1 **Original article**

2 **The *Lanata* trichome mutation increases stomatal conductance and reduces leaf** 3 **temperature in tomato**

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20 Running title: Leaf trichomes and water relations

21 **HIGHLIGHT**

22 *A monogenic mutation in tomato increases trichome density and optimizes gas*
23 *exchange and leaf temperature*

24

25

26 ABSTRACT

- 27 • *Background and aims:* Trichomes are epidermal structures with an enormous
28 variety of ecological functions and economic applications. Glandular trichomes
29 produce a rich repertoire of secondary metabolites, whereas non-glandular
30 trichomes create a physical barrier against biotic and abiotic stressors. Intense
31 research is underway to understand trichome development and function and
32 enable breeding of more resilient crops. However, little is known on how
33 enhanced trichome density would impinge on leaf photosynthesis, gas exchange
34 and energy balance.
- 35 • *Methods:* Previous work has compared multiple species differing in trichome
36 density, instead here we analyzed monogenic trichome mutants in a single
37 tomato genetic background (cv. Micro-Tom). We determined growth
38 parameters, leaf spectral properties, gas exchange and leaf temperature in the
39 *hairs absent (h)*, *Lanata (Ln)* and *Woolly (Wo)* trichome mutants.
- 40 • *Key results:* Shoot dry mass, leaf area, leaf spectral properties and cuticular
41 conductance were not affected by the mutations. However, the *Ln* mutant
42 showed increased carbon assimilation (A) possibly associated with higher
43 stomatal conductance (g_s), since there were no differences in stomatal density or
44 stomatal index between genotypes. Leaf temperature was furthermore reduced in
45 *Ln* in the early hours of the afternoon.
- 46 • *Conclusions:* We show that a single monogenic mutation can increase glandular
47 trichome density, a desirable trait for crop breeding, whilst concomitantly
48 improving leaf gas exchange and reducing leaf temperature.

49

50 *Key words:* Gas exchange, *Solanum lycopersicum*, stomatal conductance, stress, tomato
51 mutants, transpiration, water loss.

52

53 INTRODUCTION

54 Trichomes are extensions of the epidermis found in most terrestrial plants. The
55 density and type of trichomes are under strong genetic control but can vary due to
56 environmental factors (Holeski *et al.*, 2010; Thitz *et al.*, 2017). Variation is inter- and
57 intra-specific, but may occur within an individual as well, depending on leaf position
58 and developmental stage of the plant (Chien and Sussex, 1995; Vendemiatti *et al.*,
59 2017). Glandular trichomes are characterized by the ability to synthesize, store and
60 secrete a vast array of secondary metabolites, mostly terpenoids and phenylpropanoids,
61 making them appealing platforms for biotechnological applications (Tissier, 2012;
62 Huchelmann *et al.*, 2017). Furthermore, considerable interest has arisen in exploiting
63 glandular trichomes as a breeding tool to increase resistance to herbivory and thus avoid
64 excessive use of pesticides (Simmons and Gurr, 2005; Maluf *et al.*, 2007; Tian *et al.*,
65 2014; Chen *et al.*, 2018). Thus, research has focused on identifying metabolic pathways
66 within glandular trichomes (Besser *et al.*, 2009; Sallaud *et al.*, 2009; Bleeker *et al.*,
67 2012; Balcke *et al.*, 2017; Leong *et al.*, 2019).

68 However, some important gaps in the knowledge need to be resolved before
69 increased trichome density can be deployed in crops. Leaf pubescence caused by high
70 trichome density can have direct and indirect effects on photosynthesis, transpiration
71 and leaf energy balance (Ehleringer and Mooney, 1978; Bickford, 2016). On the one
72 hand, modelling work has shown that trichomes may affect gas exchange through
73 increasing the leaf boundary layer resistance (Schreuder *et al.*, 2001). The boundary
74 layer is a zone of relatively calm air that can influence the transfer of heat, CO₂ and

75 water vapour between the leaf and the environment (Schuepp, 1993). Research,
76 however, has shown that this effect is only significant under particular environmental
77 conditions (Ehleringer and Mooney, 1978; Benz and Martin, 2006). On the other hand,
78 a more pronounced effect is the increased reflectance of photosynthetically active
79 radiation (PAR) by highly pubescent leaves, which can occur by trichomes themselves
80 (Sandquist and Ehleringer, 1997, 1998) or by accumulation of water droplets (Brewer *et*
81 *al.*, 1991). Altered light absorptance can in turn affect photosynthetic rate directly
82 through decreased PAR or indirectly through increased leaf temperature (Bickford,
83 2016).

84 Tomato (*Solanum lycopersicum*) and its related wild species display great
85 diversity in density and type of trichomes: seven types of trichome, of which three
86 (types II, III and V) are non-glandular and four (types I, IV, VI and VII) correspond to
87 the glandular type (Luckwill, 1943; Glas *et al.*, 2012). Tomato has, therefore, been
88 proposed as a model for the study of trichomes (Schuurink and Tissier, 2019). Here, we
89 investigated how changes in trichome density influence leaf spectral properties, gas
90 exchange and leaf temperature. We used four tomato (cv. Micro-Tom, MT) genotypes
91 that differ in trichome density: wild-type (MT) and mutants with either decreased (*hairs*
92 *absent*, *h*), or increased (*Lanata*, *Ln* and *Woolly*, *Wo*) trichome density. We found that
93 the *Ln* mutation increased assimilation rate and stomatal conductance, while reducing
94 leaf temperature. Given the growing interest in altering trichome density in tomato and
95 other crops to increase insect resistance (Glas *et al.*, 2012), and to exploit the production
96 of secondary metabolites (Sridhar *et al.*, 2019), we discuss the physiological and
97 breeding implications of our results.

98

99 MATERIAL AND METHODS

100 *Plant material and experimental setup*

101 Seeds of tomato (*Solanum lycopersicum* L) mutants in their original background
102 were donated by Dr. Roger Chetelat (Tomato Genetics Resource Center – TGRC,
103 Davis, University of California) and then introgressed into the Micro-Tom (MT) genetic
104 background to generate near-isogenic lines (NILs). The NILs harbouring the mutations
105 *Lanata* (*La*), *Woolly* (*Wo*), *hair absent* (*h*) were generated as described by (Carvalho *et*
106 *al.*, 2011). Seeds of MT were kindly donated by Prof. Avram Levy (Weizmann Institute
107 of Science, Israel) in 1998 and kept as a true-to-type cultivar through self-pollination.
108 All the steps in the introgression process were performed in the Laboratory of Hormonal
109 Control of Plant Development (HCPD), Escola Superior de Agricultura “Luiz de
110 Queiroz” (ESALQ), University of São Paulo, Brazil.

111 Plants were grown in a greenhouse at the Federal University of Viçosa (642 m
112 asl, 20° 45’ S; 42° 51’ W), Minas Gerais, Brazil, under semi-controlled conditions.
113 Seeds were germinated in 2.5 L pots containing commercial substrate (Tropstrato® HT
114 Hortaliças, Vida Verde). Upon appearance of the first true leaf, seedlings of each
115 genotype were transplanted to pots containing commercial substrate supplemented with
116 8 g L⁻¹ 4:14:8 NPK and 4 g L⁻¹ dolomite limestone (MgCO₃ + CaCO₃).

117

118 *Biomass measurement*

119 Shoot and root biomass were measured from the dry weight of leaves, stem and
120 roots after oven-drying at 80 °C for 24 h. The measurement was performed in plants
121 40 days after germination. Leaf area was measured by digital image analysis using a
122 scanner (Hewlett Packard Scanjet G2410, Palo Alto, California, USA), and the images
123 were later
124 processed using the ImageJ (NIH, Bethesda, Maryland, USA).

125 *Leaf spectral properties*

126 Spectral analysis of leaves was performed in the attached central leaflet of the
127 fourth fully expanded leaf 56 days after germination. Leaf reflectance and transmittance
128 were measured on the adaxial side of the leaflet throughout the 280–880 nm spectrum
129 using a Jaz Modular Optical Sensing Suite portable spectrometer (Ocean Optics, Inc.,
130 Dunedin, Florida, USA).

131 *Gas exchange*

132 The net rate of carbon assimilation (A), stomatal conductance (g_s), internal CO_2
133 concentration (C_i) and transpiration (E) were measured in the third or fourth (from the
134 bottom) fully expanded leaf in all genotypes 40 days after germination. Gas exchange
135 measurements were performed using an open-flow gas exchange infrared gas analyzer
136 (IRGA) model LI-6400XT (LI-Cor, Lincoln, NE, USA). The analyses were performed
137 under common conditions for photon flux density ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, from standard
138 LiCor LED source), leaf temperature ($25 \pm 0.5^\circ\text{C}$), leaf-to-air vapor pressure difference
139 ($16.0 \pm 3.0 \text{ mbar}$), air flow rate into the chamber ($500 \mu\text{mol s}^{-1}$) and reference CO_2
140 concentration of 400 ppm (injected from a cartridge), using an area of 6 cm^2 in the leaf
141 chamber.

142 *Epidermal features analysis*

143 Dental resin impressions were used to obtain number and area of pavement cells
144 and stomatal density, following the methodology described by (Geisler *et al.*, 2000).
145 The third or fourth (from the bottom) fully expanded leaf was sampled 56 days after
146 germination for resin impressions in all genotypes. Using a spatula, the mixture of
147 Oranwash L and indurent gel (Zhermack) was applied to the adaxial and abaxial face of
148 opposite leaflets. After drying, the resin mold was removed from the leaflet surface and

filled with nail polish to create a cast that was analyzed by light microscopy (Zeiss Axioskop 40, Carl Zeiss, Jena, Germany). Images were analyzed with Image-Pro ® PLUS. Stomatal index (SI) was calculated using the formula $SI = S / (S + E) \times 100$, where S is number of stomata per area and E is number of epidermal cells per area. The analysis was performed in five plants per genotype, five images per plant, totalizing 25 images per genotype. Pavement cell area were measured for 30 pavement cells per genotype.

Leaf temperature determinations

Leaf temperature was measured in the central leaflet of the fourth fully expanded leaf 60 days after germination using an infrared thermometer (LASERGRIP GM400). Measurements were performed from 8:00 am to 6:00 pm with an interval of two hours between each measurement. A second set of measurements was carried out on thermal images obtained simultaneously with an infrared camera (FLIR systems T360, Nashua USA). All image processing and analysis was undertaken in Flir Tools software version 5.2.

Minimum epidermal conductance (g_{mim})

Analysis of minimum epidermal conductance was performed in plants 62 days after germination. The fully expanded fourth leaf was carefully removed and the petiole was sealed with silicone. The leaves were placed in zip lock bags with high CO₂ concentration. After an hour in the dark, the leaves were weighed on an analytical balance every 15 minutes for four hours. At the end of measurements, leaf area was calculated digitizing leaves with an HP Scanjet G2410 scanner (Hewlett-Packard, Palo Alto, California, USA) and performing image analysis with ImageJ. The minimum

172 cuticular conductance was calculated using the $g_{\min}$ Analysis Worksheet Tool Created
173 by Lawren Sack (University of California, Los Angeles, <http://prometheuswiki.org>).

174 *Transpirational water loss under still and turbulent air*

175 Transpirational water loss determinations were performed in 56-day old plants.
176 In order to evaluate the influence of the boundary layer on transpiration, measurements
177 were performed under either static or turbulent air conditions. Turbulent air was created
178 with a fan (ARNO, São Paulo, Brazil) placed at 1.5m distance, at the minimum
179 ventilation velocity (determined to be 10 km/h using an anemometer, Bmax,
180 Anemometer). We determined the ‘static’ air to be 1 km/h using the same instrument.
181 Pots were irrigated the night before the measurements until they reached field capacity.
182 Later the pots were covered with plastic film to reduce water loss from the substrate via
183 evaporation and then weighed. The following day, pots were weighed every two hours
184 between 8:00 am and 6:00 pm. Water loss was calculated as the difference in weight
185 every between measurements.

186 *Statistical analyses*

187 The data were obtained from the experiments using a completely randomized
188 design with the genotypes MT, *h*, *Ln* and *Wo*. Data are expressed as mean $\pm$ s.e.m. Data
189 were subjected to one-way analysis of variance (ANOVA), followed by Tukey’s
190 honestly significant difference test ($p < 0.05$) or Fisher’s Least Significance Difference
191 (LSD) test. All statistical analyses were carried out using Assistat v. 7.7 (Campina
192 Grande, Brazil).

193

194 **RESULTS**

195 *Mutations altering trichome type and density do not affect leaf area or shoot dry mass*

196 Here, we studied three near-isogenic lines (NILs) that harbor mutations affect
 197 trichome density in the genetic background of tomato cv. Micro-Tom (MT) (see
 198 Supplementary Table 1 for details). Like most cultivated tomatoes, MT displays three
 199 types of glandular trichomes (types I, VI and VII) and two types of non-glandular
 200 trichomes (III and IV) in adult leaves. Typically, total trichome density higher on the
 201 abaxial (around 250 trichomes cm⁻²) than on the adaxial (~100 trichomes cm⁻²) side of
 202 the leaf, and the most abundant on both sides is type V (Fig. 1). The *h* mutant displays a
 203 small, but significant reduction on type III and VI trichome density. Both the *Ln* and *Wo*
 204 mutants show higher trichome density on both sides of the leaf. The change, however, is
 205 not proportional among trichomes types: both *Ln* and *Wo* show preferential increases of
 206 types III and V (adaxial) and I and V (abaxial). Both genotypes also display a branched
 207 (‘ramose’) type of trichome that is absent in MT. Altogether, these changes result in a
 208 very similar ‘hairy leaf’ phenotype for *Ln* and *Wo*, with roughly three-fold increase of
 209 total trichome density on either side of the leaf.

210 Given the possibility of unrelated pleiotropic alterations caused by the
 211 mutations, we assessed other macroscopic plants traits. Except for a subtle reduction in
 212 leaf margin indentation in *Ln* (Fig. 1), we found no differences in leaf architecture, leaf
 213 area or shoot dry mass between the mutants of trichomes and MT (Fig. 1). Remarkably,
 214 all the *h* mutant showed higher root system dry mass (Fig. 1).

215

216 *Changes in trichome density do not affect the leaf spectral properties*

217 Depending on their density, trichomes may affect leaf function by altering the
 218 amount of light absorbed by leaves and/or by increasing the resistance of the boundary
 219 layer. Thus, we next measured transmittance and reflectance of photosynthetically

active radiation (PAR) on leaves of MT and the trichome mutants. The *h* mutant had a lower reflectance than the *Ln* mutant ($p=0.0068$), but neither was significantly different to MT or to the *Wo* mutant. No differences were found in leaf transmittance or absorptance in the PAR wavelength (Fig. 2). Calculation of the boundary layer resistance is problematic (see Discussion). To provide an indirect assessment of the effect of trichomes on the boundary layer, we next carried out a gravimetric determination of transpirational water loss under conditions of static (1 km h^{-1}) and turbulent (10 km h^{-1}) air. Under static air, we did not observe differences in rates of water loss between genotypes throughout the day or in transpiration calculated as water lost divided by leaf area over time (Fig. S1). Under turbulent air, water loss was lower for all genotypes, but without differences between them, except for one interval (between 12:00-14:00) for the *Ln* mutant, which showed less water loss than MT (Fig. S1).

233

234 *The Ln mutation increases photosynthetic assimilation rate and stomatal conductance*

We next investigated the effects of trichome mutations on leaf gas exchange. Both net photosynthesis rate (A) and stomatal conductance (g_s) were 29% and 38% higher in *Ln* than in MT ($p<0.0001$), respectively (Fig. 3). Internal CO_2 concentration (C_i) was higher in *h* (12%) and *Wo* (9.5%) mutants, compared with MT, but we found no difference in the C_i of *Ln* mutant (Fig. 3). Intrinsic water-use efficiency (A/g_s) was higher in MT and *Ln* than in *h* and *Wo*.

Given the changes in g_s , we determined whether trichome mutations have an impact on stomatal size and density. Stomatal density can be altered by changes on pavement cell size and density. Pavement cell density was higher in *h* and *Ln* than in MT and *Wo* on the adaxial side of the leaf (Fig. S2). Pavement cell area was higher in

245 MT than in all three trichome mutants. However, neither stomatal density nor stomatal
246 index were different between genotypes (Fig. S2). Stomatal size can influence
247 conductance, so we also determined the length and width of the guard cells. Except for a
248 significant guard cell length reduction in *Ln* (restricted to the adaxial side of the leaf),
249 no differences were found in stomatal size between genotypes (Fig. S3).

250 Stomata and cuticle are the primary leaf components responsible for maintaining
251 plant water status (Schuster *et al.*, 2016). To avoid excessive water loss, stomata close
252 in periods of water deficit. Even when stomata are closed, water loss continues at very
253 low rates through the leaf cuticle. This basal rate of water loss is expressed as the
254 minimum conductance of a leaf ($g_{\min}$). Both $g_{\min}$ and stomatal closure can be affected by
255 trichomes (Hegebarth *et al.*, 2016; Galdon-Armero *et al.*, 2018). However, we found no
256 differences in $g_{\min}$ among the genotypes evaluated in this experiment (Supplementary
257 data Fig. S4).

258

259 *The Ln mutation reduces leaf temperature during the afternoon*

260 Trichome density may potentially influence leaf temperature. To explore this
261 effect, we determined a time course of temperature using two variants of infrared
262 thermography (camera and thermometer). We found a steep increase in leaf temperature
263 over the course of the morning, reaching more than 30°C around noon (Fig. 4). From
264 then on, a further, yet smoother increase occurred until 15:00, when leaf temperature
265 reached 35°C. This, however, was not the case for *Ln* plants, which maintained a lower
266 temperature over the transition from noon to the mid-afternoon. Eventually, leaves of all
267 plants showed a steep decline in temperature at 16:30 and later (Fig. 4).

268

269 **DISCUSSION**

270 The recent years have seen a growing interest in harnessing trichomes as either a
 271 biotechnological platform for the production of valuable metabolites (Huchelmann *et*
 272 *al.*, 2017) or as an alternative, natural source of resistance to multiple biotic and abiotic
 273 stressors in crops (Glas *et al.*, 2012; Oksanen, 2018). However, some gaps in basic
 274 knowledge need to be resolved before trichome manipulation can be deployed as a
 275 breeding tool (Schuurink and Tissier, 2019; Chalvin *et al.*, 2020). Among others, an
 276 important question refers to the ecophysiological trade-off between advantages and
 277 disadvantages of increasing trichome density in crops (Bickford, 2016). Here we set out
 278 to explore this question using monogenic trichome mutants of tomato. Considerable
 279 variation in trichome type and density exists in cultivated tomato and its wild relatives,
 280 making this a suitable system for trichome study (Simmons and Gurr, 2005; Glas *et al.*,
 281 2012). Monogenic mutants have been described controlling alterations in trichome type
 282 and density (Yang *et al.*, 2011*a,b*; Chang *et al.*, 2018). Given the epistatic effects of
 283 genetic background, functional comparative studies of mutants are best performed using
 284 the same tomato cultivar (Tonsor and Koornneef, 2005).

285 Here, we analyzed trichome mutants in the same tomato cultivar (MT), thus
 286 avoiding the confounding effects of variation in genetic background (Carvalho *et al.*,
 287 2011). The only macroscopic alteration we observed was an increase in root dry weight
 288 in the *h* mutant. Although inconsistent, changes in root structure are not unusual in
 289 trichome mutants (Yang *et al.*, 2011*a*; Khosla *et al.*, 2014; Li *et al.*, 2016). In tomato
 290 plants, the expression pattern of the *Woolly* (*Wo*) gene in roots highlights a role for *Wo*
 291 in lateral root differentiation (Yang *et al.*, 2011*a*). The absence of leaf trichomes in the
 292 *tril* mutant in cucumber was accompanied by short root hairs and increases in root
 293 length and branching (Li *et al.*, 2016). On the other hand, *gl2* mutants of *Arabidopsis*
 294 exhibit glabrous leaves and excess root hair formation in primary roots (Khosla *et al.*,

295 2014). Since many genes that control trichome development play a pivotal role in
296 regulating the differentiation of the epidermis in various tissues, including roots,
297 changes in root growth and structure are not unexpected.

298 Leaf trichomes have long been known to affect light absorptance, which in turn
299 can influence photosynthetic rate and leaf temperature (Ehleringer *et al.*, 1976;
300 Ehleringer and Björkman, 1978). We only found a minor alteration in reflectance of
301 photosynthetically active radiation (PAR) between genotypes, a 1% increase in *Ln* and a
302 >1% reduction in *h* compared to MT. This is probably because even in the pubescent
303 mutants, trichome morphology cannot effect changes on reflectance. A similar result
304 was found comparing pubescent and glabrous populations of another solanaceous
305 species, *Datura wrightii* (Ii and Hare, 2004). On the other hand, closely related species
306 of *Pachycladon* with comparable trichome densities exhibited considerably different
307 spectral properties – an effect that was ascribed to variation in trichome morphology
308 (Mershon *et al.*, 2015).

309 Trichomes can indirectly affect gas exchange by altering light absorptance,
310 boundary layer resistance and/or leaf temperature. Determination of the boundary layer
311 resistance is challenging, given the large number of biophysical variables involved
312 (Schuepp, 1993). The current consensus is that the effect of trichomes is negligible
313 compared to stomatal resistance (Bickford, 2016). Comparing transpirational water loss
314 under conditions of still and turbulent air we could find no difference between
315 genotypes, except for slight decrease of water loss in *Ln* under turbulent conditions. Our
316 finding of increased *A* and *g_s* in the *Ln* mutant but not in *Wo* was surprising, given the
317 strong resemblance between both phenotypes. The molecular identity of the *Ln*
318 mutation is hitherto unknown, so this effect remains enigmatic. Also, It has been
319 proposed that trichomes could act as dew or fog collectors, wetting the leaf surface and

reducing the leaf-to-air water potential difference and thus increasing WUE (Brewer *et al.*, 1991; Brewer and Smith, 1994). Our results do not support this notion, as both *Ln* and *Wo* have similar WUE to the MT control. We could furthermore not ascribe the increased g_s in *Ln* to alterations on stomatal density or size (in fact, a slight decrease in adaxial guard cell length for the mutant). Thus, it seems plausible that *Ln* is a direct molecular regulator of stomatal opening.

Lastly, leaf temperature was lower in *Ln* during the hot hours of midday and the early afternoon. This effect was not observed in the *Wo* mutant, nor was increased temperature found for *h*, so it does not appear to be a consequence of changes in trichome density, but rather the result of evaporative cooling through increased transpiration caused by a higher g_s . Reduced leaf temperature in *Ln* could have a synergistic positive effect along with increased g_s on carbon assimilation rate (Ripley *et al.*, 1999). The temperature dependence of C_3 photosynthesis is quite steep below 20°C and above 30°C, with the optimum around 25 °C (Sage and Kubien, 2007). The reduction of more than 2°C in *Ln*, especially at the hottest hours of the day, could have a beneficial effect for field-grown tomatoes in tropical regions by potentially reducing photoinhibition.

Given the relationship between trichomes and water relations, recent research has evaluated the role of trichomes in tolerance and adaptation to water stress (Sletvold, 2011; Ewas *et al.*, 2016; Mo *et al.*, 2016; Galdon-Armero *et al.*, 2018). In *Arabidopsis lyrata*, plants with higher density of trichomes were more tolerant to drought than glabrous plants (Sletvold, 2011). In watermelon (*Citrullus lanatus*), tolerance to drought stress in wild genotypes, in relation to domesticated ones, is linked to the increased density of trichomes in the former (Mo *et al.*, 2016). In tomato, overexpression of MIXTA-like MYB transcription factor led to higher density of trichomes and tolerance

345 to water stress (Ewas *et al.*, 2016). Further work demonstrated that the ratio of
346 trichomes to stomata plays a role in avoiding excessive water loss (Galdon-armero *et*
347 *al.*, 2018). Collectively, these results make increasing glandular trichome density in
348 crops an appealing breeding prospect, for instance glandular trichome type IV, which is
349 found in wild tomato relatives but not in adult leaves of cultivated tomato (Vendemiatti
350 *et al.*, 2017).

351

352 **CONCLUSIONS**

353 Leaf trichomes are key structures regulating plant-environment interactions. The
354 use of glandular trichomes as a biotechnological platform or as a breeding tool requires
355 a deeper understanding of both the molecular control of their development and the
356 ecophysiological consequences of increased leaf pubescence. We have shown that the
357 monogenic *Lanata* mutation leads to higher assimilation rate and stomatal conductance,
358 resulting in lower leaf temperature, thus bypassing some of the undesirable
359 physiological traits usually associated with leaf pubescence for agronomic settings.
360 Cloning and molecular characterization of *Ln* should pave the way for its exploitation in
361 crop breeding.

362

363 **SUPPLEMENTARY DATA**

364 Table S1. Description of the genotypes studied in this work.

365 Table S2. Leaf area of the plants

366 Figure S1. Transpirational water loss time-courses

367 Figure S2. Leaf epidermis features

368 Figure S3. Guard cell size

369 Figure S4. Minimum leaf conductance ($g_{\min}$)

370

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372

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FIGURE LEGENDS

Figure 1. Macroscopic view of representative plants of tomato cv. Micro-Tom (MT) and the trichome mutants *hairs absent* (*h*), *Lanata* (*Ln*), *Woolly* (*Wo*). (A) Top view, bar = 5 cm. (B) Detail of leaf tips showing the difference in pilosity between genotypes, bar = 1 cm. (C) Trichome density per trichome type on the adaxial and abaxial side of fully-expanded leaves (based on Vendemiatti et al. 2017). Asterisks indicate significant differences to MT ($p < 0.05$). (D) Leaf, stem and roots dry weight determined through destructive analysis in plants 65 days after germination. Values are means $\pm$ s.e.m. (n=10). Significant differences tested with one-way ANOVA followed by Tukey's honestly significant difference (HSD) test, letters indicate significant differences ($p < 0.05$). n.s. indicates that no significant difference was found between genotypes.

Figure 2. Leaf spectral properties are not affected by trichome density in tomato. Percentage reflectance (A), transmittance (B) and absorptance (C) on leaves of Micro-Tom (MT) and the *hairs absent* (*h*), *Lanata* (*Ln*) and *Woolly* (*Wo*) mutants, measured 56 days after germination. Values are means $\pm$ s.e.m. (n=6). Significant differences tested with one-way ANOVA followed by Tukey's honestly significant difference (HSD) test,

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Figure 3. Trichome mutants affect leaf gas exchange parameters in tomato. Measurements were performed on fully expanded leaves of Micro-Tom (MT) and the *hairs absent* (*h*), *Lanata* (*Ln*) and *Woolly* (*Wo*) mutants 40 days after germination. (A) Net carbon assimilation rate (A). (B) Stomatal conductance (g_s). (C) Internal CO_2 concentration (C_i). (D) Intrinsic water-use efficiency (A/g_s) calculated using the data from panels A and B. Values are mean $\pm$ s.e.m. ($n=8$). Significant differences tested with one-way ANOVA followed by Tukey's honestly significant difference (HSD) test, letters indicate significant differences ($p < 0.05$).

Figure 4. The *Lanata* (*Ln*) mutation reduces afternoon leaf temperature. Time courses of leaf temperature on tomato cv. Micro-Tom (MT) and *hairs absent* (*h*), *Lanata* (*Ln*) and *Woolly* (*Wo*) trichome mutants using (A) infrared thermography (representative plants shown), and (B) point-and-shoot infrared thermometer. Measurements were conducted on fully expanded leaves of 60-day old plants ($n=8$). LSD: Fisher's Least Significant Difference between means.

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A

MT

*h**Ln**Wo*

B

Leaf temperature (°C)