



The balance of survival: Comparative drought response in wild and domesticated tomatoes

Yaniv Lupo^{a,b}, Menachem Moshelion^{a,*}

^a The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

^b French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boker, Israel

ARTICLE INFO

Keywords:

Drought
Transpiration
Stomatal conductance
Photosynthesis
Functional Phenotyping

ABSTRACT

Plants have the ability to undergo reversible behavioral, morphological, or physiological changes in response to environmental conditions. This plasticity enables plants to cope with uncertain environmental conditions, such as drought. A primary plastic trait is the rate of stomatal response to changes in ambient conditions, which determines the amount of water lost via transpiration, as well as levels of CO₂ absorption, growth, and productivity. Here, we examined the differences between domesticated (*S. lycopersicum* cv. M82) and wild tomato (*S. pennellii*) species and their responses to drought stress. The plants were grown in pots in a functional phenotyping platform (FPP) in a semi-controlled environment greenhouse. We found that the domesticated tomato had a higher transpiration rate (E) and higher stomatal conductance (g_s). The domesticated tomato also had greater biomass and greater leaf area under drought conditions, as compared to the wild tomato. Despite the domesticated tomato's higher E and higher g_s, there was no difference between the photosynthetic rates (A_n) of the two lines. Moreover, the wild tomato had a higher maximum rate of rubisco activity (V_{cm_{max}}), which might explain its greater leaf level and whole canopy water-use efficiency. The domesticated tomato's higher E and greater leaf area led to its earlier exposure to drought stress, as compared to the wild tomato, which maintained higher levels of soil water, enabling it to maintain steady rates of whole-canopy stomatal conductance (g_{sc}) for extended periods. The wild tomato was also more sensitive to soil water availability and lowered its maximum transpiration rate (E_{max}) at a higher soil-water-content (SWC) level compared to the domesticated species. Our results suggest that the domestication of tomatoes favored morphological/anatomical performance traits over physiological efficiency.

1. Introduction

Plants are immobile organisms and, therefore, are exposed to uncertain and unstable environmental conditions, which can become unfavorable to plant development and threaten the plant's survival. Drought is a significant environmental stress that limits crop production and endangers food security (FAO, 2017). Droughts are expected to become more extensive and intense due to climate change and global warming (Trenberth et al., 2014; Diffenbaugh et al., 2015). Drought significantly impacts plant growth and survival by inhibiting several key

physiological processes (Yan et al., 2016; Dietz et al., 2021). Plants have evolved different mechanisms to avoid the adverse effects of drought (Bacon, 2009). One such mechanism is to increase the plasticity of stomatal conductance (g_s) in response to the environment and to engage in more conservative behavior in environments in which the availability of water is unstable (Galkin et al., 2018; Hajjhashemi, 2019). However, crop plants have been bred to maximize their productivity (i.e., biomass and yield) during a growing season while increasing the amount of time their stomata are open despite environmental conditions (Bai and Lindhout, 2007). In recent years, it has become increasingly clear that

Abbreviations: An, CO₂ Assimilation Rate; Ci, Intercellular CO₂ Concentration; E, transpiration rate; E_c, transpiration rate normalized by the plant weight; E_{cm_{max}}, Midday E_c; FPP, Functional Physiological Phenotyping; g_s, Stomatal Conductance; g_{sc}, Canopy Conductance; iWUE, Intrinsic Water Use Efficiency; J_{max}, Maximum Rate of Electron Transport; PAR, Photosynthetically Active Radiation; SWC, Soil Volumetric Water Content; V_{cm_{max}}, Maximum Rate of Carbon Fixation; VPD, Vapor Pressure Deficit; WUE, Water Use Efficiency.

* Corresponding author.

E-mail address: menachem.moshelion@mail.huji.ac.il (M. Moshelion).

<https://doi.org/10.1016/j.plantsci.2023.111928>

Received 3 September 2023; Received in revised form 12 November 2023; Accepted 17 November 2023

Available online 20 November 2023

0168-9452/© 2023 Elsevier B.V. All rights reserved.

more breeding against unfavorable conditions is essential to minimize the yield gap arising from stressful growing conditions and improve world food security (Cattivelli et al., 2008; Razzaq et al., 2021; Villagómez-Aranda et al., 2022). Moreover, a better understanding of the mechanisms involved in plant drought response will enable us to breed crops that will perform well in changing environments to meet the growing demand for agricultural production (Boyer, 1982).

Maintaining high g_s benefits plants when water is not a limiting factor since g_s is correlated with high yield (Richards, 2000). Although immediate closure of stomata in response to drought helps to ensure survival, maintaining high g_s to the point of turgor loss will benefit productivity but also risks exposing the plant to more severe stress and endangering its survival (Sade et al., 2012; Claeys and Inzé, 2013). Therefore, the ideal plastic stress response is high g_s under well-watered conditions and low g_s under drought conditions (Negin and Moshelion, 2016). Moreover, g_s is tightly correlated with the CO_2 assimilation rate (A_n), as higher g_s allows for more CO_2 to enter the mesophyll and more substrate for photosynthesis. Thus, g_s is continually regulated to maximize the momentary water-use efficiency (WUE) in response to the dynamic soil-atmosphere conditions (Gosa et al., 2019). A high WUE is desirable for agriculture, as it allows crops to produce more yield using less water. The same goes for wild plants, as high WUE may improve survival under limited water conditions (Condon et al., 2004).

Conventional plant-breeding processes are based on the hybridization of parents and phenotypic selection of offspring and are relatively slow. An average breeding program for an annual crop can take 10–12 years (Kumar et al., 2015; Spindel and McCouch, 2016) depending on factors such as the target environment, the availability of genetic variation and the heritability of the trait of interest. In recent decades, the development of technologies associated with molecular markers and genomic selection has provided new tools that have enhanced classical breeding processes and made breeding for simple and complex traits more efficient (Spindel et al., 2015; Bhat et al., 2016). Today, the technical challenge of genomic selection lies in the reliability of the available phenotypic data (Rosenqvist et al., 2019). The gap between the genotypic data and the phenotypic data available to breeders creates a situation known as the genotype-phenotype gap (Orgogozo et al., 2015). The genotype-phenotype gap is even more complex when interacting with a water-limited environment. Therefore, to harness the full benefits of new genetic technologies, we must apply them with high-throughput phenotyping under different environmental conditions (Großkinsky et al., 2015). Functional physiological phenotyping (FPP) is a physiology-based, high-throughput, non-destructive and non-invasive phenotyping technique that continually measures the plant and ambient conditions (i.e., soil and atmosphere). FPP enables the detection of small changes in specific physiological traits (e.g., g_s) associated with environmental changes in general and stress conditions in particular (Gosa et al., 2019).

In this work, we investigated the plasticity of the g_s response to drought stress of a crop plant compared to a wild relative. For this investigation, we used a domesticated tomato (*S. lycopersicum* L. 'M82') and its wild relative (*S. pennellii* C.). We worked with these species for three main reasons. First, tomatoes are an important crop plant. Second, introgression lines of these two species are available for future research (Eshed and Zamir, 1995). Finally, *S. pennellii* has adapted to its native environment, the arid regions of the Andes in South America, as can be seen in its physiological drought-response patterns (Moyle, 2008; Bolger et al., 2014).

We used the FPP system to examine many plants over short (i.e., hours) and long (i.e., weeks) periods (Halperin et al., 2017; Dalal et al., 2020). With this system, we were able to simultaneously analyze continuous data during periods of optimal irrigation and drought. Analyzing continuous data enabled us to better examine the plant's response to the environment and treatments. We hypothesized that M82 would have higher absolute levels of physiological and morphological traits related to plant water balance (e.g., photosynthesis, g_s , growth rate

and WUE) and would be more efficient but more sensitive to drought than *S. pennellii*.

2. Materials and methods

2.1. Experimental setup and plant material

The experiment was conducted in a controlled greenhouse at the Faculty of Agriculture, Food and Environment in Rehovot, Israel (iCORE functional-phenotyping greenhouse) during the spring of 2017. The iCORE polycarbonate greenhouse includes cooling pads, which were activated when the temperature reached 30 °C. Climate conditions were continuously monitored by a weather station located near the plants in the greenhouse. The continuous photosynthetically active radiation (PAR) and vapor pressure deficit (VPD) measured during the experimental period are presented in Fig. 3a.

For this study, we used a domesticated tomato cultivar (*S. lycopersicum* L. 'M82') and a wild tomato species (*S. pennellii* C. LA716). Seeds from M82 and Pennellii were sprouted in a commercial germination plate for four weeks. One plant was planted in each 3.8-L pot filled with commercial growing medium (Bental 11, Tuff Merom Golan Ltd., referred to as soil) and transferred to the functional-phenotyping platform. The plant lines were separated onto different benches to prevent interactions such as changes in humidity or shading, which could be influenced by the structural differences between the wild type (Pennellii) and the cultivated line (M82). We opted for separate benches as this approach allowed us to observe the natural behavior of each variety under the specific conditions to which they are accustomed. Prior to the filling of the pots, the soil was mixed for several hours with excess water in a commercial-size mixer to achieve soil saturation (~80% volumetric water content). Each pot was put into a plastic container through a hole in its cover. A plastic cover with a hole in the middle prevented evaporation from the containers and the soil to allow the plant to emerge. The empirical investigation employed two distinct treatments: surplus irrigation and induced drought. The latter was triggered by the cessation of irrigation, as detailed in Halperin et al. (2017). Given that our substrate was characterized by high water content and the reservoir water for each pot (see below), this specific drying methodology was optimally suited to establishing the comparative conditions we desired. Specifically, it facilitated an extensive observation of plant water balance management within a soil ecosystem undergoing continuous moisture evaporation. The drought treatment started when the plants reached 650 (M82; 23/05) and 230 (Pennellii; 26/05) mL of daily transpiration. The beginning of the drought treatment was set to occur when transpiration reached about 30% of the available water. Due to M82's higher transpiration rate, it reached this point before Pennellii. The drought treatments continued until the treated plants reached 10% SWC (7 days for M82 and 12 days for Pennellii; Fig. 3f). After the irrigation was resumed, 7 days of recovery were given to each species (data not shown). Eight plants from each species were subjected to continuous irrigation throughout the experiment to create a control group. Conversely, a group comprising twenty-eight plants from each species was subjected to the drought treatment. As a result, the total experimental population comprised 72 specimens.

2.2. Functional-phenotyping platform

A functional-phenotyping platform (FPP; Plantarray®, PlantDitech Ltd., Yavne, Israel) was used to monitor plant growth and water balance through controlled tracking and measurement of the transpiration and biomass gain of each plant throughout the growing period (Fig. 1). The Plantarray® system recorded the weights of the pots and the environmental information that was registered by the sensors every 3 min. Prior to the experiment, all load cells were calibrated with 1-kg and 5-kg weights and randomly examined for reading accuracy. The data collection could be viewed in real-time through the online web-based

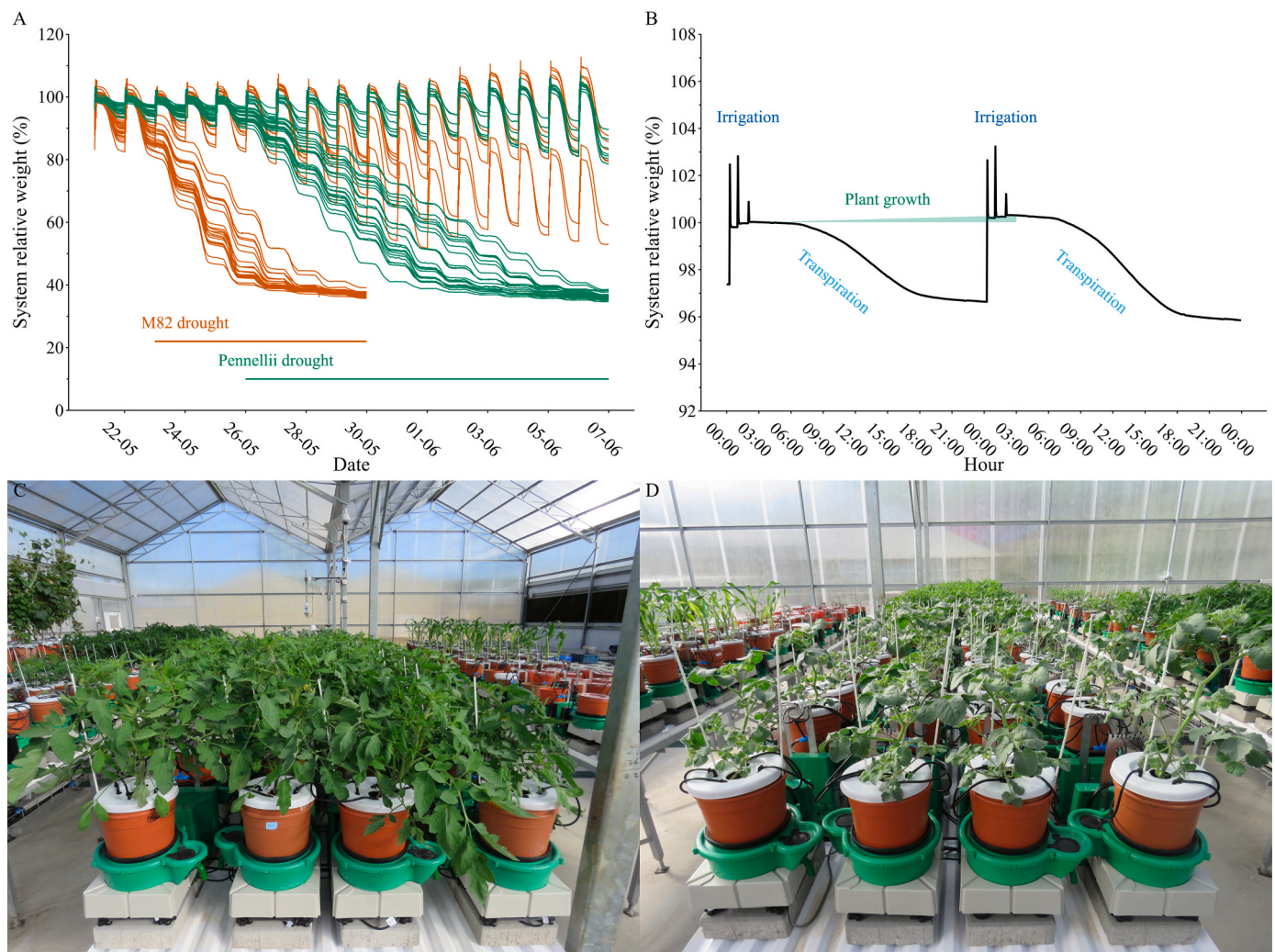


Fig. 1. Raw data from the FPP. (A) Raw data regarding the system's relative weight (plant + pot + container) throughout the experiment. Each line represents one plant; red = M82, green = Pennellii. (B) Representative two days of the system under the control condition showing the daily weight loss from transpiration, three irrigation peaks during the night and the plant growth rate calculated from the increase in the system weight. (C) M82 plants on the FPP on May 22nd. (D) Pennellii plants on the FPP on May 22nd.

software SPAC-analytics (PlantDitech).

Each plant was irrigated by four on-surface 4-L/h drippers to ensure uniform water distribution in the pots at the end of the irrigation event and prior to free drainage. Plants were irrigated in three consecutive cycles of irrigation and drainage between 24:00 and 02:00. Fertilizer (Haifa Group, Poly-Feed™, N-P-K 17–10–27 + ME) was applied with the irrigation water. After the pots had drained, the daily pre-dawn pot weight was determined as the average weight between 04:00 and 04:30. Additional water was provided during the irrigation event and was stored in the container, an additional 250 mL on average. The reservoir water ensured that water would be fully available to the well-irrigated plants throughout the following day without supplemental irrigation. This method enabled us to apply the data-analysis algorithm SPAC-analytics. Reaching an a priori-determined water level at drainage completion during the night enabled us to determine the daily plant weight gain (Figs. 1b, 3c). The whole-plant physiological measurements and system techniques are thoroughly described in Halperin et al. (2017) and Dalal et al. (2020). Briefly, the plant's weight gain (also referred to as fresh weight or biomass) was calculated at the end of each irrigation and drainage period, typically at pre-dawn (Fig. 1). This was accomplished by the system automatically comparing the container weights (subtracting all tare weights) upon drainage termination where soil reached its field capacity on consecutive days. The whole-plant

water-use efficiency (WUE) over a specific period was computed as the ratio between the sum of the daily plant fresh weight gain and the total amount of water consumed throughout this period. The momentary whole-plant transpiration rate (Transpiration) was derived by multiplying the first derivative of the measured load-cell time series by -1 . The Transpiration was then normalized by the plant's fresh weight, denoted as E_c . The canopy conductance (g_{sc}) was determined by utilizing E_c and considering the atmospheric pressure (VPD). g_{sc} measurement was conducted according to Halperin et al. (2017). The accuracy of these calculations was independently confirmed to be highly accurate by Jaramillo Roman et al. (2021). Prior to these calculations, the data time series were smoothed using the Savitzky and Golay method with a 61-data-point filtering window and a fourth-order polynomial. This step was taken to mitigate noise amplification, which increases as the sampling interval decreases.

Plants were cut at the bottom of the stem at the end of the experiment and measured for fresh weight. Then, the plants were oven-dried at 65 °C for 72 h and measured for dry weight ($n = 18$ for M82 and $n = 31$ for Pennellii). Agronomic and canopy WUE were calculated from the linear regression slope of shoot dry or fresh weight, respectively, and the total non-normalized transpiration taken during the plants' vegetative growth stage (days 13/05–06/06). Leaf area was measured at the end of a preliminary experiment under similar environmental and stress

conditions using the LI-3100 C area meter (LI-COR, Lincoln, NE, USA).

2.3. Gas-exchange measurements

The plants used for the gas-exchange measurements were grown next to the plants on the FPP, in similar pots and growing media, and received similar irrigation treatment as did the control plants on the FPP, $n = 11$ (M82) and 10 (Pennellii). Gas-exchange measurements were taken with the LI-6400XT portable gas-exchange system equipped with a 6-cm² aperture standard leaf cuvette (LI-COR, Lincoln, NE, USA). Young, fully expanded, sun-exposed leaves were measured for A–Ci curves between 10 PM and 2 PM. Measurements for M82 were taken between May 25th and June 12th. We used fixed chamber parameters as described below to avoid the differences between the days. All measurements were taken under saturating light (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; blue light was set to 10% of the photosynthetically active photon). The leaf-to-air VPD was kept between 1.5 and 2.5 kPa during all measurements. The leaf temperature for all measurements was about 25 °C. The CO₂ surrounding the leaf flux density ranged from 50 $\mu\text{mol mol}^{-1}$ to 1600 $\mu\text{mol mol}^{-1}$. The CO₂ response curves were measured at concentrations of 400, 300, 200, 150, 100, 50, 400, 600, 800, 1000, 1200, and 1600 $\mu\text{mol mol}^{-1}$, in that order.

A–Ci curves, maximum rate of carbon fixation (V_{cmax}), and maximum rate of electron transport (J_{max}) were calculated with the Plantecophys R Package, as described by (Duursma, 2015). The first measurement in each A–Ci curve (400 $\mu\text{mol mol}^{-1}$ CO₂) was used to examine the difference in gas exchange parameters between the species (Fig. 5).

2.4. Data analysis

Data from the FPP system was analyzed with the data-analysis algorithm SPAC-analytics. The data set included daily data (one value per day per plant) and momentary data acquired every 3 min (480 values per day per plant). The absence of overlapping confidence intervals indicates a significant difference at $P < 0.05$ (Fig. 4a, b). Other data analyses and data visualization were performed using R (4.2.1). Midday averages were fitted to a smooth curve using locally weighted polynomial regression (Fig. 3b, d and e). Means deemed significantly different at $P < 0.05$ were compared using Student's *t*-test or ANOVA followed by Tukey-Kramer's HSD. Analysis of covariance (ANCOVA) was used to compare the slopes in Figs. 2c and 2d.

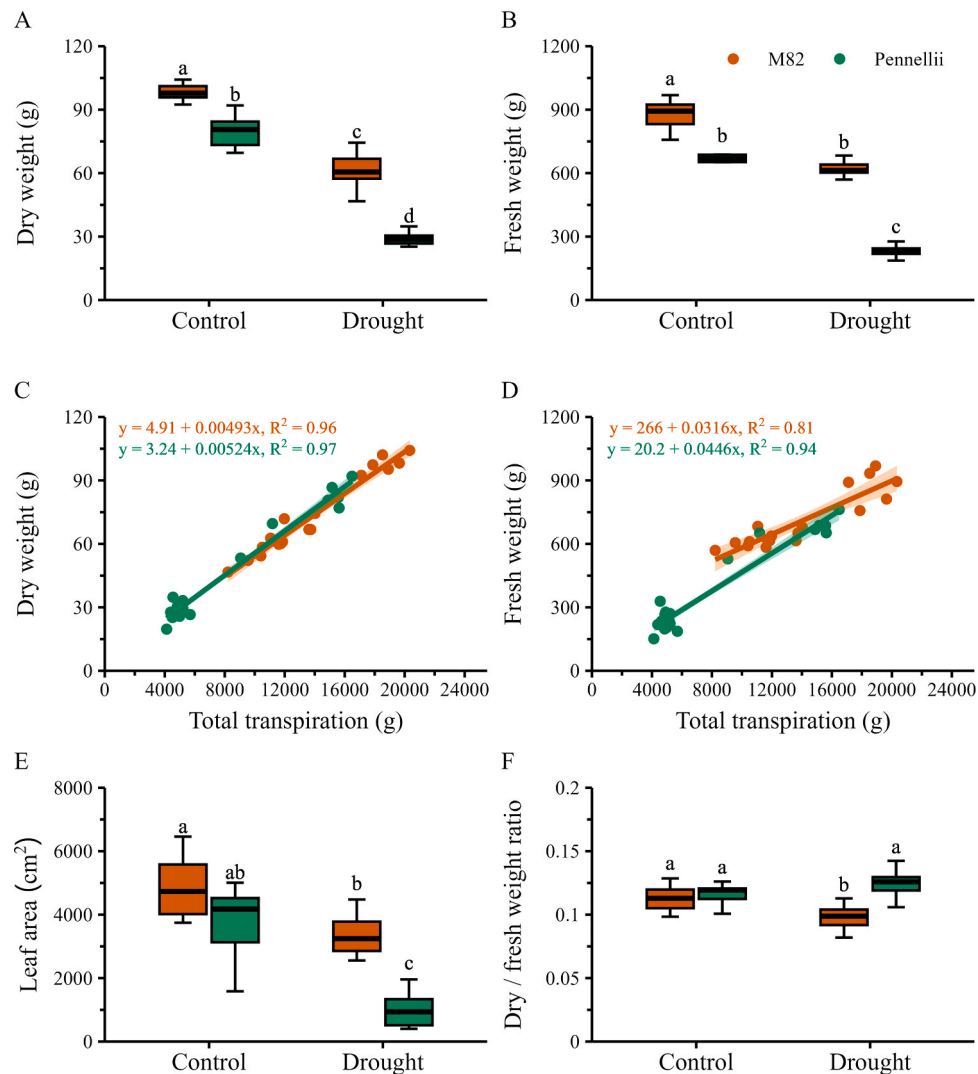


Fig. 2. Morphological parameters and water-use efficiency (WUE) at the end of the experiment. (A) Plant dry weight. (B) Plant fresh weight. (C) Agronomic WUE (dry weight vs. total transpiration). (D) Canopy WUE (fresh weight vs. total transpiration). (E) Leaf area measured in a preliminary experiment. (F) Dry-to-fresh weight ratio; red = M82, green = Pennellii. Different letters represent significant differences between species and treatments, according to Tukey's HSD, $P < 0.05$. $n_{\text{weight}} = 6$ (M82 control), 12 (M82 drought), 7 (Pennellii control) and 24 (Pennellii drought). $n_{\text{leaf area}} = 8$ (M82 control), 12 (M82 drought), 6 (Pennellii control) and 17 (Pennellii drought).

3. Results

At the end of the experiment (24 and 33 days for M82 and Pennellii, respectively), M82 had higher fresh and dry shoot weights under the control and drought treatments, as compared to Pennellii (Fig. 2a, c). Both species had lower fresh and dry weights under the drought treatment than the control treatment. M82 also had greater leaf area than Pennellii under the drought treatment. Both species had smaller leaf areas under the drought treatment than under the control condition (Fig. 2e). There were no significant differences in the agronomic WUE of the two species (0.52% for Pennellii as compared to 0.49% for M82; slope in Fig. 2c, $P = 0.28$). Pennellii had higher canopy WUE (4.46%, as compared to M82's 3.16%; slope in Fig. 2d, $P < 0.01$). M82 had a lower dry/fresh weight ratio under the drought treatment, as compared to the control and as compared to Pennellii (Fig. 2f). Taking out the drought factor on the WUE, i.e. correlating only the control plants, resulted in agronomic WUE of 0.27% ($R^2 = 0.52$), and 0.43% ($R^2 = 0.88$) for M82 and Pennellii, respectively and canopy WUE of 2.2% ($R^2 = 0.74$) for Pennellii (no correlation was found between M82's total transpiration and fresh weight; data not shown).

Comparative functional-phenotyping screening of whole-plant water-balance regulation was conducted under similar ambient VPD and PAR conditions, and those parameters were measured continuously throughout the experiment (Fig. 3; for continuous temperature and relative humidity, see Appendix Figure A1). Under the control condition, M82's absolute Transpiration (non-normalized) was higher than Pennellii's throughout the period of the study (Fig. 3b). M82 and Pennellii exhibited lower Transpiration (compared to the control) at 3 and 5 days, respectively, after drought treatment was initiated (Fig. 3b). Under well-irrigated conditions, both M82 and Pennellii exhibited an increase in Transpiration from day to day. However, throughout the experiment, M82 showed a faster rate of increase due to the fact that it was gaining mass more quickly (Fig. 3c). In response to the drought treatment, M82 stopped growing after 6 days (28/05, as calculated by the FPP). In contrast, Pennellii stopped growing after 12 days (06/06, as calculated by the FPP). When total transpiration was normalized to plant weight (E_c), the differences between M82 and Pennellii were reduced, yet M82 still had a higher E_c under the control condition (Fig. 3d). Moreover, both M82 and Pennellii exhibited decreasing E_c over the course of the experiment. A similar pattern was also revealed for the canopy stomatal conductance (g_{sc} ; Fig. 3e).

Plotting the midday E_c (E_{cmax}) against the SWC revealed the transpiration response to the reduction in SWC (Fig. 4a). We found that M82 maintained higher E_{cmax} through lower SWC, reaching the lower physiological drought point (θ_{crit} ; determined as the point at which soil water limits the transpiration needs) at 33.2% and then decreasing sharply (slope = 0.05). Pennellii, on the other hand, exhibited a much stronger water-conservative response of significantly higher θ_{crit} of SWC = 46.6%, followed by a more moderate decrease (slope = 0.02). Plotting M82's midday g_{sc} response pattern to SWC revealed a similar response pattern to E_c . Nevertheless, Pennellii's g_{sc} responded differently than its E_c , suggesting different regulations of the whole-canopy conductance under drought. To examine the g_{sc} pattern at a higher resolution, we looked at momentary g_{sc} throughout the day (Fig. 4c, d). Plants were compared 1 day before the drought was initiated (May 22nd and May 25th for M82 and Pennellii, respectively) and 5 days after irrigation was stopped. The atmospheric conditions were similar on those days (Fig. 3A). Under well-watered conditions, M82 had a higher g_{sc} than Pennellii throughout the day (Fig. 4c). In terms of transpiration, Pennellii was more sensitive to drought than M82 was (higher θ_{crit}), which enabled it to keep more available soil water for a more extended period. This allowed it to maintain a higher g_{sc} (as compared to M82) after 5 days of drought, as could be detected from the fact that there were no differences between the Pennellii control and drought treatments throughout most of the day (Fig. 4d). At the same time, throughout the day, the g_{sc} of drought-treated M82 was lower than that of the control

M82 and that of the drought-treated Pennellii (Fig. 4d).

After examining the different water regimes and biomass accumulation responses of M82 and Pennellii to the environment, we evaluated the differences between the species' leaf-level gas exchange and photosynthetic efficiency. Gas-exchange measurements at the single-leaf level revealed no differences in the A_n of M82 and Pennellii under well-irrigated conditions (Fig. 5a; for C_i , see Appendix Figure A2). M82 had higher g_s and E than Pennellii (Fig. 5b, c). This meant that Pennellii had higher intrinsic water-use efficiency (iWUE) under control conditions, as compared to M82 (Fig. 5d; see Materials and Methods for more details on the calculation of the iWUE). Moreover, Pennellii also had a higher maximum carboxylation rate (V_{cmax}) than M82 (Fig. 6b). There were no significant differences between the two species' maximum rates of electron transport (J_{max} ; Fig. 6c).

4. Discussion

In this study, we evaluated the water-balance responses of domesticated and wild tomato species to drought. The domesticated species (M82) had greater biomass, grew more quickly (measured by the FPP, see Materials and Methods and Fig. 1b), and exhibited greater g_s and total transpiration than the wild species (Pennellii). However, there were no differences in the species' photosynthetic rates per unit leaf area, which resulted in Pennellii's higher WUE (canopy and intrinsic, Fig. 2 and Fig. 5, respectively). M82 was also less sensitive to declining SWC than Pennellii, as it maintained a high level of transpiration at low SWC levels. M82's higher total transpiration, combined with its slower response to reduced SWC, led to faster and more intense drought stress for M82 compared to Pennellii.

4.1. Cultivated tomato has higher transpiration and is a drought-susceptible plant

In this work, we demonstrate that, during early growth stages, M82 (crop plant) has greater plant mass and higher total and normalized (to biomass) Transpiration due to its higher g_s , as compared to Pennellii (wild plant). High g_s can be beneficial when environmental conditions are not limiting, as they allow for more gas exchange and CO_2 fixation (Gago et al., 2016). Yet, higher transpiration often has a tradeoff with efficiency (Dalal et al., 2017). Examination of the relationship between E_{cmax} and SWC shows that Pennellii lowered its Transpiration at higher SWC and, therefore, was able to keep growing for a longer period under drought (Fig. 3c) and to maintain constant g_{sc} over those longer periods (Fig. 4b). In contrast, M82's higher g_{sc} and higher total transpiration resulted in a faster reduction in SWC under terminal drought, which reduced g_{sc} and plant growth almost immediately after the θ_{crit} of E_{cmax} was reached. M82's higher E_{cmax} combined with its late response to drought led it to experience drought stress faster than Pennellii but also allowed it to be more productive until that lower θ_{crit} point was reached.

At the leaf level, even though M82 had higher g_s and E than Pennellii, there were no differences in the A_n levels of the two species (Fig. 5a). This combination of lower transpiration and a similar CO_2 fixation rate led to Pennellii's higher iWUE (Fig. 5b), which was in agreement with its higher canopy WUE (Fig. 2b). These findings are consistent with those observed when Pennellii was compared with another cultivated tomato species (Martin et al., 1994). Moreover, under non-limiting conditions, Pennellii also exhibited more efficient CO_2 fixation (V_{cmax} ; Fig. 6b), which explains how it can fix more CO_2 at lower g_s . RuBisCO is the rate-determined enzyme in the Calvin cycle. However, it is not an efficient enzyme as it can bind O_2 , as well as it binds CO_2 (Tcherkez, 2021; Lin et al., 2022). This explains Pennellii's higher dry/fresh shoot weight ratio (Fig. 2f). Another possible explanation could be Pennellii's even distribution of stomata on the abaxial-adaxial leaf surfaces. This even distribution correlates with higher carbon isotope discrimination, indicative of improved CO_2 uptake efficiency (Muir et al., 2014).

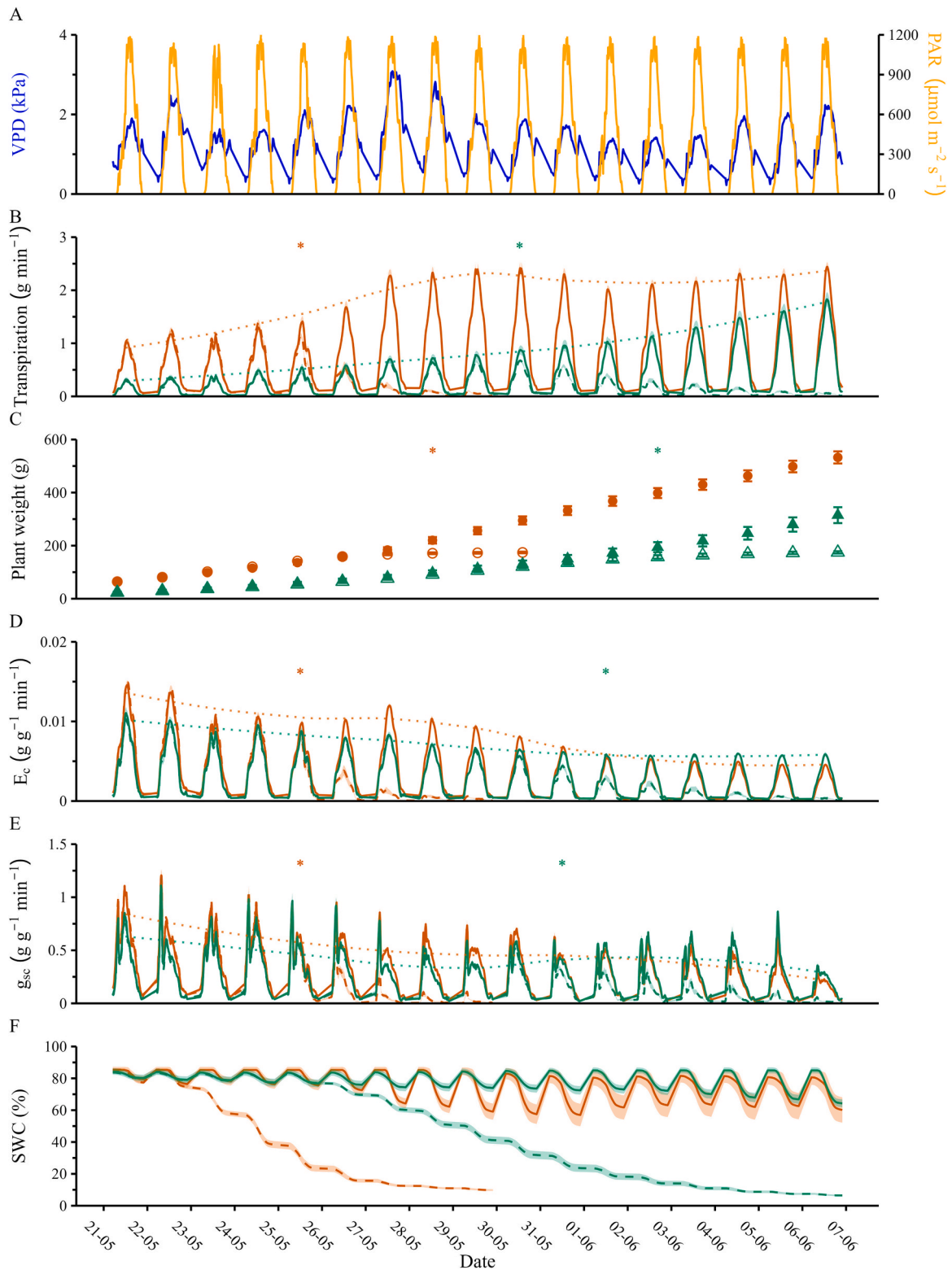


Fig. 3. Soil-plant-atmosphere-continuum (SPAC) data. (A) Vapor pressure deficit (VPD; blue line) and photosynthetic active radiation (PAR; orange line) over time. (B) Absolute transpiration rate (whole-plant water-loss rate, continuous plot) and midday average (dotted line) over time. (C) Calculated plant weight over time; circles = M82, triangles = Pennellii. (D) Transpiration rate normalized to plant weight (E_c , continuous plot) and midday average (dotted line) over time. (E) Whole-canopy stomatal conductance (g_{sc} , continuous plot) and midday average (dotted line) over time. (F) Calculated soil volumetric water content (SWC) over time. (B, D-F) Red = M82, green = Pennellii; solid lines/full symbols = control, dashed lines/empty symbols = drought. Asterisks represent the first day on which significant differences between treatments within each species were observed, according to Student's *t*-test, $P < 0.05$. $n = 7$ (M82 control), 23 (M82 drought), 7 (Pennellii control) and 24 (Pennellii drought).

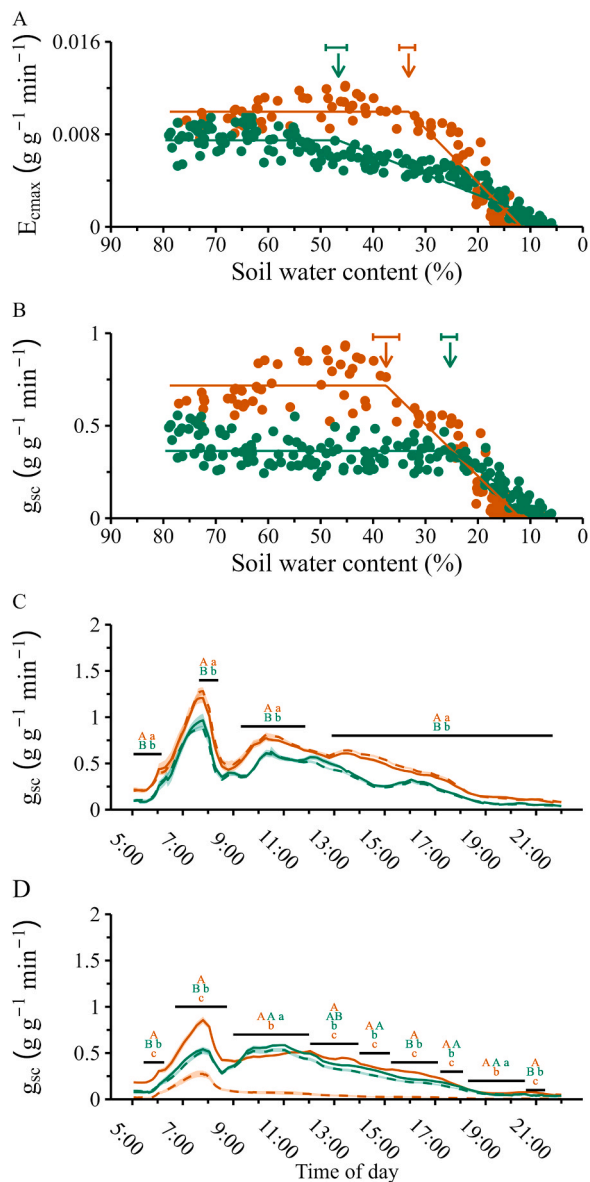


Fig. 4. Piecewise curve fit and diurnal canopy stomatal conductance (g_{sc}) at different soil water content (SWC) levels. (A) Piecewise curve fit of midday E_c (E_{cmax}) in response to SWC. (B) Piecewise curve fit of midday g_{sc} in response to SWC. (C) Diurnal g_{sc} under well-irrigated conditions (SWC = ~80%). (D) Diurnal g_{sc} 5 days after drought started (SWC at 5 AM was 16% and 39% for M82 and Pennellii, respectively). Red = M82, green = Pennellii; solid lines = control, dashed lines = drought. Arrows mark where soil water is restricted from supplying midday transpiration needs (Θ_{crit}). Bars represent upper and lower CI for Θ_{crit} . Different letters represent significant differences between species and treatments (uppercase represents control and lowercase represents drought), according to two-way ANOVA, $P < 0.05$. $n = 7$ (M82 control), 23 (M82 drought), 7 (Pennellii control) and 24 (Pennellii drought).

Pennellii was also found to have greater stomatal resistance due to thicker leaves and lower stomatal frequency compared to a domesticated species, which was attributed to Pennellii's improved drought tolerance (Kebede et al., 1994). Therefore, Pennellii is more adaptable to dry conditions, as it can fix more CO_2 and produce more biomass with less water lost to transpiration.

Taking all the above into consideration, we can say that Pennellii has a more conservative water-protective drought adaptation than M82, which involves transpiring less and responding more quickly to the depletion of water from the soil, as could be expected from a wild species

evolving toward "survivability-enhancing" behavior (Dalal et al., 2017). Pennellii is native to an arid region, in which it adapted to become drought-tolerant in order to survive (Egea et al., 2018). Kebede et al. (1994) also suggested that Pennellii's lower g_s combined with its relatively low root system are indications that Pennellii's water-saving adaptations are the foundation of its drought resistance. M82, on the other hand, is a domesticated plant bred to produce high yields under optimal conditions, which may result in it being less able to survive under prolonged drought.

4.2. M82 is a higher transpiring but less responsive and less efficient plant than Pennellii

During domestication, plants were selected for simple morphological/agronomical traits, such as size and yield. In the process of breeding for rapid growth and high yield under optimal water conditions, M82's WUE was inadvertently reduced. The physiological definition of molecular WUE (units of CO_2 gained in photosynthesis per units of H_2O lost in transpiration) measures efficiency but not productivity (Condon et al., 2004). Indeed, M82 is less efficient and has lower iWUE (A_n/g_s) but is more productive in terms of biomass gain and is reported to have higher yields in the field (Eshed & Zamir, 1995), indicating a tradeoff between efficiency and productivity.

These results match what has been found in other crops. For example, a comparison of cultivated wheat with its wild progenitors showed that the area of individual leaves and the total leaf area of seedlings increased with the shift from wild to cultivated forms. Still, that increase was coupled with a progressive reduction in the rate of photosynthesis per unit leaf area (Evans & Dunstone, 1970). Moreover, in the last century, there has been no change in the rate of photosynthesis in cereals. Yet, total photosynthesis has increased as a result of an increase in total leaf area, the daily duration of photosynthesis or leaf area, but not due to any direct improvement of photosynthesis efficiency (Richards, 2000). However, these robust biochemical traits seem to have been canalized in modern crops, leaving the question of their native plasticity unanswered.

Stomatal conductance is a highly plastic trait that allows the plant to optimize its risk/production response to a dynamically changing environment. High plasticity for this trait is beneficial as it helps the plant to avoid the adverse effects of drought as it rapidly responds to small changes in the SWC. Yet, it carries the penalty of reduced photosynthesis and production. M82 exhibited much less plasticity in this trait, compared with Pennellii, as it maintained higher transpiration, only reducing its E_{max} at a relatively low SWC, leading to rapid exposure to severe stress (Fig. 4a). In Pennellii, on the other hand, this trait was more plastic. Pennellii exhibited lowered E_{max} under higher SWC, which enabled it to reserve more water and gave it more time to adjust to the drought. At that point, the plants maintained their rate of growth (Fig. 3c) and exhibited a slower reduction in g_{sc} (Fig. 3e), which is an additional aspect of Pennellii's plastic behavior, seen in its lower Θ_{crit} of g_{sc} (Fig. 4b).

An additional plastic trait is the daily pattern of stomatal response. Under well-irrigated conditions, both lines exhibited a morning peak of high g_{sc} when VPD was relatively low, and PAR was sufficient, thus maintaining optimal behavior. This peak is referred to in the literature as the "golden hour" and correlates with tomato yield in the field (Gosa et al., 2022). Pennellii's rapid E_c response to lowered SWC enabled it to maintain the golden-hour peak for longer periods under drought, as compared with M82 (Fig. 4c, d). This behavioral tradeoff enabled Pennellii to reduce its risk through fast closure of stomata during the part of the day when VPD is higher, and although it had lower g_{sc} , it could be maintained for a longer time, which probably improves the plant's survival chances. Yet, on the other hand, this activity results in smaller plants at the end of the drought period (Fig. 2). Interestingly, under well-irrigated conditions, this daily peak was maintained in M82 at an even higher level. The availability of introgression lines between M82

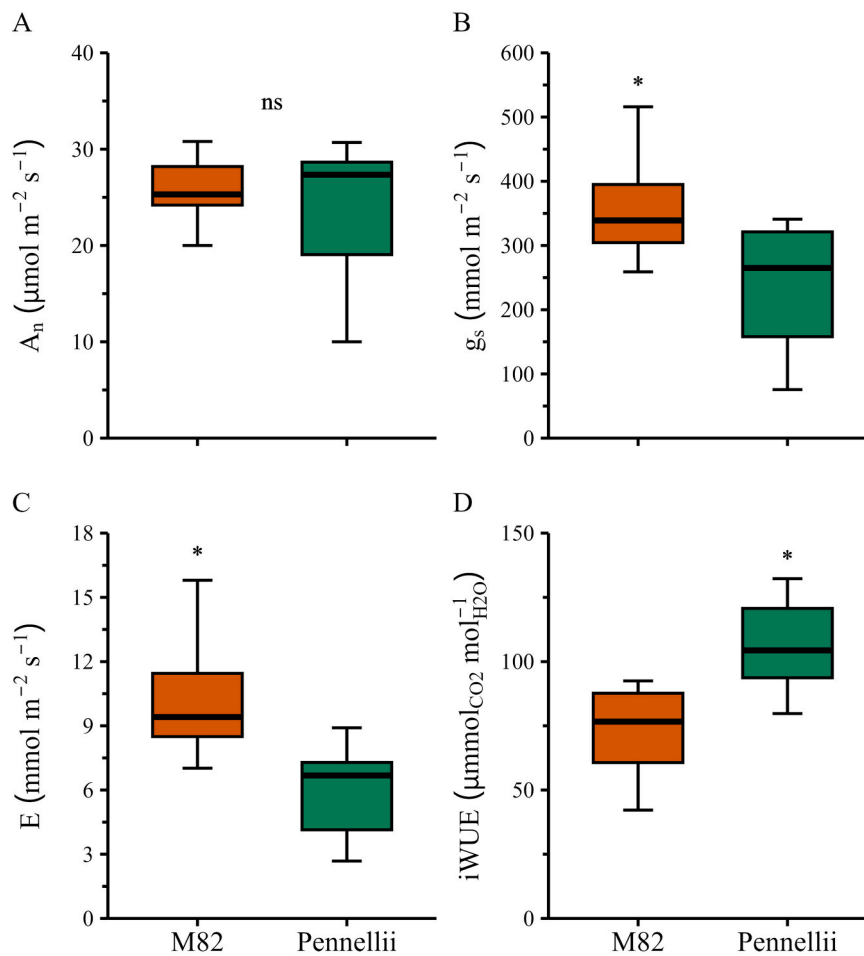


Fig. 5. Gas exchange of well-watered plants of the two species. (A) Photosynthetic assimilation rate (A_n). (B) Stomatal conductance (g_s). (C) Transpiration rate (E). (D) Intrinsic water-use efficiency (iWUE; calculated as A_n/g_s). Red = M82; green = Pennellii. Asterisks represent significant differences according to Student's *t*-test, $P < 0.05$. $n = 11$ (M82) and 10 (Pennellii).

and Pennellii can be used in future trials to evaluate the golden-hour peak effect on WUE and productivity.

Our results suggest that M82's cultivation process canalized (fixed) plastic traits, such as high g_s , low responsiveness to drought and the golden-hour peak, thereby directly improving yield under well-watered conditions (i.e., the conditions used in the breeding process). Moreover, we suggest that breeding had more of an impact on the rate of growth than on biochemical (photosynthetic) activity traits. Similar results were found in wheat, in which breeding for high yield did not result from improved photosynthesis efficiency (Driever et al., 2014). At the same time, it reduced a "protective" response to the environment while maintaining a productive response, such as the golden-hour peak, which enables dynamic WUE over the course of the day (Fig. 4a, c). Therefore, we conclude that breeding increased M82's biomass and, in doing so, also increased its total transpiration and photosynthesis, resulting in increased yield, but at the cost of a decreased ability to sense and respond to environmental changes, which put it at greater risk in stressful conditions.

These findings are congruent with our hypothesis that cultivated plants are more canalized toward production and, therefore, exhibit a less plastic stomatal response to the environment, resulting in a risk-taking response to drought. However, unlike what we hypothesized, the cultivation process does not appear to have resulted in more efficient behavior but does appear to have increased the plant size and, in doing so, increased the total transpiration and photosynthesis to support higher yields.

4.3. Functional phenotyping under stress: Should we normalize when evaluating plant performance?

Transpiration measurements are essential for evaluating plant water status and plants' responses to their environment, particularly under drought. Should we use normalized or absolute transpiration when comparing plants? Should we use leaf or whole-plant measurements to estimate drought response? Normalization of transpiration helps when comparing different plants observed at different times. However, normalization can be misleading and even inaccurate in some cases, such as when the assumptions used are not tested and proved for every examined line. For example, a broadly accepted assumption for gas-exchange calculations used in many studies over the past decades is that the relative humidity inside the substomatal cavities is near-saturated (Farquhar & Raschke, 1978). However, in *Eucalyptus pauciflora*, the relative humidity inside the intercellular air spaces at midday was found to be 90% (Canny & Huang, 2006). A recent study by Farquhar (Wong et al., 2022) also contradicted the original assumption by showing that the RH inside the substomatal cavities decreased to 80% when the air humidity was reduced.

Moreover, an improved method for calculating gas exchange was recently suggested (by the author of the original theory), which also considers the cuticular conductance to water (Márquez et al., 2021). However, this improved method is still valid only for leaf-level measurements. The selected leaves for this kind of measurement are usually young and sun-exposed and do not represent the entire plant canopy. As the plant grows, leaves mature and become less productive, and the

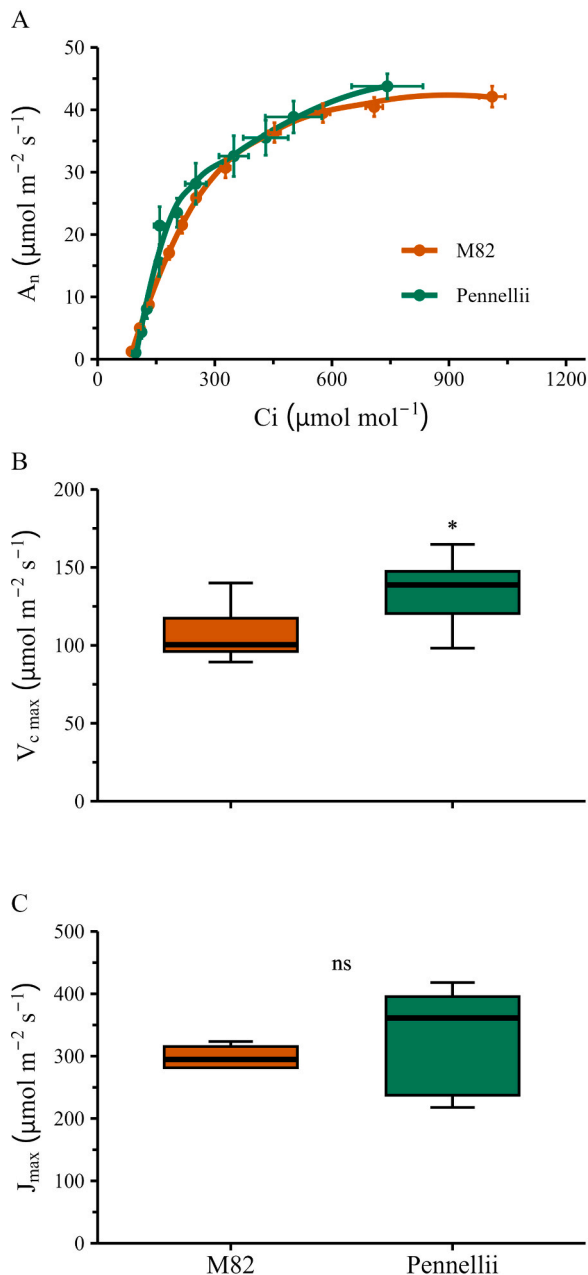


Fig. 6. CO_2 response curves of the two species. (A) A– C_i curves. (B) Maximum rate of carbon fixation ($V_{c\text{max}}$). (C) Maximum rate of electron transport (J_{max}). Red = M82; green = Pennellii. Asterisks represent significant differences according to Student's *t*-test, $P < 0.05$. $n = 11$ (M82) and 10 (Pennellii).

basal leaves are shaded, resulting in reduced g_{sc} . Therefore, using non-normalized whole plant measurements (e.g., absolute transpiration) is essential to better understand the whole plant's response to the environment based on values that need no interpretation (e.g., mL water transpired per plant per day). Moreover, absolute transpiration better demonstrates the whole plant's adaptation to the environment by showing the actual soil–plant water balance, which can also be interpreted to determine the exact irrigation amount.

For example, in this work, M82 had much higher absolute transpiration than Pennellii when there was an adequate supply of water, which led those plants to reach their pot capacity (~1200 mL) on May 30th (Fig. 3b). However, normalizing the transpiration to plant weight (E_w) or to plant weight and VPD (g_{sc}) reduced the size of the differences between the species (Fig. 3d, e), despite the big difference in their actual water

loss.

Measuring the entire plant canopy allows us to avoid the need to decide which leaf to choose for manual gas-exchange measurements, as the FPP measures the whole plant, including old and shaded leaves. The actual whole-plant g_{sc} exhibits a declining trend (Fig. 3e), resulting from the fact that many of the older leaves maintain their size and weight as their performance decreases and lower leaves become shaded. Moreover, measuring the entire canopy with no physical interference (e.g., clipping chamber, wind blowing or other effects that the manual gas-exchange apparatus imposes on a leaf) that modifies the boundary layer or any assumptions regarding the abaxial/adaxial stomatal density provides an accurate measure of g_{sc} , as recently reported for the Plantarray system (Jaramillo Roman et al., 2021). The study conducted by Halperin et al. (2017) found that a lysimetric system can accurately represent plant gas exchange parameters for young, non-woody plants. The research demonstrated a high correlation between data from a lysimetric experiment and measurements from a portable gas exchange apparatus, effectively validating the use of the lysimetric system for determining physiological parameters in such plants. Therefore, in plant-breeding programs in which many cultivars are screened in parallel and compared based on their performance, we suggest that whole-plant non-normalized behavior will be better represented by the absolute water consumption, which presents the field scenario and helps the breeder to see the actual risks and behavioral response of the different lines. Breeding for improved canopy-level WUE is essential in the face of a changing climate (Hatfield & Dold, 2019). However, if we are aiming to improve crop plant productivity, the WUE must be integrated with yield production and cannot serve as a stand-alone trait. Here, we show how high-throughput phenotyping of a crop's wild relative may gain potential for improving canopy WUE under unfavorable environmental conditions.

5. Conclusions

M82, a domesticated plant, exhibited greater mass and higher increases in absolute transpiration, which resulted in enhanced absolute photosynthesis ($A_n \times$ total plant leaf area) under non-water-limited conditions compared to its wild relative. However, M82 exhibits a more rapid reduction in soil water content under drought stress than Pennellii. Our study revealed that the domesticated crop (M82) had higher morphological and physiological traits (leaf area, g_s) rather than biochemical traits (RuBisCO efficiency). It seems that in modern breeding programs, there has been a preference for high-yielding plants, a selection that inherently involves increased transpiration and, due to the non-linear relationship between photosynthesis and stomatal conductance as elucidated by Wong et al. (1979), this has resulted in a reduction in Water Use Efficiency (WUE). Our findings suggest a tradeoff exists between a plant's productivity and adaptability to different environments. During crop domestication, this balance can shift toward productivity at the expense of responsiveness to the environment. With the increasing scarcity and cost of agricultural resources such as water and fertilizers, future breeding programs should prioritize plants' physiological adaptation to changing environments where resources may be limited.

Funding

The work was funded by the Israel Innovation Authority (grants No. 0002122 and 001897).

CRedit authorship contribution statement

Yaniv Lupo: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Menachem Moshelion:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - review & editing, Supervision, Funding

acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use Generative AI and AI-assisted technologies in the writing process. The authors take full responsibility for the content of the publication.

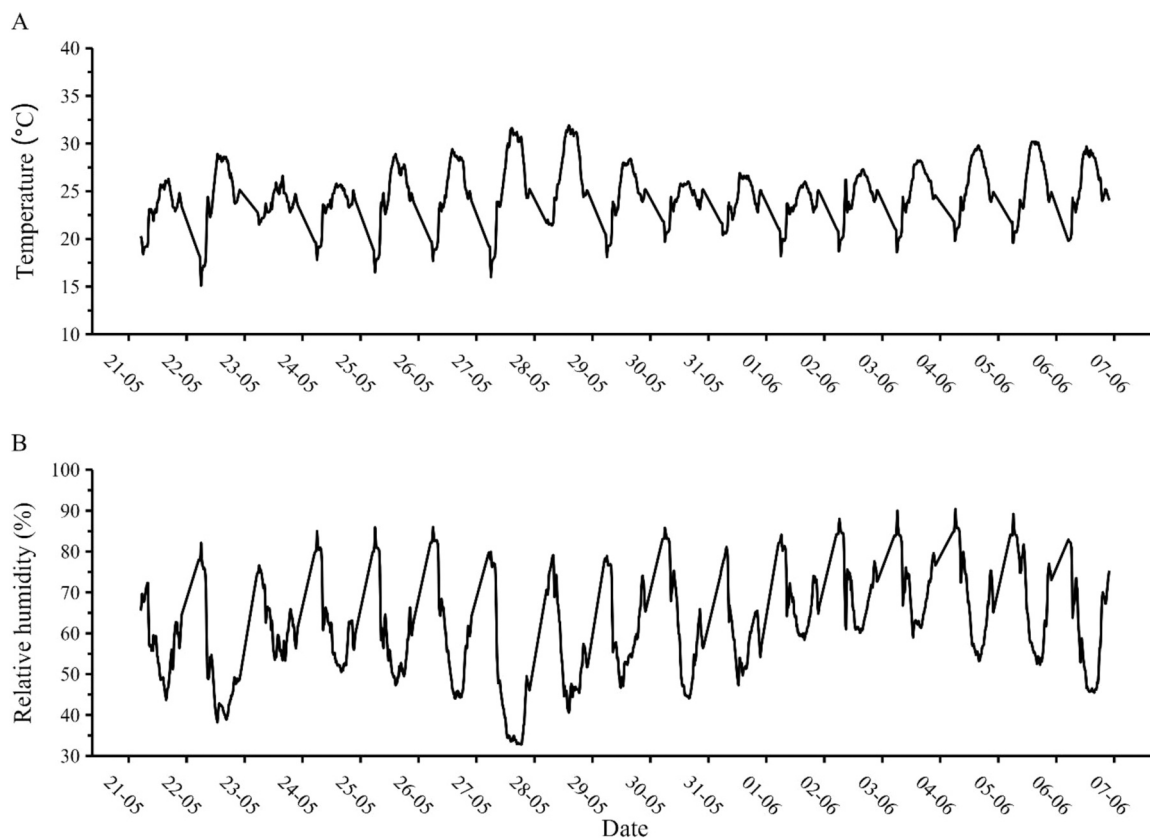
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

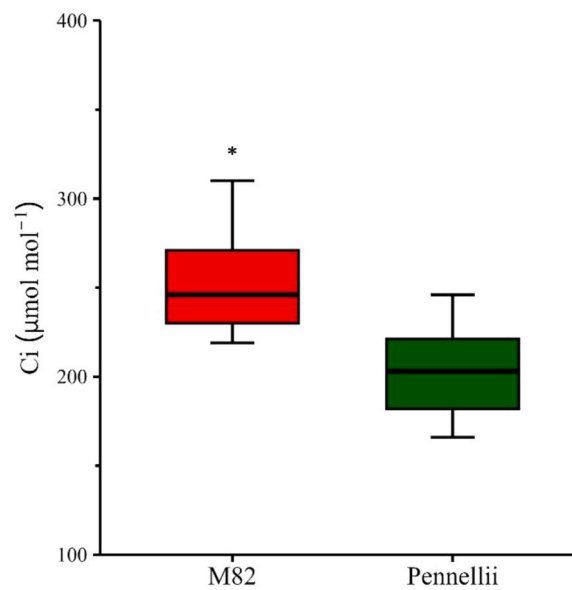
Data availability

Data will be made available on request.

Appendix



Appendix Fig. A1. Temperature and relative humidity between 5 AM and 9 PM during the days of the experiment. Climate conditions were continuously monitored by a weather station located near the plants in the greenhouse.



Appendix Fig. A2. Intercellular CO₂ concentration (Ci; μmol of CO₂ per mol of air) for M82 (red) and Pennellii (green). Asterisks represent significant differences according to Student's *t*-test, $P < 0.05$. $n = 11$ (M82) and 10 (Pennellii).

References

- M. Bacon, Water Use Efficiency in Plant Biology, John Wiley & Sons, 2009.
- Y. Bai, P. Lindhout, Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? *Ann. Bot.* 100 (2007) 1085–1094.
- J.A. Bhat, S. Ali, R.K. Salgotra, Z.A. Mir, S. Dutta, V. Jadon, A. Tyagi, M. Mushtaq, N. Jain, P.K. Singh, G.P. Singh, K.V. Prabhu, Genomic selection in the era of next generation sequencing for complex traits in plant breeding, *Front. Genet.* 7 (2016).
- A. Bolger, F. Scossa, M.E. Bolger, C. Lanz, F. Maumus, T. Tohge, H. Quesneville, S. Aalsekh, I. Sørensen, G. Lichtenstein, E.A. Fich, M. Conte, H. Keller, K. Schneeberger, R. Schwacke, I. Ofner, J. Vrebalov, Y. Xu, S. Osorio, S.A. Aflitos, E. Schijlen, J.M. Jiménez-Gómez, M. Ryngajillo, S. Kimura, R. Kumar, D. Koenig, L. R. Headland, J.N. Maloof, N. Sinha, R.C.H.J. van Ham, R.K. Lankhorst, L. Mao, A. Vogel, B. Arsova, R. Panstruga, Z. Fei, J.K.C. Rose, D. Zamir, F. Carrari, J. J. Giovannoni, D. Weigel, B. Usadel, A.R. Fernie, The genome of the stress-tolerant wild tomato species *Solanum pennellii*, *Nat. Genet.* 46 (2014) 1034–1038.
- J.S. Boyer, Plant Productivity and Environment, *Science* 218 (1982) 443–448.
- M.J. Canny, C.X. Huang, Leaf water content and palisade cell size, *N. Phytol.* 170 (2006) 75–85.
- L. Cattivelli, F. Rizza, F.-W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Marè, A. Tondelli, A.M. Stanca, Drought tolerance improvement in crop plants: An integrated view from breeding to genomics, *Field Crops Res.* 105 (2008) 1–14.
- H. Claeys, D. Inzé, The Agony of Choice: How Plants Balance Growth and Survival under Water-Limiting Conditions, *Plant Physiol.* 162 (2013) 1768–1779.
- A.G. Condon, R.A. Richards, G.J. Rebetzke, G.D. Farquhar, Breeding for high water-use efficiency, *J. Exp. Bot.* 55 (2004) 2447–2460.
- A. Dalal, Z. Attia, M. Moshelion, To Produce or to Survive: How Plastic Is Your Crop Stress Physiology? *Front. Plant Sci.* 8 (2017).
- A. Dalal, I. Shenhar, R. Bourstein, A. Mayo, Y. Grunwald, N. Averbuch, Z. Attia, R. Wallach, M. Moshelion, A Telemetric, Gravimetric Platform for Real-Time Physiological Phenotyping of Plant-Environment Interactions, *JoVE* (2020) 61280.
- K.-J. Dietz, C. Zörb, C.-M. Geilfus, Drought and crop yield, *Plant Biol.* 23 (2021) 881–893.
- N.S. Diffenbaugh, D.L. Swain, D. Touma, Anthropogenic warming has increased drought risk in California, *Proc. Natl. Acad. Sci.* 112 (2015) 3931–3936.
- S.M. Driever, T. Lawson, P.J. Andralojc, C.A. Raines, M.A.J. Parry, Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes, *J. Exp. Bot.* 65 (2014) 4959–4973.
- R.A. Duursma, Plantecophys - an R package for analysing and modelling leaf gas exchange data, *PLOS ONE* 10 (2015), e0143346.
- I. Egea, I. Albaladejo, V. Meco, B. Morales, A. Sevilla, M.C. Bolarin, F.B. Flores, The drought-tolerant *Solanum pennellii* regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration, *Sci. Rep.* 8 (2018), 2791.
- Y. Eshed, D. Zamir, An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL, *Genetics* 141 (1995) 1147–1162.
- L.T. Evans, R.L. Dunstone, Some physiological aspects of evolution in wheat, *Aust. Jnl Bio Sci.* 23 (1970) 725–742.
- FAO, The State of Food and Agriculture 2020: Overcoming water challenges in agriculture, FAO, Rome, Italy, 2017.
- G.D. Farquhar, K. Raschke, On the Resistance to Transpiration of the Sites of Evaporation within the Leaf 1, *Plant Physiol.* 61 (1978) 1000–1005.
- J. Gago, D. de M. Daloso, C.M. Figueroa, J. Flexas, A.R. Fernie, Z. Nikoloski, Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach, *Plant Physiol.* 171 (2016) 265–279.
- E. Galkin, A. Dalal, A. Evenko, E. Fridman, I. Kan, R. Wallach, M. Moshelion, Risk-management strategies and transpiration rates of wild barley in uncertain environments, *Physiol. Plant.* 164 (2018) 412–428.
- S.C. Gosa, A. Koch, I. Shenhar, J. Hirschberg, D. Zamir, M. Moshelion, The potential of dynamic physiological traits in young tomato plants to predict field-yield performance, *Plant Sci.* 315 (2022), 111122.
- S.C. Gosa, Y. Lupo, M. Moshelion, Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: New tools to support pre-breeding and plant stress physiology studies, *Plant Sci.* 282 (2019) 49–59.
- D.K. Großkinsky, J. Svenggaard, S. Christensen, T. Roitsch, Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype knowledge gap, *J. Exp. Bot.* 66 (2015) 5429–5440.
- S. Hajjhashemi, Stomatal Regulation as a Drought-tolerance Mechanism. *Molecular Plant Abiotic Stress*, John Wiley & Sons, Ltd., 2019, pp. 45–64.
- O. Halperin, A. Gebremedhin, R. Wallach, M. Moshelion, High-throughput physiological phenotyping and screening system for the characterization of plant–environment interactions, *Plant J.* 89 (2017) 839–850.
- J.L. Hatfield, C. Dold, Water-use efficiency: advances and challenges in a changing climate, *Front. Plant Sci.* 10 (2019).
- V. Jaramillo Roman, R. van de Zedde, J. Peller, R.G.F. Visser, C.G. van der Linden, E. N. van Loo, High-resolution analysis of growth and transpiration of quinoa under saline conditions, *Front. Plant Sci.* 12 (2021).
- H. Kebede, B. Martin, J. Nienhuis, G. King, Leaf anatomy of two lycopersicon species with contrasting gas exchange properties, *Crop Sci.* 34 (1994) cropscl1994.0011183x003400010019x.
- J. Kumar, A. Pratap, S. Kumar, Phenomics in Crop Plants: Trends, Options and Limitations, Springer India, New Delhi, 2015.
- M.T. Lin, H. Salihovic, F.K. Clark, M.R. Hanson, Improving the efficiency of Rubisco by resurrecting its ancestors in the family Solanaceae, *Sci. Adv.* 8 (2022), eabm6871.
- D.A. Márquez, H. Stuart-Williams, G.D. Farquhar, An improved theory for calculating leaf gas exchange more precisely accounting for small fluxes, *Nat. Plants* 7 (2021) 317–326.
- B. Martin, H. Kebede, C. Rilling, Photosynthetic differences among lycopersicon species and triticum aestivum cultivars, *Crop Sci.* 34 (1994) cropscl1994.0011183x003400010020x.
- L.C. Moyle, Ecological and evolutionary genomics in the wild tomatoes (*solanum* sect. lycopersicon), *Evolution* 62 (2008) 2995–3013.
- C.D. Muir, J.B. Pease, L.C. Moyle, Quantitative genetic analysis indicates natural selection on leaf phenotypes across wild tomato species (*solanum* sect. lycopersicon; solanaceae), *Genetics* 198 (2014) 1629–1643.

- B. Negin, M. Moshelion, The advantages of functional phenotyping in pre-field screening for drought-tolerant crops, *Funct. Plant Biol.* 44 (2016) 107–118.
- V. Orgogozo, B. Morizot, A. Martin, The differential view of genotype–phenotype relationships, *Front. Genet.* 6 (2015).
- A. Razzaq, P. Kaur, N. Akhter, S.H. Wani, F. Saleem, Next-generation breeding strategies for climate-ready crops, *Front. Plant Sci.* 12 (2021).
- R.A. Richards, Selectable traits to increase crop photosynthesis and yield of grain crops, *J. Exp. Bot.* 51 (2000) 447–458.
- E. Rosenqvist, D.K. Großkinsky, C.-O. Ottosen, R. van de Zedde, The Phenotyping Dilemma—The Challenges of a Diversified Phenotyping Community, *Front. Plant Sci.* 10 (2019).
- N. Sade, A. Gebremedhin, M. Moshelion, Risk-taking plants, *Plant Signal Behav.* 7 (2012) 767–770.
- J. Spindel, H. Begum, D. Akdemir, P. Virk, B. Collard, E. Redoña, G. Atlin, J.-L. Jannink, S.R. McCouch, Genomic Selection and Association Mapping in Rice (*Oryza sativa*): Effect of Trait Genetic Architecture, Training Population Composition, Marker Number and Statistical Model on Accuracy of Rice Genomic Selection in Elite, *Tropical Rice Breeding Lines*, *PLoS Genet* 11 (2015), e1004982.
- J.E. Spindel, S.R. McCouch, When more is better: how data sharing would accelerate genomic selection of crop plants, *N. Phytol.* 212 (2016) 814–826.
- G. Tcherkez, How atmospheric oxygen is captured by RuBisCo, *Nat. Rev. Mol. Cell Biol.* 22 (2021), 304–304.
- K.E. Trenberth, A. Dai, G. van der Schrier, P.D. Jones, J. Barichivich, K.R. Briffa, J. Sheffield, Global warming and changes in drought, *Nat. Clim. Change* 4 (2014) 17–22.
- A.L. Villagómez-Aranda, A.A. Feregrino-Pérez, L.F. García-Ortega, M.M. González-Chavira, I. Torres-Pacheco, R.G. Guevara-González, Activating stress memory: eustressors as potential tools for plant breeding, *Plant Cell Rep.* 41 (2022) 1481–1498.
- S.C. Wong, M.J. Canny, M. Holloway-Phillips, H. Stuart-Williams, L.A. Cernusak, D. A. Márquez, G.D. Farquhar, Humidity gradients in the air spaces of leaves, *Nat. Plants* 8 (2022) 971–978.
- S.C. Wong, I.R. Cowan, G.D. Farquhar, Stomatal conductance correlates with photosynthetic capacity, *Nature* 282 (1979) 424–426.
- W. Yan, Y. Zhong, Z. Shanguan, A meta-analysis of leaf gas exchange and water status responses to drought, *Sci. Rep.* 6 (2016), 20917.