The ratio of trichomes to stomata is associated with water use efficiency in *Solanum lycopersicum* (tomato)

Javier Galdon-Armero\(^1\), Mateu Fullana-Pericas\(^2\), Pere A. Mulet\(^2\), Miquel A. Conesa\(^2\), Cathie Martin\(^1\) and Jeroni Galmes\(^2\)*

\(^1\)Department of Metabolic Biology, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK, and
\(^2\)Research Group on Plant Biology under Mediterranean Conditions – INAGEA, Universitat de les Illes Balears, Carretera de Valldemossa km 7.5, 07122 Palma, Spain

Received 8 January 2018; revised 3 July 2018; accepted 10 July 2018; published online 1 August 2018.

**For correspondence (e-mail jeroni.galmes@uib.cat).**

The ratio of trichomes to stomata is associated with water use efficiency (WUE) of major crops (Antunes et al., 2013; Franks et al., 2015). In the aerial organs of Solanum lycopersicum (tomato), the epidermis is patterned with trichomes, which are epidermal outgrowths with diverse roles in the defence against biotic and abiotic stresses. The epidermis also contains stomata, which are epidermal pores that regulate gas exchange and contribute directly to the control of water status. The cuticle that covers the surface of the epidermis is a hydrophobic layer, consisting of cutin and waxes, that prevents uncontrolled water loss (Riederer and Schreiber, 2001). As a result of their function in limiting water losses, specialised structures in the epidermis are promising targets to improve the drought tolerance and water use efficiency (WUE) of major crops (Antunes et al., 2012; Galmes et al., 2013; Franks et al., 2015).

Trichomes in Solanum are multicellular and have been classified into eight different types according to the presence or absence of glandular cells, and the shape and number of cells (Luckwill, 1943; McDowell et al., 2011). Research on trichomes has traditionally focused on understanding the specialised metabolic pathways operating in glandular trichomes (Schilmiller et al., 2008; Kang et al., 2014; Spyropoulou et al., 2014); however, trichomes also play a series of important physiological roles, including tolerance to biotic and abiotic stresses, especially in terms of tolerance to insect attack (Bleeker et al., 2012; Tian et al., 2012) and drought (Hauser, 2014).

A role for trichomes in tolerance and adaptation to water stress has been reported for several species. In *Arabidopsis lyrata*, trichome production has been linked to improved performance under drought conditions (Sletvold and Agren, 2012). In tomato, SIMX1-overexpressing plants with high trichome density showed a higher tolerance to water stress compared with unmodified plants (Ewas et al., 2016). In *Citrullus lanatus* (watermelon), wild, drought-tolerant genotypes have increased trichome density compared with domesticated, drought-sensitive varieties (Mo et al., 2016). In addition, trichome formation is increased in...
plants grown under water stress, such as *Hordeum vulgare* (barley; Liu and Liu, 2016), *Solanum melongena* (aubergine; Fu et al., 2013) and *Olea europaea* (olive; Boughaleb and Hajlaoui, 2011). Trichomes may limit water loss by transpiration through an increase of the leaf–air boundary layer resistance (Palliotti et al., 1994; Guerfel et al., 2009; Mo et al., 2016). Trichomes also protect leaves from UV-related photoinhibition (Savé et al., 2000; Galmés et al., 2007a), either by reflection of UV radiation or absorption by pigmented molecules (Holmes and Keiller, 2002), and prevent leaf overheating (Ehleringer and Mooney, 1978). These findings indicate an important role for trichomes in plant–water relations.

Stomata consist of two specialised guard cells, which modulate their turgor to regulate the pore aperture in response to environmental stimuli, such as light intensity or CO₂ concentration (Hetherington and Woodward, 2003). The effect of stomatal density and size on drought tolerance has been widely studied for many species (Masle et al., 2005; Wentworth et al., 2006; Lawson and Blatt, 2014). Recent studies have shown a link between low stomatal density and improved performance under water-deficit conditions (Farber et al., 2016; Zhao et al., 2017). In addition, evidence exists that plants adjust their stomatal density under water-stress conditions (Galmés et al., 2007b; Hamanishi et al., 2012). Reductions in stomatal density in water-stressed plants have been reported in *Triticum aestivum* (wheat; Li et al., 2017) and *Spondias tuberosa* (the umbu tree; Silva et al., 2009). In contrast, for other species, stomatal density increases under drought conditions, as in *Phaseolus vulgaris* (the common bean; Gan et al., 2010) and *Leymus chiniensis* (Xu and Zhou, 2008). These contradictory observations suggest that the effect of water deficit on stomatal density differs between species, and it should be investigated on a case-by-case basis.

*Solanum pennellii* is a drought-tolerant wild tomato species that originates from the Peruvian deserts (Rick, 1973; Kahn et al., 1993; Peralta et al., 2008), with important differences at the physiological, morphological and molecular levels to the cultivated tomato, *S. lycopersicum*, including increased trichome density (Simmons and Gurr, 2005; Moyle, 2008). The *S. lycopersicum × S. pennellii* introgression line (IL) population (Eshed and Zamir, 1995; Zamir, 2001) consists of near-isogenic lines with relatively small fragments of the *S. pennellii* genome in the genetic background of the cultivated tomato, *S. lycopersicum*. This population has been used successfully before to characterise various aspects of the response of tomato to water stress (Barrios-Masias et al., 2014; Rigano et al., 2016). Therefore, the *S. lycopersicum × S. pennellii* IL population provides an excellent platform to investigate the role of differences in epidermal features on performance under water stress.

In this study, we have investigated the effect of differences in trichome density on the response to water stress using several lines from the IL population. We hypothesised that changes in trichome density, introgressed from drought-tolerant *S. pennellii*, would lead to changes in WUE, adding to the current understanding of the relationship between water stress and epidermal features, and building a foundation for improvement of tomato under drought stress. We determined that higher trichome densities result in improved WUE, especially under water-deficit conditions. We also determined the impact of water stress on the phenotype of newly developed tissues to characterise the epidermal responses to drought stress in tomato.

**RESULTS**

**Phenotypic characterisation of tomato lines under glasshouse and field conditions before drought treatment**

After a preliminary visual inspection of the 76 lines of the IL population, we chose three ILs (IL 4-1, 10-2 and 11-3) and the parental line M82, for their distinct trichome phenotypes, for further analysis.

We characterised the epidermal features of the three selected ILs (4-1, 10-2 and 11-3) and the parental line M82 in plants grown under full field capacity conditions both in the glasshouse and in the field before the onset of water-deficit conditions (Figure 1). Under glasshouse conditions, ILs 4-1 and 10-2 showed a low trichome density (TD) phenotype, whereas M82 and IL 11-3 showed a high-TD phenotype. Although field-grown plants displayed substantially higher TD than glasshouse-grown plants, IL 4-1 had a lower TD than IL 11-3 and M82, and IL 11-3 had a higher TD than ILs 10-2 and 4-1 (Figure 1a). We also observed differences among lines in their stomatal densities (SD). Under glasshouse conditions, IL 4-1 had a higher SD than M82 and IL 10-2, and M82 had a lower SD than ILs 11-3 and 4-1. In field-grown plants, no significant differences were observed between lines for SD (Figure 1b). Similar to the observations for TD, SD values were generally higher under field conditions. When TD and SD were calculated in terms of area units, we observed identical differences between lines for glasshouse-grown plants, and similar relative values for field-grown plants (Figure S1). We calculated the ratio of trichomes to stomata (T/S) as an integrative parameter for epidermal anatomy. We observed a substantially higher T/S in M82 compared with the other lines under glasshouse conditions, whereas in field plants, IL 4-1 showed a lower T/S than M82 and IL 11-3 (Figure 1c). Stomatal size (SS) showed no significant differences between lines under glasshouse conditions. Under field conditions, however, IL 10-2 had a higher SS than the other lines under study. Unlike the higher values observed for TD and SD in field-grown plants compared with
The Plant Journal, Volume 00, Number 00, 0000, Pages 000–000

Trichome/stomata ratio determines WUE in tomato

Comparative analysis of morphological parameters and water status under WW and WD conditions in the field

We evaluated the effects of differences in the densities of trichomes on water status of plants under WW and WD conditions in the field. We observed a lower midday leaf water potential ($\Psi_{leaf}$) in IL 10-2 compared with M82 and IL 11-3 under WW conditions (Table 1). Under WD conditions, IL 11-3 had higher $\Psi_{leaf}$ than IL 10-2. All lines showed a lower $\Psi_{leaf}$ under WD conditions except for IL 11-3, where no difference was observed. The leaf mass area (LMA) was lower in ILs 4-1 and 11-3 under WD conditions compared with the other lines, but no differences amongst lines under WW conditions or between WW and WD treatments were observed (Table 1). Leaf thickness (LT) was higher in IL 11-3 compared with IL 4-1 under WW conditions, but under WD conditions LT in IL 11-3 was significantly lower than in IL 10-2 (Table 1).

Whole-plant water use efficiency (WUE$_p$) showed no differences between lines under WW conditions (Table 1). Under WD conditions, WUE$_p$ was lower in IL 4-1 compared with IL 11-3. In M82 and IL 11-3, WUE$_b$ was higher in WD plants compared with WW plants.

Comparative analysis of photosynthetic parameters under WW and WD conditions in the field

The parameters $A_{Ni}$, $C_{24h}$, $g_v$, $g_{mi}$, $g_{rot}$, $V_{cmax}$ and WUE$_i$ showed no significant differences between lines under WW conditions in the field (Table 2). In contrast, under WD conditions, IL 4-1 had a significantly lower WUE$_i$ than the other lines. IL 11-3 had a significantly higher WUE$_i$ in WD compared with WW conditions (Table 2).

The leaf carbon isotope composition ($\delta^{13}C$) showed no significant differences between lines within each treatment or between treatments for any line; however, leaf $\delta^{13}C$ was correlated with WUE$_b$ ($R^2 = 0.67$, $P = 0.01$; Figure S5a). We also observed a tight positive correlation ($R^2 = 0.68$, $P = 0.01$) between WUE$_i$ and WUE$_b$ (Figure S5b).

Relationships between epidermal features and photosynthetic parameters

We found an inverse correlation between TD and $g_s$ ($R^2 = 0.58$, $P = 0.03$; Figure 3a), and we also observed a positive correlation between SD per unit area and $g_s$ ($R^2 = 0.56$, $P = 0.03$; Figure 3b). Therefore, changes in the density of trichomes and stomata had opposite effects at the gas-exchange level. Importantly, TD was positively correlated with WUE$_i$ ($R^2 = 0.88$, $P = 0.00$; Figure 3c). SD showed no correlation with WUE$_i$. As expected from the observed relationship between WUE$_i$ and WUE$_b$ (Figure S5b), TD was positively correlated with WUE$_b$ ($R^2 = 0.59$, $P = 0.03$; Figure S6a). Interestingly, SD was negatively correlated with WUE$_b$ ($R^2 = 0.50$, $P < 0.05$; Figure S6b). The correlation between TD and WUE$_i$ and WUE$_b$ was not significant, but we observed an inverse association between TD and SD in WD plants ($R^2 = 0.94$; $P = 0.03$; Figure 2d).

© 2018 The Authors, The Plant Journal published by John Wiley & Sons Ltd and Society for Experimental Biology, The Plant Journal, (2018), 96, 607–619
Figure 1. Initial morphological characterization of lines M82, 4-1, 10-2 and 11-3 grown under glasshouse (GH) and field (F) conditions before the onset of drought treatment. (a) Trichome density (TD), (b) stomatal density (SD), (c) trichome-to-stomata ratio (T/S) and (d) stomatal size (SS) are expressed as mean ± SE of between three and six replicates per line and treatment. TD and SD were calculated as a percentage of the total number of epidermal cells. SS was calculated as pore length. Different letters denote statistically significant differences by Tukey’s analysis (P < 0.05) within glasshouse-grown plants (lower case) and field-grown plants (upper case). For panels (a)–(d), purple bars represent M82, turquoise represents 4-1, red represents 10-2 and green represents 11-3, with lighter and darker shades representing GH and F conditions, respectively. Representative scanning electron micrographs for each line: (e) M82, (f) 4-1, (g) 10-2 and (h) 11-3.
was maintained when TD was expressed per unit area (Figure S7), but this was not the case for the correlation between SD and WUEb, which was only observed when SD was expressed as a percentage of epidermal cells. Finally, a strong positive association was found between T/S and WUEi ($R^2 = 0.86$, $P = 0.00$; Figure 4a) and WUEb ($R^2 = 0.72$, $P = 0.01$; Figure 4b).

**DISCUSSION**

**The development of leaf trichomes is influenced by water availability**

We observed a 10- to 15-fold higher TD in leaves grown under field conditions compared with glasshouse-grown plants (Figures 1a and S1). These differences were likely to have resulted from differences in the age of the plant as well as changes in environmental conditions between the glasshouse and the field. Trichome development is reported to change with the age of the plant (Telfer et al., 1997; Vendemiatti et al., 2017), with higher TD observed in the late leaves of tomato (Gurr and McGrath, 2001).

Moreover, environmental factors, such as temperature, photoperiod, light intensity or soil humidity have direct effects on trichome development in several species, including tomato (Wellso and Hoxie, 1982; Gianfagna et al., 1992; Chien and Sussex, 1996; Souza et al., 2016). Despite the dramatic change between environmental conditions in the glasshouse and the field, the relative differences in TD were conserved between lines (Figure 1a), indicating strong genetic control of trichome development in the selected ILs. We observed a 1.5- to 5.0-fold higher SD in field-grown plants (Figure 1b). These differences could also be a function of the age of the plant when leaves were sampled (Ceulemans et al., 1995) as well as the environmental conditions (Beerling and Chaloner, 1993; Rogiers et al., 2011). Unlike the observation for TD, relative differences in SD between lines were not conserved in different environments, pointing to the differential regulation of TD and SD.

We assessed the changes in leaf anatomy in WD plants compared with WW plants. We observed a higher TD in ILs 4-1 and 11-3 under WD conditions compared with WW plants.
Table 1 Morphological and water status characterisation of lines M82, 4-1, 10-2 and 11-3 under well-watered (WW) and water deficit (WD) conditions in the field. Midday leaf water potential ($\Psi_{\text{leaf}}$), leaf mass per area (LMA), leaf thickness (LT) and plant-level water use efficiency (WUE$_{\text{p}}$) are shown. Values are means ± SE of three to four replicates per line and treatment. Different letters denote statistically significant differences by Tukey analysis ($P < 0.05$) within each treatment between lines, and asterisks denote statistically significant differences ($P < 0.05$) between treatments for each line.

| Acc. | $\Psi_{\text{leaf}}$ MPa | LMA g m$^{-2}$ | LT mm | WUE$_{\text{p}}$ g kg$^{-1}$ H$_2$O | WW | WD |
|------|-----------------|--------------|-------|-------------------------------|----|----|
| M-82 | -0.74 ± 0.04$^b$ | 79.45 ± 9.56$^a$ | 0.70 ± 0.05$^b$ | 0.76 ± 0.12$^a$ | -1.01 ± 0.07$^{ab}$ | 96.01 ± 3.41$^b$ | 0.76 ± 0.04$^{ab}$ | 1.14 ± 0.09$^{ab}$ |
| 4-1  | -0.86 ± 0.06$^{ab}$ | 58.12 ± 4.33$^a$ | 0.63 ± 0.03$^b$ | 0.74 ± 0.04$^a$ | -1.18 ± 0.05$^{ab}$ | 63.83 ± 6.68$^b$ | 0.66 ± 0.06$^{ab}$ | 0.79 ± 0.02$^c$ |
| 10-2 | -0.99 ± 0.04$^b$ | 82.37 ± 6.58$^a$ | 0.69 ± 0.01$^b$ | 0.89 ± 0.1$^a$ | -1.21 ± 0.07$^{ab}$ | 98.65 ± 5.72$^a$ | 0.81 ± 0.02$^{ab}$ | 1.12 ± 0.17$^{ab}$ |
| 11-3 | -0.74 ± 0.07$^b$ | 71.20 ± 4.45$^a$ | 0.80 ± 0.03$^a$ | 0.85 ± 0.05$^e$ | -0.93 ± 0.08$^a$ | 64.18 ± 6.35$^c$ | 0.63 ± 0.03$^{ab}$ | 1.36 ± 0.06$^{ab}$ |

Table 2 Photosynthetic characterization of lines M82, 4-1, 10-2 and 11-3 under well-watered (WW) and water deficit (WD) conditions in the field. Net photosynthetic rate ($A_N$), daily carbon fixation rate ($C_{\text{fix}}$), stomatal conductance ($g_s$), mesophyll conductance ($g_m$), total leaf conductance ($g_l$), maximum velocity of Rubisco carboxylation ($V_{\text{max}}$) and intrinsic water use efficiency (WUE$_{\text{i}}$) are shown. Values are means ± standard error of four replicates per line and treatment. Different letters denote statistically significant differences by Tukey analysis ($P < 0.05$) within each treatment between lines, and asterisks denote statistically significant differences ($P < 0.05$) between treatments for each line.

| Acc. | $A_N$ $\mu$mol CO$_2$ m$^{-2}$ sec$^{-1}$ | $C_{\text{fix}}$ $\mu$mol H$_2$O m$^{-2}$ day$^{-1}$ | $g_s$ $\mu$mol H$_2$O m$^{-2}$ sec$^{-1}$ | $g_m$ $\mu$mol CO$_2$ m$^{-2}$ sec$^{-1}$ | $g_l$ $\mu$mol CO$_2$ m$^{-2}$ sec$^{-1}$ | $V_{\text{max}}$ $\mu$mol CO$_2$ m$^{-2}$ sec$^{-1}$ | WUE$_{\text{i}}$ $\mu$mol CO$_2$ mol$^{-1}$ H$_2$O |
|------|-----------------|--------------|-------|-------------------------------|----|----|----------|---------|
| WW   |                 |               |       |                               |    |    |          |         |
| M-82 | 13.22 ± 1.16$^a$ | 0.34 ± 0.03$^a$ | 0.222 ± 0.046$^a$ | 0.087 ± 0.015$^a$ | 0.049 ± 0.006$^a$ | 304.05 ± 57.19$^a$ | 66.22 ± 11.52$^a$ |
| 4-1  | 15.31 ± 1.35$^a$ | 0.34 ± 0.06$^b$ | 0.272 ± 0.027$^a$ | 0.088 ± 0.004$^a$ | 0.056 ± 0.003$^a$ | 349.38 ± 107.21$^a$ | 57.09 ± 4.68$^a$ |
| 10-2 | 16.87 ± 1.26$^a$ | 0.42 ± 0.04$^a$ | 0.229 ± 0.026$^a$ | 0.123 ± 0.015$^a$ | 0.063 ± 0.004$^a$ | 335.96 ± 49.79$^a$ | 75.88 ± 7.33$^a$ |
| 11-3 | 15.82 ± 1.43$^a$ | 0.44 ± 0.05$^a$ | 0.243 ± 0.026$^a$ | 0.102 ± 0.017$^a$ | 0.061 ± 0.010$^a$ | 336.77 ± 38.46$^a$ | 65.60 ± 2.11$^a$ |
| WD   |                 |               |       |                               |    |    |          |         |
| M-82 | 11.52 ± 0.80$^a$ | 0.31 ± 0.04$^a$ | 0.165 ± 0.021$^a$ | 0.084 ± 0.010$^a$ | 0.046 ± 0.005$^a$ | 271.97 ± 22.89$^a$ | 71.44 ± 4.26$^{ab}$ |
| 4-1  | 12.53 ± 0.80$^a$ | 0.30 ± 0.03$^a$ | 0.233 ± 0.009$^a$ | 0.083 ± 0.016$^a$ | 0.052 ± 0.006$^a$ | 217.25 ± 26.34$^a$ | 53.74 ± 2.76$^a$ |
| 10-2 | 13.20 ± 1.59$^a$ | 0.38 ± 0.03$^a$ | 0.184 ± 0.014$^a$ | 0.101 ± 0.018$^a$ | 0.052 ± 0.006$^a$ | 253.63 ± 49.58$^a$ | 71.60 ± 5.29$^{ab}$ |
| 11-3 | 15.57 ± 2.38$^a$ | 0.39 ± 0.03$^a$ | 0.187 ± 0.034$^a$ | 0.143 ± 0.044$^a$ | 0.061 ± 0.013$^a$ | 321.88 ± 52.84$^a$ | 84.52 ± 6.92$^{ab}$ |
conditions (Figure 2a). Increases in TD with herbivore and water stress have been reported previously in several species (Traw and Bergelson, 2003; Bjorkman et al., 2008; Fu et al., 2013), as part of the adaptive stress response. In fact, transcriptomic studies in water-stressed Arabidopsis thaliana plants showed an upregulation of genes related to trichome initiation and morphogenesis (TT8, BRICK1, KAK), but not of genes involved in stomatal initiation (Bechtold et al., 2016). Not all the lines in this study showed uniform responses, with IL 4-1 showing bigger changes upon WD treatment (lower SS, higher TD) (Figure 2). This could be explained by a greater inability of IL 4-1 to control water loss, as indicated by its lower WUEb and WUEi (Tables 1 and 2), leading to more severe physiological stress in this line and, subsequently, a stronger response to WD. In any case, these leaf adaptive changes did not account for an increase in WUE (Tables 1 and 2), probably because of a lower overall TD in IL 4-1.

We observed an inverse association between trichome density and stomatal density under WD conditions (Figure 2d), in agreement with previous reports in Nicotiana tabacum (tobacco) and tomato (Glover et al., 1998; Glover, 2000). Developmentally, trichomes and stomata originate from protodermal cells (Morohashi and Grotewold, 2009; Pillitteri and Dong, 2013), and the inverse association observed suggests that the regulation of their development might be linked. Similar relationships have been found in trichome mutants of A. thaliana (Bean et al., 2002), where altered trichome phenotypes affected
stomatal patterning. In aubergine, increases in TD have been associated with increases in SD (Fu et al., 2013), in contrast to our observations, indicating that there may be different developmental associations even between related Solanum species. The correlation was not observed when TD and SD per area were used, suggesting that TD and SD as percentage of epidermal cells might give a better representation of the developmental changes in the epidermis. The fact that TD and SD were not correlated when both WW and WD plants were considered (Figure 2d) might be a result of the lack of genetic differences in SD, as only TD was clearly different between the assessed lines (Figures 1b and S1b). However, the observed TD–SD association suggests that the developmental response of the leaf to drought stress involves changes in the determination of cell fate in the whole epidermal tissue, simultaneously affecting TD and SD, and this might occur through different regulatory mechanisms under different water regimes. In conclusion, we observed an important effect of water availability on leaf anatomy and the determination of epidermal features.

**Variation amongst the ILs and the potential for developing drought-tolerant varieties**

The highly inbred tomato cultivar M82 has traditionally been used as a check variety in breeding programmes (Grandillo et al., 1999) and as a reference cultivar for scientific research, used in relation to the response to water stress as a drought-sensitive cultivar (Iovieno et al., 2016), in contrast with the drought-tolerant S. pennellii (Egea et al., 2018). The IL population has been used extensively for functional and physiological studies (Steinhauser et al., 2011; Chitwood et al., 2013; de Oliveira Silva et al., 2018), and the natural variation within the IL population provides an excellent platform to investigate the role of differences in epidermal features on performance under water stress.

The intrinsic water use efficiency (WUE<sub>i</sub>) is an important target for crop improvement with respect to drought tolerance, although it needs to be considered carefully as it might not be directly related to improved fruit productivity (Blum, 2005, 2009). We observed a lower WUE<sub>i</sub> for IL 4-1 under WD conditions compared with the other lines under study (Table 2), whereas WUE<sub>i</sub> in IL 11-3 was higher under WD compared with WW conditions (Table 2). This increase in WUE<sub>i</sub> has been reported in drought-tolerant varieties in several crops (Guha et al., 2010; Fracasso et al., 2016; Liu et al., 2016), although it might not always have a positive effect on fruit yield. Interestingly, none of the ILs under study were considered before for WUE improvement as a result of the lack of genetic differences in SD, as only TD was clearly different between the assessed lines (Figures 1b and S1b). However, the observed TD–SD association suggests that the developmental response of the leaf to drought stress involves changes in the determination of cell fate in the whole epidermal tissue, simultaneously affecting TD and SD, and this might occur through different regulatory mechanisms under different water regimes. In conclusion, we observed an important effect of water availability on leaf anatomy and the determination of epidermal features.

The highly inbred tomato cultivar M82 has traditionally been used as a check variety in breeding programmes (Grandillo et al., 1999) and as a reference cultivar for scientific research, used in relation to the response to water stress as a drought-sensitive cultivar (Iovieno et al., 2016), in contrast with the drought-tolerant S. pennellii (Egea et al., 2018). The IL population has been used extensively for functional and physiological studies (Steinhauser et al., 2011; Chitwood et al., 2013; de Oliveira Silva et al., 2018), and the natural variation within the IL population provides an excellent platform to investigate the role of differences in epidermal features on performance under water stress.

The intrinsic water use efficiency (WUE<sub>i</sub>) is an important target for crop improvement with respect to drought tolerance, although it needs to be considered carefully as it might not be directly related to improved fruit productivity (Blum, 2005, 2009). We observed a lower WUE<sub>i</sub> for IL 4-1 under WD conditions compared with the other lines under study (Table 2), whereas WUE<sub>i</sub> in IL 11-3 was higher under WD compared with WW conditions (Table 2). This increase in WUE<sub>i</sub> has been reported in drought-tolerant varieties in several crops (Guha et al., 2010; Fracasso et al., 2016; Liu et al., 2016), although it might not always have a positive effect on fruit yield. Interestingly, none of the ILs under study were considered before for WUE improvement as a result of the lack of genetic differences in SD, as only TD was clearly different between the assessed lines (Figures 1b and S1b). However, the observed TD–SD association suggests that the developmental response of the leaf to drought stress involves changes in the determination of cell fate in the whole epidermal tissue, simultaneously affecting TD and SD, and this might occur through different regulatory mechanisms under different water regimes. In conclusion, we observed an important effect of water availability on leaf anatomy and the determination of epidermal features.

The highly inbred tomato cultivar M82 has traditionally been used as a check variety in breeding programmes (Grandillo et al., 1999) and as a reference cultivar for scientific research, used in relation to the response to water stress as a drought-sensitive cultivar (Iovieno et al., 2016), in contrast with the drought-tolerant S. pennellii (Egea et al., 2018). The IL population has been used extensively for functional and physiological studies (Steinhauser et al., 2011; Chitwood et al., 2013; de Oliveira Silva et al., 2018), and the natural variation within the IL population provides an excellent platform to investigate the role of differences in epidermal features on performance under water stress.

The intrinsic water use efficiency (WUE<sub>i</sub>) is an important target for crop improvement with respect to drought tolerance, although it needs to be considered carefully as it might not be directly related to improved fruit productivity (Blum, 2005, 2009). We observed a lower WUE<sub>i</sub> for IL 4-1 under WD conditions compared with the other lines under study (Table 2), whereas WUE<sub>i</sub> in IL 11-3 was higher under WD compared with WW conditions (Table 2). This increase in WUE<sub>i</sub> has been reported in drought-tolerant varieties in several crops (Guha et al., 2010; Fracasso et al., 2016; Liu et al., 2016), although it might not always have a positive effect on fruit yield. Interestingly, none of the ILs under study were considered before for WUE improvement as they showed no differences in δ<sup>13</sup>C compared with M82 (Xu et al., 2008), in agreement with our results, so this epidermis-based analysis provides an alternative path for increased WUE. Therefore, the genomic region
have been suggested for trichomes in plant drought tolerance (Galmès et al., 2007a; Bougheleb and Hajlaoui, 2011). On the basis that TD is negatively associated with $g_s$ (Figure 3a), our data suggest that trichomes in tomato might play a role in avoiding excessive water loss by changing the resistance of the boundary layer, as proposed in previous studies (Guerfel et al., 2009; Mo et al., 2016). Another possible explanation for the correlation between TD and WUE involves the mutually exclusive developmental association between trichomes and stomata. Increased trichome initiation as part of the response to drought (a possibility supported by expression analysis in Arabidopsis; Bechtold et al., 2016), could lead to lower SD that could lead to an improved WUE. Genes involved inABA signalling, known to play a role in the drought response, are expressed in trichomes (Ren et al., 2010; Daszkowska-Golec, 2016). For example, the tomato homologue of the WRKY transcription factor ABA OVERSENSITIVE 3 (AtABO3) (Ren et al., 2010) or the bZIP transcription factor ABRE BINDING FACTOR 1 (AtABF1) (Yoshida et al., 2015) are both expressed in trichomes in tomato according to the available RNAseq data (Spyropoulou et al., 2014). Changes in TD could lead to changes in the transcript abundance of these or other ABA-related factors. In fact, the expression level of SIABO3 is slightly higher in leaves of IL 11-3 compared with leaves of ILs 4-1 and 10-2 according to the available RNAseq data (Chitwood et al., 2013), and SIABF1 is located in the genomic region introgressed from S. pennelli in IL 11-3, indicating a possible role for trichome-expressed ABA-related genes in the observed drought response.

Whole-plant water use efficiency ($WUE_b$) was also correlated with SD (Figure S6b), although the impact of SD on WUE was lower than that of TD, because there was no correlation between WUE and SD at the leaf level, and when correlation coefficients for TD-$WUE_b$ and SD-$WUE_b$ were compared, the effect of SD on $WUE_b$ was lower than that of TD (Figures 3 and S6). The fact that both stomata and trichomes were involved in the drought response was not surprising, given the developmental link that we observed under WD conditions (Figure 2d) and the direct role of stomata in gas exchange (Figure 3b). In fact, the ratio of trichomes to stomata ($T/S$), which gives information about the relationship between both structures, showed a strong correlation with both WUE, and $WUE_b$ (Figure 4). In addition to this, $T/S$ is unitless and is not expressed either in terms of leaf area or percentage of epidermal cells, thereby overcoming the differences observed in correlations between developmental and photosynthetic traits. It is interesting to note that $T/S$ becomes a more prominent parameter under WD conditions, when both TD and SD change together (Figure 2d), whereas under WW conditions, when there is no correlation between them (Figure 2d) or significant changes between lines (Figure S4), the genotype-specific TD is likely to be the main player in the relationship between epidermis and WUE. In conclusion, $T/S$ plays an important role in the efficiency by which water is used by tomato, and differences in $T/S$ could be used to develop more drought-tolerant tomato varieties.

**Experimental Procedures**

**Preliminary characterization of epidermal cells**

From a visual inspection of 76 S. lycopersicum cv. M82 × S. pennelli ac. LA716 ILs (Eshed and Zamir, 1995), grown under glasshouse conditions, we selected four lines (ILs 4-1, 10-2 and 11-3, and M82) displaying a clear visual trichrome phenotype. IL 4-1 (LA4048) had an apparent lower trichome density (TD) than the parental line M82, IL 10-2 (LA4089) had a hairless-like phenotype, and IL 11-3 (LA4092) had an apparently higher TD compared with the parental line M82 (LA3475). We characterised the trichome densities of the adaxial and abaxial sides of leaves from the four lines under study. This analysis indicated similar values for TD on both sides of the leaf in all four lines and a strong correlation ($R^2 = 0.92, P = 0.04$) between the values on both sides of the leaf (Figure S8), which allowed us to work with values on the adaxial surface in all future investigations.

The epidermal features of these four lines were characterised further using scanning electron microscopy (SEM) of plants grown both under glasshouse and field conditions. For glasshouse assays, three or four plants per line were grown under water field capacity at the John Innes Centre (https://www.jic.ac.uk), using natural light with an average temperature of between 20 and 22°C. The terminal leaflet of the first leaf of 4-week-old plants was excised, and inter-vein sections of approximately 0.5 × 0.5 cm were taken as samples. These sections were vacuum-fixed in a glutaraldehyde 2.5% cacodylate solution and dehydrated through an ethanol series. Samples were dried in a Leica CPD300 critical-point dryer (Leica Microsystems, http://www.leica.com), to avoid the collapse of trichomes, and were gold-coated before imaging in a Zeiss Supra 55 VP SEM (Zeiss, https://www.zeiss.com) at 20 kV. The terminal leaflet of the first leaf of 4-week-old plants was excised, and inter-vein sections of approximately 0.5 × 0.5 cm were taken as samples. These sections were vacuum-fixed in a glutaraldehyde 2.5% cacodylate solution and dehydrated through an ethanol series. Samples were dried in a Leica CPD300 critical-point dryer (Leica Microsystems, http://www.leica.com), to avoid the collapse of trichomes, and were gold-coated before imaging in a Zeiss Supra 55 VP SEM (Zeiss, https://www.zeiss.com) at 20 kV and under high-vacuum conditions, generating between eight and 15 micrographs of approximately 0.3 mm² per sample of the adaxial surface.

Characterisation under field conditions was carried out in the experimental plot at the University of the Balearic Islands (UIB, http://www.uib.eu). The environmental conditions during plant growth are detailed in the next section. Six plants were sampled for each line under study. Terminal leaflets of fully expanded leaves at the same position were excised and sections of 0.5 × 0.5 cm were taken as samples. The adaxial surface of these sections was imaged at 20 kV, without coating, inside a Hitachi 3400N variable pressure SEM (Hitachi High-Technology, https://www.hitachi-hightech.com). We generated between eight and 10 micrographs of approximately 0.3 mm² per sample, and trichomes, stomata and pavement cells were counted manually. In both analyses, trichome and stomatal densities were expressed both as a percentage of epidermal cells and as density per area. Trichomes were classified in types and had their length measured. For trichome density, all types of trichome were considered together. The ratio of trichomes to stomata was calculated as trichome density divided by stomatal density.

**Field growth conditions and drought treatment**

Seeds from the four lines were germinated and grown for 4 weeks in glasshouses at the University of the Balearic Islands (UIB) with natural light and average maximum temperatures of 25°C in
March–April 2016. Twelve plants per line were placed outdoors in the UIB experimental field for acclimation for 2 weeks before being transferred to 50-L pots containing a mixture of bog peat-based horticultural substrate (Prohumin-Potting Soil Klaasmann-Deilmann; Projar, https://www.projar.es) and perlite (granulometry A13; Projar) in a 4 : 1 proportion (v/v). The environmental conditions from June to September 2016 were those typical for a Mediterranean summer: with average daily temperatures of 22.8 ± 0.3, 25.7 ± 0.3, 25.0 ± 0.2 and 22.7 ± 0.4°C; average daily minimum temperatures of 16.5 ± 0.5, 20.3 ± 0.3, 19.5 ± 0.4 and 17.8 ± 0.4°C; and average daily maximum temperatures of 31.1 ± 0.4, 35.5 ± 0.4, 33.5 ± 0.3 and 34.7 ± 0.6°C, respectively, for June, July, August and September. Plants were watered to field capacity every other day and fertilised weekly with 50% Hoagland’s solution for 2 months before beginning the drought treatment.

From 11 July, plants were subjected to two different water regimes: WW and WD. Watering regimes and plant water consumption were monitored by weighing and watering the potted plants every 2 days. For the WW treatment, four plants per line were maintained at field capacity, with a pot water content ranging between 100% field capacity just after irrigation (corresponding to 9.3 L of water per pot) and 69.3 ± 0.1% field capacity (on average throughout the treatment period) (Figure S9). For the WD treatment, the irrigation of four plants was progressively reduced until halving the pot water content compared with the WW plants. Then, WD plants were maintained at a pot water content ranging between 0.2 and 0.1% field capacity before irrigation and 46.3 ± 0.1% field capacity after irrigation (Figure S9). The four remaining plants per line were used for biomass-related measurements. The total water supplied and dry biomass of each plant was recorded upon experiment completion (Table S2). Three weeks were allowed from treatment application for the development of new leaves under the new water regime before any measurement was performed.

All leaf-based measurements and samples were taken from the terminal leaflets of the youngest fully expanded leaves generated after application of the treatment. Epidermal features (trichome and stomatal densities, stomatal size and ratio of trichomes to stomata) were evaluated as described for glasshouse-grown plants (‘Preliminary characterization of plant material’ section).

**Plant water status and growth-related measurements**

Plant water status was measured as midday leaf water potential (Ψleaf) (n = 4 per line and treatment) using a Scholander pressure chamber (Soilmoisture Equipment Corp., https://www.soilmoisture.com), as described by Turner (1988).

For the calculation of WUEb, four plants per line were harvested at the time of drought treatment. Seventy-four days after treatment application, four plants per line and per treatment (WW and WD) were harvested. Leaves, shoots and roots were oven-dried separately at 60°C and weighed (dry weight, DW). Biomass production during the drought treatment was calculated as the difference between the DW of the plants harvested at the end of the experiment (DWfinal) and the DW of the plants harvested before the treatment (DWinitial). Water consumption was monitored every other day by weighing the pots containing the plants, and the total water consumption (TWC) of each plant was estimated from these values. WUEb was calculated as follows: WUEb = (DWfinal − DWinitial)/TWC.

**Leaf morphological determinations**

Leaf thickness (LT) was determined for the middle part of the terminal leaflet of a young fully-expanded leaf with callipers, avoiding regions of the leaf with major veins. Leaf mass area (LMA) was calculated from the same terminal leaflet, as the dry mass to leaf area ratio. Dry mass was measured by weighing after oven-drying leaves at 60°C for 48 h. The leaf area was digitally measured from pictures of the leaves using IMAGEJ 1.49 (National Institutes of Health, https://imagej.nih.gov/ij/) before drying. Both LT and LMA were measured for one leaf per plant (n = 4 per line and treatment).

**Leaf gas exchange and chlorophyll a fluorescence**

Measurements were performed with an open infrared gas-exchange analyser equipped with a leaf chamber fluorometer (LI-6400-40; LI-COR, https://www.licor.com) from 09:00 to 12:00 h and from 16:00 to 19:00 h in the first 2 weeks of August 2016. Preliminary tests confirmed non-significant differences between morning and afternoon measurements. The conditions in the chamber consisted of leaf temperatures of 31-33°C, a vapour pressure deficit of 2.0-3.0 kPa and an air flow of 500 μmol (air) min⁻¹. For net CO₂ assimilation rate-sub stomatal CO₂ concentration (AN-Ci) curves, the ambient concentration of CO₂ in the chamber (Ci) was set at 400, 0, 50, 100, 200, 300, 600, 900, 1500, 2000 and 4000 μmol CO₂ mol⁻¹ air, at a saturating photosynthetic photon flux density (PPFD) of 1500 μmol m⁻² sec⁻¹ (with 10% blue light), allowing 4 min between measurements for the chamber to reach a steady state. Corrections for CO₂ leakage in and out of the leaf chamber of the Li-6400-40 were applied to all gas-exchange data, as described by Flexas et al. (2007). Mesophyll conductance to CO₂ (gₘ) was estimated according to (Harley et al., 1992) as:

\[ g_m = AN/C_i = \left(1^{\ast}ETR + 8(AN + RL_i)/ETR - 4(AN + RL_i) \right), \]

where \(1^\ast\) is the chloroplast CO₂ compensation point in the absence of day respiration, ETR is the electron transport rate and RL is the rate of non-photorespiratory CO₂ evolution under light. ETR and RL were calculated as described by Galmes et al. (2011). \(1^\ast\) was retrieved from in vitro-based measurements for S. lycopersicum by Hermida-Carrera et al. (2016), but adjusted for the leaf temperature during the measurement.

Total leaf conductance (gₜot) was calculated assuming the stomatal conductance (gₛ) and mesophyll conductance (gₘ) were in series, such that: gₜot = 1/(1/gₘ + 1/gₛ).

AN-Ci curves were transformed into AN-chloroplastic CO₂ concentration (Cₐ) curves using estimated values of gₘ. From AN-Cₐ curves, the maximum velocity of Rubisco carboxylation (Vₗ₉₉₉₉) was calculated as described by Bernacchi et al., 2002, but using specific values of Rubisco kinetics for S. lycopersicum adjusted to the leaf temperature during the measurements (Hermida-Carrera et al., 2016). The intrinsic water use efficiency (WUE) was calculated as the ratio between the net photosynthetic rate (AN) and the stomatal conductance (gₛ).

We determined the daily carbon fixation rate (C₀) by measuring the net CO₂ exchange rate at 2-h intervals over 24 h. These measurements were performed after drought treatment (n = 4 per line and per treatment) using an open infrared gas-exchange analyser equipped with a clear chamber (Li-6400-40; LI-COR). Three measurements were performed under ambient CO₂ and light levels, averaged per plant and per time point. The daily fixation rate was calculated as the integral value for the curve generated by the point measurements.

**Leaf δ¹³C isotope composition**

The dried leaves used to calculate LMA were ground to fine dust for the determination of carbon isotopic composition. Samples...
were subjected to combustion in an elemental analyser (Thermo Flash EA 1112 Series; ThermoFisher Scientific, http://www.thermoisher.com) and CO₂ was injected into a continuous-flow isotope ratio mass spectrometer (Thermo-Finnigan Delta XP; ThermoFisher Scientific). Peach leaf standards (NIST 1547) were run every six samples. The standard deviation of the analysis was <0.2%.

Statistical analysis

The differences between lines, treatments and interactions were assessed by univariate analysis of variance (ANOVA). Significant differences between means were determined by a post-hoc Tukey’s test (P < 0.05). The relationship between variables in each experiment was determined by correlation coefficient (R²). The analyses were performed using r 3.2.2 (R Core Team, https://www.r-project.org).

ACKNOWLEDGEMENTS

We thank Kim Findlay, Elaine Barclay and Ferran Hierro for the technical support in SEM image acquisition. We are grateful to Cyril Douthie for LI-COR technical assistance. This article benefited from insightful discussions with Prof. James Brown (JIC). We appreciate the financial support of the Rotation Studentship from the John Innes Foundation, the Institute Strategic Program Understanding and Exploiting Plant and Microbial Secondary Metabolism (BB/J004596/1) from the UK Biotechnology and Biological Sciences Research Council (BBSRC) to JIC, as well as the project AGL2013–42364R (Plan Nacional, Spain) awarded to Dr Galmés and the European funded COST ACTION FA1106 QualityFruit. We appreciate the financial support of the Rotation Studentship from the John Innes Foundation, the Institute Strategic Program Understanding and Exploiting Plant and Microbial Secondary Metabolism (BB/J004596/1) from the UK Biotechnology and Biological Sciences Research Council (BBSRC) to JIC, as well as the project AGL2013–42364R (Plan Nacional, Spain) awarded to Dr Galmés and the European funded COST ACTION FA1106 QualityFruit. We are grateful to Mr Miquel Truyols and collaborators of the UIB Experimental Field and Glasshouses (UIB Grant 15/2015) for their support of our experiments.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Figure S1. Initial morphological characterization of lines M82, 4-1, 10-2 and 11-3 under field conditions (F) before the onset of drought treatment.

Figure S2. Percentage of trichome types and trichome length in plants grown under glasshouse conditions.

Figure S3. Stomatal density in lines M82, 4-1, 10-2 and 11-3 under water-deficit (WD) and well-watered (WW) conditions in the field.

Figure S4. Trichome and stomatal densities in lines M82, 4-1, 10-2 and 11-3 under water-deficit (WD) and well-watered (WW) conditions in the field, expressed in terms of area.

Figure S5. Correlations between carbon isotope composition and intrinsic water use efficiency, and between intrinsic water use efficiency and plant-level water use efficiency, in lines M82, 4-1, 10-2 and 11-3.

Figure S6. Relationship between epidermal features and plant-level water use efficiency (WUE) in plants under well-watered (WW) and water-deficit (WD) conditions in the field.

Figure S7. Correlations between trichome density expressed per unit area and water use in lines M82, 4-1, 10-2 and 11-3 under WW and WD conditions.

Figure S8. Trichome densities on abaxial and adaxial sides of leaves of lines M82, 4-1, 10-2 and 11-3 grown under glasshouse conditions.

Figure S9. Evolution of the pot water content during the experiment for the well-watered (WW, blue) and water-deficit (WD, red) plants.

Table S1. Leaf morphological traits and photosynthetic characterization of the lines M82, 4-1, 10-2 and 11-3 under field conditions before the onset of the drought treatment.

Table S2. Dry biomass and total water supplied to plants upon completion of the experiment for lines M82, 4-1, 10-2 and 11-3.

REFERENCES

Antunes, W.C., Provart, N.J., Williams, T.C.R. and Loureiro, M.E. (2012) Changes in stomatal function and water use efficiency in potato plants with altered sucrolytic activity. Plant Cell Environ. 35, 747–759.

Barrios-Masias, F.H. and Jackson, L.E. (2014) California processing tomatoes: morphological, physiological and phenological traits associated with crop improvement during the last 80 years. Eur. J. Agron. 53, 45–55.

Barrios-Masias, F.H., Chetelat, R.T., Gruikel, N.E. and Jackson, L.E. (2014) Use of introgression lines to determine the ecophysiological basis for changes in water use efficiency and yield in California processing tomatoes. Funct. Plant Biol. 41, 119–132.

Bean, G.J., Marks, M.D., Hulskamp, M., Clayton, M. and Croxdale, J.L. (2002) Tissue patterning of Arabidopsis cotyledons. New Phytol. 153, 461–467.

Bechtold, U., Penfold, C.A., Jenkins, D.J., et al. (2016) Time-series transcriptomics reveals that AGAMOUS-LIKE22 affects primary metabolism and developmental processes in drought-stressed Arabidopsis. Plant Cell, 28, 345–366.

Beeling, D.J. and Chalonier, W.G. (1993) The impact of atmospheric CO₂ and temperature changes on stomatal density: observation from Quercus robur lammas leaves. Ann. Bot. 71, 231–235.

Bernacchi, C.J., Portis, A.R., Nakano, H., van Cammerer, S. and Long, S.P. (2002) Temperature response of mesophyll conductance. Implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiol. 130, 1992–1998.

Bokrman, C.J., Dalin, P. and Ahnke, K. (2008) Leaf trichome responses to herbivory in willows: induction, relaxation and costs. New Phytol. 179, 176–184.

Bleecker, P.M., Mirabella, R., Diegarde, P.J., VanDoorn, A., Tissier, A., Kant, M.R., Prins, M., de Vos, M., Haring, M.A. and Schuurink, R.C. (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proc. Natl Acad. Sci. USA, 109, 20124–20129.

Blum, A. (2005) Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56, 1159–1168.

Blum, A. (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop. Res. 112, 119–123.

Bouhalleb, F. and Hajlaoui, H. (2011) Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmita and Chemlali). Acta Physiol. Plant. 33, 53–65.

Caruso, G., Gomez, L.D., Ferriello, F., et al. (2016) Exploring tomato Solanum pennelli introgression lines for residual biomass and enzymatic digestibility traits. BMC Genet. 17, 56.

Ceulemans, R., Van Praet, L. and Jiang, X.N. (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Crop. Res. 112, 119–123.

Chitwood, D.H., Kumar, R., Headland, L.R., et al. (2008) Leaf trichome responses to her-
water loss and induces genes involved in amino acid and ethylene/jasmonic metabolism under dehydration. Sci. Rep. B, 2791.

Ehleringer, J.R. and Mooney, H.A. (1978) Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. Oecologia, 37, 183–200.

Eshed, Y. and Zamir, D. (1995) An introgression line population of Lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics, 141, 1147–1162.

Ewas, M., Gao, Y.Q., Wang, S.C., et al. (2016) Manipulation of SIMXI for enhanced carotenoid accumulation and drought resistance in tomato. Sci. Bull. 61, 1413–1418.

Farber, M., Attila, Z. and Weiss, D. (1994) Cytokinin activity increases stomatal density and transpiration rate in tomato. J. Exp. Bot. 47, 6351–6362.

Farquhar, G. and Richards, P. (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Funct. Plant Biol. 11, 539–552.

Flexas, J., Diaz-Espejo, A., Berry, J.A., Cifre, J., Galvis, J., Kaldenhoff, R., Medrano, H. and Ribas-Carbo, M. (2007) Analysis of leakage in IRGA’s leaf chambers of open gas exchange systems: quantification and its effects on photosynthesis parameterization. J. Exp. Bot. 58, 1533–1543.

Fracasso, A., Trindade, L.M. and Damacci, S. (2016) Drought stress tolerance strategies revealed by RNA-Seq in two sorghum genotypes with contrasting WUE. BMC Plant Biol. 16, 115.

Franka, P.J., Doheny-Adams T, W., Britton-Harper, Z.J. and Gray, J.E. (2015) Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytol. 207, 188–195.

Fu, Q.S., Yang, R.C., Wang, H.S., Zhao, B., Zhou, C.L., Ren, S.X. and Guo, Guerfel, M., Baccouri, O., Boujnah, D., Charmentier, S., Zamir, D. and Tanksley, S.D. (2016) Identification of novel QTLs for drought tolerance in Arabidopsis thaliana. Plant Physiol. 171(4), 1429–1436.

Hauser, M.T. (2014) Molecular basis of natural variation and environmental control of trichome patterning. Front. Plant Sci. 5, 320.

Hermeda-Carrera, C., Kapralov, M.V. and Galmes, J. (2016) Rubisco catalytic properties and temperature response in crops. Plant Physiol. 171(4), 2549–2561. https://doi.org/10.1104/pp.16.01846

Hetherington, A.M. and Woodward, F.I. (2003) The role of stomata in sensing and driving environmental change. Nature, 424, 901–908.

Holmes, M.G. and Keiller, D.R. (2002) Effects of pomegranate and pomegranate leaves on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant Cell Environ. 25, 85–93.

Iovieno, P., Punzo, P., Guida, G. et al. (2016) Transcriptomic changes drive physiological responses to progressive drought stress and rehydration in tomato. Front. Plant Sci. 7, 371.

Kahn, T.L., Fender, S.E., Bray, E.A. and O’Connell, M.A. (1993) Characterization of expression of drought- and abscisic acid-regulated tomato genes in the drought-resistant species Lycopersicon pennellii. Plant Physiol. 103, 597–605.

Kang, J.H., MRoberts, J., Shi, F., Moreno, J.E., Jones, A.D. and Howe, G.A. (2014) The flavonoid biosynthetic enzyme chalcone isomerase modulates terpenoid production in glandular trichomes of tomato. Plant Physiol. 164, 1161–1174.

Lawson, T. and Blatt, M.R. (2014) Stomatal size, shape, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol. 164, 1558–1570.

Li, Y., Li, H., Li, Y. and Zhang, S. (2017) Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. Crop J. 5, 231–239.

Liu, X. and Liu, C. (2016) Effects of Drought-Stress on Fusarium Crown Rot Development in barley. PLoS ONE 11, e0167304.

Liu, E.K., Mei, X.R., Yan, C.R., Gong, D.Z. and Zhang, Y.G. (2016) Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes. Agric. Water Manag. 167, 75–85.

Luckwill, L. (1943) The genus Lycopersicon: a historical, biological, and taxonomic survey of the wild and cultivated tomatoes. Aberdeen: The University Press.

Martin, B. and Thorstenson, Y.R. (1988) Stable carbon isotope composition (δ13C), water use efficiency, and biomass productivity of Lycopersicon esculentum, Lycopersicon pennellii, and the F(1) hybrid. Plant Physiol. 98, 213–217.

Masle, J., Gilmore, S.R. and Farquhar, G.D. (2005) The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature, 436, 866-870.

McDowell, E.T., Kaptayn, J., Schmidt, A., et al. (2011) Comparative functional genomic analysis of Solanum glandular trichome types. Plant Physiol. 155, 524–539.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.-M. and Bota, J. (2015) From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J. 3, 220–228.

Mo, Y., Yang, R., Liu, L., Gu, X., Yang, X., Wang, Y., Zhang, X. and Li, H. (2016) Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. Plant Growth Regul. 79, 229–241.

Morohashi, K. and Grootewold, E. (2009) A systems approach reveals regulatory circuitry for Arabidopsis trichome initiation by the GL3 and GL1 selectors. PLoS Genet, 5, e1000396.

Moyle, L.C. (2008) Ecological and evolutionary genomics in the wild tomatoes (Solanum sect. Lycopersicon). Evolution, 62, 3095–3103.
Trichome/stomata ratio determines WUE in tomato

Niinemets, U., Diaz-Espejo, A., Flexas, J., Galves, J. and Warren, C.R. (2009) Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. J. Exp. Bot. 60, 2249–2270.

de Oliveira Silva, F.M., Lichtenstein, G., Alsekh, S., et al. (2018) The genetic architecture of photosynthesis and plant growth-related traits in tomato. Plant Cell Environ. 41, 327–341.
Palliotti, A., Bongi, G. and Rocchi, P. (1994) Peltate trichomes effects on photosynthetic gas exchange of Olea Europaea L. leaves. Life Sci. Adv. Plant Physiol. 13, 35–44.
Peralta, I.E., Spooner, D.M. and Knapp, S. (2008) Taxonomy of wild tomatoes and their relatives (Solanum sect Lycopersicoides, sect. Juglandifolia, sect. Lycopersicon; Solanaceae). Syst. Bot. Monographs, 84, 186.
Pillitteri, L.J. and Dong, J. (2013) Stomatal development in Arabidopsis. Arabidopsis Book, 11, e0182.
Ren, X., Chen, Z., Liu, Y., Zhang, H., Zhang, M., Liu, Q., Hong, X., Zhu, J.K. and Gong, Z. (2010) ABOS, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J. 63, 417–429.
Rick, C.M. (1973) Potential genetic resources in tomato species: clues from observations in native habitats. In Genes, Enzymes, and Populations (Srb, A.M. ed.). Boston, MA: Springer US, pp. 255–269.
Riederer, M. and Schreiber, L. (1973) Potential genetic resources in tomato species: clues from observations in native habitats. In Genes, Enzymes, and Populations (Srb, A.M. ed.). Boston, MA: Springer US, pp. 255–269.
Riederer, M. and Schreiber, L. (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. J. Exp. Bot. 52, 2023–2032.
Rigano, M.M., Arena, C., Di Matteo, A., Sellitto, S., Frusciante, L. and Barone, A. (2016) Eco-physiological response to water stress of drought-tolerant and drought-sensitive tomato genotypes. Plant Biosystems, 150, 662–691.
Rogiers, S.Y., Hardie, W.J. and Smith, J.P. (2011) Stomatal density of grapevine leaves (Vitis vinifera L.) responds to soil temperature and atmospheric carbon dioxide. Aust. J. Grape Wine Res. 17, 147–152.
Save, R., Biel, C. and de Herralde, F. (2000) Leaf Pubescence, Water Relations and Chlorophyll Fluorescence in Two Subspecies of Lotus Creticus L. Biol. Plant. 43, 239–244.
Schlimmer, A.L., Last, R.L. and Pichersky, E. (2008) Harnessing plant trichome biochemistry for the production of useful compounds. Plant J. 54, 702–711.
Silva, E.C., Nogueira, R.J.M.C., Vale, F.H.A., Araujo, F.P.D. and Pimenta, M.A. (2009) Stomatal changes induced by intermittent drought in four umbrae tree genotypes. Braz. J. Plant. Physiol. 21, 33–42.
Simmons, A.T. and Gurr, G.M. (2005) Trichomes of Lycopersicon species and their hybrids: effects on pests and natural enemies. Agric. For. Entomol. 7, 265–276.
Sletvold, N. and Agren, J. (2012) Variation in tolerance to drought amongst Scandinavian populations of Arabidopsis lyrata. Evol. Ecol. 26, 559–577.
Souza, M.A.A.D., Santos, L.A.D., Brito, D.M.C.D., Rocha, J.F., Castro, R.N., Fernandes, M.S. and Souza, S.R.D. (2016) Influence of light intensity on glandular trichome density, gene expression and essential oil of menthol mint (Mentha arvensis L.). J. Essent. Oil Res. 28, 138–145.
Sypropoulou, E., Haring, M. and Schuurink, R. (2014) RNA sequencing on Solanum lycopersicum trichomes identifies transcription factors that activate terpene synthase promoters. BMC Genom. 15, 402.
Steinhauser, M.C., Steinhauser, D., Gibson, Y., Bolger, M., Arraival, S., Usadel, B., Zamir, D., Fernie, A.R. and Stitt, M. (2011) Identification of enzyme activity Quantitative Trait Loci in a Solanum lycopersicum X Solanum pennellii Intergeneration Line population. Plant Physiol. 157, 998–1014.
Teller, A., Bolman, K.M. and Poethig, R.S. (1997) Phase change and the regulation of trichome distribution in Arabidopsis thaliana. Development, 124, 645–654.
Tian, D., Tooker, J., Peiffer, M., Chung, S. and Felton, G. (2012) Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (Solanum lycopersicum). Planta, 236, 1053–1066.
Traw, M.B. and Bergelson, J. (2003) Interactive effects of jasmonic acid, salicylic acid, and gibberellin induction of trichomes in Arabidopsis. Plant Physiol. 133, 1367–1375.
Turner, N.C. (1988) Measurement of plant water status by the pressure chamber technique. Irrig. Sci. 9, 289–308.
Vendemiatti, E., Zsoog, A., Silva, G.F.F.E., de Jesus, F.A., Cutri, L., Figueiredo, C.R.F., Tanaka, F.A.O., Nogueira, F.T.S. and Peres, L.E.P. (2017) Loss of typeIV glandular trichomes is a heterochronic trait in tomato and can be reverted by promoting juvenility. Plant Sci. 259, 35–47.
Wells, O.G. and Hoxie, R.P. (1982) The Influence of environment on the expression of trichomes in wheat. Crop Sci. 22, 879–886.
Wentworth, M., Murchie, E.H., Gray, J.E., Villegas, D., Pastenes, C., Pinto, M. and Horton, P. (2006) Differential adaptation of two varieties of common bean to abiotic stress: acclimation of photosynthesis. J. Exp. Bot. 57, 699–709.
Xu, Z. and Zhou, G. (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J. Exp. Bot. 59, 3317–3325.
Xu, X., Martin, B., Comstock, J.P., Vision, T.J., Tauer, C.G., Zhao, B., Pausch, R.C. and Knapp, S. (2008) Fine mapping a QTL for carbon isotope composition in tomato. Theor. Appl. Genet. 117, 221.
Yoshida, T., Fujita, Y., Murayama, K., Mogami, J., Todaka, D., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2015) Four Arabidopsis AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. Plant Cell Environ. 38, 35–49.
Zamir, D. (2001) Improving plant breeding with exotic genetic libraries. Nat. Rev. Genet. 2, 983–989.
Zhao, L., Li, Y., Xie, Q. and Wu, Y. (2017) Loss of CDK;2 increases both cell division and drought tolerance in Arabidopsis thaliana. Plant J. 91, 616–628.

© 2018 The Authors. The Plant Journal published by John Wiley & Sons Ltd and Society for Experimental Biology., The Plant Journal, (2018), 96, 607–619