

1 **Transpiration efficiency variations in the pearl millet**

2 **reference collection PMiGAP**

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15

16 **Abstract**

17 Transpiration efficiency (TE), the biomass produced per unit of water transpired, is a key
18 trait for crop performance under limited water. As water becomes scarce, increasing TE
19 would contribute to increase crop drought tolerance. This study is a first step to explore pearl
20 millet genotypic variability for TE on a large and representative diversity panel. We analyzed
21 TE on 538 pearl millet genotypes, including inbred lines, test-cross hybrids, and hybrids bred
22 for different agroecological zones. Three lysimeter trials were conducted in 2012, 2013 and
23 2015, to assess TE both under well-watered and terminal-water stress conditions. We

24 recorded grain yield to assess its relationship with TE. Up to two-fold variation for TE was
25 observed over the accessions used. Mean TE varied between inbred and testcross hybrids,
26 across years and was slightly higher under water stress. TE also differed among hybrids
27 developed for three agroecological zones, being higher in hybrids bred for the wetter zone,
28 underlining the importance of selecting germplasm according to the target area.
29 Environmental conditions triggered large genotype-by-environment interactions, although
30 TE showed some moderately high heritability. Transpiration efficiency was the second
31 contributor to grain yield after harvest index, hence its relevance in pearl millet breeding
32 programs. Future research on TE in pearl millet should focus (i) on investigating the causes
33 of its plasticity i.e. the GxE interaction (ii) on studying its genetic basis and its association
34 with other important physiological traits.

35 **Keywords:** Climate change, vapor pressure deficit, lysimeter, breeding, drought, food
36 security

37

38 **Introduction**

39 Pearl millet (*Pennisetum glaucum*) is one of the most drought tolerant cereals. It is grown as
40 fodder and rainfed cereal in arid and semi-arid regions, where annual rainfall is low and
41 intermittent. As the length of the cropping season is very limited in these regions, the
42 interruption of rainfall at the end of the rainy season can lead to terminal drought stress
43 (from flowering through grain filling). This abiotic stress has the most radical impact on pearl
44 millet grain and forage yield, as well as yield stability (Bidinger and Hash, 2004; Kholova and
45 Vadez, 2012; Mahalakshmi et al., 1987; Winkel et al., 1997). In this context, having higher
46 transpiration efficiency (TE), i.e. the amount of biomass produced per unit of water
47 transpired, could be a key in improving drought tolerance. Indeed, a higher TE can contribute
48 to a slower rate of soil moisture depletion, and thus leave a higher total soil water content for
49 the grain filling stage (Vadez et al., 2013), a phase that is critical for the drought tolerance of
50 this crop (Bidinger et al., 1987). This trait is known to be a complex term that is dependent,
51 not only on physiological factors but also environmental variables (Sinclair, 2012). Given this,
52 it seems worthwhile to measure this trait under several environmental conditions and

53 explore the range of genetic variation and putative genotype-by-environment interactions
54 for that trait in a pearl millet diversity panel.

55 Measuring TE has been the object of a lot of research in the last three or four decades or so,
56 much of it focusing on either transient leaf-based measurement or use of proxies. More
57 recently, a gravimetric method using lysimeters has been developed, where TE is measured
58 as the ratio of biomass accumulated per unit of water transpired over the entire crop cycle
59 (Vadez et al., 2014). This method enabled the assessment of genetic variation for TE and
60 identify whether there are any significant interactions between genotype and environment.
61 It also allows to evaluate all the components of the Passioura equation (TE, water use, and
62 harvest index, Passioura (1977)), and to test their relationships to yield on the same plants
63 using a large set of germplasm (Vadez et al., 2014). This has been observed previously on
64 sorghum and groundnut collections (Vadez et al., 2011a, 2011b; Vadez and Ratnakumar,
65 2016). The precedence of certain components was either dependent on the genetic
66 background of used material (Vadez et al., 2011a) or on environmental conditions (Vadez
67 and Ratnakumar, 2016).

68 A few studies have been carried out in lysimeters with an assessment of TE on pearl millet
69 panel (Beggi et al., 2015; Choudhary et al., 2020; Pilloni, 2022; Vadez et al., 2013). However,
70 the genotypic differences were examined on very limited panels of genotypes. The first
71 objective of our study was then to evaluate TE diversity in a larger and more diversified
72 panel, including inbred lines from the PMiGAP collection (Pearl Millet Inbred Germplasm
73 Association Panel), testcross hybrids from this panel, and hybrids targeted for different
74 agroecological zones in India. The second objective was to compare genotypic responses in
75 different environments. Finally, the last objective was to assess the influence of TE on yield
76 across a range of water treatments, environments, and germplasm. Practically, we assessed
77 TE, grain yield and related traits under different conditions: (i) under well-watered and
78 terminal water-stress conditions for the same panel and season, (ii) between two seasons on
79 the same panel and under the same irrigation regime; (iii) between a population of inbreds
80 and hybrids, during the same season and under the same irrigation regime.

81 **Material and methods**

82 **Plant material and experiments**

83 Three lysimeter experiments were carried out in the International Crops Research Institute
84 for the Semi-Arid Tropics (ICRISAT, Patancheru, Hyderabad, India) in 2012 (Exp. 1), in 2013
85 (Exp. 2) and 2015 (Exp. 3).

86 Weather data was recorded during the trial periods: minimum temperatures were 12.2°C,
87 11°C and 12.8°C in 2012, 2013 and 2015 respectively, while maximum temperatures were
88 40.2°C, 41.2°C and 43.2°C. Relative humidity (RH) was measured between 7am and 5pm. The
89 minimum RH was 35%, 38% and 43% in 2012, 2013 and 2015 respectively, while the
90 maximum RH was 94% in 2012 and 2013, 98% in 2015. The vapor pressure deficit (VPD)
91 was calculated using the method proposed by the FAO. Daily VPD ranged between 2.63 to
92 6.72 kPa in 2012, 0.66 to 6.73 in 2013 and 0.72 to 7.52 kPa in 2015. The plant material
93 belonged to the PMiGAP panel, a collection of pearl millet germplasm representing most of
94 the genetic diversity in pearl millet (Supplementary Table 1). In Exp. 1, 206 test-crosses
95 hybrids of the PMiGAP using 843-22A as a tester were assayed in the lysimeter. In Exp. 2, 70
96 test-crosses in common with Exp. 1 were studied, and grown under two irrigation regimes,
97 i.e. a fully irrigated treatment (well-watered, WW) and a terminal water-stress (WS). In Exp.
98 3, 235 PMiGAP inbred lines and 43 hybrids were selected. These hybrids were bred for three
99 agroecological production zones of India that differ in annual rainfall: i) 14 hybrids developed
100 for the driest production zone of India with less than 400 mm annual rainfall (A1 zone); ii)
101 13 hybrids developed for intermediate rainfall (A zone); iii) 13 hybrids developed for rainfall
102 above 400 mm (B zone). The boundaries of these breeding target zones had been described
103 previously (Gupta et al., 2013).

104

105 **Table 1. One way ANOVA for the main traits in each experiment.**

	Experiment	Exp. 1	Exp. 2	Exp. 2	Exp. 3	Exp. 3
	Irrigation	WW	WW	WS	WW	WW
	Plant material	Test-cross	Test-cross	Test-cross	Inbred	Hybrid
SUMTR	Mean	25.8	38.58	18.84	25.9	26.99
	Min	12.55	19.44	13.31	12.12	17.12
	Max	36.78	54.84	23.94	38.5	36.18
	F-value	3.533	1.471	1.308	2.123	2.026
	Pr(>F)	< 2.2E-16	ns	7.22E-02	6.06E-14	1.13E-03
		***		.	***	**
	H²	0.52	NA	NA	0.41	0.37
TOTDW	Mean	65.25	123.31	66.24	72.01	79.49
	Min	34.56	59.7	42.9	24.18	30.61
	Max	92.49	184.10	89.18	117.67	127.63
	F-value	2.652	1.417	1.363	2.233	2.423
	Pr(>F)	<2.20E-16	ns	4.59E-02	1.02E-15	5.63E-05
		***		***	***	***
	H²	0.45	NA	0.28	0.42	0.41
TE	Mean	2.55	3.22	3.54	2.79	2.95
	Min	1.73	2.26	2.24	1.27	1.53
	Max	3.49	4.51	4.79	4.39	4.31
	F-value	1.388	0.883	1.482	1.693	1.841
	Pr(>F)	4.98E-04	ns	1.60E-02	1.57E-07	4.30E-03
		***		*	***	**
	H²	0.3	NA	0.29	0.36	0.35
HI	Mean	0.153	0.354	0.218	0.2	0.347
	Min	0.001	0.150	0.021	0.001	0.077
	Max	0.328	0.580	0.406	0.436	0.546
	F-value	1.8735	2.0932	3.0628	3.3977	1.1033
	Pr(>F)	6.13E-11	0.009	2.00E-10	<2.20E-16	ns
		***	**	***	***	
	H²	0.36	0.79	0.48	0.53	NA
GRAIN	Mean	9.91	42.24	7.81	14.57	28.7
YIELD	Min	0.08	6.2	1.03	0.1	3.62
	Max	26.31	84.96	18.69	48.03	59.99
	F-value	1.688	1.484	2.4534	2.4905	1.8096
	Pr(>F)	4.918e-08	ns	3.52e-07	<2.2e-16	0.00537
			***	***	***	**

Experiment	Exp. 1	Exp. 2	Exp. 2	Exp. 3	Exp. 3	
Irrigation	WW	WW	WS	WW	WW	
Plant material	Test-cross	Test-cross	Test-cross	Inbred	Hybrid	

	H²	0.34	NA	0.43	0.45	0.35

106 Mean, minimal and maximal values, and one-way ANOVA table showing F-value, p-values and
107 broad sense heritability H² for sum of transpiration (SUMTR, kg), total dry weight (TOTDW,
108 g), transpiration efficiency (TE, g biomass.kg⁻¹ water), harvest index (HI), and grain yield (g.
109 plant-1) grouped by irrigation applied (well-watered WW or water stressed WS), by year of
110 experiment (Exp 1 - 2012, Exp 2 - 2013, and Exp 3 - 2015) and by type of germplasm used
111 (test-cross, inbreds, hybrids). Symbols *, **, and *** denote significance at p<0.05, p<0.01,
112 and p<0.0001, respectively. Acronym 'ns' means non-significant.

113 **Soil filling and growth conditions in lysimeters**

114 The lysimetric system consisted in a set of cylindrical PVC tubes (25 cm diameter, 2m length).
115 The platform was located outdoor and was equipped with a rainout shelter that could be
116 moved above the crop in case of rain. Each tube was filled with Alfisol, collected from the
117 ICRISAT farm. The same lysimeters have been used in different experiments and soil was not
118 replaced between trials. For that to happen, the lysimeter platform had been treated as a field
119 and had followed typical cereal-legume rotations. The soil used to fill the lysimeters had been
120 initially sieved, and fertilized with sterilized farm manure (at a rate of 2:50 w:w) and with
121 DAP and muriate of potash (both at a rate of 200 mg.kg⁻¹) (Vadez et al., 2014). For each new
122 experiment, DAP and muriate of potash was added at a rate of 2g per lysimeter. Urea (46%
123 N) was added as a top-dressing at a rate of 2 g per lysimeter. The length of lysimeters was
124 designed to be deep enough so that rooting depth would not be limiting in this type of soil.

125 The tops of the cylinders were equipped with metal collars and chains to allow PVC tubes to
126 be lifted for weighing. Each tube, weighing between 160 and 165 kg, was raised with a block-
127 chained pulley. Between the rings of the cylinder and the pulley, a S-type load cell was
128 inserted (200 kg load capacity and 20 g precision; Mettler-Toledo, Geneva, Switzerland). The
129 same weighing protocol was applied among experiments, following procedures previously

130 described (Vadez et al., 2011b, p. 20). A few centimeters only separated the lysimeters from
131 one another so that the planting density was close to 10 plants m⁻², which is typical of pearl
132 millet fields in India. Because of possible border effects, replications were set in parallel to
133 the walls of the trenches. There were five rows of cylinders in each trench, each row
134 representing typically one replication.

135 All genotypes were sown after wetting the tubes, on 16 February 2012, 14 February 2013,
136 and 18 February 2015, with a rate of 4–5 seeds per hill and 3 hills per tube. Seedlings were
137 thinned a first time to one seedling per hill at about 10 DAS, and then to one plant per tube at
138 14 DAS. The three experiments were designed as an alpha lattice. Exp 1 was an Alpha lattice
139 with 4 blocks of 65 entries and 5 replications of each accession. Exp 2 design was an Alpha
140 lattice with 2 blocks of 35 entries and 5 replications for each accession for the WS treatment
141 and 2 replications for each accession for the WW treatment. Exp 3 was designed as an Alpha
142 lattice with 4 blocks of 70 entries and 5 replications for each accession.

143 **Application of irrigation regimes and measurements**

144 The tubes were weighed six times in Exp 1 (27, 35, 42, 49, 57, 82 DAS). In Exp 2, the number
145 of weighing was dependent on the water regime applied: four times in WS condition (21, 32,
146 48, 81 DAS) and seven times in WW condition (22, 27, 35, 42, 50, 55, and 81 DAS). Finally,
147 the tubes were weighed seven times in Exp 3 (29, 34, 42, 48, 55, 69, 94 DAS). Prior to the first
148 weighing of the lysimeters, these were abundantly watered and left to drain for two nights
149 and one day to reach field capacity. Also, prior to weighing, a plastic sheet and a 2-cm layer
150 of beads was applied on top of the tubes to limit soil evaporation. The first lysimeter weight
151 then represented the field capacity weight and was used as a benchmark for re-watering of
152 the WW condition. Lysimeters were watered after each weighing to bring back the cylinder
153 weight to field capacity weight minus 2kg to avoid drainage. Each cylinder then had an
154 estimated 12-13 L of available soil water and re-watering was done up to about 85% field
155 capacity. The lysimeters under WS conditions were treated as those under WW conditions
156 until cessation of irrigation. At that time, a last 3L irrigation was provided to each WS tube.
157 Transpiration values were calculated for each cylinder between two weighing

158 measurements, by subtracting the weight measured at the last and the current weight, minus
159 the added water.

160 **Harvest procedure and statistical analysis**

161 Plants were grown to maturity in all three trials and harvested at the last weighing. The entire
162 aerial part of the plant (grain and forage) was harvested and dried for 3 days in a forced-air
163 oven at 70°C. Stem, leaf, panicle, and grain weights were measured separately for each
164 cylinder. The total dry weight (TOTDW, g biomass) was obtained by adding the weights of
165 leaves, stems, and panicle. In these three experiments, the roots were not harvested, so that
166 the estimation of total dry weight was based on the shoot part only. However, it was
167 previously discussed that the root part would likely not affect the TE comparison among
168 genotypes (Vadez et al., 2011b). At the end of the experiment, the sum of transpiration
169 (SUMTR, kg water used) was determined by summing the transpiration values measured
170 every week over the entire crop cycle. Transpiration efficiency (TE, g.kg⁻¹) was estimated as
171 the ratio of total dry weight to the sum of transpiration values. The harvest index (HI) was
172 calculated by dividing the grain weight (g) to total biomass (g). The grain yield was estimated
173 as the grain weight per plant (g. plant⁻¹). Datasets were checked for outlier measurements.
174 Data points were deleted if their values were outside the range of two times the standard
175 deviation around the grand mean. We described the variables grouped by year, irrigation
176 regime and material type (testcrosses, inbreds, hybrids). Data were analyzed by one-way
177 ANOVA on each group to visualize TE variation among genotypes under the same conditions.
178 The results of this ANOVA were used to calculate the broad-sense heritability of TE in each
179 condition using the following formula $H^2 = (\text{Sum Sq}) / (\text{Sum Sq} + \text{Residuals})$ to obtain an
180 estimation of the phenotypic variance attributable to an overall genetic variance. We used a
181 two-way ANOVA in two scenarios: (i) on common genotypes in the same year (Exp 2) with
182 two irrigation regimes to analyze the G effect and the GxIrrigation treatment interaction
183 effect; and (ii) on common genotypes with the same water treatment in two seasons (Exp 1
184 and Exp 2) to assess the G effect and the GxYear interaction effect. As the inbreds and
185 testcross hybrids (Exp3) had no accessions in common, this analysis could not be carried out.
186 Finally, regression analyses and Pearson correlation tests were carried out in three scenarios

187 (i) between the TE component variables within each group, i.e. dry weight, and transpiration
188 within the same year or irrigation (ii) between TE measurements across different years or
189 irrigations on common material and (iii) and between grain yield and the yield component
190 according to the Passioura equation (water use, harvest index and TE, (Passioura, 1977)).

191

192 **Results**

193 **TE varied among genotypes and was heritable**

194 By and large, all traits measured (dry weight, transpiration, TE, HI, grain yield), showed
195 significant differences among genotypes during Exp 1 in 2012. TE was moderately heritable
196 in these conditions ($H^2 = 0.3$, Table 1). Dry weight and transpiration were strongly correlated
197 with each other ($R = 0.89$, $p\text{-value} < 2.2\text{e-}16$, Fig 1a). During Exp 2 in 2013, most traits showed
198 no difference among entries under WW regime, TE and grain yield included (Table 1). Yet,
199 there were only two repetitions to test this condition, compared with five repetitions in the
200 other cases. The TE components under WW regime showed the same strong correlation as
201 in Exp 1 ($R = 0.83$, $p\text{-value} < 2.2\text{e-}16$ for 2013, Fig 1b). For the same year under the WS regime,
202 the TE differences observed among entries were significant, and the trait was moderately
203 heritable ($H^2 = 0.29$, Table 1). The correlation between dry weight and transpiration was
204 weaker under WS ($R = 0.44$, $p\text{-value} = 1.273\text{e-}4$, Fig 1c) than under WW regime. During the
205 Exp 3 in 2015, significant differences among genotypes were observed for all traits in hybrid
206 and inbred panels, except HI which was not significant in the hybrid group. Generally,
207 differences among genotypes were more pronounced in the hybrid group than in the inbred
208 group. TE was heritable in both hybrid and inbred panels ($H^2 = 0.36$ for inbreds, $H^2 = 0.45$ for
209 hybrids, Table 1). Biomass production and transpiration were highly correlated for both
210 scenarios, with $R = 0.76$ ($p\text{-value} = 1.08\text{e-}06$) for hybrids and $R = 0.67$ ($p\text{-value} < 2.2\text{e-}16$) for
211 inbred lines (Figs 1d and 1e). More precisely, significant differences in TE were observed
212 among hybrids developed according to their targeted agroecological zones. The TE gradient
213 among these hybrid groups followed the same trend as annual rainfall: the drier the
214 agroecological zone, the greater the evaporative demand, the lower the TE. In addition,

215 genotypic differences were observed among individuals from the same zone in the driest
216 zones A1 (p-value= 0.01), while wetter zones A and B showed no significant TE differences
217 among genotypes (Fig 2).

218

219 **Fig 1. Relationship between total dry weight (g biomass) and sum of transpiration (g**
220 **water)** a. with 206 germplasm entries in Exp 1 under well-watered conditions, b. with 70
221 entries in Exp 2 under well-watered conditions and c. with 70 entries in Exp 2 under water-
222 stressed conditions, d. 43 hybrids in Exp 3 and with e. 235 inbred lines in Exp 3. Data are the
223 mean of five replicated (a,c,d,e) or two replicated lysimeter-grown plants per genotype (b).

224 **Fig 2. Transpiration Efficiency (TE, in g biomass. kg water⁻¹) in different hybrid groups.**
225 TE was measured under well-watered conditions for hybrid groups bred for three different
226 target zones: A1 is the driest zone with less than 400mm of annual rainfall (14 hybrids), A is
227 the intermediate annual rainfall zone (13 hybrids), B is the most humid zone, with an annual
228 rainfall above 400 mm (13 hybrids). Data are the mean of five replicated lysimeter-grown
229 plants per genotype.

230

231 **TE varied slightly between years and irrigation regimes**

232 Focusing on the impact of years on TE variation, we observed that, all genotypes combined,
233 total dry weight per plant was almost twice as high in 2013 (123 g.plant⁻¹) as in 2012 (65
234 g.plant⁻¹). Total transpiration was only 41% higher (38.6 kg in 2013 vs. 25.8 kg in 2012).
235 Overall transpiration efficiency was therefore 1.3 times higher in 2013 than in 2012 (3.22 g
236 biomass.kg water⁻¹ vs 2.55 g biomass.kg water⁻¹). Grain yield followed the same trend as TE,
237 with a four-fold higher value in 2013 (42.2 g. plant⁻¹) than in 2012 (9.9 g. plant⁻¹, Table 1). It
238 was in line with the important year effect observed (F-value = 354, p-value<2.20E-16, Table
239 2). The variability of weather conditions could potentially be a source of explanation for the
240 difference observed between Exp 1 and Exp 2. Minimum and maximum temperatures were
241 similar in both years (Fig 3a). However, RH was higher in 2013 for 73% of the duration of the
242 experiment, or 60 days out of the 82-day trial period. This overall higher RH was associated
243 with precipitation-induced humidity peaks during the 2013 field experiment, whereas these
244 peaks were absent in 2012 (Fig 3b). This variability resulted in a higher VPD in 2012 than in
245 2013, over 67% of the duration of the experiment, created more restrictive conditions. In the
246 meantime, mean TE per genotype were correlated across years (R=0.25, p = 4,76e-2, Fig 4a),

247 which also reflected the large GxYear interaction for TE (F-value = 1.5045, p= 0.0124, Table
248 2).

249 **Table 2. Two-way ANOVA summary table for Experiments 1 and 2.**

	Season (Exp 1 WW/Exp 2 WW)	Treatment (Exp 2 WW/ Exp 2 WS)
G F-value	0.5932	1.2557
Pr(>F)	ns.	ns.
T F-value	354.08	38.06
Pr(>F)	<2.20E-16 ***	1.538e-09 ***
G*T F-value	1.5045	0.9038
Pr(>F)	0.0124 *	ns.

250 The table presents F-value and p-values for genotype effect (G), treatment (T) or season (S) effect,
251 and the genotype-by-season (G*S) or genotype-by-treatment (G*T) interaction effect on transpiration
252 efficiency (TE). Season effect was tested with data of Experiments 1 and 2. Water treatment effects
253 were tested in Experiment 2 with two treatments (well-watered conditions WW, watered stressed
254 conditions WS).

255

256 **Fig 3. Environmental conditions (temperature, relative humidity and rain) during the**
257 **different trials.** (a) Minimum and maximum temperature (°C), (b) Relative humidity (%)
258 and rain (mm) measured by the local weather station in Patancheru, India, during the trial
259 period of the three experiments (Exp 1 2012, Exp 2 2013, and Exp 3 2015). The trial period
260 is defined in days after sowing. The sowing date were 16 February 2012, 14 February 2013,
261 and 18 February 2015.

262

263 Regarding the irrigation regimes applied in 2013, total dry weight production per plant was
264 two-fold higher under WW than under the WS regime, all genotypes combined (123 g. plant⁻¹
265 versus 66 g. plant⁻¹). Slightly more than twice as much water was needed to produce biomass
266 under WW conditions than under WS conditions, resulting in a mean TE that was only slightly
267 higher (7.5%) under WS than under WW (3.54 and 3.22 g.kg⁻¹ respectively). This was

268 reflected by the large irrigation effect observed in Table 2 (F-value = 38.06, p-value = 1.538e-
269 09). As expected, grain yield was higher under WW (42.2 g. plant-1 versus 7.8 g. plant-1,
270 Table 1). Mean TE of each entry was not correlated across irrigation regimes ($R=-0.096$, p-
271 value = 0.81, Fig 4b). On the other hand, the G and G*Irrigation effects were comparable (F-
272 value(G) = 1.2557, F-value(GxI) = 0.9038, p-value>0.05).

273

274 **Fig 4. TE relationships across years and water treatments.** Relationship between
275 transpiration efficiency (g. biomass. kg water⁻¹) a) in Exp 1 and Exp 2 under well-watered
276 conditions on 70 common entries, and b) in Exp 2 under well-watered (WS) and watered-
277 stressed conditions (WW). Data are the means of five replicated (in Exp 1 and Exp 2 WS) or
278 two replicated lysimeter-grown plants per genotype (Exp 2 WW).

279

280 Concerning the type of germplasm used in Exp 3, the overall total dry weight was higher for
281 hybrid (mean 79.5 g. plant⁻¹) than for inbred lines (mean 72.0 g. plant⁻¹). This higher biomass
282 production was combined with higher transpiration for hybrids (27.0 kg) than inbreds (25.9
283 kg). This resulted in a higher TE for hybrids group (2.95 g. plant⁻¹) than for inbreds (2.79 g.
284 plant⁻¹), and a higher grain yield that was on average doubled for the hybrid group (28.7 g.
285 plant⁻¹) compared with inbreds (14.6 g. plant⁻¹, Table 1). However, these observations could
286 be linked to a few high-impact individuals that affects the comparison of the whole panel.
287 Indeed, a T-test showed that the mean TE of the 20 best hybrids was 3.90 g.kg⁻¹,
288 i.e. significantly lower than the mean TE of the 20 best inbreds (4.11 g.kg⁻¹, $P< 0.001$).

289

290

291 **TE was correlated with the residual yield unexplained by HI**

292 As expected, HI was the main factor explaining yield from the Passioura equation, owing to a
293 degree of autocorrelation between HI and yield. Grain yield was also significantly related to
294 TE in all studied years, with the same correlation value under WW ($R=0.27$, $p\text{-value}=5.64e-$

295 3) and WS ($R=0.25$, p -value = $1.69e-05$) in Exp 2 as well as in Exp 1 ($R=0.30$, p -value < $2.2e-16$, Table 3). The correlation was much stronger during the Exp 3, with $R=0.45$ (p -value < $2.2e-16$) on inbred population and $R=0.67$ (p -value < $2.2e-16$) on hybrid population. To 298 remove the confounding effect of HI, the residuals of the linear relationship between grain 299 yield and HI were computed as the Euclidian distance between observed yield values and 300 predicted values from the regression line. These residuals were then plotted against the other 301 possible explanatory factors from Passioura equation (TE and Water Use). The residual yield 302 showed a higher correlation with TE (from 0.22 to 0.6, Table 3) than with water use (from - 303 0.33 to 0.42, Table 3), suggesting that grain yield differences, besides HI, were mainly 304 explained by TE and followed as a second order of importance by Water Use.

305
306 **Table 3. Regression analysis between grain yield and components of Passioura**
307 **equation.**

Experiment	Exp. 1	Exp. 2	Exp. 2	Exp. 3	Exp. 3
Irrigation	WW	WW	WS	WW	WW
Plant material	Test-cross	Test-cross	Test-cross	Inbred	Hybrid
Grain yield ~ HI	0.9	0.79	0.93	0.89	0.87
Grain yield~ TE	0.3	0.27	0.25	0.45	0.67
Grain yield ~ WU	-0.11	-0.083	-0.088	-0.015	0.28
Residual grain yield	0.22	0.34	0.6	0.5	0.51
unexplained by HI ~ TE					
Residual grain yield	-0.0066	-0.33	0.027	0.42	0.28
unexplained by HI ~WU					

308 Harvest index (HI), transpiration efficiency (TE) and water use (WU) were regressed against
309 grain yield. Then the residual grain yield variations unexplained by harvest index were
310 plotted against TE and SUMTR. The panel was grouped by year (Exp 1, Exp 2, Exp 3), by
311 irrigation applied (well-watered conditions WW or water stress conditions WS) and by type
312 of germplasm used (test-cross, inbreds, hybrids).

313 Discussion

314 In this study, pearl millet material was phenotyped during three seasons in a lysimetric
315 platform for TE variation by following up the water uptake during the whole crop cycle. To
316 our knowledge, our study is the first to report variation for TE in a large set of pearl millet
317 germplasm.

318 TE variation among entries due to genetic effects

319 Our results showed a significant variation of TE among entries in the panel for most of the
320 cases studied, except under WW conditions in Exp 2. Yet, this WW condition had only two
321 repetitions instead of five compared to other experiments. The heritability values suggested
322 that TE variation among individuals is moderately determined by a genetic component in
323 each condition studied (from $H^2=0.29$ in Exp 2 under WS with test-crosses to $H^2=36$ in Exp 3
324 under WW with inbreds). Beggi and colleagues (2015) noted a significant variation in TE
325 among 15 genotypes in the case of phosphorus-poor soil, under well-irrigated and water
326 stress conditions. Vadez et al. (2013), focused on temporal differences in water uptake,
327 reported significant differences in TE among 8 genotypes. The values mentioned here under
328 WS and WW (2.30 – 4.79 g. kg⁻¹ water) were above the range found by Beggi et al., 2015 (1.23
329 – 1.82 g. kg⁻¹ water) and that in Vadez et al., 2013 (2.19 – 2.95 g. kg⁻¹ water), hence this
330 current work reports a higher range of variation, of approximately two folds. Differences in
331 the mean values across trials could be related to differences in environmental conditions
332 (different trial periods and locations). For instance, the trial of Beggi et al. (2015) was carried
333 out under higher evaporative demand. In relation to these TE variations here, dry weight and
334 total transpiration were not always tightly correlated for some conditions (Fig 1).
335 Transpiration efficiency being the slope of the linear regression between dry weight and total
336 transpiration, a loose correlation highlighted that certain genotypes could reach a high
337 biomass at relatively low water cost, hence had higher TE, than other genotypes.

338 Variation due to genetic factors was also found in Exp 3 among hybrids that differ genetically
339 in their adaptation zone. Indeed, the group of hybrids adapted for the A1 driest zone showed
340 a variation in TE among entries, underlying the importance of selecting the best elements

341 adapted to the target production environment. On the contrary, hybrids adapted to A and B
342 wetter zones showed little or no difference among genotypes: the more arid and restrictive
343 the environment, the greater the differences among genotypes. Curiously, if we focus on the
344 mean TE of hybrids across agroecological zones, we observed that the wetter the zone, the
345 higher the TE. This is surprising, given that TE is often considered as a measure of a
346 genotype's drought tolerance, and we could have expected hybrids from the driest zone to
347 have higher TE. Zone A1 has high VPD conditions and poor, sandy soil (Yadav, 2016). In this
348 scenario, a strategy to conserve water that could easily evaporate may not be suitable.
349 Instead, it is likely that genotypes adapted to this zone may have developed a rather
350 opportunistic strategy of using the available water as quickly as possible before it evaporates,
351 and then banking on high tillering characteristics and small grains to limit the impact of
352 intermittent drought (van Oosterom et al., 2003). Some genotypes, such as H77-833/2, are
353 known to adopt this type of strategy (Kholova et al., 2010a, 2010b; Vadez et al., 2013). This
354 would imply maintaining transpiration even if VPD is high, which would decrease TE (Sinclair
355 et al., 2012), in line with our results here. Additional research would then be necessary to
356 compare water use strategies, i.e. opportunist versus conservative, in hybrids adapted to dry
357 or wet zones.

358 Independently of this water use strategy hypothesis, our results show that the notion of TE
359 as a mirror of plant performance under water stress should be revisited to determine
360 whether it is an appropriate method for measuring drought tolerance in pearl millet. In
361 addition, the characterization of pearl millet production zones as A1, A, B could also be
362 reviewed. These zones have been determined on the basis of annual rainfall in Indian regions
363 and pearl millet growing areas. It has remained unchanged since 1979 and is still considered
364 a reference in most pearl millet breeding programs. Recently, a more advanced
365 characterization based on crop modeling of these production zones has been established
366 (Garin et al., 2023). This work has allowed a more precise delimitation of five new target
367 production environments in the pearl millet growing zone in India. Testing TE variation
368 according to this new zonation would also be a way of refining our inter-zone observations.
369 Finally, the fact that some of the variation in TE appears to be due to moderate genetic effects

370 highlights the need for further study towards a genome-wide association analysis to
371 determine the genetic basis of TE variation.

372 **TE variation due to the Genotype x Environment interaction**

373 As expected, we observed a strong environmental effect on TE, whether by year or irrigation.
374 Looking more closely at Exp 2, the TE difference between irrigation treatments was only
375 7.5%. This was a smaller difference than reported earlier in sorghum where TE under WS
376 was 12.5% higher than under WW conditions in this drought-tolerant C4 crop (Vadez et al.,
377 2011b). Although not significant here, genotype effect and genotype-irrigation interaction
378 showed almost equal F-values, suggesting that both factors had equal importance on TE
379 variation. This genotypic effect was significant in Exp 1 and Exp 2 under WS conditions with
380 five replicates, and insignificant during Exp 2 under WW conditions with two replicates. In
381 this context, we can presume that we didn't capture all TE's existing genetic diversity in Exp
382 2 under WW conditions and this lack of significance biased the inter-irrigation analysis.

383 On the other hand, the genotype-year interaction was more clearly significant. As TE depend
384 partially on environmental variables such as atmospheric CO₂ concentration and
385 atmospheric vapor pressure deficit, the effect of the year was likely linked to the different
386 meteorological conditions observed over the two years of experimentation.

387 In summary, TE differences induced by GxE interactions occurred in our field-like trials, in
388 particular Genotype x Year interaction. It therefore appears to be an important component
389 to consider in capturing TE variation. Further work is needed on the phenotypic plasticity of
390 TE expressed in different environments in the potential perspective of TE-based selection.
391 However, based on our results here, it would show that GxE interactions were still weaker
392 than the differences induced by genotypic effects. This finding is in line with the TE
393 evaluation cited above, where the genotype-treatment interaction was not significant in all
394 cases studied (Beggi et al., 2015; Pilloni, 2022; Vadez et al., 2013). From these initial
395 observations, we can draw one main conclusion for future TE phenotyping on a panel of pearl
396 millet. Repeating the experiment over several years seems essential to consider weather
397 conditions and limit their impact on TE variations, to take into account GxE interactions in
398 TE assessment.

399 **TE, the second contributor to grain yield**

400 Whatever the origins of the variation, TE was an important factor to consider for pearl millet
401 yield. After removing the autocorrelation HI effect, TE explained a large portion of the
402 remaining grain yield variations in all conditions studied, highlighting the benefit of selecting
403 germplasm based on high TE for breeding programs. This result is in line with the result
404 found on other crops such as sorghum panel (Vadez et al., 2011b). This previous result on
405 sorghum was really dependent on the genetic background of the germplasm studied (Vadez
406 et al., 2011a). Here the genetic background of the pearl millet genotypes was not analyzed
407 and would be a way of improving our knowledge.

408 In summary, improvement of TE-based selection in pearl millet could be based on several
409 levers of action, such as (i) improve our knowledge on TE plasticity on the new target
410 production environments to reduce the G*E interaction (ii) investigate the genetic basis of
411 TE in pearl millet on the panels presented here and relate this to other crops. A comparative
412 study of sorghum and pearl millet on the genetic basis of TE is currently the object of
413 additional studies.

414

415 **Conflict of interest**

416 The authors have no conflicts to declare.

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429

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493

494 **Supporting information**

495 **S1 Table. List of germplasm tested.** The table indicates the year when it was tested, the
496 type of genetic material (Test-cross hybrid, inbred, hybrid), the Generation Challenge
497 Program (GCP) entry number, the test-cross pedigree and the breeding target zone when
498 applicable.

499 **S2 Table. Value of traits measured in the different trials.** Besides genotype name,
500 experiment, germplasm types the table reports transpiration, total dryweight, TE, harvest
501 index (HI) and grain yield.

502

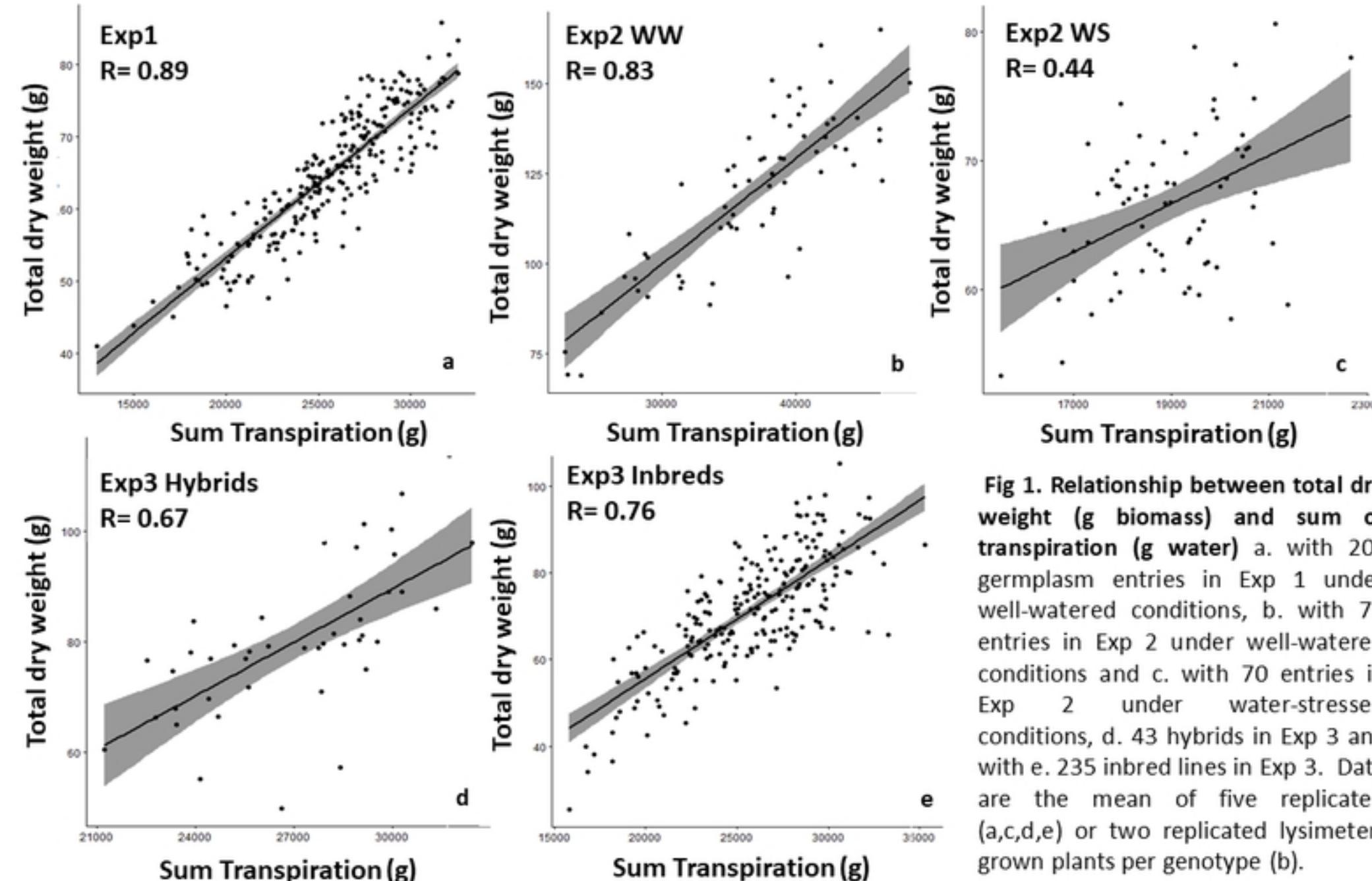


Fig 1. Relationship between total dry weight (g biomass) and sum of transpiration (g water) a. with 206 germplasm entries in Exp 1 under well-watered conditions, b. with 70 entries in Exp 2 under well-watered conditions and c. with 70 entries in Exp 2 under water-stressed conditions, d. 43 hybrids in Exp 3 and with e. 235 inbred lines in Exp 3. Data are the mean of five replicated (a,c,d,e) or two replicated lysimeter-grown plants per genotype (b).

Figure 1

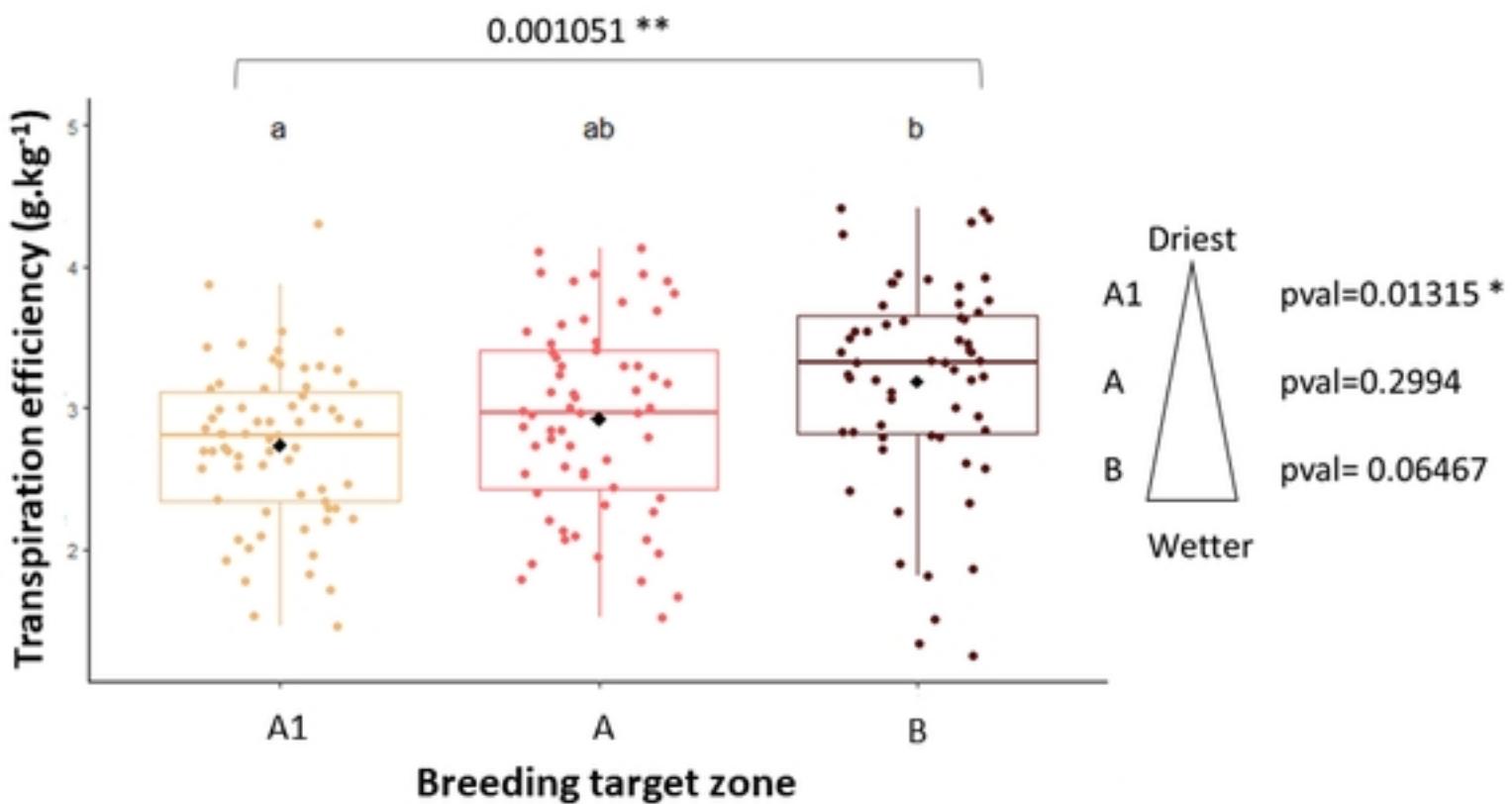


Fig 2. Transpiration Efficiency (TE, in $\text{g biomass. kg water}^{-1}$) in different hybrid groups. TE was measured under well-watered conditions for hybrid groups bred for three different target zones: A1 is the driest zone with less than 400mm of annual rainfall (14 hybrids), A is the intermediate annual rainfall zone (13 hybrids), B is the most humid zone, with an annual rainfall above 400 mm (13 hybrids). Data are the mean of five replicated lysimeter-grown plants per genotype.

Figure 2

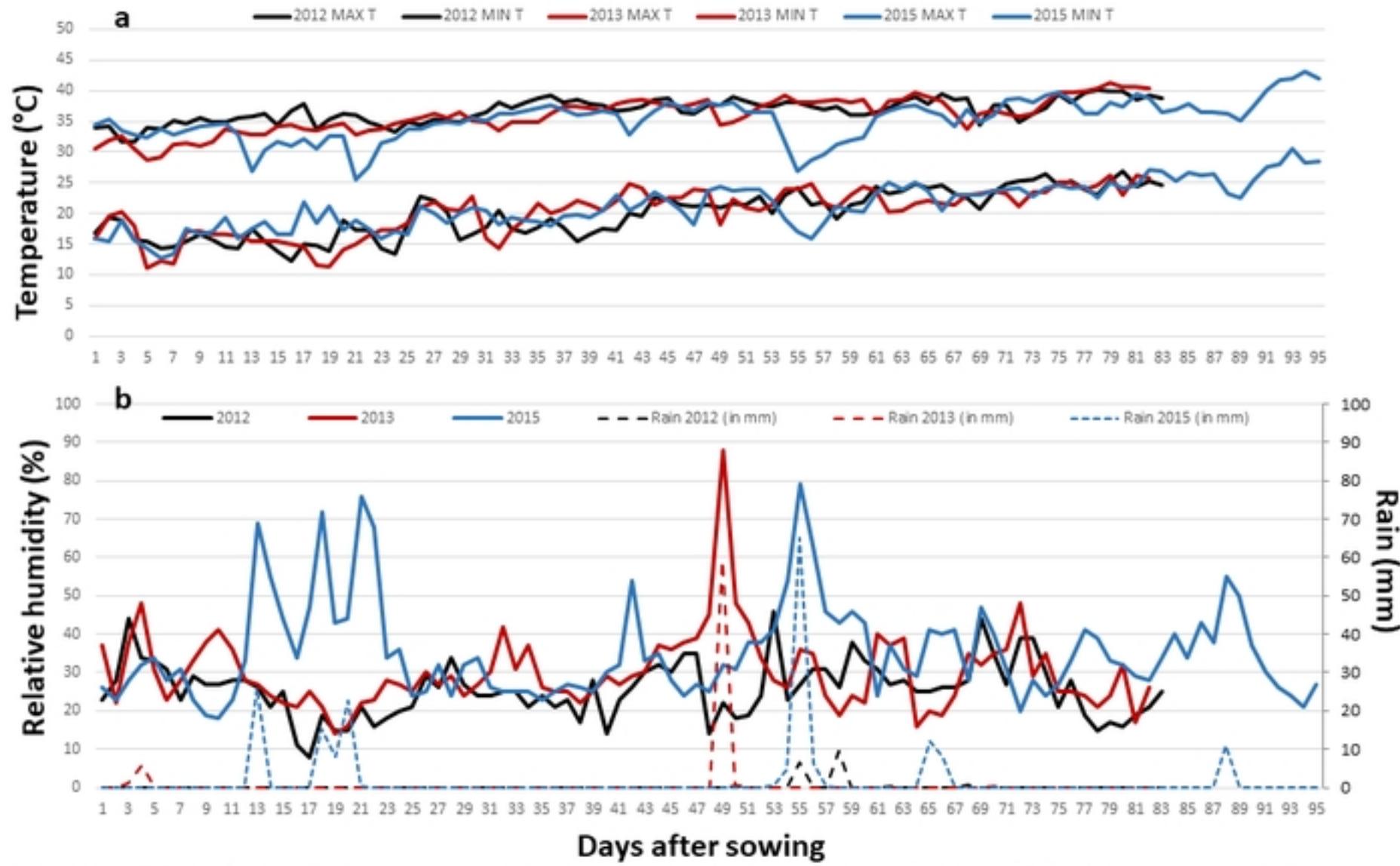


Fig 3. Environmental conditions (temperature, relative humidity and rain) during the different trials. (a) Minimum and maximum temperature (°C), (b) Relative humidity (%) and rain (mm) measured by the local weather station in Patancheru, India, during the trial period of the three experiments (Exp 1 2012, Exp 2 2013, and Exp 3 2015). The trial period is defined in days after sowing. The sowing date were 16 February 2012, 14 February 2013 and 18 February 2015.

Figure 3

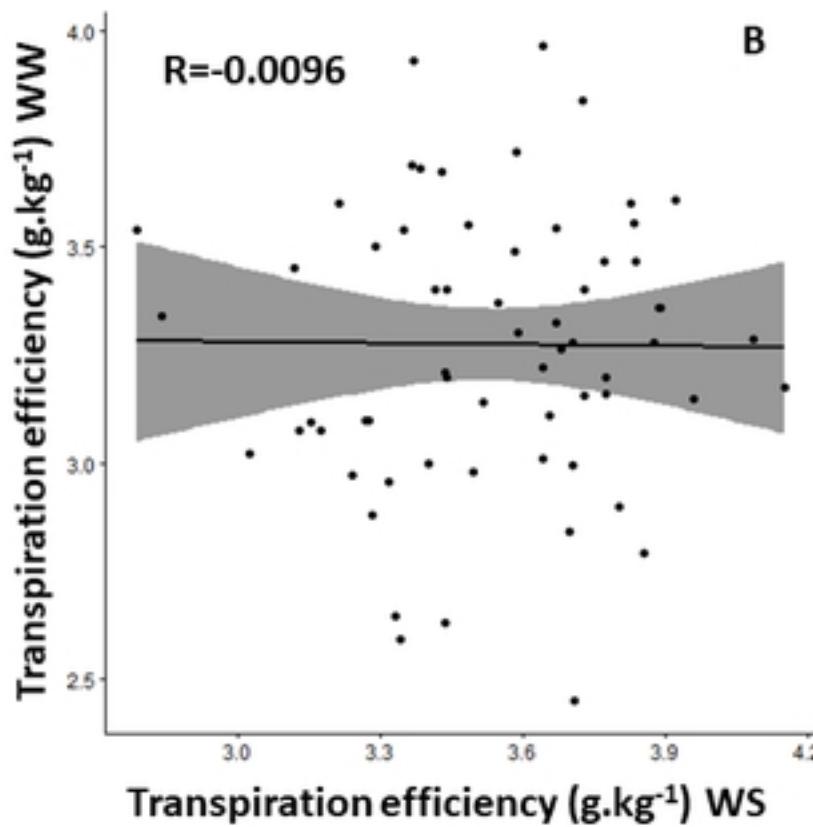
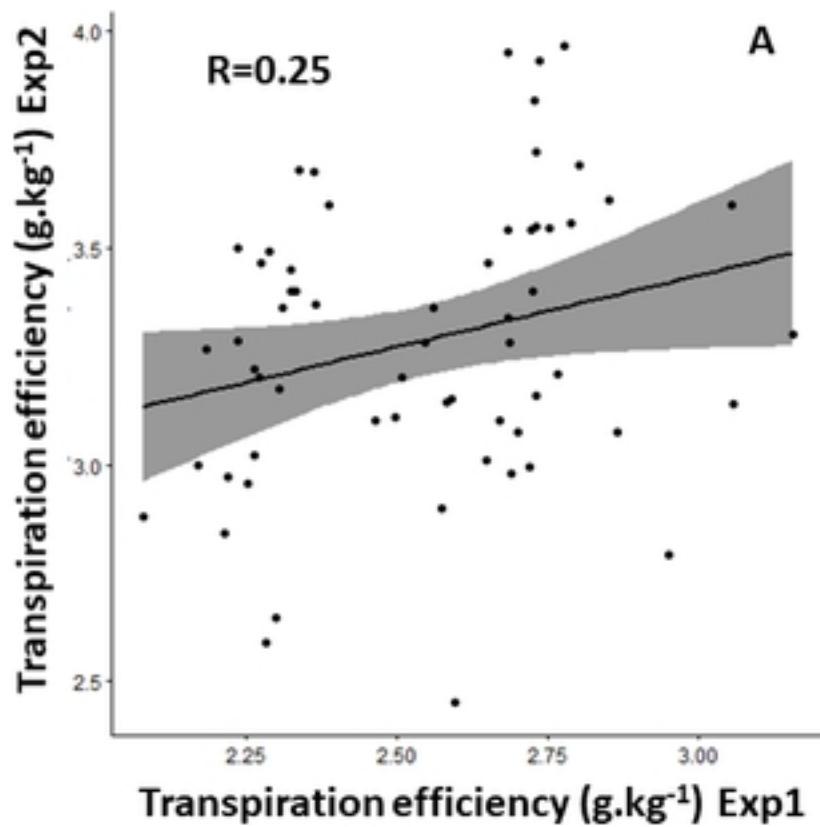


Fig 4. TE relationships across years and water treatments. Relationship between transpiration efficiency (g. biomass. kg water⁻¹) a) in Exp 1 and Exp 2 under well-watered conditions on 70 common entries, and b) in Exp 2 under well-watered (WS) and watered-stressed conditions (WW). Data are the means of five replicated (in Exp 1 and Exp 2 WS) or two replicated lysimeter-grown plants per genotype (Exp 2 WW).