ET_{ref} was strong and significantly negative in the rainy season of India ($r = -0.65$, $p < 0.01$, Figure 5c). That is, the genotypes that most transpired in response to ET_{ref} had the lowest WUE_{bm} in this case. All these results held whether ET_{ref} was calculated via either the APSIM model or the Penman Monteith equation corrected for leaf area (Figure S4b,c).

3.4 | The Positive Genetic Link Between WUE_{bm} and the Sensitivity of Transpiration to ET_{ref} in Dry Seasons May Stem From Lower VPD and Increased Light Penetration in the Canopy of Most Sensitive Genotypes

A first possible explanation of above results is that transpiration appreciably decreased VPD inside the canopy during the day, thereby affecting WUE_{bm} differentially between genotypes. Intra-canopy VPD was measured in a field trial in Senegal and in a glasshouse experiment in France. In the field trial, the measured VPD was lower within the canopy than in the air, to a greater extent in high than in low plant density. In high-density canopies, this difference became significant from Day 42 onwards (Figure 6a), when LAI was approximately 2.9 (measured on Day 45). In low-density canopies, it became significant 14 days later (Day 56) when LAI was also close to 3 (3.2 on Day 53) (Figure 6a). Differences in VPD were maximum in the morning and early afternoon (average difference of 0.97 kPa, $p = 0.05$ over this period, Figure 6b). Differences in VPD between canopy and air were also observed in the glasshouse experiment during a 15-days period, with a mean difference of 0.63 kPa (0.55–0.93 kPa, $p = 0.001$, Figure S6). Overall, VPD within the canopy was lower than that sensed by upper leaves in direct relation to open air, and more so in dense canopies. This was likely due to the intra canopy transpiration because differences nullified during the night. Genotypes that most transpired under high evaporative demand were, therefore, less penalised than expected in terms of WUE_{bm}.

Light penetration in the canopy is also a possible driver for the positive genetic relationship between sensitivity to evaporative demand and WUE_{bm}. In the field experiment in Senegal, light

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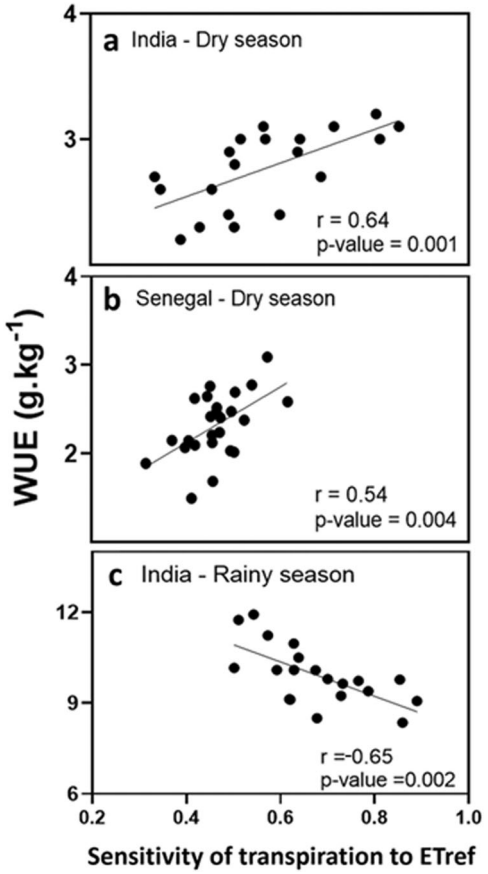

FIGURE 5 | Water use efficiency (WUE) plotted against the sensitivity of the transpiration to ETref (see Figure 2). (a) 20 genotypes of panel A in the dry season, India ($r = 0.64$, $p = 0.0001$). (b) 27 genotypes of panel B in the dry season, Senegal ($r = 0.54$, $p = 0.004$). (c) 20 genotypes of the panel A in the wet season, India ($r = -0.65$, $p < 0.01$). Data are means of 4 replications per genotype and treatment ($p < 0.0001$).

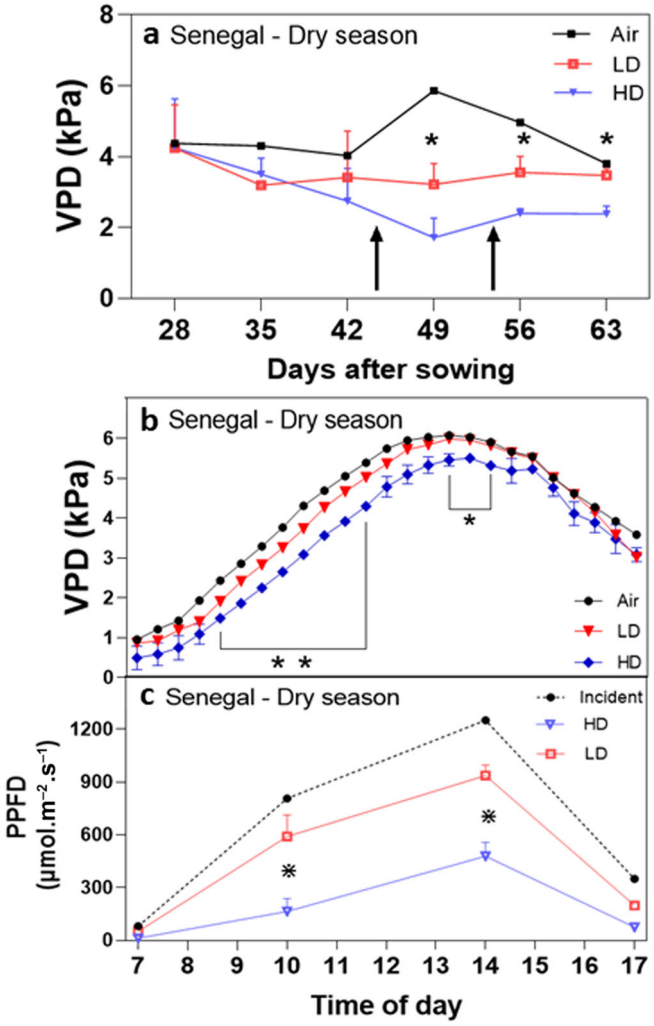

FIGURE 6 | (a) Vapour pressure deficit (VPD) measured in the air and within canopies with high and low densities (11 and 22 plants/m^2 respectively, HD and LD), as a function of time after sowing in the dry season field trial in Senegal. Black arrows in (a) represent dates at which LAI was measured (45 and 53 days after sowing), and stars indicates significant differences between HD and LD (paired *t*-test, $p < 0.001$). (b) Daily time course of VPD during the 4th week of the same field experiment from 7am to 7 pm, where stars indicate significant differences between HD and LD (paired *t*-test, $p < 0.001$). (c) Photosynthetically active Photon Flux Density (PPFD) at 4 time points across the same day in the two densities. Stars indicates significant differences between HD and LD (paired *t*-test, $p < 0.0001$). Each data point is the average of sensor data collected in three plots for each of the densities. [Color figure can be viewed at wileyonlinelibrary.com]

measurement at ground level on Day 44 showed that light was indeed appreciable within the canopy, to a larger extent for canopies with low than high density, at 10am (488 vs. 165 µmol m^{-2} s^{-1} respectively) and 2 pm (958 vs. 479 µmol m^{-2} s^{-1} respectively) (Figure 6c). This light penetration had a significant genotypic variation at ground level (45 DAS) and mid-height canopy (60 DAS) (*t*-test, $p < 0.0001$), which correlated with WUE_{bm} in the dry season (Figure 7a,b). Genotypes with the highest light intensity within the canopy (i.e. those that most let the light penetrate at mid-canopy level) at 60 DAS when LAI was 3.9 were those that had highest WUE_{bm} ($r = 0.44$, $p = 0.009$) (Figure 7b). At this phenological stage, the genotypic correlation was observed with light intensity at mid-canopy level and not at ground level where light was close to zero because studied canopies had intercepted nearly all incident light. At earlier stage (45 DAS, LAI = 2.9), a similar positive genotypic correlation between WUE_{bm} and light intensity at ground level was observed ($r = 0.56$, $p = 0.04$) (Figure 7a). Hence, genotypes that most transpired at a given evaporative demand were also those for which more light reached leaves in the lower canopy, potentially increasing the photosynthesis of these leaves. Because the intra-canopy VPD was lower than air VPD (Figure 6a,b), the response of transpiration rate to ET_{ref} had a higher effect on biomass than on transpiration, thereby increasing WUE_{bm} (Figure S7a). This was not observed in the rainy season (Figure S7b), probably explaining the observed negative genotypic effect of transpiration on WUE_{bm}.

3.5 | Differences in WUE_{bm} and in the Sensitivity of Transpiration to ET_{ref}, and Their Link, May Stem From Genotypic Differences in Canopy Architecture

We then tested if differences in traits related to plant architecture could explain the differences in light penetration. For this,

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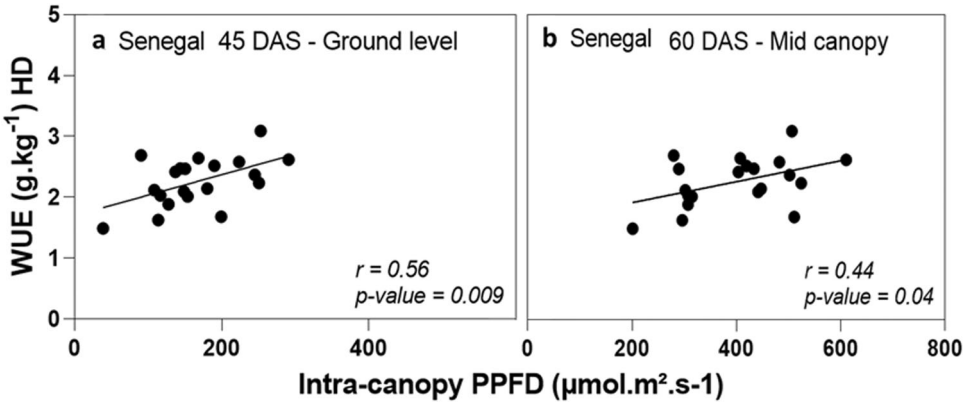

FIGURE 7 | Water use efficiency (WUE) measured in lysimeters plotted against the amount of light measured in the canopy in the adjacent field trial during the Senegal dry season, at (a) 45 DAS (ground level) and (b) 60 DAS (mid-canopy level). Data are the mean of four replications for WUE and three replications for light measurements.

we re-used the data from a recent paper where genotypic differences in several three-dimensional (3D) architecture traits were measured, in particular the leaf inclination and the average surface angle (Xue et al. 2025 for method details). We found that WUE_{bm} was positively and significantly related to a higher leaf inclination (more erected leaves) (Figure 8a, $r = 0.71$, $p = 0.006$). Similarly, WUE_{bm} was positively and significantly related to a higher average surface angle (Figure S8A). Results also showed that low sensitivity to the evaporative demand (high slopes ETr-ET_{ref}) was positively related to leaf inclination (Figure 8b, $r = 0.67$, $p = 0.01$) or average surface angle (Figure S8b), that is, genotypes that responded the most to the evaporative demand where those with a canopy architecture with erected leaves and allowing more light penetration.

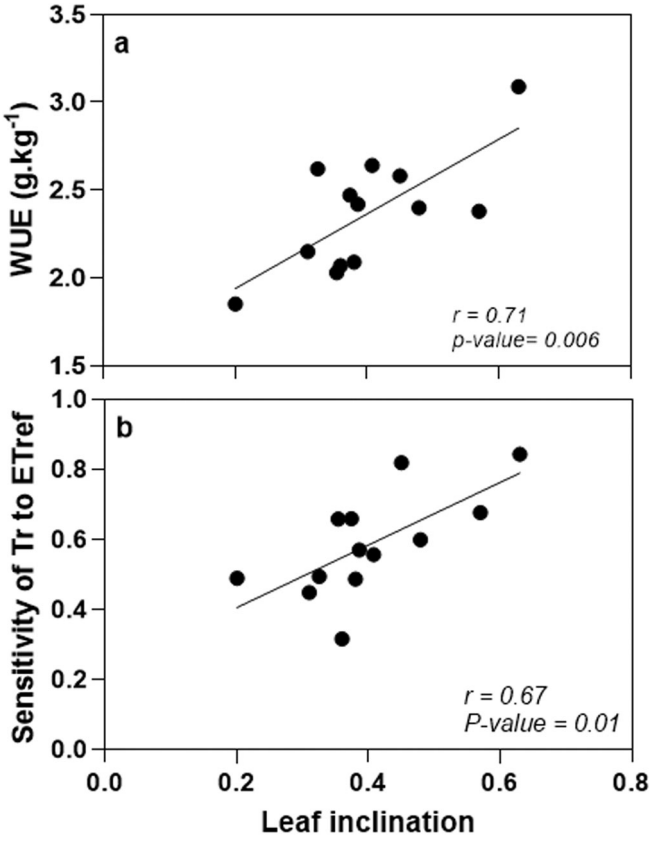

FIGURE 8 | Water use efficiency (WUE, a) and sensitivity of transpiration to ET_{ref} (b), both measured using the lysimetric experiment data from Senegal, as a function of the leaf inclination trait obtained from 3D scan images of plants generated in the glasshouse experiment in France. Dots represents genotypic means for the 13 genotypes common to these two experiments. Linear regressions shows positive and significant relations. Relationship coefficients and p-values are displayed.

4 | Discussion

Five novel results, some unexpected, came from this study:

i. Transpiration responses to ET_{ref} provided a more accurate and comprehensive fit than that to VPD, in the comparison of genotypes. This departs from most earlier studies in which the evaporative demand was taken into account via VPD alone as the main driver for transpiration, whereas light is an essential component of it.

ii. No significant breakpoints were observed in the response curves of transpiration to evaporative demand, despite extensive testing. While this result was unexpected, it adds new insights, particularly for comparing genotypes and also regarding physiological controls. We propose that this absence may be linked to the decoupling of air and intra-canopy VPD, maintaining a high response at elevated evaporative demand.

iii. A significant genetic variability was observed for transpiration at a given evaporative demand, with up to a four-fold difference between genotypes. This level of variability, observed only when using ET_{ref}, has not been reported before.

iv. Counter intuitively and departing from earlier expectations, genotypes with the lowest sensitivity to ET_{ref} (highest slope ETr-ET_{ref}) had the highest WUE_{bm} during the dry season with highest light intensity, and the lowest WUE_{bm} during the rainy season with lower light intensity.

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v. High WUE_{bm} and low sensitivity to ETref were associated with more erect canopies (higher leaf inclination, higher average surface angle). High WUE_{bm} also correlated positively with more light penetration to the lower canopy. This challenges existing views on the genetic variability of WUE_{bm}, its possible underlying physiological explanation, and its environmental interactions.

These results, together with the recent viewpoint on the transpiration limitation trait (Gleason et al. 2025), call for a re-visit at the way this trait is approached and analyzed, to better harness and target its potential.

4.1 | ET_{ref} Rather Than VPD

Our study considered the evaporative demand as ET_{ref}, calculated via the Penman-Monteith equation or APSIM model and which includes light as a factor, as the main driver for transpiration. This may explain why transpiration better fitted to ET_{ref} than to VPD. By using ETref, we not only improved the description of the immediate environment experienced by the plants, but also make possible to integrate data from multiple experiments, avoiding the confounding effects of VPD, temperature, and light.

Why different shapes of the response of transpiration to evaporative demand were observed between earlier results in growth chamber and in our outdoors study? We raise the possibility that this is due to the difference in light intensities between the two conditions. Under relatively low light and high air movement (high coupling) (Jarvis and Mcnaughton 1986; Li et al. 2017), as in a growth chamber, the decrease in stomatal conductance with high VPD directly translates into a reduction in transpiration rate. In conditions with high light and lower wind, uncoupling occurs between stomatal conductance and transpiration for most species, so leaf temperature increases with stomatal closure, thereby increasing leaf-to-air vapour pressure difference, largely decreasing the effect of stomatal closure on transpiration rate (Chaves et al. 2016). It was unexpected that a good relationship between transpiration and air VPD was still observed in our experiments in India, as it did in former studies (Kar et al. 2020; Kholova et al. 2010a). This was probably due to the correlation between light intensity and VPD, observed in our study, but potentially not in other climatic conditions, for example in regions where the wind brings either dry air from continental areas, or wet air from the sea, depending on its direction, with an unchanged light intensity (Salah and Tardieu 1996). Indeed, this correlation was not observed in the Senegal experiment.

4.2 | Simple Linear Rather Than Segmented Linear Regression

The absence of a breakpoint in relationships may be, partially, a consequence of the fact that the Penman Monteith's evaporative demand ETref was taken, in which light is taken into account as a key driver for transpiration. The discrepancy with earlier results may also be due to the fact that relationships were established at canopy level, so intra-canopy VPD increases less than air VPD. In this view, the breakpoint would be a trend for isolated plants grown in growth chambers, whereas the decoupling between air and intra canopy VPD would contribute to maintain the high response at high evaporative demand.

It was considered by Sinclair and other authors that the differential responses to evaporative demand is both characterised by stomatal closure which occurs at differently high VPD for each genotype (VPD breakpoints) and by differences in the slopes before and after the breakpoint, thereby resulting in a genetic variability of responses (e.g. Fletcher et al. 2008; Kholova et al. 2010a). Here, quadratic terms of regressions, all positive when significant, indicated a concave rather than a convex relationship. Several studies carried outdoors also showed linear responses, even when the evaporative demand was expressed via VPD only (Tharanya et al. 2018; Devi and Reddy 2018). In the same way, when a confusion of effect between temperature and VPD was avoided, only a small proportion of wheat genotypes presented a plateau under high evaporative demand (Tamang et al. 2022). Other studies have shown that certain genotypes that earlier showed a breakpoint in the transpiration response to an increased VPD, lost this breakpoint when the transpiration response to VPD is measured under higher temperature (Pradhan et al. 2019; Shekoofa et al. 2021). This could have in part been the case in our study. Importantly, some of the genotypes that had a linear response in our study did show a marked plateau at high VPD in a study in growth chamber (Karthika et al. 2019). Hence, a different representation emerges from studies carried out under high evaporative demand outdoors compared with those in growth chamber. Because no plateau for transpiration at high evaporative demand was observed, the cause of the genetic variability of the evapotranspiration could not be stomatal closure at high VPD, but rather a difference in transpiration rate over the whole range of evaporative demand. Indeed, an appreciable genetic variability for stomatal conductance was observed in maize, even at relatively low evaporative demands, with high heritability and consistent QTLs (Alvarez Prado et al. 2017; Welcker et al. 2011). The results presented here are important because they offer a quite different view of how stomatal control works under high evaporative demand. Having breakpoints in the transpiration response to an increase in the evaporative demand implies that there is a transient partial closure of stomata that result in a transpiration plateau or a slower increase in transpiration past this breakpoint. Such transient closure of stomata would imply rapid physiological processes, most likely of hydraulic nature. Hydraulic conductance of the xylem vessels, aquaporins, root radial conductance and other factors could be involved in these physiological processes, and these have been the object of much research to decipher the underlying physiological cause of stomata closure at high evaporative demand. In the absence of any breakpoint, the whole idea of a transient control of stomata aperture when the evaporative demand increases is dismissed. In the absence of a breakpoint, differences in stomatal conductance at high evaporative demand would be caused by inherent physiological processes that would not be triggered by changes in the evaporative demand. Some of these processes could also involve hydraulic conductance differences of different plant compartments.

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4.3 | A Large Genetic Variability of the Response of Transpiration to Evaporative Demand in Natural Conditions, From Plants in Canopy

This study is to our knowledge the most thorough observation of a genetic variability in the response of transpiration to the evaporative demand in a canopy, and the first to consider evaporative demand via two of its components, VPD and light intensity. This is not solely an extension of results from indoor to field studies because conditions were markedly different from those indoors. Plants in growth chambers are well separated from one another and have all leaves exposed to similar light and VPD (predominance of diffuse light, low competition for light and similar VPD all over). This differs from plants grown in canopies, where the leaves in the lower canopy may be exposed to a lower VPD than the upper leaves because of canopy transpiration, and a lower light because of self-shading. Provided they receive sufficient light, leaves in the lower canopy, with partly closed stomata and facing a milder VPD than in open air, may have a higher WUE_{int} than upper leaves, which may eventually increase WUE_{bm}. We observed significant linear relationships between evapotranspiration and ET_{ref} of each of the 47 tested genotypes, with a genetic variability in slopes in both panels A and B. This genetic variability of slopes were possibly due to stomatal behaviour, but also to other factors such as the canopy architecture. Given the high heritability of slopes, this genetic variability may be used in breeding to for design genotypes for either high transpiration rate (suited to favourable environmental scenarios) or lower transpiration rate (suited to drought-prone areas). Notably, the accessions of panel A had a lower genetic variability and heritability for slopes than the germplasm of panel B. Panel A include elite breeding lines from the ICRISAT breeding programme that were mostly bred for the rainy season, which could have explained this lower variation and would suggest that the response of transpiration to evaporative demand may have faced a selection pressure. Accessions from panel B, on the contrary, were germplasm accessions with diverse geographic origins.

4.4 | The Sign of the Relationship Between WUE_{bm} and the Response of Evapotranspiration to the Evaporative Demand Was Season-Dependent

WUE_{bm} was either positively correlated to the slope of the evapotranspiration response to ET_{ref} in seasons of high evaporative demand, or negatively correlated in seasons of low evaporative demand. This contrasts with earlier results showing that WUE_{bm} was related to transpiration restriction under high VPD (Sinclair et al. 2005; Ryan et al. 2016). We propose that differences in WUE_{bm} may be related to differences in the distribution of the light resource in the lower canopy. During the dry season with high VPD and light, genotypes with the highest slopes of the evapotranspiration response to ET_{ref} had highest WUE_{bm} and also allowed more light to penetrate deeper in the lower canopy. A lower VPD within the canopy may increase the WUE_{bm} of leaves transpiring within the canopy, during seasons facing high VPD, because WUE is inversely related to VPD (Sinclair et al. 1984; Condon et al. 2002). We propose that observed differences in WUE_{bm} among genotypes were, in part, a consequence of the proportion of transpiration, hence photosynthesis, contributed by leaves from the lower canopy. The large and heritable genotypic variation response of evapotranspiration to ET_{ref} supports this hypothesis. Our interpretation is that a stronger evapotranspiration response would come from more leaves in the lower canopy actively participating in plant transpiration, and creating a positive feedback on WUE by releasing water vapour inside the canopy, contributing to increasing RH% and/or decreasing inner canopy temperature, then decreasing inner canopy VPD, as measured in the field and in the glasshouse (Figures 6b and S6), and finally increasing WUE_{int} of leaves inside the canopy and then increasing whole plant WUE_{bm}.

On the contrary, during the rainy season with less incident light and VPD, a higher slope of the ETr response to ETref correlated to a lower WUE_{bm}. Our interpretation is that, since light resource was lower in that season (Figure 1), it may have been insufficient to penetrate deeply in the lower canopy, so both transpiration and light interception essentially involved the top leaves in the canopy, i.e. those exposed more to air VPD of the environment, average VPD value that were still above 2 kPa during that season. Hence, we propose here that differences in canopy architecture allowed variations in the light available in the lower canopy, which boosted transpiration under high evaporative demand, and increased WUE because of a higher proportion of transpiration benefitting from milder VPD conditions allowed by dense canopies. From an agronomic standpoint, our results also show the interest of using denser canopy to force milder VPD condition in the canopy and higher WUE, as shown in a recent study (Pilloni et al. 2024). The trade-off for these benefits would be under low light conditions that would not provide sufficient light to penetrate inside the canopy and benefit inner canopy leaves from a milder microclimate, or under drought conditions.

4.5 | 3D Architecture Traits May Explain Genotypic Differences in WUE_{bm} and Sensitivity to ET_{ref}

Genotypes with highest WUE_{bm} in high VPD seasons were those for which more light was available in the lower canopy. This likely created a positive feedback loop, whereby light triggered stomatal opening and photosynthesis, which in turn generated water vapour that increased RH% and decreased both temperature and VPD, and eventually increased WUE_{bm}. These interpretations are supported by recent reports (Jaikumar et al. 2025). Architectural features could be involved in this variability of response. Indeed plant architecture strongly influences variables such as light interception (Falster and Westoby 2003; Duursma et al. 2012; Gitz et al. 2015) and radiation use efficiency (RUE) (George-jaeggli et al. 2013; Truong et al. 2015; Perez et al. 2019). Other studies have also shown a better efficiency of water use in varieties allowing a better distribution of the light resource in other species (Falster and Westoby 2003; Lee and Tollenaar 2007). Our findings of a positive relationship between WUE_{bm}, the degree of sensitivity to ET_{ref}, and the leaf inclination, supports this hypothesis. These results are consistent with the finding of significant relationships between PAR

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measured in the field at mid altitude in canopies and WUE_{bm}. From a breeding point of view, selecting genotypes with erected leaves (Jaikumar et al. 2021, 2025) may be a way of boosting WUE_{bm} in this crop.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Figure S1: Evapotranspiration measured on the lysimetric platform as a function of the ETref (calculated via the Penman-Monteith equation) or the VPD during the two 2018 lysimeters experiments in India for the 20 genotypes individually. Red triangle and circle represent HD and LD treatments during the summer season respectively. Blue rhombs and squares represent HD and LD treatments during the rainy season respectively. Regressions considers all treatments and seasons and black, green and orange lines represents the adjusted model with linear, polynomial and segmental regressions respectively. The corresponding coefficient or relation (r) are displayed in the same color. **Supporting Figure S2:** Evapotranspiration measured on the lysimetric platform as a function of the ETref (calculated via the Penman-Monteith equation) or the VPD during the 2021 experiment in Senegal for all the genotypes of panel B taken individually. Blue triangle and circles represent the high and low density canopies respectively. Regressions considers all treatments and black, green and orange lines represents the adjusted model with linear, polynomial and segmental regressions respectively. The corresponding coefficient or relation (r) are displayed in the same color. **Supporting Figure S3:** Leaf area index (LAI) measured 33 (a) and 40 (b) days after sowing in the 2018 dry season field experiment as a function of the slope of the transpiration response to evaporative demand (ETref) from the adjacent lysimetric experiment. (India, Panel A). **Supporting Figure S4:** Linear regression of the slopes values generated with Kc method as a function of the values of the slopes generated with APSIM for the dry season experiment in India (r= 0.99) (a). Water use efficiency (WUE) as a function of the slope generated by the regression of the measured evapotranspiration against the ETref calculated with the Penman-Monteith corrected with a crop constant (Kc) for the 20 genotypes of the panel A in the dry (b) and rainy (c) season in India. **Supporting Figure S5:** Water use efficiency (WUE) plotted against the slope of the time course of transpiration rate during the 3 hours preceding the maximum transpiration in the indoor lysimeter experiment (Montpellier, France, 9 genotypes from panel B). **Supporting Figure S6:** Vapor pressure deficit

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(VPD) measured in air and within canopies with high and low densities (12 and 24 plants/m² respectively, HD and LD), as a function of time after sowing in the glasshouse experiment (Montpellier, France, 2 genotypes from panel B). Stars indicates significant differences between air and HD VPD. **Supporting Figure S7:** Normalized biomass and evapotranspiration (ET) plotted against the slopes generated by the regression of measured evapotranspiration and ET_{ref} during the dry (a) and wet (b) seasons in India. **Supporting Figure S8:** Water use efficiency (WUE, a) and sensitivity of transpiration to ETref (b), both measured using the lysimetric experiment data from Senegal, as a function of the average surface angle trait measured from the 3D scan images of plants generated in a glasshouse experiment in France. Dots represents genotypic means for the 13 genotypes common to these two experiments. Linear regressions show positive and significant relations. Relation coefficients and *p*-values are displayed. **Supporting Table S1:** Values of slopes, r coefficient, significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms for all genotypes from the panel A tested in India. Regressions are ETr values from the lysimeters as a function both ETref (calculated via APSIM software) or VPD (Calculated with T° et RH data from the adjacent meteorological station). *, p-value < 0.05, **, p-value < 0.01, *** p-value < 0.001, ****, p-value < 0.0001. **Supporting Table S2:** Values of slopes, r coef., significance of the regressions, quadratic terms of the regressions and significance of the quadratic terms in all genotypes from the panel B. Regressions are ETr values from the lysimeters as a function of Etref calculated via the Penman-Monteith equation corrected with a crop constant (Kc). *, p-value < 0.05, **, p-value < 0.01, *** p-value < 0.001, ****, p-value < 0.0001.

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