Effects of Superhydrophobic Sand Mulching on Evapotranspiration and Phenotypic Responses in Tomatoes (*Solanum lycopersicum*) under Normal and Reduced Irrigation

Kennedy Odokonyero, Adair Gallo Jr., Vinicius Dos Santos, and Himanshu Mishra[*]

King Abdullah University of Science and Technology, Water Desalination and Reuse Center, Division of Biological and Environmental Sciences and Engineering, Thuwal 23955 - 6900 Saudi Arabia

Correspondence Email: [*]Himanshu.Mishra@kaust.edu.sa

Abstract

Irrigated agriculture in arid and semi-arid regions is a vital contributor to the global food supply; however, these regions endure massive evaporative losses that are compensated by unsustainable freshwater withdrawals. Plastic mulches have been used to curtail evaporation, improve water-use efficiency, and ensure food–water security, but they are non-biodegradable and their disposal is unsustainable. We recently developed superhydrophobic sand (SHS), which comprises sand grains with a nanoscale wax coating that could offer a more sustainable mulching solution. Here, the effects of adding a 1.0 cm-thick layer of SHS mulch on the evapotranspiration and phenotypic responses of tomato (*Solanum lycopersicum*) plants are studied under normal and reduced irrigation. Under both irrigation regimes, SHS mulching suppressed evaporation and enhanced transpiration by 78% and 17%, respectively relative to the bare soil. Overall, SHS mulching enhanced root xylem vessel diameter, stomatal aperture, stomatal conductance, and chlorophyll content index by 21%, 25%, 28%, and 23%, respectively. Total fruit yields, total dry mass, and harvest index increased in SHS-mulched plants by 33%, 20%, and 16%, respectively than in bare soil. These findings demonstrate the potential of SHS to boost irrigation efficiency in water-limited environments and provide mechanistic insights behind yield enhancement by SHS mulching.

31

KEYWORDS: evapotranspiration; *Solanum lycopersicum*; superhydrophobic sand; transpiration efficiency; chlorophyll content index; stomatal conductance; harvest index.

INTRODUCTION

Irrigated agricultural lands are of vital importance, as they represent 20% of the total cultivated land but contribute over 40% of the global food production (WWAP, 2009). Irrigated agriculture has been expanding to feed the growing global population; for example, India, China, and Brazil have witnessed a 30% (Jain et al., 2020), 52% (Zhu et al., 2013), and 52% (Carvalho et al., 2020) increase in cultivated areas under irrigation in the last 50 years, respectively. Further, between 2012 and 2030, the global irrigated land area is expected to increase by over $\approx$30%, from 310 Mha to 402 Mha (Darko et al., 2016). These food production trends are sustainable only when freshwater is abundant. However, groundwater resources have been extensively exploited for irrigation in densely populated arid and semi-arid regions and are thus rapidly declining (Steward et al., 2013, Watto et al., 2018b, Watto et al., 2018a, Scanlon et al., 2012, Famiglietti, 2014). Therefore, water consumption patterns in irrigated agriculture must be studied, and new technologies must be created to achieve global water–food security.

Water applied to topsoil is lost via evaporation, transpiration, and percolation (Sutanto, 2012). High temperatures and dry winds in arid and semi-arid regions lead to substantial evaporative and transpiration losses (Al-Naizy, 2012, Balugani et al., 2017), whereas percolation is due to the poor water-holding capacity of sandy soils (Lehmann et al., 2019, Or and Lehmann, 2019). These water losses are compensated via irrigation that, due to the sheer size of agricultural operations, claims the lion's share (approximately $\approx$80%) of global annual freshwater consumption (2500 km^3/yr) (Shiklomanov, 2000, Hoekstra and Mekonnen, 2012). Whereas evaporation and percolation do not directly contribute to photosynthesis during plant growth, transpiration is crucial in maintaining the optimal temperature required for plants' metabolic processes (Grill and Ziegler, 1998, Hetherington and Woodward, 2003). Transpiration thus cannot be reduced while high crop productivity is maintained without genetic engineering. Percolation could be reduced by applying a water barrier layer beneath the plant root zone, but this process is labor-intensive

2

63   and expensive (Nkurunziza et al., 2019). Thus, many researchers have focused on reducing

64   evaporative losses.

65   Together, evaporation and transpiration constitute evapotranspiration (ET), a

66   process that plays an important role in irrigation and agriculture practices (Hatfield and

67   Dold, 2019, Rawitz and Hadas, 1994). The importance of transpiration is reflected in the

68   water-use efficiency (WUE) of plants, i.e., the ratio of the total biomass (root and shoot)

69   formed to the cumulative water transpired (Kadam et al., 2015), and the transpiration

70   efficiency (TE), i.e., the ratio of the shoot biomass produced to the cumulative water

71   transpired (Vadez et al., 2014). The TE represents an aspect of WUE that depends on the

72   water-conducting (i.e., hydraulic) potential of a plant to facilitate shoots' physiological

73   processes that enhance biomass under different soil water scenarios(Vadez et al., 2014).

74   Therefore, the TE is of interest in water-scarce environments where crops are most

75   sensitive to soil moisture (Condon et al., 2002, Sinclair, 2012). Transpiration also

76   influences the plants' reproductive efficiency, defined by the harvest index (HI) entailing

77   the ratio of total yield to total vegetative biomass produced, pinpointing the plant resources

78   invested in yield production (Porker et al., 2020, Unkovich et al., 2010). Hence, whereas

79   water uptake and efficient use of water are critical for plant growth and biomass

80   accumulation, the development and growth of reproductive organs determines the HI,

81   which is critical to crop yield (Hammer et al., 2021).

82   Notably, evaporation is a wasteful component of ET and should be minimized.

83   Mulching, i.e., applying a vapor diffusion barrier on the topsoil to curtail water

84   evaporation, has been proven to enhance soil moisture content and provide enhanced

85   transpiration (Moitra, 1996, Zhang et al., 2018, Farzi et al., 2017), plant biomass, and yields

86   (Ramalan, 2000, Zhang, 2017a, Mukherjee, 2010). Mulching has been demonstrated to

87   enhance leaf chlorophyll content (Wang et al., 2015), photosynthesis (Niu et al., 2020,

88   Zhang et al., 2019), and TE (Balwinder-Singh et al., 2011), as well as improve plant root

89   growth and roots' architectural and anatomical properties, such as root diameter and late

90   xylem vessel diameter, which improves water and nutrient uptake from the soil (Zhan et

91   al., 2019, Larsson and Jensen, 1996). Low-density polyethylene sheets approximately 0.1

92   mm thick have been used extensively for mulching in developed countries (Kasirajan and

93   Ngouajio, 2012). Their application in the developing world is also on the rise, e.g.,

94   consumption in China is set to exceed two million tons per year by 2024 (Liu et al., 2014,

95   Wang et al., 2013). Despite their benefits (Zhang, 2017b, Zhang et al., 2019, Balwinder-

96   Singh et al., 2011, Yin W, 2019, Li, 2004), plastics are non-biodegradable, and their

97   eventual disposal into landfills is unsustainable (Kasirajan and Ngouajio, 2012, Halley et

98   al., 2001). Recent findings on the leaching of phthalates from plastic mulches into soils and

99   their adverse effects on the soil quality and microbial activity over the long term are also

100   concerning (Shah and Wu, 2020). Therefore, sustainable mulching technologies are

101   needed.

102   We recently developed superhydrophobic sand (SHS) mulch technology (Mishra

103   et al., 2017, Gallo Jr et al., 2021a), a nature-inspired material comprising common sand

104   gains or sandy soils coated with a nanoscale layer of paraffin wax (wax to sand mass ratio

105   is 1:1000). The micro- and nanoscale surface roughness of the sand grains/particles and the

106   hydrophobic nature of wax induce superhydrophobicity (Arunachalam et al., 2019). When

107   laid on topsoil with a sub-surface irrigation system, a 5–10 mm-thick SHS layer can

108   insulate the wet soil from solar radiation and dry air, thereby acting as a diffusion barrier,

109   which reduces evaporation (Gallo Jr et al., 2021b). Multi-year field trials of SHS on

110   tomatoes (*Solanum lycopersicum*), wheat (*Triticum aestivum*), and barley (*Hordeum*

111   *vulgare*) under naturally arid land conditions in Saudi Arabia have revealed significant

112   enhancements in plant growth and yields (Gallo Jr et al., 2021a). Compared with plastic

113   mulch, SHS is cheaper and more environmentally friendly, as it is made from readily

114   abundant sands and paraffin wax that is easily degraded by soil microbes (Marino, 1998,

115   Roper, 2004). After nine months of use, the wax is decomposed by microbial activity and

116   solar radiation and incorporated into the soil after the crop cycle without affecting soil

117   microbial compositions, obviating landfilling (Gallo Jr et al., 2021a). Despite these

118   promising field results, quantitative insights into the effects of SHS on ET and the

119   phenotypic traits responsible for yield enhancement are lacking.

120   This work therefore aims to quantify the effects of mulching with SHS on tomato

121   plants by pinpointing the ET dynamics and consequent plant phenotypes, including plant

122   height, stomatal conductance, stomatal pore size (aperture), leaf chlorophyll content, fruit

123   yields, HI, TE, fresh mass, dry mass, xylem vessel and root diameter. These effects are

4

124   considered in plants grown in controlled growth chambers under normal (**N**) and reduced

125   (**R**) irrigation scenarios at 100% field capacity and 50% of field capacity, respectively.

126

MATERIALS AND METHODS

127

Plants and SHS mulch

128

129   The tomato plants (*Solanum lycopersicum*), Seminis variety (St. Louis, Missouri, US),

130   were purchased from local seed stores in Jeddah, Saudi Arabia. The SHS was produced

131   from common sand and paraffin wax following the optimized protocol detailed in previous

132   work (Mishra et al., 2017, Gallo Jr et al., 2021a); readers are referred to these works for

133   details on SHS production. Briefly, sand was added to an organic solvent containing

134   dissolved wax. The solvent was then removed by changing the pressure and temperature

135   and condensed for reuse, leaving behind the SHS.

136

Plant growth conditions, treatment, and experimental design

137

138   The tomato seeds were sown in plastic trays using a potting mix from Stender AG

139   (Schermbeck, Germany) and grown in Percival growth chambers. After four weeks, the

140   seedlings were transplanted into pots 1,870 cm^3 in volume (15 cm top diameter $\times$ 10.5 cm

141   bottom diameter $\times$ 14.5 cm height) containing approximately 2.4 kg of local sandy soil.

142   The pots were watered via sub-surface irrigation to two field capacity levels every two

143   days: 100% field capacity for **N** irrigation (i.e., the maximum soil moisture content after

144   drainage of excess water from fully saturated potted soil) and 50% of this field capacity for

145   **R** irrigation. The irrigation level of each pot was determined gravimetrically. Thirty-two

146   pots were prepared, including 16 with plants and 16 without plants, the latter of which were

147   used to quantify evaporative losses.

148   The pots were separated into two groups: those comprising SHS and those

149   containing only bare soil. In each group, half of the pots were subjected to **N** irrigation

150   while the other half was maintained under **R** irrigation. For pots containing SHS, a 1.0 cm-

151   thick layer of SHS was applied to the potting soil (approximately 265 g SHS/pot). Thus,

152   there were four treatment combinations (SHS-**N**, bare soil-**N**, SHS-**R**, and bare soil-**R)**,

153   each with two replicates with and without plants. All pots were subjected to complete

154   randomization in a 2 $\times$ 2 factorial design involving two experimental factors (i.e., soil

155   mulching and irrigation regime), each with two levels and four replicates. During the

156   growth period, nutrient solutions were applied to each pot every two weeks comprising in

157   rates per kg of dry soil: 200 mg N/kg, 250 mg P/kg, 200 mg K/kg, 150 mg Ca/kg, 30 mg

158   S/kg, 2 mg Cu/kg, 4 mg Zn/kg, 3 mg Mn/kg, 0.5 mg B/kg, and 0.25 mg Mo/kg. The plants

159   were grown for 98 days under a 14/10 h light/dark photoperiod using artificial fluorescent

160   lighting at 800 µmol m^{-2} s^{-1} of photosynthetically active radiation, a day/night temperature

161   of 28/20°C ± 2°C, and 60 ± 2% air relative humidity.

162

163   **Evapotranspiration (ET)**

164   The ET was partitioned into evaporation and transpiration by performing gravimetric

165   measurements of pots every two days until the final harvest. The water loss from each pot

166   was monitored, and water was added to compensate for the water lost. The daily ET was

167   recorded as the total water lost from each pot containing plants between the time of

168   irrigation to the time of weighing as:

169

170   *ET = (Initial weight of pot with plant − final weight of pot with plant)/(time between*

171   *measurements).*   (Eq. i)

172

173   **Evaporation**

174   Under the assumption that the pots with and without plants experienced a similar amount

175   of evaporation, the daily water loss by evaporation was estimated using pots without plants

176   as:

177

178   *Evaporation = (Initial weight of pot without plant − final weight of pot without plant)/ (time*

179   *between measurements).*   (Eq. ii)

180

181   **Transpiration**

182   The daily transpiration was determined as:

183

184   *Transpiration = (ET − Evaporation).*   (Eq. iii)

185

6

Soil water content (SWC)

The total available soil water content (SWC; i.e., the field capacity) was maintained at a relatively constant level by compensating for the water lost through ET after each gravimetric measurement scheduled at two-day interval, as has been done by prior researchers (Ray and Sinclair, 1998, Pellegrino et al., 2004).

Stomatal conductance and leaf chlorophyll content

The leaf stomatal conductance was determined using an AP4 Porometer (Delta T, Cambridge, UK). Measurements were performed on three young but fully expanded leaves once a week (between 10:00 and 12:00), and the mean conductance for each treatment combination was calculated. The leaf chlorophyll content index (CCI) was measured using a CCM-200 Chlorophyll Content Meter (Optic-Sciences, Inc. Hudson NH03051, USA), with measurements being performed on three young but fully expanded leaves.

Leaf stomatal pore and root anatomical features

Microscopic analyses of leaf stomata and root anatomic structures were performed on samples collected on the final date of harvest. Two fully expanded upper leaves were cut from each plant (between 11:00 and 11:30) and immediately immersed in 70% ethanol inside 50 ml centrifuge tubes until they were analyzed. After two weeks, the preserved leaf tissues were washed with deionized water three times, added to 40 ml of concentrated sodium hypochlorite, and left to stay for approximately 4 hours. The leaf tissues were re-washed with deionized water, and fresh 70% ethanol was again added onto the samples. This clearing process removed the chlorophyll from the leaves and rendered them white in appearance. The stomata of the cleared leaves were then observed using a digital microscope (Leica DVM6) equipped with the tools to measure the length and width of the stomatal pores. The total stomatal aperture area was calculated using the formula for the area of an ellipse, as

$Stomatal\ aperture\ area = \pi \times a \times b,$ (Eq. iv)

7

216   where *a* and *b* represent the semi-major axis (or radius) and semi-minor axis (or width) of

217   the ellipse, respectively.

218   Root samples 10 cm in length from the growing tip were cut using a razor, washed in

219   deionized water, and fixed in 70% ethanol until their microscopic analysis. Two hand-cut

220   root tissues were made per plant and their cross-sections were analyzed using the same

221   digital microscope to measure diameter of the xylem vessels and the total root.

222

223   **Plant growth, fruit yield, and biomass**

224   The plant height was measured every week during the 98–day experimental period.

225   Tomatoes were harvested and weighed at the time of harvest. During the final harvest, the

226   shoots and roots of each plant were weighed, and then put in paper bags and oven-dried at

227   105 ℃ for four days to determine their dry mass.

228

229   **Harvest index (HI) and transpiration efficiency (TE)**

230   After harvest, the HI was calculated for each plant as the ratio of total fruit yield (fruit mass

231   per plant) to total fresh mass (shoot and roots) per plant, as:

232

233    $$HI = \frac{Total\ fruit\ yield}{Total\ fresh\ biomass}. \hspace{3cm} \text{(Eq. v)}$$

234

235   The TE of each plant was calculated as the ratio of dry shoot biomass in grams to the

236   amount of water transpired in kilograms, as:

237

238    $$TE = \frac{Shoot\ dry\ biomass\ produced\ (g)}{mass\ of\ water\ transpired\ (kg)}. \hspace{2cm} \text{(Eq. vi)}$$

239

240   **Data analysis**

241   Data analysis and graphical presentations were performed using Origin Pro Software (2019

242   version). A three-way analysis of variance (ANOVA) was used to analyze the effects of

243   soil mulching, irrigation regimes, growth stage (i.e., time), and their interactions on ET.

244   For the variables averaged across time (i.e., time-independent), a two-way factorial

245   ANOVA was performed to determine the effects of soil mulching, irrigation regimes, and

8

246   their interactions. In each analysis, mean comparisons were done using the Tukey test at

247   the $p < 0.05$ level of statistical significance.

248

249   **RESULTS**

250   **Plant growth**

251   Representative snapshots of experimental plants grown under each condition (i.e., bare soil

252   or soil containing SHS and **N** or **R** irrigation) at different weeks during their growth, from

253   transplanting (week 0) to fruit ripening (week 12), are shown in **Fig. 1**. Overall, the plants

254   grown in soils containing SHS were larger and appeared healthier than those grown in bare

255   soil.

256
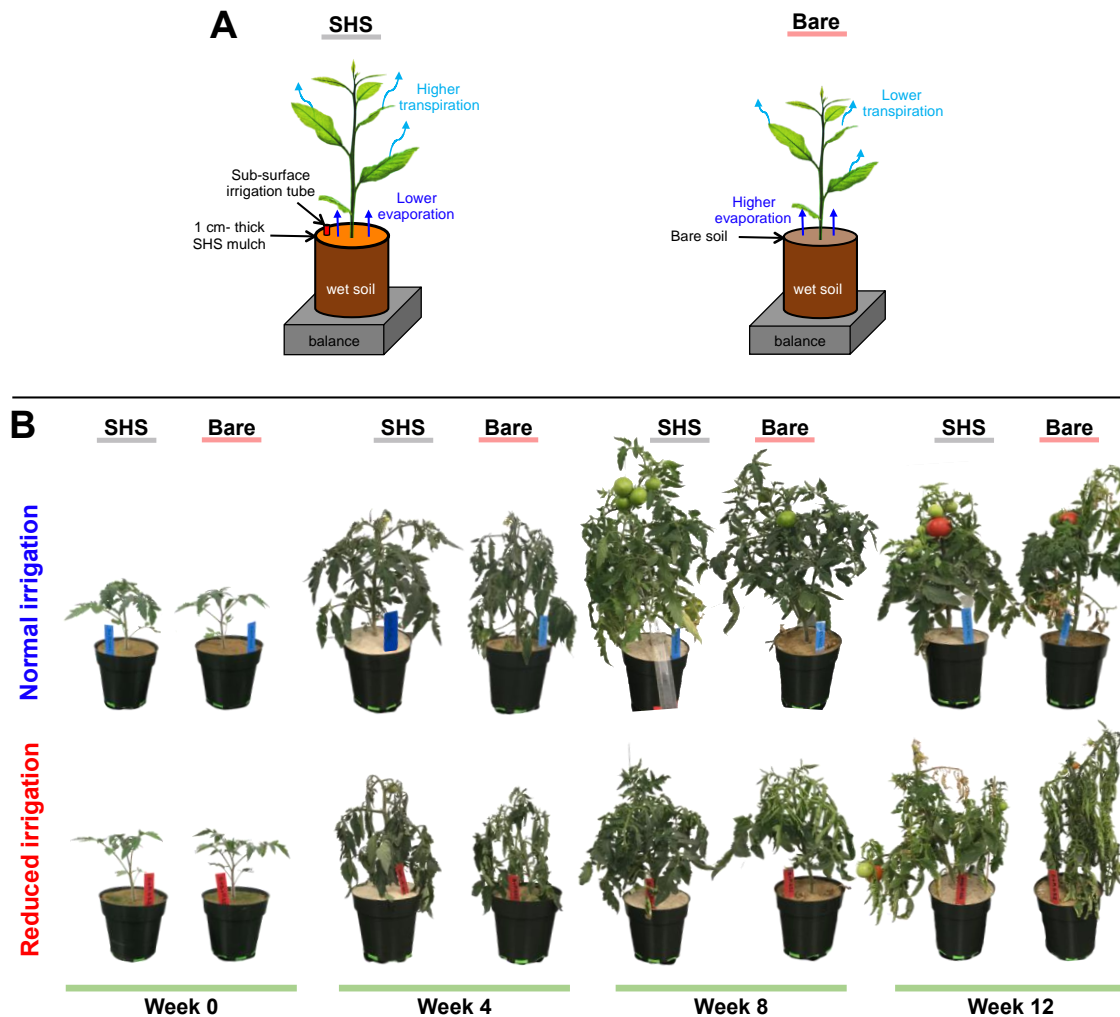
257

258

259 **Fig. 1. Effects of SHS mulching on plants.** (**A**) Schematics of the evaporation and transpiration pathways

260 and their impacts on plants. (**B**) Representative photographs of tomato plants grown in controlled growth

261 chambers mulched with 1-cm-thick superhydrophobic sand (SHS) and bare soil plants under normal (**N**) and

262 reduced (**R**) irrigation. Tomato plants were transplanted and grown for 98 days until the final fruit and

263 biomass harvest. Each treatment combination involved four (*n* = 4) replicate plants.

264

265 **Soil water content**

266 The daily SWC corresponding to each irrigation level is detailed in **Fig. 2A**. For both **N**

267 and **R** irrigation strategies (i.e., 100% and 50% field capacity, respectively), the SWC was

268 slightly increased during days 40–98 to compensate for the increase in plant size and water

269 demand. The slight fluctuations in SWC below the initial field capacities observed at five

270 occasions (i.e., between 30–50 days) represent five days when pot weights were recorded

271 without adding water until the following day due to unavoidable circumstances. However,

272 the plants were not severely stressed on these occasions, as the available SWC was enough

273 to meet the ET demand of the plants until the next day when water was added to the pots.

274 When averaged across both mulched and bare soils, the mean SWC was 0.62 ± 0.01 and

275 0.33 ± 0.01 kg/pot under **N** and **R** irrigation, respectively. This result also demonstrates

276 that plant growth could be maintained under 50% of normal irrigation (i.e., the **R** irrigation

277 strategy).

278

279 **Evapotranspiration**

280 The daily ET significantly differed between the **N** and **R** irrigation scenarios and accounted

281 for approximately 50% of the daily SWC on average, as shown in **Fig. 2B**. Under **R**

282 irrigation, ET differed between mulched and bare soils, notably during days 10–35 and 45–

283 75; no significant differences were found under **N** irrigation.

284

285 **Soil evaporation and transpiration**

286 Throughout the growth period, the daily evaporation was significantly lower from soil

287 containing SHS than bare soil under **N** and **R** irrigation, as shown in **Fig. 2C**. Consequently,

288 plants in mulched soil had higher daily transpiration than in bare soil under both irrigation

289 scenarios, as shown in **Fig. 2D**.

290
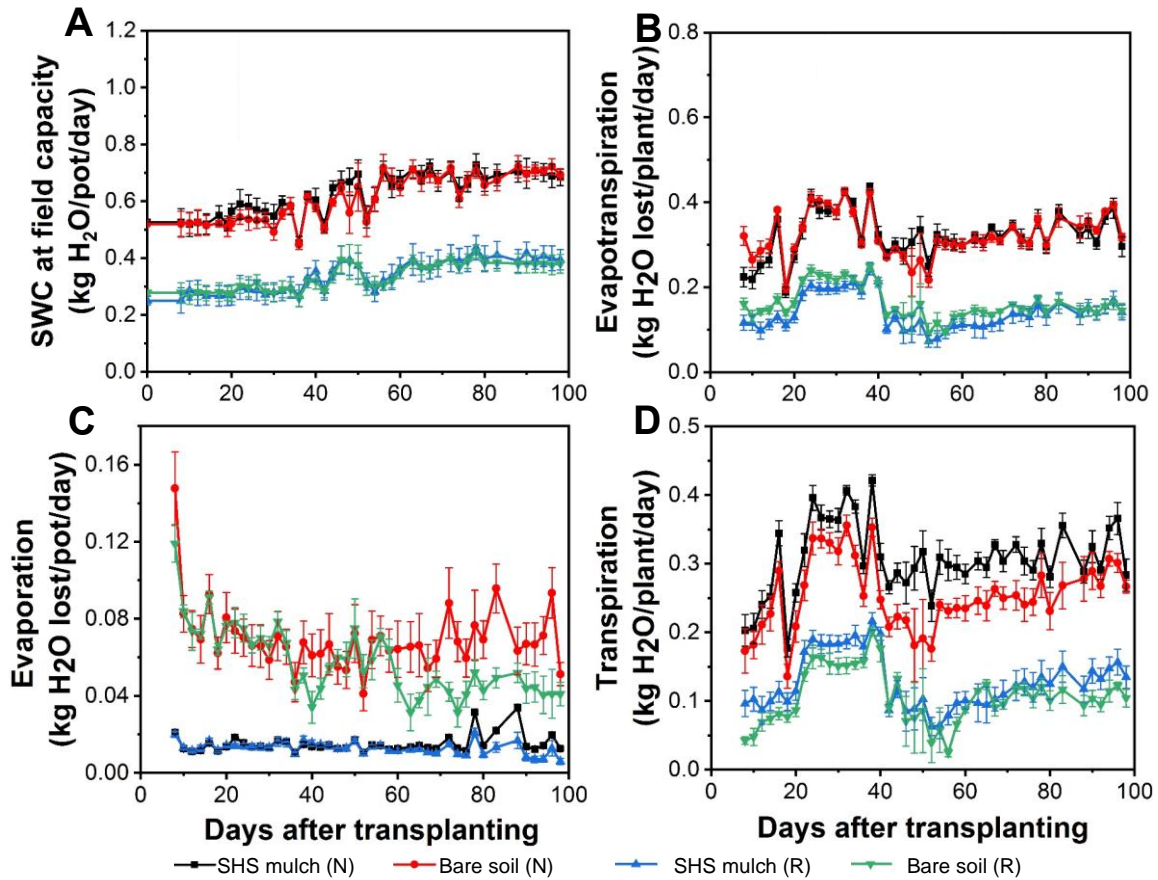
Fig. 2. (**A**) Soil water content (SWC) in pots maintained at field capacity for each treatment and irrigation regime, (**B**) daily changes in ET, (**C**) evaporation from the soil, and (**D**) transpiration between SHS-mulched and bare soils under **N** and **R** irrigation strategies throughout plant growth. Each data point is a mean of four ($n = 4$) replicates; error bars are standard errors ($\pm$SE) of the means.

Cumulative evapotranspiration

There was no significant difference in the cumulative ET loss between mulched and bare soils under **N** irrigation; both had a mean cumulative ET of 13.9 ± 0.7 kg/plant over the 98 days of observation, as shown in **Fig. 3A**. Under **R** irrigation, the cumulative ET was 13% higher in SHS-mulched soil in than bare soils (with mean values of 6.97 ± 0.57 and 6.06 ± 0.78 kg/plant, respectively).

11

Cumulative soil evaporation and transpiration

306 In SHS-mulched soils, that total cumulative evaporation (and transpiration) accounted for

308 5% (95%) and 9% (91%) of the total ET under **N** and **R** irrigation, respectively; in bare

309 soils, evaporation (and transpiration) accounted for 21.5% (78.5%) and 31.5% (65.5%) of

310 the total ET under **N** and **R** irrigation, respectively (**Figs. 3B and 3C**). Overall, SHS

311 mulching suppressed evaporation and enhanced transpiration by the same magnitude of

312 78% and 17%, respectively under both **N** and **R** irrigation relative to the bare soils.

313

Plant height

315 Throughout the growth period, the maximum plant height in SHS-mulched soil

316 significantly exceeded that in bare soil by 9% ($p = 0.005$) and 11% ($p < 0.05$) under **N** and

317 **R** irrigation, respectively (**Fig. 3D**). In particular, a more rapid increase in the plant height

318 was observed during 10–45 days after transplanting; after 70 days, plant height started to

319 level off.

320

Dependence of evapotranspiration on plant growth stage

322 The daily mean ET, evaporation, and transpiration during days 0–30, 31–60, and 61–98

323 are compiled in **Table 1**. Overall, the mean ET, evaporation, and transpiration increased

324 with time; significant differences were present in the mean ET and transpiration between

325 each timeframe ($p < 0.05$). However, no significant difference was found in the mean

326 evaporation from bare soil with time under **N** irrigation ($p > 0.05$), as evidenced by the

327 three-way ANOVA results. Significant two-way interactions were present between

328 mulching and irrigation regimes on transpiration ($p < 0.05$) and between irrigation regime

329 and growth stage on ET, evaporation, and transpiration ($p < 0.05$).

330
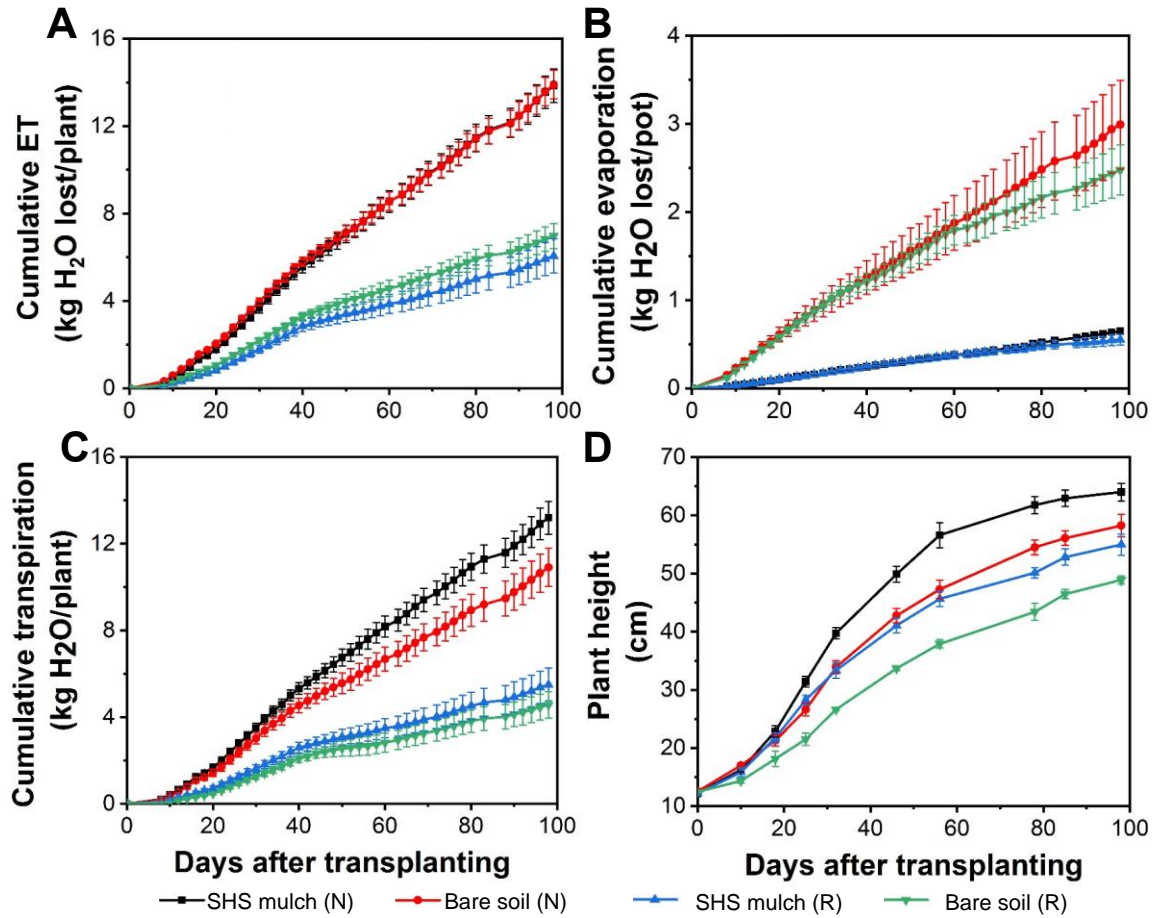
Fig. 3. (**A**) Cumulative evapotranspiration (ET), (**B**) evaporation, (**C**) transpiration, and (**D**) plant height during plant growth under contrasting soil mulching treatments (i.e., SHS-mulched vs. bare soil) and irrigation regimes (i.e., **N** and **R**). Each data point is a mean of four ($n = 4$) replicates; error bars represent the $\pm$SE of the mean.

337 **Table 1. Effects of soil mulching (M), irrigation regimes (W), growth stage (S)– shown by the three different time periods, and**

338 **their interactions on mean evapotranspiration (ET), evaporation, and transpiration.**

	ET (kg/plant)			Evaporation (kg/pot)			Transpiration (kg/plant)		
Treatments	0–30 days	31–60 days	61–98days	0–30 days	31–60 days	61–98 days	0–30 days	31–60 days	61–98 days
SHS mulch (N)	3.66±0.14	4.89±0.21	5.29±0.22	0.19±0.02	0.21±0.01	0.27±0.01	3.48±0.13	4.68±0.20	5.02±0.22
Bare soil (N)	3.93±0.14	4.59±0.16	5.34±0.15	0.93±0.11	0.93±0.16	1.12±0.18	3.00±0.21	3.66±0.20	4.23±0.30
SHS mulch (R)	1.79±0.14	2.07±0.24	2.20±0.33	0.18±0.40	0.20±0.02	0.18±0.02	1.61±0.13	1.86±0.23	2.02±0.31
Bare soil (R)	2.24±0.15	2.39±0.17	2.40±0.11	0.92±0.02	0.86±0.08	0.68±0.01	1.32±0.15	1.57±0.22	1.74±0.07

3-way ANOVA	ET	Evaporation	Transpiration
M (df =1)	ns	266 (0.0*)	25.0 (0.0*)
W (df =1)	702 (0.0*)	5.63 (0.02)	500 (0.0*)
S (df = 2)	33.0 (0.0*)	ns	26.0 (0.0*)
M×W (df = 1)	ns	ns	5.28 (0.03)
M×S (df = 2)	ns	ns	ns
W×S (df = 2)	15.0 (0.0*)	3.49 (0.04)	7.74 (0.0*)
M×W×S (df = 2)	ns	ns	ns

339 Data presented in the upper rows indicate mean values (±SE) of total ET, evaporation, and transpiration at three time periods (0–30, 31–60, and 61–98 days after

340 transplanting) for each treatment combination: SHS-mulched mulch under **N** irrigation (SHS mulch-**N**); bare soil under **N** irrigation (Bare soil-**N**); SHS-mulched

341 mulch under **R** irrigation (SHS mulch-**R**); and bare soil under **R** irrigation (Bare soil-**R**). The lower rows indicate three-way ANOVA statistics for the effects of

342 **M**, **W**, **S**, and their interactions on total ET, evaporation, and transpiration. Significant results are represented by *F* values followed by their respective *p*-values (*p*

343 < 0.05) in parentheses; ns = non-significant results; 0.0* represents *p*-values < 0.001; df = degrees of freedom.

344   **Effects of SHS mulching on physiological traits, biomass yield, and fruit yield**

345   The performed two-way ANOVA demonstrated the significant effect of SHS mulching on

346   total evaporation and transpiration ($p < 0.05$) but no significant effect of SHS mulching on

347   the total ET, as presented in **Table 2**. In addition to the changes in ET, evaporation,

348   transpiration, and plant height demonstrated in **Figs. 2–3**, SHS mulching impacted other

349   plant responses such as stomatal conductance and aperture, CCI, TE, fruit yields, HI, fresh

350   mass, dry mass, root xylem vessel, and total root diameter, as presented in **Figs. 4−7**.

351

352   **Table 2. Results of two-way ANOVA test showing effects of soil mulching (M),**

353   **irrigation regime (W), and their interactions (M × W) on plant traits measured.**

Source of variation	df	Total ET	Total evaporation	Total transpiration	Stomatal conductance	Chlorophyll content index, CCI
M	1	ns	92.7 (0.0*)	12.3 (0.0*)	17.5 (0.0*)	62.6 (0.0*)
W	1	197 (0.0*)	ns	232 (0.0*)	23.5 (0.0*)	17.4 (0.0*)
M×W	1	ns	ns	ns	ns	ns
Error		0.002	0.196	0.849	15810	26.584

Source of variation	df	Plant height	Shoot fresh mass	Root fresh mass	Total fresh mass	Shoot dry mass
M	1	11.7 (0.01)	51.4 (0.0*)	15.8 (0.0*)	53.2 (0.0*)	21.5 (0.0*)
W	1	43.4 (0.0*)	89.9 (0.0*)	29.9 (0.0*)	111 (0.0*)	80.3 (0.0*)
M×W	1	ns	12.5 (0.0*)	ns	11.2 (0.0*)	7.65 (0.02)
Error		6.468	103.310	5.971	114.8	5.619

Source of variation	df	Root dry mass	Total dry mass	Transpiration efficiency, TE	Fruit yields per plant	Harvest Index, HI
M	1	20.6 (0.0*)	14.1 (0.0*)	ns	6.19 (0.03)	15.6 (0.0*)
W	1	ns	36.2 (0.0*)	45.1 (0.0*)	23.3 (0.0*)	12.2 (0.0*)
M×W	1	ns	ns	ns	ns	ns
Error		0.792	10.518	0.1326	1541	0.004

354   Data presented are ***F*** values from two-way ANOVA and p-values for significant results ($p < 0.05$) in

355   parentheses; ns = non-significant results. 0.0* represents *p*-values < 0.001; df = degrees of freedom.

356

357

Main effects of soil mulching and irrigation regimes

A 27.7% higher stomatal conductance was present in SHS-mulched plants than those in bare soil across irrigation regimes ($p = 0.001$; **Fig. 4A-i**). Stomatal conductance was also 31.4% higher under **N** irrigation than under **R** irrigation ($p < 0.05$; **Fig. 4A-ii**). Similarly, leaf CCI was 22.8% higher in SHS-mulched plants than those in bare soil ($p < 0.05$; **Fig. 4B-i**). Irrespective of mulch treatment, leaf CCI was 12.7% higher under **N** irrigation than **R** irrigation ($p = 0.001$; **Fig. 4B-ii**). Fruit yield, defined by the total fruit mass per plant, was 32.8% higher in mulched soil than bare soil ($p = 0.03$; **Fig. 4C-i**) and 55% higher under **N** irrigation than **R** irrigation ($p < 0.05$; **Fig. 4C-ii**).

The relationship between fruit yield and total fresh mass (i.e., the HI) was characterized by 16% higher HI in mulched soil than bare soil ($p = 0.002$; **Fig. 4D-i**). Regardless of the mulch treatment, using **R** irrigation decreased the HI by 14% ($p = 0.004$; **Fig. 4D-ii**). No significant difference in the ratio of shoot dry mass to total water transpired per plant (i.e., the TE) was present between plants grown in mulched and bare soils ($p = 0.697$; **Fig. 4E-i**). However, the TE was 51.4% lower under **N** irrigation than **R** irrigation ($p < 0.05$; **Fig. 4E-ii**).

In terms of biomass allocation, plants in mulched soil had 21% more root fresh mass than those in bare soil ($p = 0.002$; **Fig. 4F-i**); plants under **N** irrigation had 28% more root fresh mass than their **R** irrigation counterparts ($p < 0.05$; **Fig. 4F-ii**). The root dry mass was 33.5% higher in mulched soil than bare soil ($p < 0.05$; **Fig. 4G-i**), while it was 14% higher under **N** irrigation than **R** irrigation ($p = 0.046$; **Fig. 4G-ii**). The total dry mass in mulched soil increased by 20% over that in bare soil ($p = 0.003$; **Fig. 4H-i**), while **N** irrigation resulted in 30% higher total dry mass than under **R** irrigation ($p < 0.05$; **Fig. 4H-ii**).

16
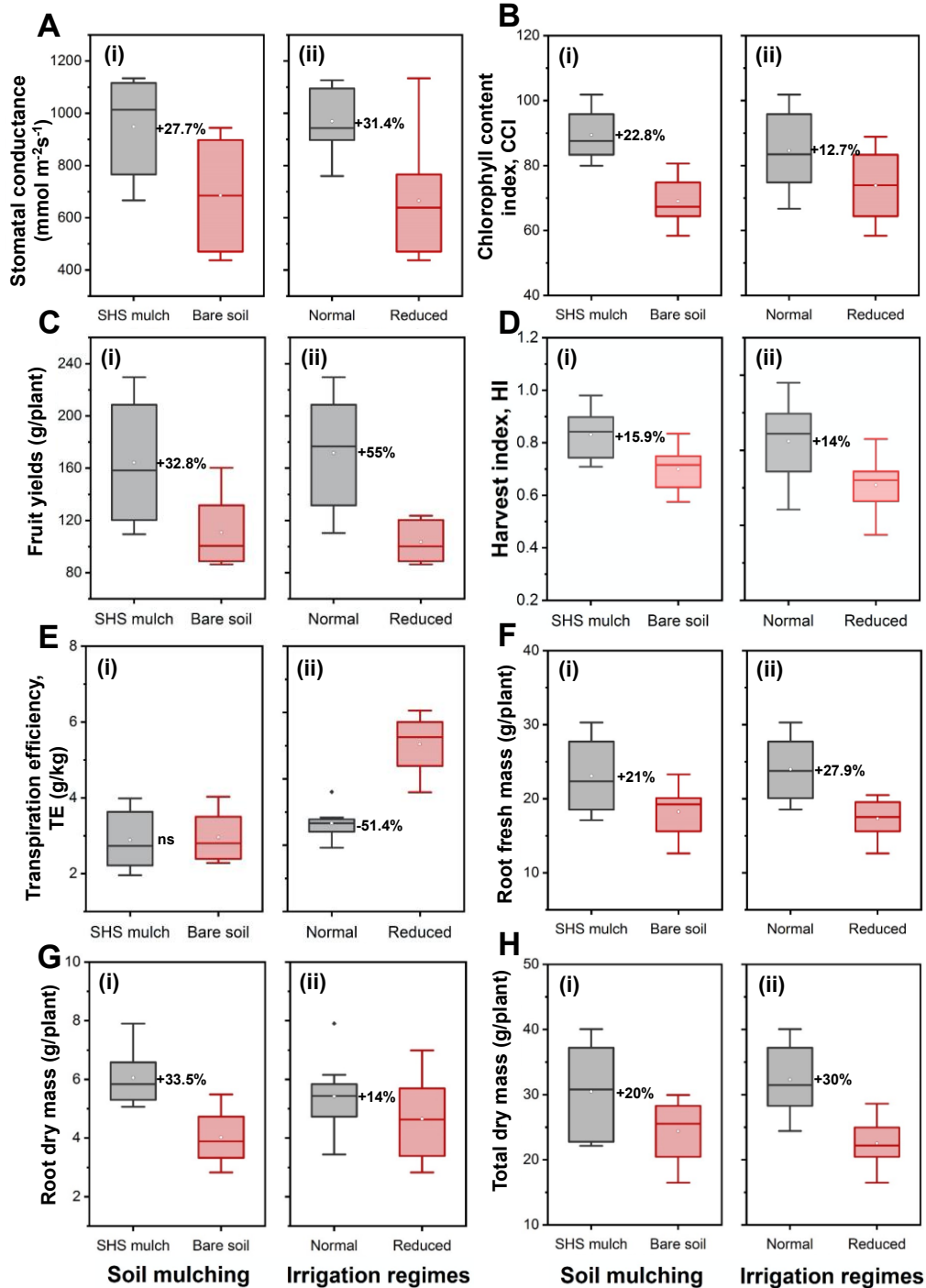
Fig. 4. ANOVA results showing effects of (**i**) soil mulching (SHS mulch vs. bare soil) and (**ii**) irrigation regimes (**N** vs. **R**) on (**A**) leaf stomatal conductance; (**B**) CCI; (**C**) fruit yields per plant; (**D**) HI; (**E**) TE; (**F**) root fresh mass; (**G**) root dry mass; and (**H**) total dry mass per plant. Each box represents the data distribution from eight plants ($n = 8$) with the median along the mid-line. The white dot inside the box represents the mean value, the upper and lower sections of the box represent the 25% and 75% confidence intervals,

17

390 respectively, the whiskers on the box represent the 1.5 interquartile range, and dots outside the box indicate

391 outliers. ns: no significant difference between treatments.

392

393 **Interaction effects of mulching and irrigation regimes**

394 There was also a significant interaction effect of mulching and irrigation on shoot fresh

395 mass, shoot dry mass, and total fresh mass per plant. The shoot fresh mass was 26.6%

396 higher in mulched soil than bare soil under **N** irrigation, whereas under **R** irrigation, the

397 shoot fresh mass was 13% higher in mulched soil than bare soil (**Fig. 5A**; $p < 0.05$, $p = $

398 0.019, respectively). The shoot dry mass increased in mulched soil by 27% compared with

399 that in bare soil under **N** irrigation; under **R** irrigation, shoot dry mass was 12% higher in

400 mulched soil than bare soil (**Fig. 5B**; $p = 0.001$, $p = 0.046$, respectively). Under **N**

401 irrigation, SHS mulching increased the total fresh mass per plant by 26.5%; under **R**

402 irrigation, the total fresh mass per plant was 13.5% higher in mulched soil than bare soil

403 (**Fig. 5C**; $p < 0.05$, $p = 0.037$, respectively).

404
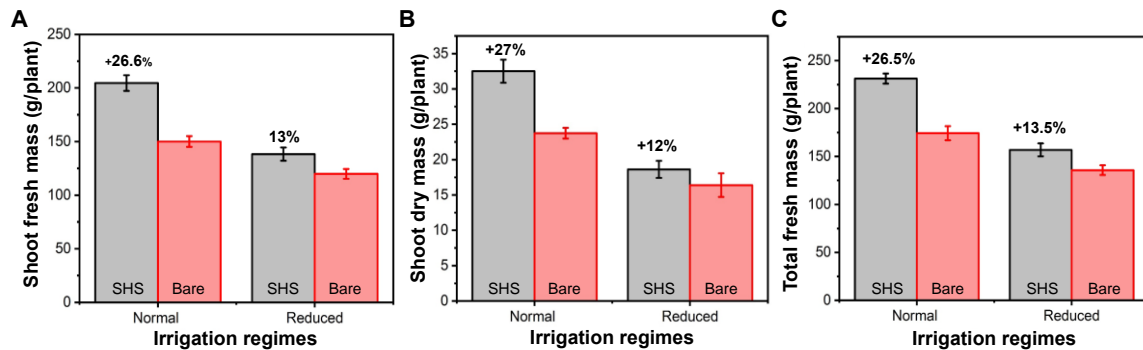
405

406

407 **Fig. 5.** Two-way interaction effects of soil mulching (SHS vs. bare soil) and irrigation regimes (**N** vs. **R**) on

408 (**A**) shoot fresh mass, (**B**) shoot dry mass, and (**C**) total fresh mass per plant. Each bar represents the mean

409 value of four replicates ($n = 4$), and each error bar represents the SE of the means of four samples.

410

411 **Leaf stomatal status**

412 Microscopy revealed that the stomatal pores of plants grown in mulched soils (**Fig. 6A-i**

413 **& iii**) were larger than those in bare soils (**Fig. 6A-ii & iv**); the mean stomatal aperture

414 (area) was 25% larger regardless of the irrigation regime ($p = 0.0019$; **Fig. 6B-i**).

18

415   Furthermore, the mean stomatal aperture was 34.6% higher under **N** irrigation than **R**

416   irrigation ($p < 0.05$; **Fig. 6B-ii**).

417

418
419
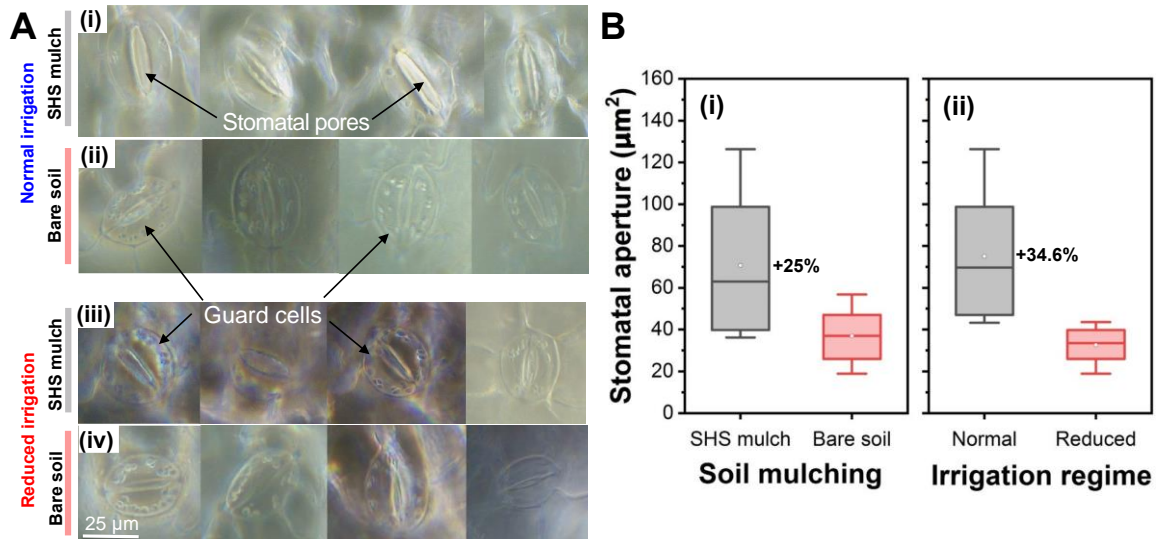
420   **Fig. 6.** Microscopic analysis of leaf stomatal pores in response to soil mulching and irrigation regimes as on

421   the final date of plant harvest. (**A**) Leica DVM6 microscopic image of leaf stomata for plants grown under **N**

422   and **R** irrigation in (**i, iii**) SHS-mulched and (**ii, iv**) bare soils. (**B**) Boxplots showing the mean stomatal

423   aperture (area) for plants grown in (**i**) SHS and bare soil, and (**ii**) under **N** and **R** irrigation. Each box

424   represents the data distribution from 16 leaf samples ($n = 16$) with the median along the mid-line. The white

425   dot inside the box represents the mean value, the upper and lower sections of the box represent the 25% and

426   75% confidence intervals, respectively, and the whiskers on the box represent the 1.5 interquartile range.

427

428   **Root anatomical structure**

429   Finally, root anatomical features were measured to determine the shoot–root feedback

430   linkages with the observed trends in ET as a function of mulching and irrigation scenarios;

431   these results are summarized in **Figs. 7A-i–7A-iv**. The xylem vessel diameter was 21%

432   larger for roots grown in mulched soil than bare soil ($p = 0.001$; **Fig. 7B-i**). However, the

433   difference in diameter under differing irrigation scenarios was not statistically significant

434   ($p = 0.343$; **Fig. 7B-ii**). Additionally, the root diameter increased in mulched soil by 28%

435   relative to bare soil ($p = 0.0177$; **Fig. 7C-i**), whereas it decreased from **R** irrigation to **N**

436   irrigation by 20% ($p = 0.0067$; **Fig. 7C-ii**).
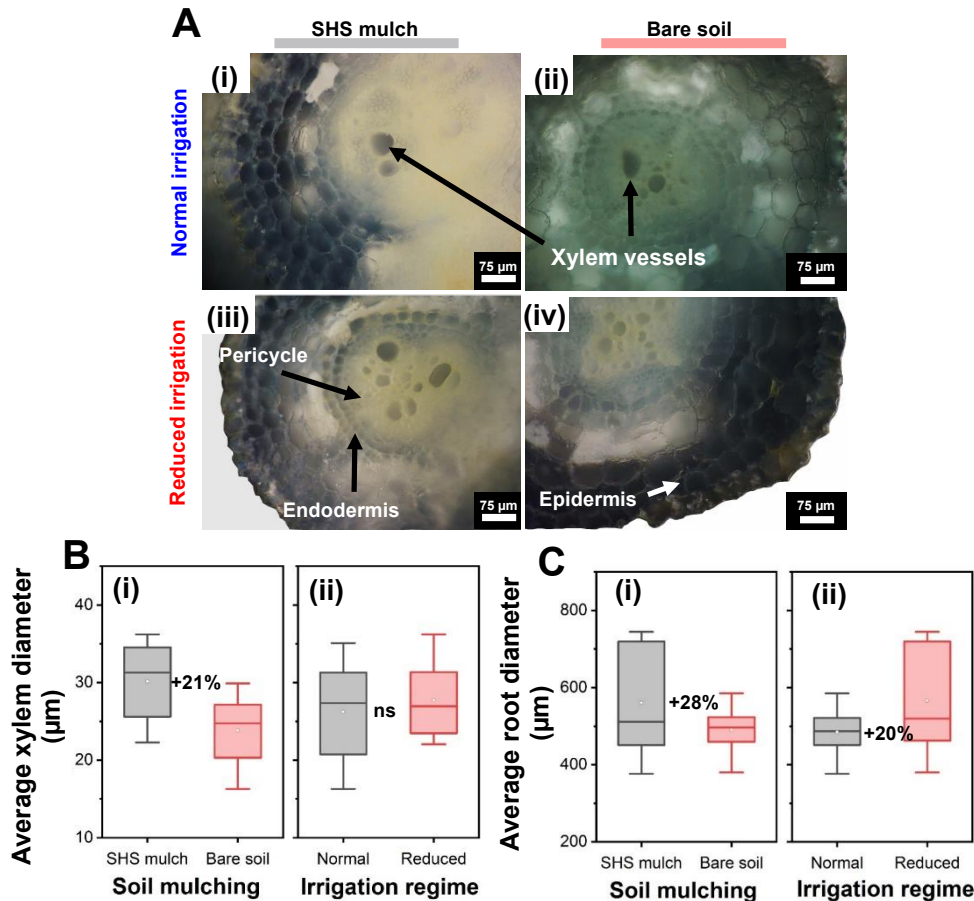
Fig. 7. (**A, i–iv**) Cross-sections of tomato roots from contrasting soil mulching and irrigation scenarios. (B-C) Box plots showing the effects of (i) SHS mulching and (ii) irrigation regimes on (**B**) average xylem vessel diameter and (**C**) average root diameter. Each box represents the data distribution from 16 root samples ($n = 16$) with the median along the mid-line. The white dot inside the box represents the mean value, the upper and lower sections of the box represent the 25% and 75% confidence intervals, respectively, the whiskers on the box represent the 1.5 interquartile range, and dots outside the box indicate outliers. ns: no significant difference between treatments.

DISCUSSION

To clarify the effects of SHS mulching presented on ET fluxes and phenotypic traits of tomato plants grown under N and R irrigation, the mechanistic insights behind SHS mulching and their various impacts on plants and interrelationships are analyzed in this section. The physical mechanism behind the ability of SHS to reduce evaporation from soil is based on the Laplace pressure (i.e., the pressure difference across a curved liquid

20

453   surface), which depends on the surface tension of water and the curvature of the liquid−air

454   interface (Domingues et al., 2018, Das et al., 2019, Gallo Jr et al., 2021a). When water is

455   supplied through sub-surface irrigation, it rises upward through the soil by capillarity and

456   contacts the SHS layer on the topsoil. The curvature of the air–water interface touching the

457   SHS grains forms a convex shape; the capillary action that drives the rise of water in soils

458   prevents it from wetting the SHS layer, creating a diffusion barrier (Gallo Jr et al., 2021a,

459   Gallo Jr et al., 2021b). By physically covering the topsoil, the mulch also insulates the soil

460   from solar radiation and wind, thereby further reducing the capacity for evaporation losses

461   (Kader et al., 2017, Gallo Jr et al., 2021a). With less evaporation, the soil moisture content

462   rises, allowing for higher plant transpiration.

463   A significant causal relationship was present between the stomatal conductance and

464   aperture (**Fig. 8A**) and between transpiration and stomatal conductance (**Fig. 8B**), total

465   fresh mass (**Fig. 8C**), total dry mass (**Fig. 8D**), and fruit yield (**Fig. 8E**). These relationships

466   suggest that the soil moisture-retaining function of SHS augments a chain of plant-related

467   responses that affect stomatal opening and closure, which directly influence transpiration

468   rates. Plants can alter their stomatal pore aperture by actively adjusting the turgor pressure

469   within their guard cells (Blatt, 2000), which moderates the exchange of gases such as water

470   vapor, carbon dioxide (CO_2), and oxygen between the leaf interior and the atmosphere

471   (Kollist et al., 2014, Lawson and Vialet-Chabrand, 2019, Franks and Farquhar, 2007).

472   Enhanced water availability in the soil increases the guard cell turgor pressure, leading to

473   a larger stomatal aperture and increased stomatal conductance of water vapor, which

474   enhances the transpiration and CO_2 uptake rates for photosynthesis (Bertolino et al., 2019,

475   Condon et al., 2002, Putra et al., 2012). This rationale underpins the observed increase in

476   transpiration (**Figs. 2−3**; **Table 1**), stomatal conductance (**Fig. 4A**), and stomatal aperture

477   (**Fig. 6**) when using SHS mulch.

478
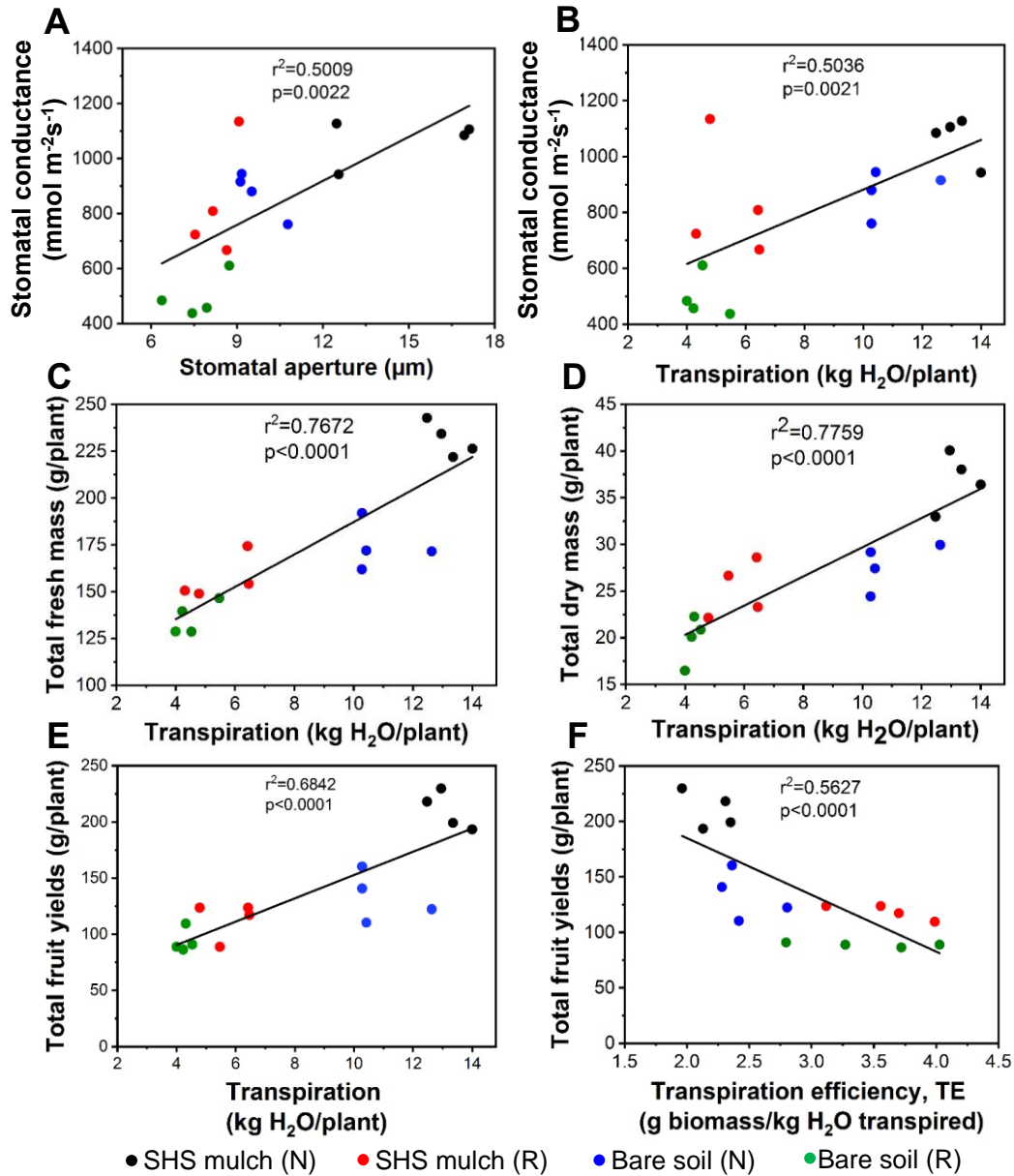
Fig. 8. Regression analyses showing the relationship between (**A**) leaf stomatal conductance and stomatal aperture, (**B**) stomatal conductance and total transpiration, (**C**) total fresh mass and total transpiration, (**D**) total dry mass and total transpiration, (**E**) total fruit yield per plant and total transpiration, and (**F**) total fruit yield per plant and TE. Each dot on the graph indicates an individual plant grown in SHS mulch or bare soil under **N** or **R** irrigation.

Aboveground physiological processes, including stomatal responses, are governed by a feedback mechanism linked closely with the hydraulic conductivity of the soil water

22

488   in the root zone (Putra et al., 2012, Tardieu and Parent, 2017, Zarebanadkouki et al., 2016,

489   Bertolino et al., 2019, Adachi et al., 2010, Chaves et al., 2002). This hydraulic conductivity

490   is dependent on the diameter of the roots and the xylem vessels (Niklas, 1985, Comas et

491   al., 2013). Here, larger xylem vessel and root diameters were present in mulched soil than

492   in bare soil (**Fig. 7**). Consistent with these findings, larger xylem vessel and root diameters

493   have been shown to develop in wheat plants in response to increased SWC caused by

494   mulching (Zhan et al., 2019), increasing WUE and shoot biomass (Kadam et al., 2015).

495   Although small-diameter roots maintain plant growth during a drought, especially at soil

496   depths with available water (Comas et al., 2013), several researchers have suggested that

497   large-diameter roots are more instrumental in resource uptake and plant growth under water

498   scarcity (Larson and Funk, 2016, Kadam et al., 2015). The latter agrees with the observed

499   results; the roots had a larger average diameter under **R** irrigation than **N** irrigation. A larger

500   diameter may promote root penetrative ability or soil exploration in dry soils (Clark et al.,

501   2003) and enhance root longevity (McCormack et al., 2012). These results indicate the

502   important role that root architecture plays in resource acquisition and conservation to

503   facilitate aboveground processes that determine productivity in plants.

504   The root–shoot hydraulic system conducts nutrients and water via the transpiration

505   stream to the leaves; these nutrients (e.g., nitrogen) support plant growth and the

506   photosynthetic process. Nitrogen availability is related to the total chlorophyll content in

507   plant leaves (Bassi et al., 2018). The higher the chlorophyll content, the more efficient its

508   photosynthetic capacity is, causing increased growth, yields, and biomass (Condon et al.,

509   2004, Haefele et al., 2009). Here, a higher CCI and higher transpiration due to SHS

510   mulching contributed to higher fruit yield and biomass production, leading to a higher HI.

511   The fruit mass per plant was negatively correlated with TE (**Fig. 8F**), indicating that fruit

512   yields increased under low TE. This finding contradicts the proposal that higher TE is

513   critical in increasing crop yields in water-limited environments (Christy et al., 2018,

514   Sinclair, 2012, Rebetzke et al., 2002, Coupel-Ledru et al., 2016, Condon et al., 2004, Vadez

515   and Ratnakumar, 2016). However, TE is a physiologically complex trait that results from

516   a combination of multiple physiological parameters, such as photosynthesis, stomatal

517   conductance, mesophyll conductance (i.e., the diffusion of CO_2 from sub-stomatal cavities

518   to the sites of carboxylation in the chloroplasts) (Flexas and Medrano, 2002, Flexas et al.,

23

519 2008), and other conditions that determine carbon balance and growth in plants (Natarajan

520 et al., 2021). Consequently, the conditions responsible for the expression of high TE in one

521 situation may be associated with a low TE in another environment (Sinclair, 2012).

522 A prolonged stomatal opening and higher stomatal conductance account for lower

523 TE (Flexas et al., 2008); mulched soils retain more SWC and maintain stomatal openings

524 longer than bare soils, leading to a low TE (Balwinder-Singh et al., 2011). However, no

525 significant difference in TE was present between SHS-mulched and bare soils, despite their

526 difference in stomatal conductance. However, lower stomatal conductance was associated

527 with higher TE under **R** irrigation than under **N** irrigation. These findings indicate the trade-

528 off required between stomatal conductance and carboxylation efficiency to improve TE

529 and yields. Unlike increasing the stomatal conductance, increasing the mesophyll

530 conductance increases photosynthesis and TE without increasing transpiration because the

531 CO_2 diffusion pathway involving mesophyll conductance is not shared with that of

532 transpired water (Flexas et al., 2008, Ouyang et al., 2017, Flexas et al., 2013, Barbour et

533 al., 2010).

534 Future investigations are needed to examine physiological traits associated with

535 enhanced TE under different environmental conditions. For example, experiments

536 resolving instantaneous leaf gas exchange could help determine photosynthesis and

537 conductance (stomatal vs. mesophyll conductance), whereas measurements of carbon

538 isotope composition ($\delta^{13}C$; a surrogate trait that integrates TE and variation in root traits

539 and plasticity) could enhance understanding of how aboveground and belowground

540 processes shape plant responses and yield performance under different water regimes.

541 Determining the genetic factors that can maximize transpiration and carbon assimilation to

542 enhance photosynthetic output would complement the water-saving benefit of SHS

543 mulching to improve yields in arid land agriculture.

544

545 **CONCLUSIONS**

546 In conclusion, by combining a broad set of techniques to investigate the effects of SHS

547 mulching on ET fluxes and plant responses, this study revealed that adding a thin (1.0-cm

548 thick) layer of SHS dramatically decreased water evaporation from the topsoil, thereby

549 boosting transpiration under both irrigation scenarios studied. The enhanced transpiration,

550 in turn, increased plant biomass and fruit yield. Furthermore, this study facilitated

551 understanding the mechanistic insights behind increased transpiration, biomass, and fruit

552 yield owing to the root–shoot hydraulic processes accounting for enhanced water uptake

553 and stomatal regulation. The quantitative assessment of ET, evaporation, and transpiration

554 presented here will aid crop WUE assessments and field water optimization and

555 management using SHS mulching, irrigation systems design, and agricultural regimes in

556 arid and semi-arid regions.

557

558 **ACKNOWLEDGMENTS**

561

562 **FUNDING**

565

566 **AUTHOR CONTRIBUTIONS**

567 **KO:** Designed and performed the experiments, including soil preparation, planting,

568 harvests, data collection, and analysis; manuscript writing and revision. **AGJ:** Assisted

569 with the experimental design, data analysis, and manuscript revision. **VD:** Collected the

570 evapotranspiration data and assisted in plant harvest and biomass determination. **HM:**

571 Supervised this research and revised the manuscript.

572

573 **DATA AVAILABILITY STATEMENT**

574 All data supporting the findings reported in this study are available in the paper. **HM** and

575 **AGJ** have filed a US patent (US20200253138A1) for the SHS used in this work, while

576 **KO** and **VD** have no competing financial interest in this work.

577

578 **ORCID:** Himanshu Mishra (https://orcid.org/0000-0001-8759-7812)

REFERENCES

Adachi, S., Tsuru, Y., Kondo, M., Yamamoto, T., Arai-Sanoh, Y., Ando, T., Ookawa, T., Yano, M. & Hirasawa, T. 2010. Characterization of a rice variety with high hydraulic conductance and identification of the chromosome region responsible using chromosome segment substitution lines. *Annals of Botany,* 106, 803-11.

Al-Naizy, R., Simonet, D. 2012. Analysis of Water Evaporation and Drift Losses During Irrigation in Semi-arid Areas of Sharjah (UAE) and Riyadh (KSA). *Natural Resources Research,* 21, 285-299.

Arunachalam, S., Das, R., Nauruzbayeva, J., Domingues, E. M. & Mishra, H. 2019. Assessing omniphobicity by immersion. *Journal of Colloid and Interface Science,* 534, 156-162.

Balugani, E., Lubczynski, M. W., Reyes-Acosta, L., Van Der Tol, C., Frances, A. P. & Metselaar, K. 2017. Groundwater and unsaturated zone evaporation and transpiration in a semi-arid open woodland. *Journal of Hydrology,* 547, 54-66.

Balwinder-Singh, Eberbach, P. L., Humphreys, E. & Kukal, S. S. 2011. The effect of rice straw mulch on evapotranspiration, transpiration and soil evaporation of irrigated wheat in Punjab, India. *Agricultural Water Management,* 98, 1847-1855.

Barbour, M. M., Warren, C. R., Farquhar, G. D., Forrester, G. & Brown, H. 2010. Variability in mesophyll conductance between barley genotypes, and effects on transpiration efficiency and carbon isotope discrimination. *Plant, Cell & Environment,* 33, 1176-1185.

Bassi, D., Menossi, M. & Mattiello, L. 2018. Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Scientific Reports,* 8.

Bertolino, L. T., Caine, R. S. & Gray, J. E. 2019. Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. *Frontiers in Plant Science,* 10.

Blatt, M. R. 2000. Cellular signaling and volume control in stomatal movements in plants. *Annual review of cell and developmental biology,* 16, 221-241.

Carvalho, D., Martins, R., Santos, J., Teles, G., Gentile, M. & Oliveira, M. 2020. Evolution and current scenario of irrigated area in Brazil: Systematic data analysis. *Revista Brasileira de Engenharia Agrícola e Ambiental,* 24, 505-511.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osorio, M. L., Carvalho, I., Faria, T. & Pinheiro, C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany,* 89, 907-916.

Christy, B., Tausz-Posch, S., Tausz, M., Richards, R., Rebetzke, G., Condon, A., Mclean, T., Fitzgerald, G., Bourgault, M. & O'leary, G. 2018. Benefits of increasing transpiration efficiency in wheat under elevated CO2 for rainfed regions. *Global Change Biology,* 24, 1965-1977.

Clark, L., Whalley, W. & Barraclough, P. 2003. How do roots penetrate strong soil? *Roots: The Dynamic Interface Between Plants and the Earth.* Springer.

Comas, L. H., Becker, S. R., Cruz, V. V., Byrne, P. F. & Dierig, D. A. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science,* 4.

Condon, A. G., Richards, R. A., Rebetzke, G. J. & Farquhar, G. D. 2002. Improving Intrinsic Water-Use Efficiency and Crop Yield. *Crop Science,* 42, 122-131.

Condon, A. G., Richards, R. A., Rebetzke, G. J. & Farquhar, G. D. 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany,* 55, 2447-2460.

Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., Doligez, A. & Simonneau, T. 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences,* 113, 8963-8968.

Darko, R. O., Yuan, S., Hong, L., Liu, J. & Yan, H. 2016. Irrigation, a productive tool for food security – a review. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science,* 66, 191-206.

Das, R., Arunachalam, S., Ahmad, Z., Manalastas, E. & Mishra, H. 2019. Bio-inspired gas-entrapping membranes (GEMs) derived from common water-wet materials for green desalination. *Journal of Membrane Science,* 588, 117185.

Domingues, E. M., Arunachalam, S., Nauruzbayeva, J. & Mishra, H. 2018. Biomimetic coating-free surfaces for long-term entrapment of air under wetting liquids. *Nature Communications,* 9, 3606.

Famiglietti, J. S. 2014. The global groundwater crisis. *Nature Climate Change,* 4, 945-948.

Farzi, R., Gholami, M., Baninasab, B. & Gheysari, M. 2017. Evaluation of different mulch materials for reducing soil surface evaporation in semi-arid region. *Soil Use and Management,* 33, 120-128.

Flexas, J. & Medrano, H. 2002. Drought‐inhibition of photosynthesis in C3 plants: stomatal and non‐stomatal limitations revisited. *Annals of botany,* 89, 183-189.

Flexas, J., Niinemets, Ü., Gallé, A., Barbour, M. M., Centritto, M., Diaz-Espejo, A., Douthe, C., Galmés, J., Ribas-Carbo, M. & Rodriguez, P. L. 2013. Diffusional conductances to CO 2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynthesis Research,* 117, 45-59.

Flexas, J., Ribas-Carbo, M., Diaz-Espejo, A., Galmes, J. & Medrano, H. 2008. Mesophyll conductance to CO2: current knowledge and future prospects. *Plant, Cell & Environment,* 31, 602-21.

Franks, P. J. & Farquhar, G. D. 2007. The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology,* 143, 78-87.

Gallo Jr, A., Odokonyero, K., Mousa, M. A., Reihmer, J., Al-Mashharawi, S., Marasco, R., Manalastas, E., Morton, M. J., Daffonchio, D., Mccabe, M. F. & Mishra, H. 2021a. Superhydrophobic sand mulches increase agricultural productivity in arid regions. *arXiv e-prints*, arXiv: 2102.00495. [Preprint].

Gallo Jr, A., Tavares, F., Das, R. & Mishra, H. 2021b. How particle–particle and liquid–particle interactions govern the fate of evaporating liquid marbles. *Soft Matter (DOI: 10.1039/D1SM00750E).*

Grill, E. & Ziegler, H. 1998. A plant's dilemma. *Science,* 282, 252-253.

Haefele, S. M., Siopongco, J. D. L. C., Boling, A. A., Bouman, B. A. M. & Tuong, T. P. 2009. Transpiration efficiency of rice (Oryza sativa L.). *Field Crops Research,* 111, 1-10.

Halley, P., Rutgers, R., Coombs, S., Kettels, J., Gralton, J., Christie, G., Jenkins, M., Beh, H., Griffin, K. & Jayasekara, R. 2001. Developing biodegradable mulch films from starch‐based polymers. *Starch‐Stärke,* 53, 362-367.

Hammer, G. L., Cooper, M. & Reynolds, M. P. 2021. Plant production in water-limited environments. *Journal of Experimental Botany*, 72, 5097-5101.

Hatfield, J. L. & Dold, C. 2019. Water-Use Efficiency: Advances and Challenges in a Changing Climate. *Frontiers in Plant Science,* 10.

Hetherington, A. M. & Woodward, F. I. 2003. The role of stomata in sensing and driving environmental change. *Nature,* 424, 901-908.

Hoekstra, A. Y. & Mekonnen, M. M. 2012. The water footprint of humanity. *Proceedings of the National Academy of Sciences,* 109, 3232-3237.

Jain, R., Kishore, P. & Singh, D. 2020. Irrigation in India: Status, challenges and options. *Journal of Soil and Water Conservation,* 18.

Kadam, N. N., Yin, X. Y., Bindraban, P. S., Struik, P. C. & Jagadish, K. S. V. 2015. Does Morphological and Anatomical Plasticity during the Vegetative Stage Make Wheat More Tolerant of Water Deficit Stress Than Rice? *Plant Physiology,* 167, 1389-U448.

Kader, M. A., Senge, M., Mojid, M. A. & Ito, K. 2017. Recent advances in mulching materials and methods for modifying soil environment. *Soil & Tillage Research,* 168, 155-166.

Kasirajan, S. & Ngouajio, M. 2012. Polyethylene and biodegradable mulches for agricultural applications: a review. *Agronomy for Sustainable Development,* 32, 501-529.

Kollist, H., Nuhkat, M. & Roelfsema, M. R. G. 2014. Closing gaps: linking elements that control stomatal movement. *New Phytologist,* 203, 44-62.

Larson, J. E. & Funk, J. L. 2016. Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. *New Phytolologist,* 210, 827-38.

Larsson, L. & Jensen, P. 1996. Effects of mulching on the root and shoot growth of young black currant bushes (Ribes nigrum). *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 46, 197-207.

Lawson, T. & Vialet-Chabrand, S. 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytolologist,* 221, 93-98.

Lehmann, P., Berli, M., Koonce, J. E. & Or, D. 2019. Surface Evaporation in Arid Regions: Insights From Lysimeter Decadal Record and Global Application of a Surface Evaporation Capacitor (SEC) Model. *Geophysical Research Letters,* 46, 9648-9657.

Li, F.-M. W., Ping; Wang, Jun; Xu, Jin-Zhang. 2004. Effects of irrigation before sowing and plastic film mulching on yield and water uptake of spring wheat in semiarid Loess Plateau of China. *Agricultural Water Management,* 67, 77-88.

Liu, E., He, W. & Yan, C. 2014. 'White revolution'to 'white pollution'—agricultural plastic film mulch in China. *Environmental Research Letters,* 9, 091001.

Marino, F. 1998. *Biodegradation of Paraffin Wax.* Master of Engineering, McGill University.

Mccormack, L. M., Adams, T. S., Smithwick, E. A. & Eissenstat, D. M. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist,* 195, 823-831.

Mishra, H., Reihmer, J. & Gallo Jr., A. 2017. *Compositions and methods relating to functionalized sands (U.S. Patent and Trademark Office; U.S. Department of Commerce, Provisional Patent Application #62550264;* https://patents.google.com/patent/WO2018091986A1/en*).*

Moitra, R., Gosh, D. C. & Sarkar, S. 1996. Water use pattern and productivity of rainfed yellow sarson (Brassica rapa L. var glauca) in relation to tillage and mulching. *Soil and Tillage Research,* 38, 153-160.

Mukherjee, A. K., M; Sarkar, S. 2010. Role of irrigation and mulch on yield, evapotranspiration rate and water use pattern of tomato (Lycopersicon esculentum L.). *Agricultural Water Management,* 98, 182-189.

Natarajan, S., Basnayake, J., Lakshmanan, P. & Fukai, S. 2021. Genotypic variation in intrinsic transpiration efficiency correlates with sugarcane yield under rainfed and irrigated field conditions. *Physiologia Plantarum,* 172, 976-989.

Niklas, K. J. 1985. The evolution of tracheid diameter in early vascular plants and its implications on the hydraulic conductance of the primary xylem strand. *Evolution,* 39, 1110-1122.

Niu, L., Yan, Y. Y., Hou, P., Bai, W. B., Zhao, R. L., Wang, Y. H., Li, S. K., Du, T. S., Zhao, M., Song, J. Q. & Zhou, W. B. 2020. Influence of plastic film mulching and planting density on yield, leaf anatomy, and root characteristics of maize on the Loess Plateau. *Crop Journal,* 8, 548-564.

Nkurunziza, L., Chirinda, N., Lana, M., Sommer, R., Karanja, S., Rao, I., Romero Sanchez, M. A., Quintero, M., Kuyah, S., Lewu, F., Joel, A., Nyamadzawo, G. & Smucker, A. 2019. The Potential Benefits and Trade-Offs of Using Sub-surface Water Retention Technology on Coarse-Textured Soils: Impacts of Water and Nutrient Saving on Maize Production and Soil Carbon Sequestration. *Frontiers in Sustainable Food Systems,* 3.

Or, D. & Lehmann, P. 2019. Surface Evaporative Capacitance: How Soil Type and Rainfall Characteristics Affect Global-Scale Surface Evaporation. *Water Resources Research,* 55, 519-539.

Ouyang, W. J., Struik, P. C., Yin, X. Y. & Yang, J. C. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany,* 68, 5191-5205.

Pellegrino, A., Lebon, E., Voltz, M. & Wery, J. 2004. Relationships between plant and soil water status in vine (Vitis vinifera L.). *Plant and Soil,* 266, 129-142.

Porker, K., Straight, M. & Hunt, J. R. 2020. Evaluation of G × E × M Interactions to Increase Harvest Index and Yield of Early Sown Wheat. *Frontiers in Plant Science,* 11.

Putra, E., Wahab, Z., Abdullah, N. A. & Saleh, G. 2012. Stomatal Morphology, Conductance and Transpiration of Musa sp. cv. Rastali in Relation to Magnesium, Boron and Silicon Availability. *American Journal of Plant Physiology,* 7, 84-96.

Ramalan, A. A. & Nwokeocha, C. U. 2000. Effects of furrow irrigation methods, mulching and soil water suction on the growth, yield and water use efficiency of tomato in the Nigerian Savanna. *Agricultural Water Management,* 45, 317-330.

Rawitz, E. & Hadas, A. 1994. Efficient Use of Water in Rain-Fed Agriculture. *In:* Tanji, K. K. & Yaron, B. (eds.) *Management of Water Use in Agriculture.* Berlin, Heidelberg: Springer Berlin Heidelberg.

Ray, J. D. & Sinclair, T. R. 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *Journal of Experimental Botany,* 49, 1381-1386.

Rebetzke, G. J., Condon, A. G., Richards, R. A. & Farquhar, G. D. 2002. Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Science,* 42, 739-745.

Roper, M. M. 2004. The isolation and characterisation of bacteria with the potential to degrade waxes that cause water repellency in sandy soils. *Australian Journal of Soil Research,* 42, 427-434.

Scanlon, B. R., Faunt, C. C., Longuevergne, L., Reedy, R. C., Alley, W. M., Mcguire, V. L. & Mcmahon, P. B. 2012. Groundwater depletion and sustainability of irrigation in the US High Plains and Central Valley. *Proceedings of the National Academy of Sciences,* 109, 9320-9325.

Shah, F. & Wu, W. 2020. Use of plastic mulch in agriculture and strategies to mitigate the associated environmental concerns. *Advances in Agronomy, Vol 164,* 164, 231-287.

Shiklomanov, I. A. 2000. Appraisal and Assessment of World Water Resources. *Water International,* 25, 11-32.

Sinclair, T. R. 2012. Is transpiration efficiency a viable plant trait in breeding for crop improvement? *Functional Plant Biology,* 39, 359-365.

Steward, D. R., Bruss, P. J., Yang, X., Staggenborg, S. A., Welch, S. M. & Apley, M. D. 2013. Tapping unsustainable groundwater stores for agricultural production in the High Plains Aquifer of Kansas, projections to 2110. *Proceedings of the National Academy of Sciences,* 110, E3477-E3486.

Sutanto, S., Wenninger, J., Coenders-Gerrits, A., Uhlenbrook, S. 2012. Partitioning of evapotranspiration into transpiration, soil evaporation and interception: A comparison between isotope measurements and a HYDRUS-1D model. . *Hydrology and Earth System Sciences,* 16, 2605–2616.

Tardieu, F. & Parent, B. 2017. Predictable 'meta-mechanisms' emerge from feedbacks between transpiration and plant growth and cannot be simply deduced from short-term mechanisms. *Plant, Cell & Environment,* 40, 846-857.

Unkovich, M., Baldock, J. & Forbes, M. B. 2010. Chapter 5 - Variability in Harvest Index of Grain Crops and Potential Significance for Carbon Accounting: Examples from Australian Agriculture. *Advances in Agronomy.*

Vadez, V., Kholova, J., Medina, S., Kakkera, A. & Anderberg, H. 2014. Transpiration efficiency: new insights into an old story. *Journal of Experimental Botany,* 65, 6141-6153.

Vadez, V. & Ratnakumar, P. 2016. High transpiration efficiency increases pod yield under intermittent drought in dry and hot atmospheric conditions but less so under wetter and cooler conditions in groundnut (Arachis hypogaea (L.)). *Field Crops Research,* 193, 16-23.

Wang, C., Wang, H., Zhao, X., Chen, B. & Wang, F. 2015. Mulching affects photosynthetic and chlorophyll a fluorescence characteristics during stage III of peach fruit growth on the rain-fed semiarid Loess Plateau of China. *Scientia Horticulturae,* 194, 246-254.

Wang, J., Luo, Y., Teng, Y., Ma, W., Christie, P. & Li, Z. 2013. Soil contamination by phthalate esters in Chinese intensive vegetable production systems with different modes of use of plastic film. *Environmental Pollution,* 180, 265-273.

Watto, M. A., Bashir, S. & Niazi, N. K. 2018a. Pakistan heading for groundwater crisis. *Nature,* 554, 300-300.

Watto, M. A., Mugera, A. W., Kingwell, R. & Saqab, M. M. 2018b. Re-thinking the unimpeded tube-well growth under the depleting groundwater resources in the Punjab, Pakistan. *Hydrogeology Journal,* 26, 2411-2425.

WWAP 2009. The United Nations World Water Development Report 3: Water in a changing world.

Yin W, F. Z., Hu F, Yu A, Zhao C, Chai Q, And Coulter J. A. 2019. Innovation in alternate mulch with straw and plastic management bolsters yield and water use efficiency in wheat-maize intercropping in arid conditions. *Nature,* 9.

Zarebanadkouki, M., Ahmed, M. A. & Carminati, A. 2016. Hydraulic conductivity of the root-soil interface of lupin in sandy soil after drying and rewetting. *Plant and Soil,* 398, 267-280.

Zhan, A., Liu, J. L., Yue, S. C., Chen, X. P., Li, S. Q. & Bucksch, A. 2019. Architectural and anatomical responses of maize roots to agronomic practices in a semi-arid environment. *Journal of Plant Nutrition and Soil Science,* 182, 751-762.

Zhang, H. M., Huang, G. H., Xu, X., Xiong, Y. W. & Huang, Q. Z. 2018. Estimating Evapotranspiration of Processing Tomato under Plastic Mulch Using the SIMDualKc Model. *Water,* 10.

Zhang, H. X., Yunwu; Huang, Guanhua; Xu, Xu; Huang, Quanzhong. 2017a. Effects of water stress on processing tomatoes yield, quality and water use efficiency with plastic mulched drip irrigation in sandy soil of the Hetao Irrigation District. *Agricultural Water Management,* 179, 205-214.

Zhang, P. W., Ting; Cai, Tie; Ali, Shahzad; Han, Qingfang; Ren, Xiaolong; Jia, Zhikuan. 2017b. Plastic-Film Mulching for Enhanced Water-Use Efficiency and Economic Returns from Maize Fields in Semiarid China. *Frontiers in plant science,* 8, 512.

Zhang, X. D., Yang, L. C., Xue, X. K., Kamran, M., Ahmad, I., Dong, Z. Y., Liu, T. N., Jia, Z. K., Zhang, P. & Han, Q. F. 2019. Plastic film mulching stimulates soil wet-dry alternation and stomatal behavior to improve maize yield and resource use efficiency in a semi-arid region. *Field Crops Research,* 233, 101-113.

Zhu, X., Li, Y., Li, M., Pan, Y. & Shi, P. 2013. Agricultural irrigation in China. *Journal of Soil and Water Conservation,* 68.