

KAÍK FARIA DE SOUZA

USING *Solanum pennellii* INTROGRESSION SUBLINES TO UNDERSTAND THE PHOTOSYNTHETIC ALTERATIONS ASSOCIATED WITH CHROMOSOME 2 QTL IN TOMATO

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Plant Physiology Graduate Program to obtain the degree of *Magister Scientiae*.

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ABSTRACT

SOUZA, Kaik Faria de, M.Sc., Universidade Federal de Viçosa, May, 2023. **Using *Solanum pennellii* introgression sublines to understand the photosynthetic alterations associated with chromosome 2 QTL in tomato.** Adviser: Adriano Nunes Nesi. Co-adviser: Rebeca Patrícia Omena-Garcia

Some portion of the tomato's (*Solanum lycopersicum*) genome is involved on the regulation of photosynthesis. However, due to the complexity, the gene function and the regulation of mechanisms related to photosynthesis are still not fully understood. One of the ways to measure photosynthetic efficiency is through the CO₂ assimilation rate (*A*). Some genomic regions of chromosome 2, termed BINs, have been associated with photosynthesis performance. These BINs were defined through studies with introgression lines (ILs). Thus, looking to investigate the phenotypes related to *A* and associated with the chromosome 2, one IL (IL2-5) and three subILs (IL2-5-2, IL2-5-6, and IL2-5-12) were selected. They delimit overlapping regions in the chromosome 2. These ILs also cover the BIN 2K, which has previously been associated with increases in *A*, starch content and increases in RuBisCO content. Thus, plants of the selected IL and subILs were exposed to an atmosphere enriched with 800 μmol CO₂ mol⁻¹ and a control atmosphere (400 μmol CO₂ mol⁻¹) aiming to unravel the mechanisms associated with increases in *A* and to search new genomic regions of tomato. Additionally, the used lines were subjected to drought stress in order to describe the behavior of this genotypes under this condition. The results suggested that the increase in *A* may be regulated by changes in the biochemical step of photosynthesis, evidenced by the improve in the electron transport rate (*ETR*) while diffusive mechanisms remaining stable. Experiments were established in 2 different years and evidences highlight the region covered by the SubILs (IL 2-5-2 and IL 2-5-6) as a candidate region for gene selection of photosynthesis regulation in tomato. In addition, the IL 2-5-2 kept its leaf water potential higher when compared to M82 when exposed to moderate drought. It did not differ in control conditions.

Keywords: Photosynthesis. Elevated CO₂. Drought. Introgression lines.

RESUMO

SOUZA, Kaik Faria de, M.Sc., Universidade Federal de Viçosa, maio de 2023. **Using *Solanum pennellii* introgression sublines to understand the photosynthetic alterations associated with chromosome 2 QTL in tomato.** Orientador: Adriano Nunes Nesi. Coorientadora: Rebeca Patrícia Omena-Garcia

Uma porção do genoma do tomate (*Solanum lycopersicum*) está provavelmente envolvido na regulação da fotossíntese. Entretanto, devido a complexidade, a função de genes e a regulação de mecanismos relacionados a fotossíntese ainda não são completamente compreendidos. Uma das maneiras de mensurar a eficiência fotossintética é através da assimilação de CO₂ (*A*). Algumas regiões genômicas do cromossomo 2, denominadas BINs, têm sido associadas à performance fotossintética. Esses BINs foram definidos por meio de estudos com linhas de introgressão (ILs). Desse modo, buscando investigar os fenótipos relacionados com *A* associados ao cromossomo 2, foram selecionadas uma IL (IL2-5) e três subILs (IL2-5-2, IL2-5-6, e IL2-5-12) que delimitam regiões de interseção do cromossomo 2. Essas ILs também abrangem o BIN 2K, que foi previamente associado a aumentos em *A*, conteúdo de amido e aumento no conteúdo de RuBisCO. Plantas da IL e subILs selecionadas foram submetidas a uma atmosfera enriquecida com 800 µmol CO₂ mol⁻¹ e uma atmosfera controle (400 µmol CO₂ mol⁻¹) esperando desvendar os mecanismos associados ao aumento em *A* e buscar novas regiões genômicas do tomateiro. Adicionalmente, as linhas utilizadas foram submetidas ao estresse hídrico com objetivo de descrever o comportamento desses genótipos em condição de seca. Os resultados sugerem que o aumento em *A* pode ser regulado pela etapa bioquímica da fotossíntese, evidenciado pelo aumento na taxa de transporte de elétrons (ETR) enquanto mecanismos difusivos permaneceram estáveis. Experimentos foram implantados em dois anos diferentes e evidências destacaram a região que abrange as SubILs (IL 2-5-2 e IL 2-5-6) como uma região candidata para seleção de genes da regulação da fotossíntese em tomate. Adicionalmente, a IL 2-5-2 manteve seu potencial hídrico foliar mais alto que M82 quando expostos a seca moderada. Em condição controle, não houve diferença significativa.

Palavras-chave: Fotossíntese. Elevado CO₂. Seca. Linhagens de introgressão.

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1 INTRODUCTION

Tomato is a widely cultivated plant whose, according to the Food and Agricultural Organization of the United Nations (FAO), represents more than 15% of the world's vegetable production (<https://www.fao.org/faostat>). Consequently, it is widely used as a research model plant. Some aspects of the species facilitate genetic and physiological studies. They are related to its short life cycle (90 to 120 days) and reproductive characteristics once it can reproduce by a cross and self-pollination. The tomato plant is also considered relatively easy to transform and its genome is already completely sequenced (Rothan et al., 2019; Sato et al., 2012).

The domesticated tomato (*Solanum lycopersicum*) is a member of the Solanaceae family and was first reported in South America (Bolivia, Chile, Peru, and Ecuador). Also, other weedy species from the same family escaped worldwide are reported (Peralta et al., 2008). The stress-tolerant wild tomato with green fruits *Solanum pennellii* is known for its large genetic variability. Its origin also comes to Andean South America. This species has agronomic value when functioning as a donor for *Solanum lycopersicum* (Bretó et al., 1993; Bolger et al., 2014). The crossing between *Solanum pennellii* and *Solanum lycopersicum* (cv M82) allowed the development of a population of 76 Introgression lines (ILs) and each obtained almost isogenic line contains a small chromosome fragment from the *S. pennellii* LA0716 inserted in the *S. lycopersicum* M82 background (Eshed and Zamir, 1995).

This population provide tomatoes the genetic variability which was decreased during vegetable domestication. That comes as a powerful tool for plant breeding programs (Eshed and Zamir, 1995; Zamir, 2001). It allowed great advance in quantitative trait loci (QTL) mapping through the molecular marchers. Actually, there are more than 3000 mapped QTLs obtained through the use of the 76 previously generated *S. pennellii* ILs. Besides, over 350 traits have been pointed out, such as plant morphology, abiotic stress tolerance, enzyme activities, and yield (Alseekh et al., 2013). Illustrating, the QTL (Brix 9-2-5) of *S. pennellii* contains a genomic region of 484 base pairs encoding an apoplastic invertase exclusive to flowers and fruits. The generated protein is associated with the substrate biding-site of the enzyme. The obtained results are fruits with higher glucose and fructose contents and, consequently, higher Brix (Fridman et al., 2000). Leaf metabolites quantitative trait

loci (mQTLs) in tomatoes have recently been demonstrated (Oliveira-Silva et al., 2017; Nunes-Nesi, 2019). In such a manner QTL mappings are important and recurrent in tomato species (Rothan et al., 2019). Even though the tomato genome is largely studied, the functions of specific genes are not fully understood (Sato et al., 2012). Aiming to better disclose the gene specificities Alseekh et al. (2013) generated and described 285 sublines (subILs) available for QTL or gene mapping, up to date covering 75% of the *S. pennellii* genome.

Oliveira-Silva et al (2017) mapped 41 QTLs termed BINs distributed in 33 overlapping regions by using the 76 mentioned *S. pennellii* IL population. Of those, 11 BINs stood out as interesting for selecting candidate genes involved in the regulation of photosynthesis in tomato. The *S.pennellii* variability could help to explain the regulation of the photosynthetic related processes and their interactions on the leaf of tomato plants. There was one specific chromosome's 2 region, named BIN 2K (south marker: SGN-M46; north marker SGN-M122, for details see Oliveira et al., 2017 supplementary data - tomato-EXPEN 2000 mapping population available at <http://solgenomics.net>), responsible for changes in starch levels and increments on the CO₂ assimilation rate (A).

The identification of QTLs that control photosynthesis is crucial to selecting valuable accesses for plant breeding programs, as it is related to yield (Dariva et al., 2020). The improvement of the photosynthetic capacity can be achieved in many ways such as through increasing light capture and carbon fixation (Lana-Costa et al., 2020), by improving sunlight conversion rate and mesophyll conductance, or even via modulation of the photorespiration pathway (Singer et al., 2019; Ort et al., 2015). More recently, the high photosynthesis attributed to the BIN 2K has been studied by Lana-costa et al (2020). The high photosynthetic rates were associated with photochemical (maximum carboxylation velocity and maximum electron transport rate) and biochemical, such as changes in RuBisCO content (Lana-Costa et al., 2020).

The photosynthetic process is directly influenced by the ambient concentration of CO₂, especially in plants that do not have a carbon concentrating mechanism, like C3 plants as tomatoes. Furthermore, drought is one of the stresses that most affect the growth, development, and production of plants. Under drought conditions, plants close their stomata, in order to maintain cell turgescence. However, this comes at a

cost to photosynthesis once CO₂ capture is compromised (Ahuja et al., 2010). This becomes even more important in a climate changing world in which these stressful situations will be more often.

For all that, in order to further investigate the photosynthesis on BIN 2K region and, additionally, a larger portion of the chromosome 2, we carefully elected one IL (IL 2-5) (Eshed and Zamir, 1995), previously associated to photosynthetic metabolism (Oliveira-Silva et al., 2017; Lana-Costa et al., 2020, and 3 subILs (IL 2-5-2; IL 2-5-6; IL 2-5-12) described by Alseekh et al (2013) which have a region of overlap with BIN 2K and IL. The plants were cultivated in open top chambers (OTCs) under ambient (aCO₂ ~400 μmol CO₂ mol⁻¹) and elevated (eCO₂ ~800 μmol CO₂ mol⁻¹) CO₂ atmosphere, and submitted to drought stress. This study proposes an evaluation of carbon uptake traits, including biomass, anatomical, and growth-related parameters on the mentioned lines. We also launch the hypothesis that there is an intersection region on the *S.pennellii* subILs associated with efficiency in the photosynthetic process.

2 MATERIAL AND METHODS

2.1 Plant material

The *S.pennellii* introgression lines population was generated by Eshed and Zamir et al (1995) and the subILs were described by Alseekh et al (2013). They are the IL 2-5 and the subILs 2-5-2, 2-5-6, and 2-5-12. These lines have been selected once they have previous associations with enhanced photosynthesis and cover the BIN 2K genomic region. The IL and subILs were compared against the control genotype *S. lycopersicum*, cultivar M82. Pictures of the lines and a scheme of the genomic regions delimited by the selected lines highlighting the BIN 2K are demonstrated in the figure 1.



Figure 1: Adapted from Aloseekh et al. (2013). Tomato's Chromosome 2 (red bar) location, size and identity of 1 *S. pennellii* LA0716 introgression line (IL 2-5) (dark green bar) and 3 sub-ILs (IL2-5-2; IL2-5-6; IL2-5-12) (light green bars) anchored to the tomato linkage map. The blue dash indicates the genomic region BIN 2K (Oliveira-Silva et al., 2017) previously associated with increases in the CO₂ assimilation rate and starch content. The yellow (BIN 2J) dash indicates the genomic region delimited by the overlapping of the introgression lines and sublines. The Tomato-EXPEN 2000 mapping population is available at (<http://solgenomics.net/>). Map distances are indicated in centiMorgans (cM). Tomato 45-days-old plants grown under elevated CO₂. (A) *Solanum lycopersicum* M82; (B) IL 2-5; (C) subIL 2-5-2; (D) subIL 2-5-6; (E) subIL 2-5-12.

2.2 Experimental conditions for elevated CO₂

The seeds of the first season experiment were germinated in a greenhouse at Viçosa-MG (642 m in altitude, 20°45` S latitude and 42°51` W longitude). Rooted seedlings were transferred to 3 dm³ pots filled with commercial substrate Tropstrato HT Via Verde®. Each plant was supplemented with 8 g of fertilizer (N:4; P₂O₅:14; K₂O:8). They grew under naturally fluctuating conditions of light intensity ($\pm 1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), temperature of $\pm 30^\circ\text{C}$, and relative air humidity of $\pm 60\%$. After 10 days of acclimatizing period the seedlings were then transferred to the OTCs with the following specifications, 1,1m diameter and 1,2m height. The experiment was implemented in four OTCs. Two were continuously maintained at the current ambient

level of CO₂ (400 μmol CO₂ mol⁻¹) and the other two were maintained at elevated levels of CO₂ (800 μmol CO₂ mol⁻¹). The CO₂ concentration in the chambers was monitored during the whole day with a portable CO₂ meter AZ-77535 for 21 days. The CO₂ injection worked from 6 a.m. to 6 p.m.

2.3 Environmental conditions for drought stress

Seedlings of the second season experiment were transferred to 3 dm³ pots filled with commercial substrate Tropstrato HT Via Verde[®] in the same location indicated above. Each one was supplemented with 8 g of fertilizer (N:4; P₂O₅:14; K₂O:8). The plants grew under naturally fluctuating conditions of light intensity ($\pm 1000 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$), temperature of $\pm 30^\circ\text{C}$, and relative air humidity of $\pm 60\%$. After 10 days of acclimatizing period the seedlings were then transferred to the already specified OTCs. The experiment was implemented in four OTCs. The plants were randomized and half were daily irrigated and the other half were exposed to 11 days of drought treatment.

2.4 Gas exchange and chlorophyll a fluorescence measurements

The net CO₂ assimilation rate (A), stomatal conductance to water vapor (g_s), and internal CO₂ concentration (C_i), transpiration (E) were executed simultaneously by adopting an open-flow infrared gas-exchange analyzer system equipped with an integrated fluorescence chamber (IRGA, LI-COR Inc. LI-6400XT; NE). The environmental conditions in the 2 cm² leaf chamber were maintained at 25 °C, leaf-to-air vapor pressure deficit ranged from 1,2 to 1,8 kPa, flow rate of 300 mol s⁻¹, 0,5 stomatal ratio for amphistomatic leaves, and saturating light intensity of 1000 μmol photons m² · s⁻¹. Also, the amount of blue light was set in 10% of photosynthetic photon flux density (PPFD) in order to maximize stomatal opening. These measurements were performed from 7 am to 1 pm. From that, we calculated the intrinsic water-use efficiency (WUE_i), defined as the ratio between A and g_s , and the water-use efficiency (WUE), defined as the ratio between A and E . The rates of dark respiration (R_d) were determined from 8 p.m. to 2 a.m.

In terms of fluorescence parameters in dark-adapted leaves, saturating white light pulses of $8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied for 0.8 s to determine maximum fluorescence emission (F_m). In light-adapted leaves, the steady-state fluorescence yield (F_s) was measured and followed by a saturating white light pulse ($8000 \mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s) for the determination of the light-adapted maximum fluorescence (F_m'). The actinic light was then turned off, and far-red illumination was applied ($2 \mu\text{mol m}^{-2} \text{s}^{-1}$) to measure the light-adapted initial fluorescence (F_0') (Aucique-Perez et al., 2014). The values were used to determine the coefficients of photochemical quenching (q_p), non-photochemical quenching (q_n) and the electron transport rate (ETR), as previously described (Aucique-Perez et al., 2014; Schreiber et al., 1995).

2.5 Growth-related analyses

Plants were measured in four days intervals, starting with the transplanting date. The final height (measured from the base to the tip of the terminal bud) was determined at the end of the experiment. The relative growth rate in height (RGR-h) is calculated according to Hunt (1982) (equation 1). Using a caliper, the stem diameter was measured three times throughout the experiment, first on the day of transplanting and ten days after transplanting and then, at the end of the experiment.

The dry matter weight (DW) and fresh matter weight (FW) were measured when plants reached 45 days old. Leaves, stems, and roots were separately placed and weighted for determining FW, then taken to an oven with forced air circulation at a temperature of 60°C until reaching constant weight, around seven days, for determining DW. Nine leaf discs with a pre-determined area were taken to a forced-air circulation oven at the temperature of 60°C as well. The specific leaf area (SLA) was resolved according to equation (2) (Hunt, 2002). Subsequently, total leaf area (TLA) was estimated from the SLA and DW according to equation (3) (Mielke et al., 1995).

(Equation 1) $\text{RGR (cm.cm}^{-1}\text{.day)} = \frac{\ln(\text{final height (cm)}) - \ln(\text{initial height (cm)})}{\text{Number of days}}$

(Equation 2) $SLA (m^2.g^{-1}) = \text{Area of the leaf discs } (m^2) / \text{Discs dry matter } (g)$

(Equation 3) $TLA (m^2) = \text{Leaf DW } (g) * \text{Area of the leaf discs } (m^2) / \text{Discs dry matter } (g)$

2.6 Leaf water potential measurements

The leaf water potential was determined at both predawn (LWP_{pd}) and midday (LWP_m) using a Scholander pressure chamber (Model 1000, PMS instruments, USA). The penultimate leaflet was detached and placed into the chamber for the measurements (Scholander et al., 1964). 45 days-old plants were evaluated after four and eight days of stress.

2.7 Relative water content analyses

The relative water content (RWC) was determined by detaching and immediately weighing nine leaf discs (Fresh weight - FW) from the base, middle, and apex of the plant. Then discs were placed in Petri dishes filled with distilled water for four hours to obtain the turgid weight (TW). The dry weight (DW) was measured after three days in a forced air circulation oven at the temperature of 60°C. Finally, the RWC was calculated according to equation (4) (Sharp et al., 1989).

(Equation 4) $RWC = (FW-DW)/(TW-DW) * 100$

2.8 Water loss determination

For water loss measurements, the penultimate leaflet of the third completely expanded leaf was detached from five different plants at 90 days-old growth under control conditions and the petiole floated in MES-KCl buffer (5 mM KCl, 10 mM MES, 50 μ M $CaCl_2$, pH 6.15). They were incubated under continuous illumination (120 μ E.m⁻² s⁻¹) at 25°C for at least 2 hours to induce stomatal opening. Next, the water loss was determined through gravimetric over four hours at 25 minutes intervals.

Water loss was then calculated as a percentage of the initial fresh weight (Omena-Garcia et al., 2019; Robledo et al., 2020).

2.9 Leaf anatomy analyses

Fully expanded mid-leaflets were harvested and fixed in FAA₅₀ solution for 24 h then transferred to a 50% ethyl ethanol solution. Pieces of the central region were dehydrated in ethanolic series and merged in methacrylate (LeicaHistoresin, LEICA, Chicago, IL, USA). Blocks were sectioned through an automatic rotative microtome (Leica RM 2155, UK). The sections were colored using toluidine blue and placed in synthetic resin (Permount, FISHER SCIENTIFIC, Fair Lawn, NJ, USA). Four sections per biological replicate (five replicates per genotype) were photographed (10x objective) in a microscope (Zeiss Scope A1) equipped with AxioCam 105 color image capture system. The leaf thickness, palisade parenchyma (PP) and spongy parenchyma (SP) thickness, upper epidermis (UE), and lower epidermis (LE) thickness were measured using the Image-Pro Plus software. Four measures per section were taken so consisting in a mean value of 16 observations per parameter per replicate.

For leaf venation leaflets from the mid-region were detached and merged in methanol for 48 h. Then, samples were transferred to lactic acid and stocked in an oven at a temperature of 65 °C for 24 h. Five biological replicates were photographed (10x objective) in a microscope (Zeiss Scope A1) equipped with AxioCam 105 color image capture system.

2.10 Statistical Analyses

The experiments were conducted in a completely randomized design. An analysis of variance (ANOVA) was applied to the data set which was performed assuming random effects for genotype (G) x atmosphere environment ([CO₂]) interaction and for genotype (G) x water availability (WA) interaction. For each variable, means were calculated for each of the lines and the M82 control and compared by Tukey's test at 5% probability ($p < 0.05$). The variability of the results is expressed as the mean \pm standard error (SE). Five biological replicates (n = 5) were

utilized for each analysis in elevated CO₂, except for the dry matter accumulation, specific leaf area, and total leaf area (n = 6). For the drought experiment six biological replicates (n = 6) were used, but for gas exchange and fluorescence (n = 4). Statistical analyses and charts were executed on the R statistical software (www.r-project.org).

3 RESULTS

3.1 Photosynthetic related parameters in elevated CO₂

The CO₂ assimilation rates were higher in IL2-5 and IL2-5-6 than the parental M82 in both conditions ambient CO₂ (aCO₂) and elevated CO₂ atmosphere (eCO₂) (Figure 2A). Besides, the IL2-5 and IL2-5-6 also exhibited superior g_s in comparison with M82 in aCO₂, what did not happen in eCO₂ that g_s rates did not vary significantly among the lines (Figure 2C). Additionally, the IL2-5-6 exhibited lower g_s in eCO₂ versus itself in aCO₂. An opposite pattern was observed in the IL2-5-12 (Figure 2C). The IL2-5-12 displayed higher R_d when compared to M82 in both conditions.

The IL2-5-6 also exhibited greater F_v'/F_m' (Figure 3A) and q_n (Figure 3C) than M82 in aCO₂ and eCO₂. The ETR differs among the lines as well with the IL2-5 and IL2-5-2 standing against M82 in both conditions.

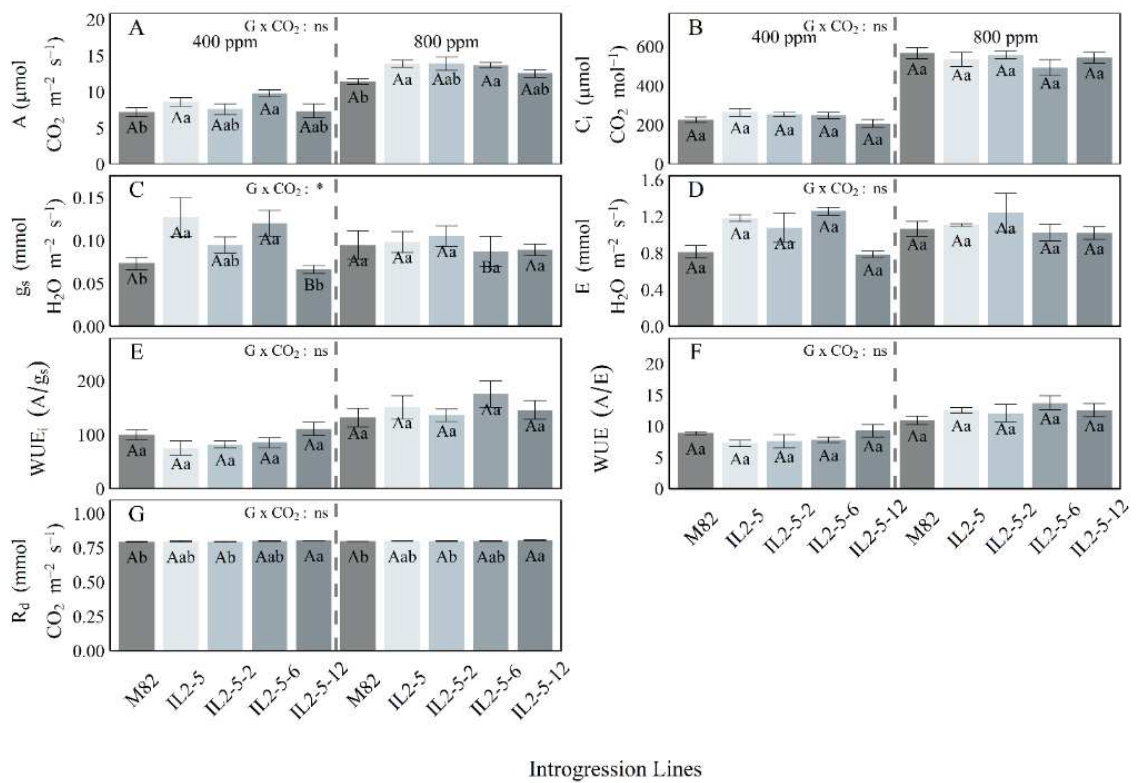


Figure 2: Gas exchange of tomato plants treated with two different CO₂ concentrations (control - 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; and elevated CO₂ atmosphere - 800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) for 21 days growth in open top chambers. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO₂ assimilation rate (A); internal CO₂ concentration (B); stomatal conductance to water vapor (C); transpiration (D); intrinsic water-use efficiency (E); water-use efficiency (F); rates of dark respiration (G). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 5$).

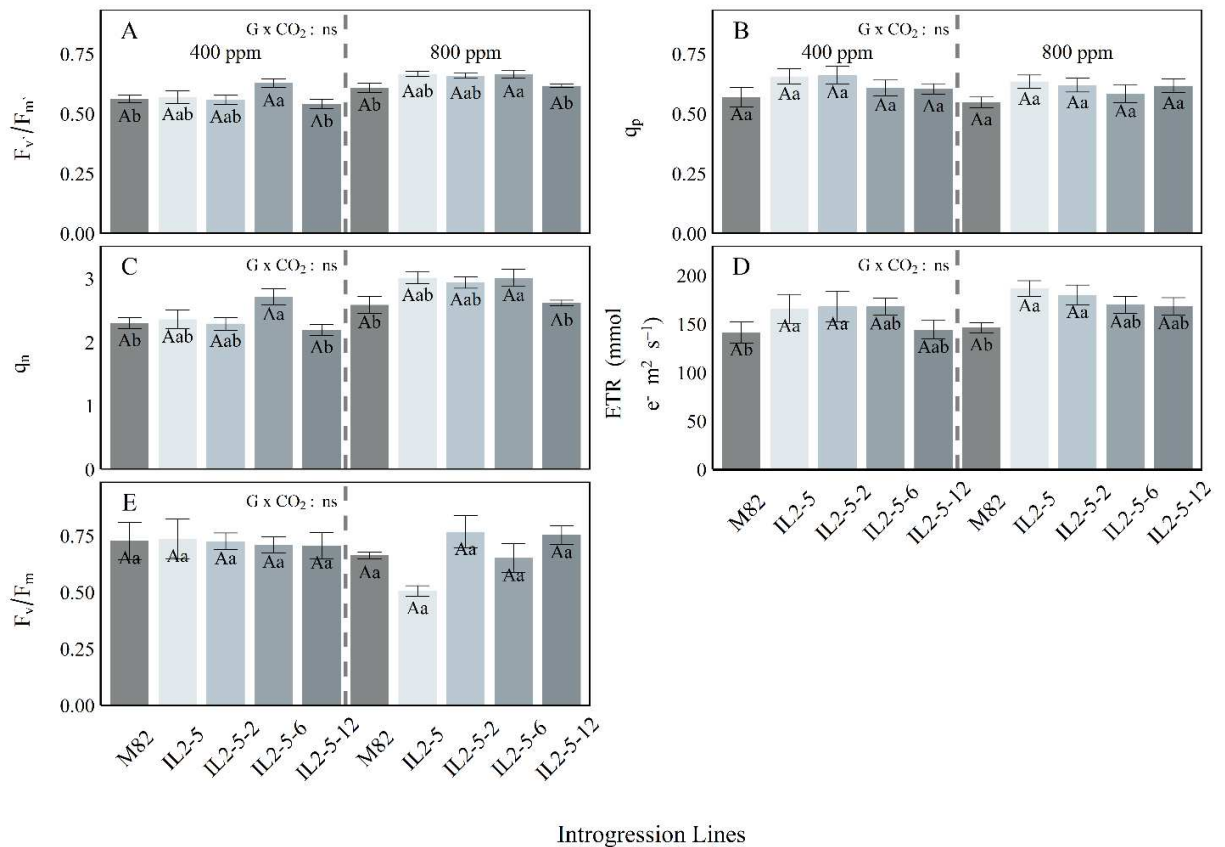


Figure 3: Fluorescence and *ETR* of tomato plants treated with two different CO₂ concentrations (control - 400 μmol CO₂ mol⁻¹; and elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alosekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. PSII maximum efficiency (**A**); photochemical quenching (**B**); non-photochemical quenching (**C**); the electron transport rate (**D**); Maximum quantum efficiency of PSII photochemistry (**E**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 5$).

3.2 Dry matter accumulation and growth in elevated CO₂

The IL2-5-2 displayed lower stem DW than M82 in aCO₂ (Figure 4D). Additionally, the IL2-5-2 exhibited higher root DW than M82 in eCO₂. After plants were taken to chambers with eCO₂ atmosphere, the IL2-5, and IL2-5-2, presented higher relative growth rates in height (RGR-h) than M82 (Figure 5A). The lines IL2-5, IL2-5-2, and IL2-5-6 increased their RGR-h (Figure 5A) and their height (Figure 5B) in eCO₂ when comparing to themselves in aCO₂. The IL2-5 exhibited smaller stem diameter than M82 in aCO₂ and eCO₂ (Figure 5D).

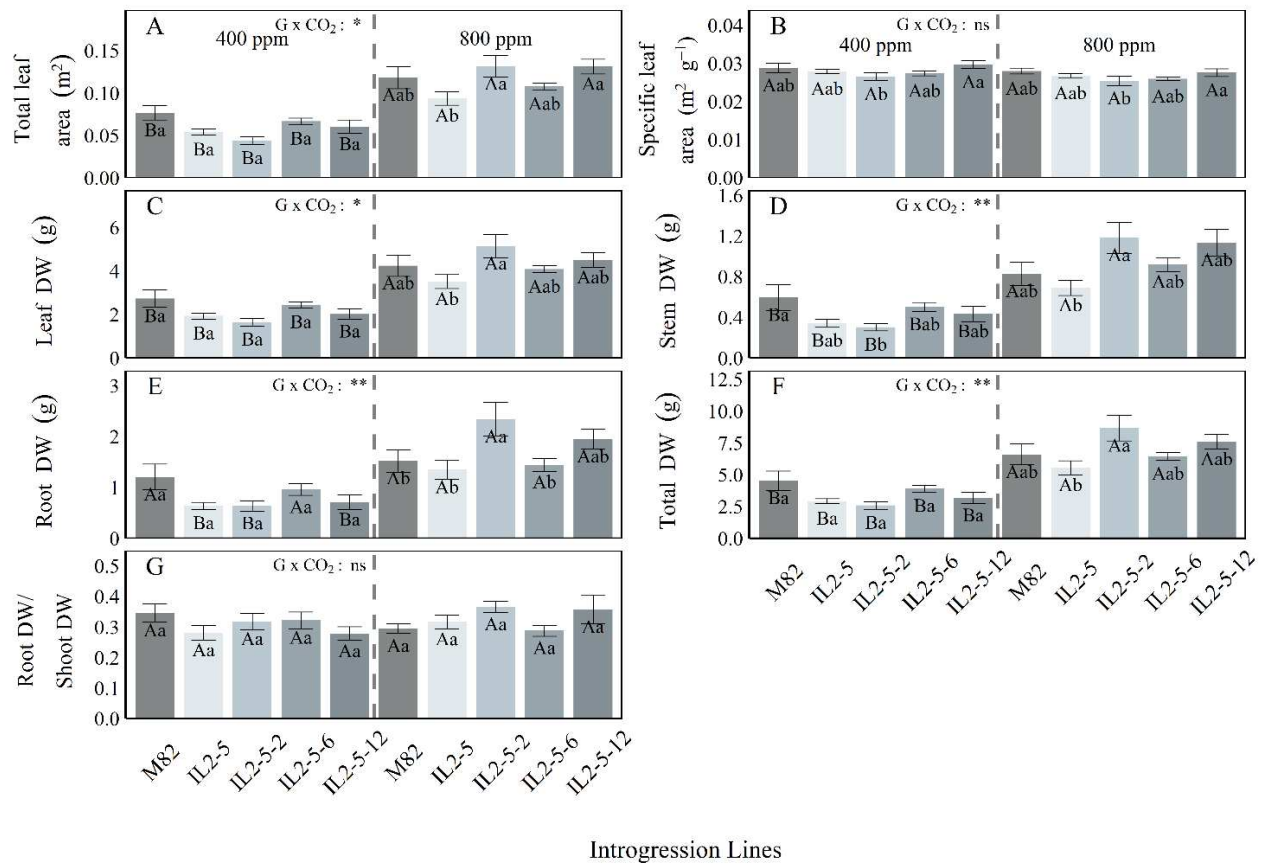


Figure 4: Dry matter accumulation of tomato plants treated with two different CO₂ concentrations (control - 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; and elevated CO₂ atmosphere - 800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Total leaf area (A); specific leaf area (B); leaf dry weight (C); stem dry weight (D); root dry weight (E); total dry weight (F); root dry weight / shoot dry weight (G). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 6$).

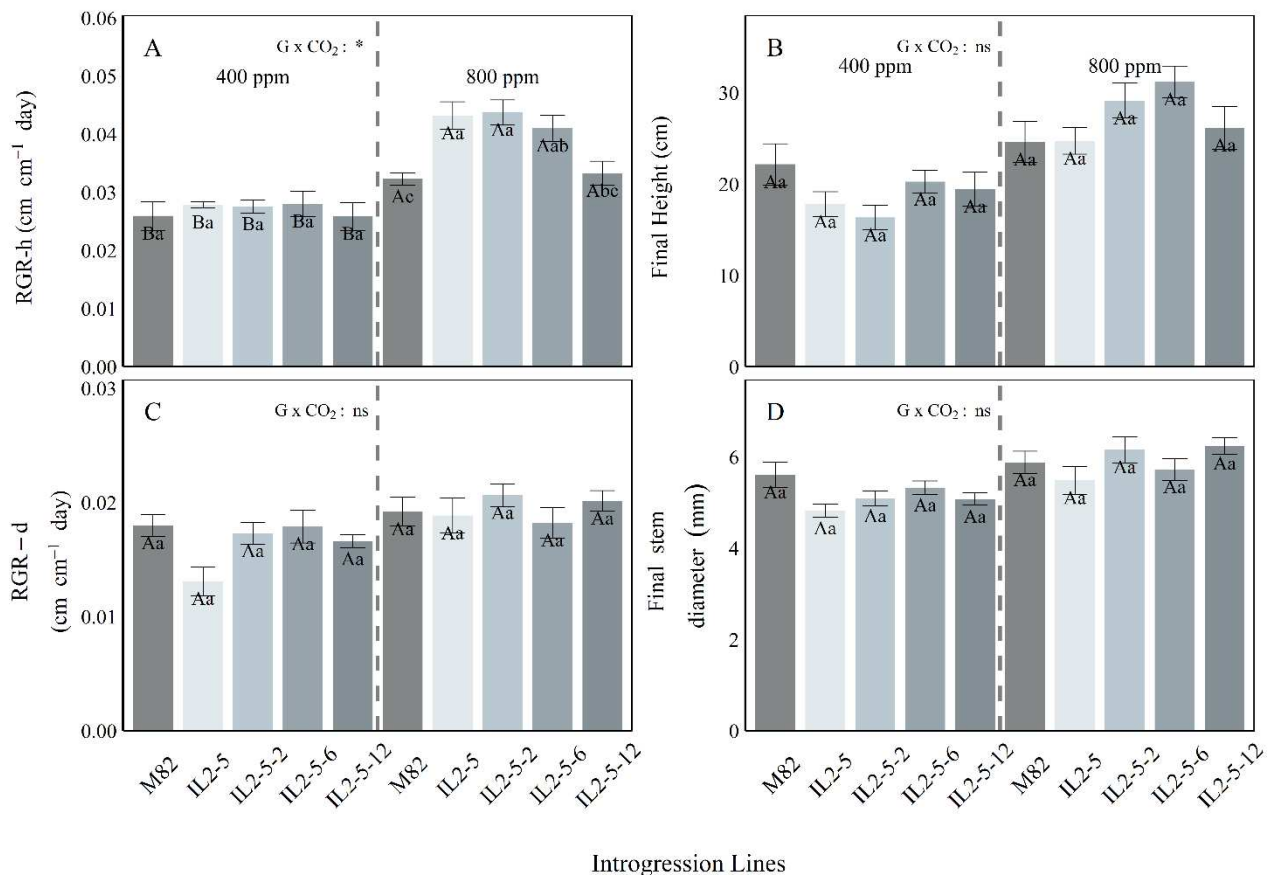


Figure 5: Growth traits of tomato plants treated with two different CO₂ concentrations (control - 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; and elevated CO₂ atmosphere - 800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Relative growth rate in height (A); final height (B); relative growth rate in stem diameter (C); stem diameter (D). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) \times atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 5$).

3.3 Leaf anatomical traits in elevated CO₂

Considering the leaf anatomy parameters, only the palisade parenchyma (PP) thickness vary among the lines. The IL2-5-12 exhibited a thicker PP than M82 in eCO₂ (Figure 6C). Furthermore, IL 2-5-6 and IL2-5-12 exhibited a thicker PP in eCO₂ against themselves in aCO₂ (Figure 6C).

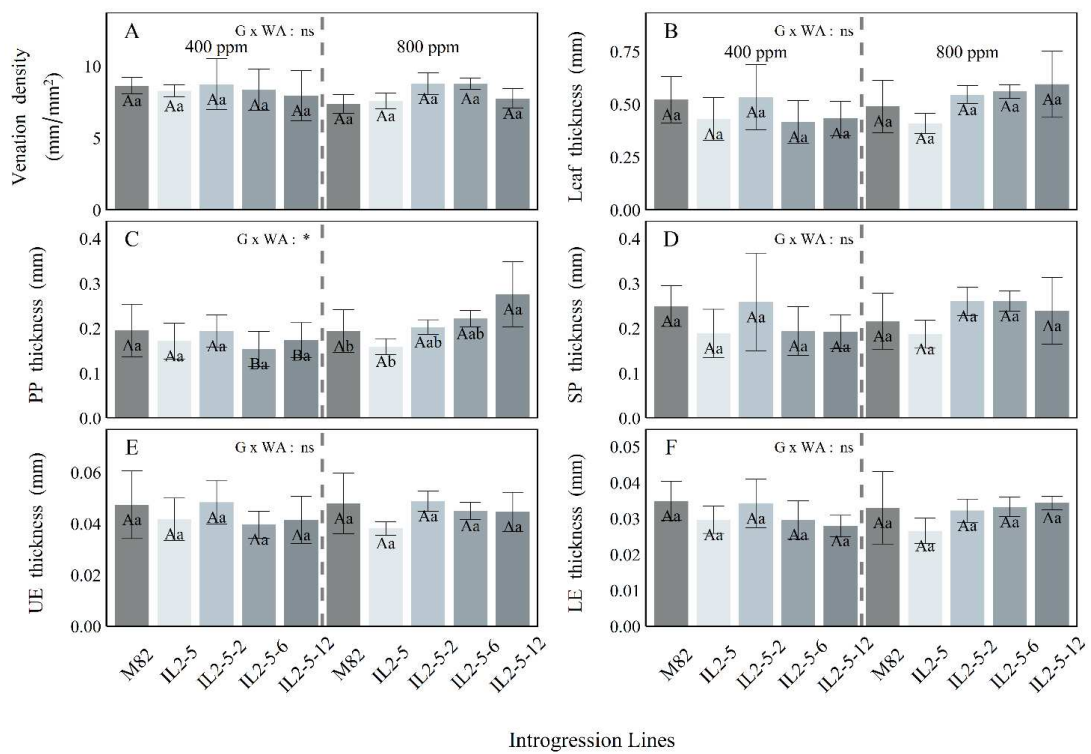


Figure 6: Leaf anatomical traits of tomato plants treated with two different CO₂ concentrations (control - 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; and elevated CO₂ atmosphere - 800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three sublILs (IL2-5-2; IL2-5-6; IL2-5-12) (Aseeikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Leaf venation density (A); leaf thickness (B); palisade parenchyma thickness (C); spongy parenchyma thickness (D); upper epidermis thickness (E); lower epidermis thickness (F). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 5$).

3.4 Photosynthesis-related parameters under different drought levels

After four days under drought, the photosynthesis-related parameters did not differ among the lines (Figure 7), but was reduced in all lines under drought, with exception of WUE and WUE_i . Nonetheless, the IL2-5-12 stands out as higher ETR (Figure 8D) and q_p (Figure 8B) than the parental M82 under both control and four-day drought stress condition. Then, under eight days of drought, the ETR was higher for the IL2-5-2 against M82 (Supplementary Figure 4D).

Under 11 days of drought, the M82 plants exhibited higher WUE_i than the IL 2-5-6 and IL 2-5-12 (Supplementary figure 5E). The M82 also had greater WUE than IL 2-5-6 and IL 2-5-12 in drought conditions. These two lines also exhibited the lowest WUE performance under drought when compared to control conditions

(Supplementary figure 5F). In this way, the IL2-5-12 displayed lower F_v/F_M than M82 in 11 days under drought. In addition, all lines but M82 and 2-5-2 decreased their F_v/F_M in drought conditions (Supplementary figure 6E).

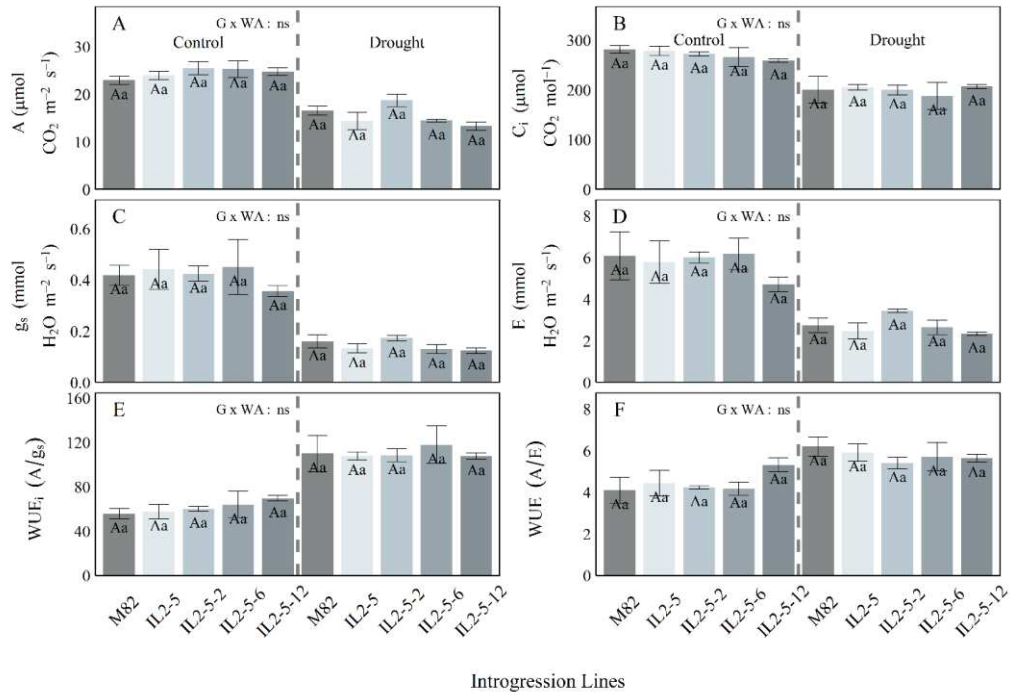


Figure 7: Gas exchange of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - four days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO₂ assimilation rate (A); internal CO₂ concentration (B); stomatal conductance to water vapor (C); transpiration (D); intrinsic water-use efficiency (E); water-use efficiency (F). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).

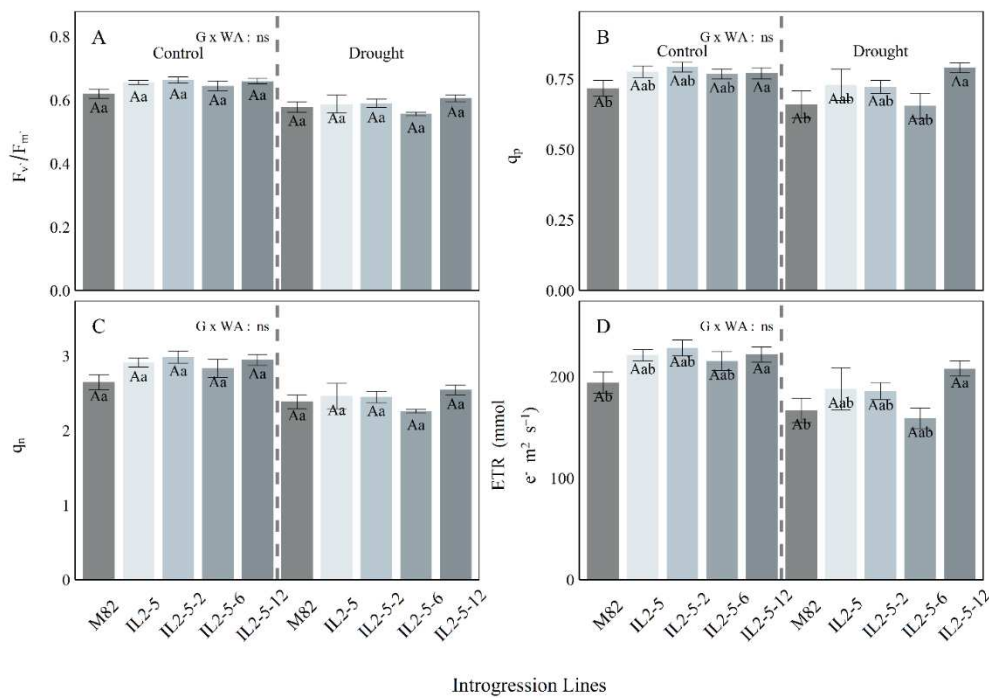


Figure 8: Chlorophyll a fluorescence and ETR of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - four days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. PSII maximum efficiency (**A**); photochemical quenching (**B**); non-photochemical quenching (**C**); the electron transport rate (**D**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (-); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).

3.5 Plant water status in response to drought stress

The leaf water potential was determined at both predawn (LWP_{pd}) and at midday (LWP_m) (Figure 9). Under four days of drought the IL 2-5-2 presented a higher LWP_{pd} than M82 (Figure 9A). In control condition, no difference was observed. Also, all lines presented higher LWP_{pd} when irrigated (Figure 9A). At midday, any of the studied lines differed from the parental M82 at any condition. In addition, there was no interaction between control and drought (Figure 9C).

When exposed to eight days of drought, no line differed in LWP_{pd} when compared to M82 at both condition. However, all of them decreased their LWP_{pd} when in water restriction (Figure 9B). At noon, no interaction was observed in LWP_m at control and drought treatment (Figure 9D). The relative water content for the lines did not differ from the parental in both condition. In addition, no difference was

observed in the RWC when comparing drought and control conditions (Supplementary figure 7). No line differed from the parental M82 still visual differences were observed. The IL 2-5-2 stood out as exhibiting higher leaf water loss than M82 and the IL 2-5-12 and IL 2-5-6 displayed the lower values (Supplementary figure 8).

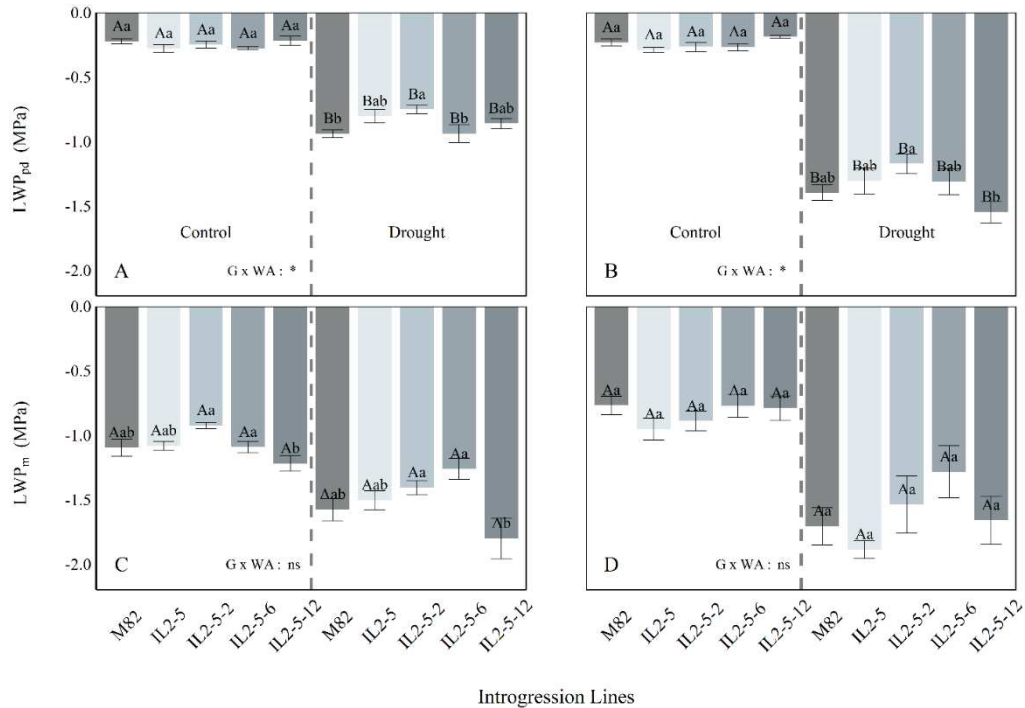


Figure 9: Leaf water potential of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - four days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Leaf water potential at pre-dawn (**A**); leaf water potential at midday (**B**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (-); significant at 1% (-+). Values are presented as mean \pm SE ($n = 6$).

3.6 Dry matter accumulation and growth of ILs under drought stress

The dry matter accumulation, specific leaf area (Figure 10), and growth-related parameters (Figure 11) were influenced by the drought treatment. Nevertheless the genotypes did not differ amongst them. The lines did not differ from the parental M82 in any condition for TLA (Figure 10A), Leaf DW (Figure 10C), Root DW (Figure 10E), Total DW (Figure 10F), and Root DW/Shoot DW ratio (Figure 10G). Reduced SLA was observed in all lines when compared to the parental genotype (Figure 10B). The accumulation of DW in the stem was superior in the lines

IL2-5, IL2-5-2, IL2-5-6, and IL2-5-12 compared to M82 in both irrigation conditions (Figure 10D).

Before plants were exposed to water restriction, IL2-5 and IL2-5-6 exhibited higher RGR-h than M82 (Figure 11A). Whatsoever, IL2-5-12 displayed lower RGR-h before treatment than the parental M82 (Figure 11A). The M82 individuals were shorter than the IL2-5, IL2-5-2, and IL2-5-6 ones at the end of the experiment in irrigated and non-irrigated plants (Figure 11C). Also, the IL2-5-2 showed higher RGR-d than M82 in both conditions (Figure 11D).

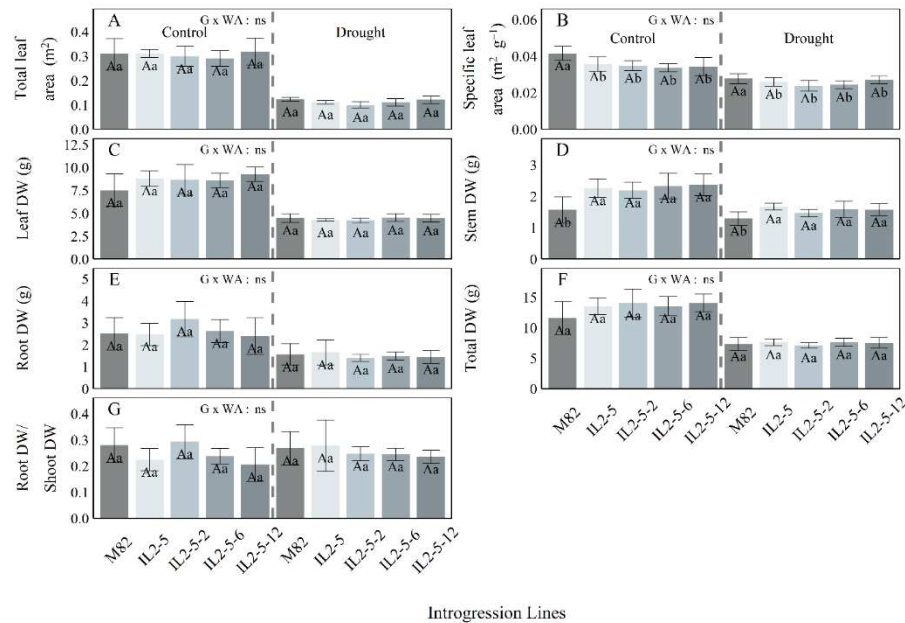


Figure 10: Dry matter accumulation of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - 11 days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Aalseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Total leaf area (A); specific leaf area (B); leaf dry weight (C); stem dry weight (D); root dry weight (E); total dry weight (F); root dry weight / shoot dry weight (G). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 6$).

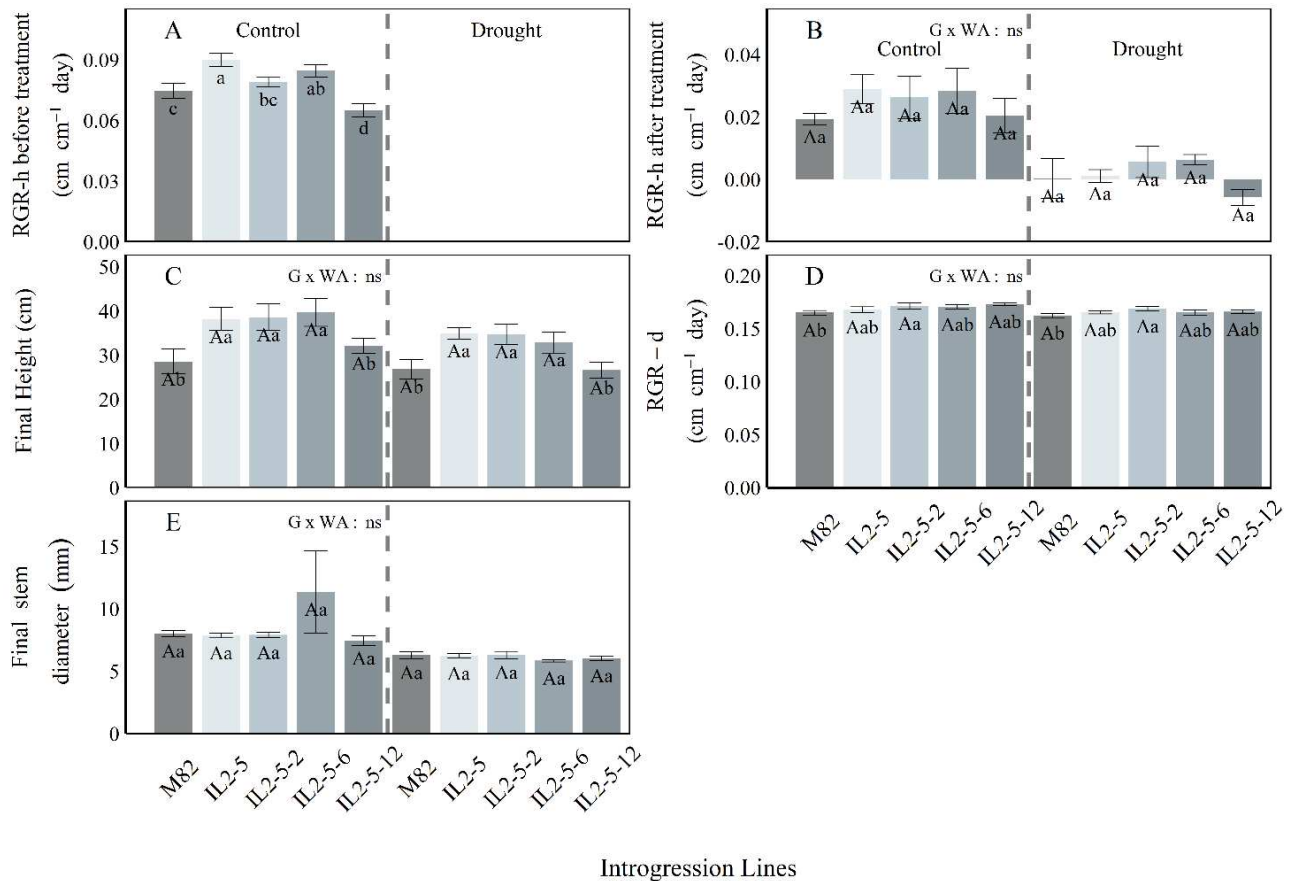


Figure 11: Growth of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - 11 days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Relative growth rate in height before treatment (A); Relative growth rate in height after treatment (B); height (C); relative growth rate in stem diameter (D); stem diameter (E). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (+); significant at 1% (**). Values are presented as mean \pm SE ($n = 6$).

4 DISCUSSION

4.1 QTLs associated with high photosynthetic efficiency in chromosome 2 of *Solanum pennellii*

The IL2-5 and IL2-6 were reported as genomic regions of the tomato's chromosome 2 responsible for the regulation of CO₂ assimilation rate (*A*) (Oliveira et al., 2017). The overlapping of *S. pennellii* introgressed genomic regions in these lines delimits a region in the chromosome 2 named BIN 2K that is also associated with starch accumulation in leaves (Oliveira et al., 2017). In order to better understand the regulation of the mechanisms behind the higher photosynthetic efficiency observed in these lines, Lana-Costa et al (2020) investigated the BIN 2K under elevated CO₂ atmosphere, which conditions lead to reduced biochemical limitations of photosynthesis (Von Caemmerer and Farquhar, 1981). The obtained results revealed changes in the biochemical machinery (maximum carboxylation velocity, and Rubisco content), and *ETR* at the same time the diffusive components remained unaltered.

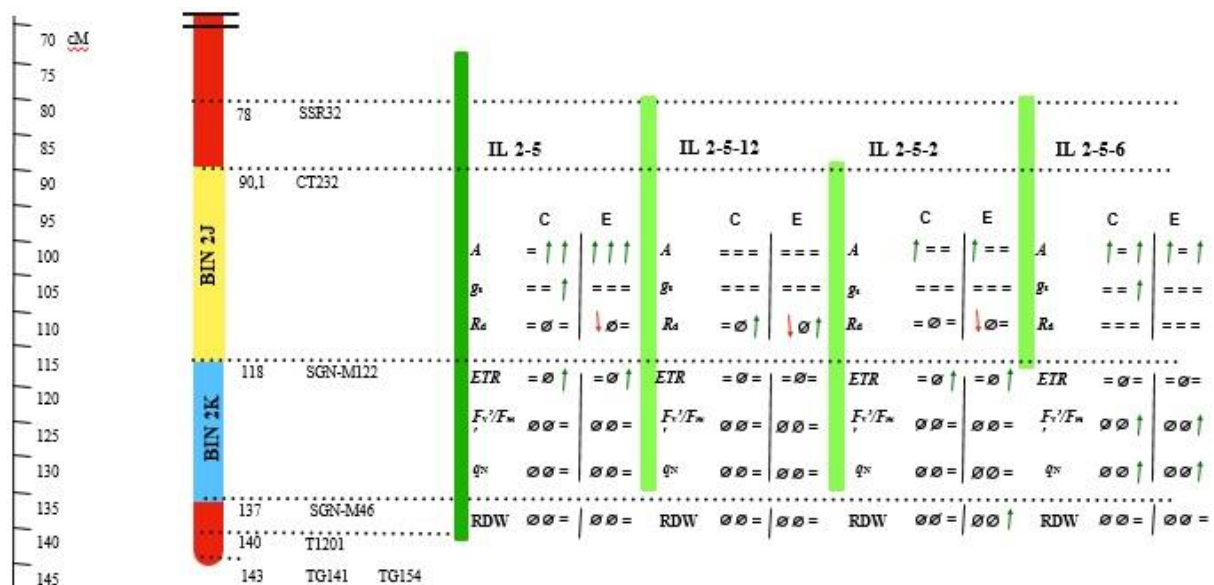


Figure 12: Quantitative trait locus map of significant changes in lines growing under control atmosphere - 400 μmol CO₂ mol⁻¹ and elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹. Tomato's Chromosome 2 (red bar) genomic regions of 1 *S. pennellii* LA0716 introgression lined (IL2-5; dark green bar) and 3 subILs (IL2-5-2; IL2-5-6; IL2-5-12; light green bars) anchored to the tomato linkage map. The blue dash indicates the genomic region named BIN 2K previously associated with increases in the CO₂ assimilation rate and starch content (Oliveira-Silva et al., 2017). The yellow dash (BIN 2K1) indicates the genomic region delimited by the overlapping of the IL2-5-2 and IL2-5-6. The turquoise (BIN 2K2) dash is delimited by the overlapping of the IL2-5 and IL 2-5-2. The Tomato-EXPEN 2000

mapping population is available at (<http://solgenomics.net/>). Map distances are indicated in centiMorgans (cM). CO₂ assimilation rate (*A*); stomatal conductance to water vapor (*g_s*); rates of dark respiration (*R_d*); the electron transport rate (*ETR*) PSII maximum efficiency (*F_v'/F_m'*); non-photochemical quenching (*q_N*); root dry weight (RDW); control atmosphere - 400 μmol CO₂ mol⁻¹ (C); elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹ (E). Changes in comparison to M82 (*p* < 0.05) by the Tukey test are illustrated by the arrows (green means increase and red decrease). Equal sign means no changes. The crossed circle means no data available. Each column represent data from different experiments, available at the supplementary material.

The ILs and subILs evaluated in the present of study did not exactly delimit the overlapping of BIN 2K, but the genomic region is covered by the IL2-5, IL2-5-2 and IL2-5-12. The overlapping regions of the studied introgression lines and sublines delimit an additional BIN, the BIN 2J. A total of 29 traits related to the photosynthetic performance of tomato plants were evaluated and associated with these genomic regions. In this way, looking for differences against M82, the lines and subILs displayed significant variation in 10 traits, including the *A*, *ETR*, and root dry weight (Supplementary table 2), which were eligible for QTL selection. The criteria for QTL selection used in this study were two: (i) the genomic region covered by at least two ILs displaying the same differences against M82 and (ii) the results repeated in at least two independent experiments. For the elevated CO₂ treatment, we gathered data available from three experiments (experiment 1 - supplementary figure 1; experiment 2 - supplementary figure 2 and supplementary table 1; and experiment 3 - supplementary table 3) executed in three different years for further comparative physiological, biometric, and genomic analyses. In this study, the use of plants from *S. pennellii* ILs and subILs grown under elevated CO₂ condition reveals a new QTL associated with increases in the *A* and in the *ETR* (BIN 2J) (Figure 12).

The *S. pennellii* has been reported as presenting lower *A* than *S. lycopersicum* M82 when grown in similar environments (Carrari et al., 2003; Nunes-Nesi et al., 2005). However, from a bulk of 76 *S. pennellii* ILs, 16 displayed higher *A* than M82 and no ILs presented lower *A*. (Oliveira-Silva et al., 2017). In agreement, in this study, no ILs and subILs exhibited lower *A* and three out of five presented higher *A* than M82 (Figure 12). The higher *A* observed in some of these ILs was partially related to stomatal conductance (Oliveira-Silva et al., 2017). However, it was later found that higher *A* on the BIN 2K was better explained by changes in the higher maximum carboxylation velocity, maximum electron transport rate, and increases in the RuBisCO content (Lana-Costa., 2020). The present study confirmed the

importance of the ETR (Figure 3) on the regulation of photosynthesis associated with chromosome 2 once diffusive mechanisms remain unclear.

4.2 QTLs associated with drought stress responses in chromosome 2 of *Solanum pennellii*

In general, drought can be very harmful to life especially to plants that are sessile organisms. Further, C3 plants like tomatoes are more sensitive to water deficit once they have no CO₂ concentration mechanism. The use of ILs plays an important role in this subject through accessing the wild variability. New populations of introgressed lines on tomato beyond the *S. pennellii* were recently reported (REF...). The *Solanum sitiens* is a tomato relative from the Atacama desert known as wild nightshade and have been studied for drought tolerance in tomato (Chetelat et al., 2019). Additionally, a new population from *Solanum lycopersicoides* was also described by Powell et al., (2022). However, the stress-tolerant wild tomato *S. pennellii* is largely reported in studies regarding drought conditions once its origin also comes to the arid environment Andean South America (Bolger et al., 2014). Working as a donor for *S. lycopersicum*, the *S. pennellii* ILs could be useful for plant breeding under drought in a climate-changing world. A comparative analysis has been placed in ILs under water limited conditions. The water use efficiency was associated to the ratio of trichomes to stomata, indicating an important role in drought tolerance on this species (REF...). Additionally, the trichome density and stomatal size were traits that differ from the parental *S. lycopersicum*. On the other hand, photosynthetic parameters like as assimilation of CO₂ and g_s remained unaltered (Galdon-Armero et al., 2018) just like in the present study (Figures 7, Supplementary figures 3, and 5). In addition, the drought tolerance in tomato was also associated to lower stomatal density (Chanamé, 2016).

The *S. pennellii* was previously compared to the *S. lycopersicum* to understand the photosynthetic mechanisms and changes were observed in the stomatal frequency and distribution (REF...). Beyond, differences were observed in the leaf thickness and air space volume in the leaf tissue (Kebede et al, 1994). Changes in anatomic traits as the palisade parenchyma thickness were also observed in the present study (Figure 6). Additionally, Kebede et al (1994) noted that

the trichomes were fewer and longer in the *S. pennellii*, the chlorophyll amount and RuBisCO activity were lower than *S. lycopersicum*, even the *A* remained the same. In the current study the RubisCO activity and quantification were not performed, but the chlorophyll *a* fluorescence parameters were altered in some level (Figures 4, 9, and 13).

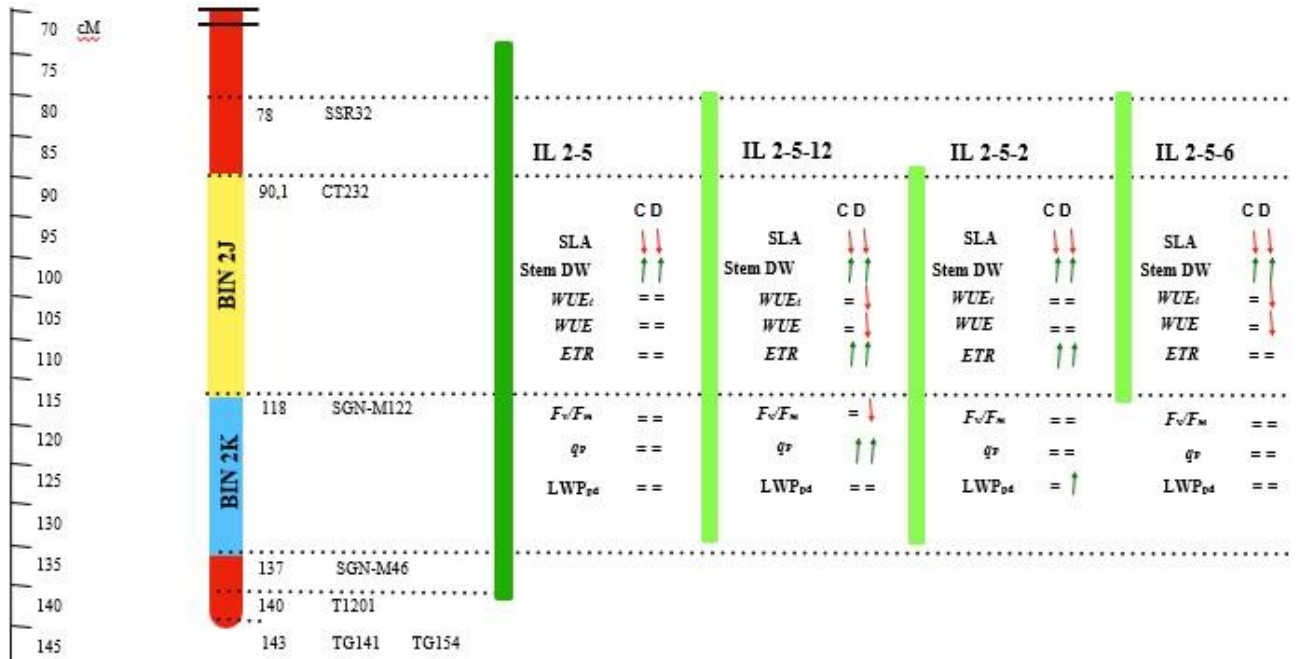


Figure 13: Quantitative trait locus map of significant changes. Tomato's Chromosome 2 (red bar) genomic regions of *S. pennellii* LA0716 introgression lined (IL2-5; dark green bar) and three sublines (IL2-5-2; IL2-5-6; IL2-5-12; light green bars) anchored to the tomato linkage map. The blue dash indicates the genomic region named BIN 2K previously associated with increases in the CO₂ assimilation rate and starch content (Oliveira-Silva et al., 2017). The yellow dash (BIN 2K1) indicates the genomic region delimited by the overlapping of the IL2-5-2 and IL2-5-6. The turquoise (BIN 2K2) dash is delimited by the overlapping of the IL2-5 and IL 2-5-2. The Tomato-EXPEN 2000 mapping population is available at (<http://solgenomics.net/>). Map distances are indicated in centiMorgans (cM). Specific leaf area (SLA); dry weight (DW); intrinsic water-use efficiency (WUE_i); water-use efficiency (WUE); the electron transport rate (ETR); maximum quantum efficiency of PSII photochemistry (F_v/F_m); photochemical quenching (q_p); leaf water potential at pre-dawn (LWP_{pd}); control - daily-irrigated plants (C); drought - non-irrigated plants (D). Changes in comparison to M82 ($p < 0.05$) by the Tukey test are illustrated by the arrows (green means increase and red decrease). Equal sign means no changes, available at the supplementary material.

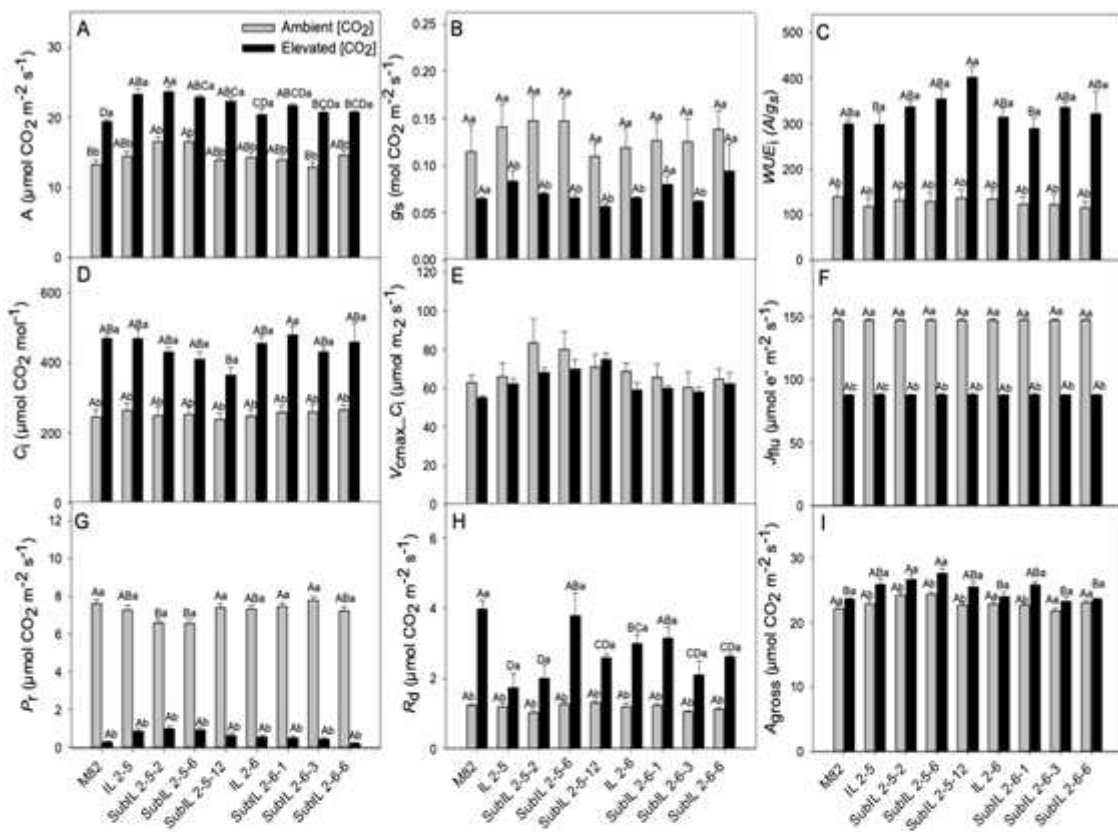
Once the *S. pennellii* is originated from arid regions and has already been reported as drought-tolerant, the present study focused on a large description of the behavior of the introgression lines and sublimes (IL2-5, IL2-5-2, IL2-5-6, and IL2-5-12) under drought. The photosynthetic mechanisms played on different role under this condition when compared to the parental M82 (Figures 7, and Supplementary figures 3- 6). Additionally, the biomass accumulation and growth parameters also displayed no difference under drought and control (Figure 10 and 11). The difference was

observed in the LWP_{pd} (Figure 9) with the IL2-5-2 outstanding versus M82 under drought. Also, the IL2-5-6 and IL2-5-12 exhibited lower WUE and WUE_i when compared to M82 under 11 days of drought (Supplementary figure 6).

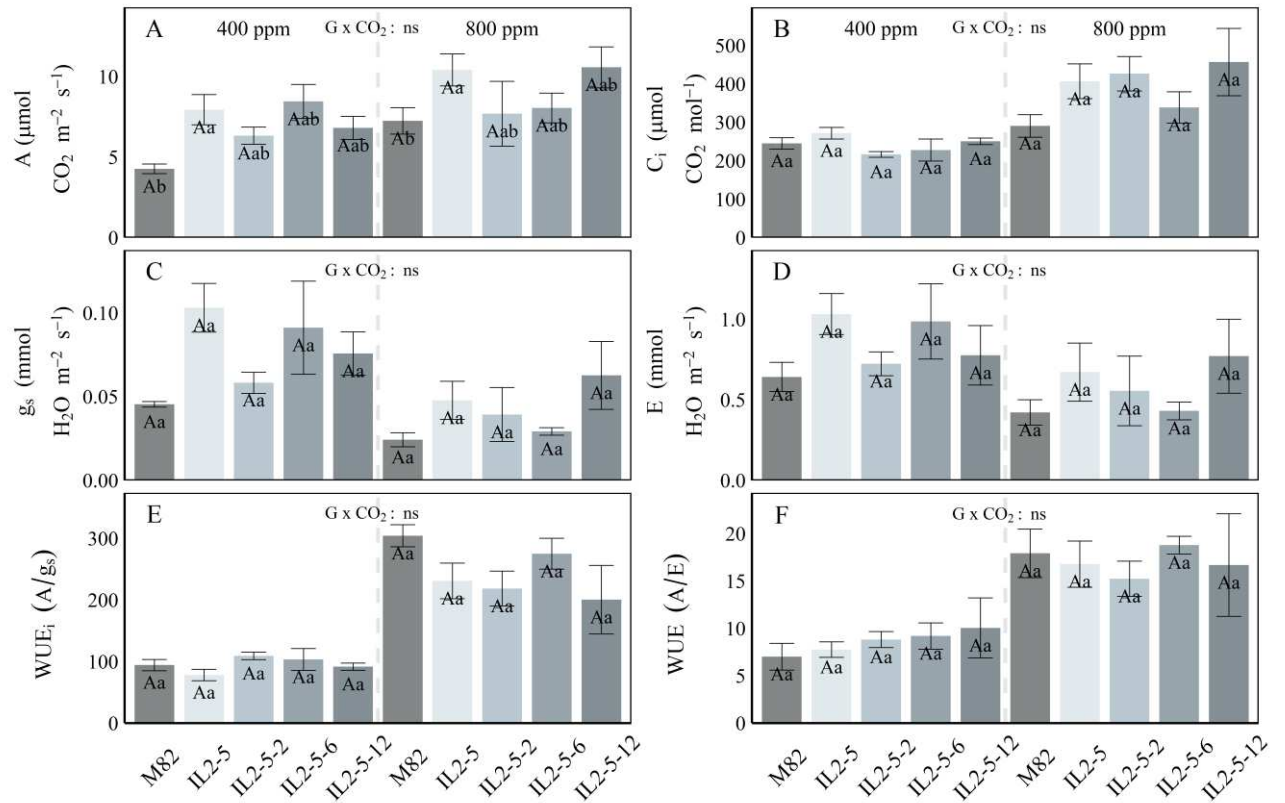
CONCLUSIONS

Taken together, our results show that alterations in the photosynthetic capacity of subILs are explained by *ETR* and both characteristics are associated with two regions of chromosome 2 of *S. pennellii*, BIN 2K and BIN 2J. Furthermore, the intersection of the introgression lines allowed the bidding the genomic region, the BIN 2J associated with increases in the stem dry matter (Stem DW) and *ETR* and decreases in the SLA. Even though the present study accomplished some answers behind the drought tolerance in the *S. pennellii* ILs, further studies are necessary to completely unravel the tolerance mechanisms on tomato. Anatomic and stomatal analyses could strongly support the understanding of it and support plant breeding programs focused on Solanaceae. Additionally, it is important to identify the genes from the studied BINs to better specify the genetic basis for the high photosynthesis and drought resistance in tomato plants.

SUPPLEMENTARY MATERIAL



Supplementary figure 1: Photosynthesis of tomato plants treated with two different CO₂ concentrations (control - 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; and elevated CO₂ atmosphere - 800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) for 21 days. The IL 2-5, IL 2-6 (Eshed and Zamir, 1995), six subILs (IL2-5-2; IL2-5-6; IL2-5-12; IL2-6-1; IL2-6-3; IL2-6-6) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Net CO₂ assimilation rate (A), stomatal conductance to water vapor (B), intrinsic water-use efficiency (C) internal CO₂ concentration (D), maximum carboxylation velocity (E), electron transport rate (F), photorespiration (G), rates of dark respiration (H), gross CO₂ assimilation rate (I). Capital letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Lowercase letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. When there is no difference among lines neither treatments letters are hide. Values are presented as mean \pm SE (n) = (4).



Supplementary figure 2: Gas exchange of tomato plants treated with two different CO₂ concentrations (control - 400 μmol CO₂ mol⁻¹; and elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO₂ assimilation rate (A); internal CO₂ concentration (B); stomatal conductance to water vapor (C); transpiration (D); intrinsic water-use efficiency (E); water-use efficiency (F). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x atmosphere environment (CO₂) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE (n = 3).

Supplementary table 1: Gas exchange of tomato plants treated with two different CO₂ concentrations (control - 400 μmol CO₂ mol⁻¹; and elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹) for 21 days. The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO₂ assimilation rate (*A*); internal CO₂ concentration (*C_i*); stomatal conductance to water vapor (*g_s*); transpiration (*E*); intrinsic water-use efficiency (*WUE_i*); water-use efficiency (*WUE*). Lowercase letters compare means that differ between the genotypes within a treatment ($P < 0,05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($P < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) × (CO₂) atmosphere environment interaction. Non-significative (ns); significative at 5% (*); significative at 1% (**). Values are presented as means ±SE (n = 3).

Trait	G x CO ₂	Control - 400 μmol CO ₂ mol ⁻¹					Elevted CO ₂ - 800 μmol CO ₂ mol ⁻¹				
		M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12	M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12
<i>A</i> (μmol CO ₂ m ⁻² s ⁻¹)	ns	4,27 ± 0,30 Ab	7,94 ± 0,94 Aa	6,33 ± 0,53 Anb	8,46 ± 1,05 Anb	6,81 ± 0,72 Anb	7,25 ± 0,82 Ab	10,41 ± 1,00 Aa	7,69 ± 2,01 Anb	8,05 ± 0,93 Anb	10,57 ± 1,27 Anb
<i>C_i</i> (μmol CO ₂ mol ⁻¹)	ns	245,22 ± 14,69 An	271,83 ± 14,98 An	216,62 ± 7,44 An	227,93 ± 28,60 An	250,91 ± 8,50 An	290,83 ± 29,37 An	406,96 ± 45,23 An	426,54 ± 44,74 An	338,76 ± 40,98 An	457,09 ± 87,55 An
<i>g_s</i> (μmol H ₂ O m ⁻² s ⁻¹)	ns	0,045 ± 0,002 An	0,103 ± 0,015 An	0,058 ± 0,006 An	0,091 ± 0,028 An	0,076 ± 0,013 An	0,024 ± 0,004 An	0,048 ± 0,011 An	0,034 ± 0,016 An	0,029 ± 0,002 An	0,063 ± 0,020 An
<i>E</i> (mmol H ₂ O m ⁻² s ⁻¹)	ns	0,642 ± 0,091 An	1,033 ± 0,128 An	0,725 ± 0,074 An	0,987 ± 0,234 An	0,777 ± 0,185 An	0,422 ± 0,078 An	0,673 ± 0,181 An	0,556 ± 0,216 An	0,432 ± 0,056 An	0,772 ± 0,230 An
<i>WUE_i</i> (A/ <i>g_s</i>)	ns	94,47 ± 9,11 An	78,37 ± 9,35 An	109,36 ± 6,14 An	103,58 ± 17,85 An	91,86 ± 5,80 An	304,43 ± 17,82 An	231,06 ± 28,93 An	218,75 ± 28,26 An	275,25 ± 25,11 An	200,59 ± 55,74 An
<i>WUE</i> (A/ <i>E</i>)	ns	7,02 ± 1,42 An	7,76 ± 0,82 An	8,83 ± 0,85 An	9,20 ± 1,39 An	10,06 ± 3,16 An	17,92 ± An	16,79 ± 2,44 An	15,24 ± 1,86 An	18,79 ± 0,94 An	16,69 ± 5,41 An

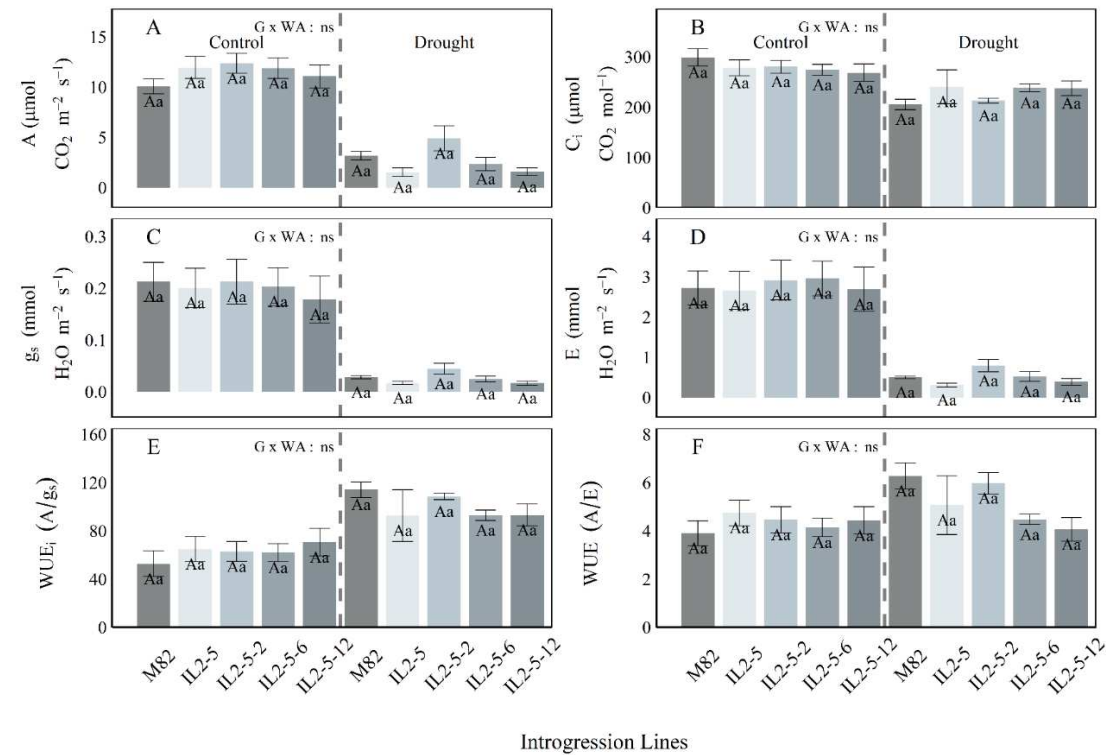
Supplementary table 2: Photosynthesis related parameters of tomato plants treated with two different CO₂ concentrations (control - 400 μmol CO₂ mol⁻¹; and elevated CO₂ atmosphere - 800 μmol CO₂ mol⁻¹) for 21 days. The table displays data from the IL2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82). CO₂ assimilation rate (*A*); internal CO₂ concentration (*C*_i); stomatal conductance to water vapor (*g*_s); transpiration (*E*); intrinsic water-use efficiency (*WUE*_i); water-use efficiency (*WUE*); rates of dark respiration (*R*_d); the electron transport rate (*ETR*); PSII maximum efficiency (*F*_v/*F*_m); Maximum quantum efficiency of PSII photochemistry (*F*_v/*F*_m) photochemical quenching (*q*_P); non-photochemical quenching (*q*_N); total leaf area (TLA); specific leaf area (SLA); dry weight (DW); relative growth rate in height (RGR-h); relative growth rate in stem diameter (RGR-d); palisade parenchyma (PP); spongy parenchyma (SP); upper epidermis (UE); lower epidemic (LE). Lowercase letters compare

Trait	G x CO ₂	Control - 400 μmol CO ₂ mol ⁻¹					Elevated CO ₂ - 800 μmol CO ₂ mol ⁻¹				
		M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12	M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12
<i>A</i> (μmol CO ₂ m ⁻² s ⁻¹)	ns	7,24 ± 0,62 Ab	8,62 ± 0,62 Aa	7,61 ± 0,73 Aab	9,85 ± 0,47 Aa	7,38 ± Aab	11,44 ± 0,41 Ab	13,91 ± 0,45 Aa	13,94 ± 0,93 Aab	13,69 ± 0,40 Aa	12,56 ± 0,57 Aab
<i>C</i> _i (μmol CO ₂ mol ⁻¹)	ns	227,87 ± 13,78 Aa	264,61 ± 21,28 Aa	255,88 ± 10,80 Aa	248,67 ± 15,30 Aa	208,01 ± 19,56 Aa	566,08 ± 28,90 Aa	534,55 ± 34,69 Aa	557,08 ± 18,97 Aa	493,39 ± 40,00 Aa	542,67 ± 28,34 Aa
<i>g</i> _s (μmol H ₂ O m ⁻² s ⁻¹)	*	0,074 ± 0,007 Ab	0,128 ± 0,023 Aa	0,095 ± 0,009 Aab	0,120 ± 0,015 Aa	0,066 ± 0,005 Bb	0,095 ± 0,016 Aa	0,099 ± 0,012 Aa	0,106 ± 0,012 Aa	0,087 ± 0,018 Ba	0,089 ± 0,007 Aa
<i>E</i> (mmol H ₂ O m ⁻² s ⁻¹)	ns	0,815 ± 0,068 Aa	1,186 ± 0,035 Aa	1,078 ± 0,161 Aa	1,260 ± 0,043 Aa	0,787 ± 0,034 Aa	1,067 ± 0,083 Aa	1,106 ± 0,014 Aa	1,243 ± 0,209 Aa	1,026 ± 0,091 Aa	1,020 ± 0,067 Aa
<i>WUE</i> _i (A/ <i>g</i> _s)	ns	100,23 ± 8,27 Aa	75,34 ± 12,87 Aa	81,98 ± 6,77 Aa	85,74 ± 9,32 Aa	110,82 ± 12,17 Aa	132,03 ± 17,71 Aa	151,08 ± 21,22 Aa	136,27 ± 12,11 Aa	175,72 ± 24,19 Aa	146,09 ± 17,21 Aa
<i>WUE</i> (A/ <i>E</i>)	ns	8,89 ± 0,23 Aa	7,28 ± 0,55 Aa	7,63 ± 1,09 Aa	7,84 ± 0,41 Aa	9,28 ± 0,98 Aa	10,91 ± 0,68 Aa	12,58 ± 0,46 Aa	12,11 ± 1,42 Aa	13,73 ± 1,14 Aa	12,55 ± 1,04 Aa
<i>R</i> _d (mmol CO ₂ m ⁻² s ⁻¹)	ns	0,794 ± 0,003 Ab	0,797 ± 0,004 Aab	0,795 ± 0,005 Ab	0,801 ± 0,002 Aab	0,805 ± 0,001 Aa	0,799 ± 0,001 Ab	0,800 ± 0,001 Aab	0,800 ± 0,002 Ab	0,798 ± 0,003 Aab	0,808 ± 0,002 Aa
<i>ETR</i> (mmol e ⁻ m ⁻² s ⁻¹)	ns	141,33 ± 0,09 Ab	165,93 ± 0,15 Aa	168,16 ± 0,10 Aa	168,19 ± 0,13 Aab	144,35 ± 0,09 Aab	146,44 ± 0,13 Ab	186,70 ± 0,09 Aa	180,28 ± 0,09 Aa	170,11 ± 0,13 Aab	168,63 ± 0,04 Aab
<i>F</i> _v / <i>F</i> _m	ns	0,563 ± 0,015 Ab	0,570 ± 0,026 Aab	0,560 ± 0,019 Aab	0,629 ± 0,016 Aa	0,541 ± 0,019 Ab	0,601 ± 0,020 Ab	0,667 ± 0,010 Aab	0,659 ± 0,011 Aab	0,666 ± 0,015 Aa	0,617 ± 0,006 Ab
<i>F</i> _v / <i>F</i> _m	ns	0,729 ± 0,083 Aa	0,736 ± 0,089 Aa	0,727 ± 0,036 Aa	0,711 ± 0,036 Aa	0,707 ± 0,058 Aa	0,664 ± 0,015 Aa	0,507 ± 0,023 Aa	0,769 ± 0,072 Aa	0,652 ± 0,064 Aa	0,754 ± 0,042 Aa
<i>q</i> _P	ns	0,570 ± 0,028 Aa	0,657 ± 0,022 Aa	0,661 ± 0,033 Aa	0,608 ± 0,042 Aa	0,605 ± 0,037 Aa	0,548 ± 0,022 Aa	0,635 ± 0,028 Aa	0,620 ± 0,037 Aa	0,583 ± 0,022 Aa	0,617 ± 0,029 Aa
<i>q</i> _N	ns	2,30 ± 0,09 Ab	2,36 ± 0,15 Aab	2,29 ± 0,10 Aab	2,71 ± 0,13 Aa	2,19 ± 0,09 Ab	2,59 ± 0,13 Ab	3,01 ± 0,09 Aab	2,94 ± 0,09 Aab	3,01 ± 0,13 Aa	2,62 ± 0,04 Ab
TLA (m ²)	*	0,077 ± 0,008 Ba	0,054 ± 0,004 Ba	0,044 ± 0,005 Ba	0,067 ± 0,004 Ba	0,061 ± 0,008 Ba	0,118 ± 0,013 Aab	0,094 ± 0,008 Ab	0,132 ± 0,013 Aa	0,108 ± 0,004 Aab	0,131 ± 0,009 Aa
SLA (m ² g ⁻¹)	ns	0,029 ± 0,001 Aab	0,028 ± 0,001 Aab	0,027 ± 0,001 Ab	0,027 ± 0,001 Aab	0,030 ± 0,001 Aa	0,028 ± 0,001 Aab	0,027 ± 0,000 Aab	0,025 ± 0,001 Ab	0,026 ± 0,000 Aab	0,028 ± 0,001 Aa
Leaf DW (g)	*	2,73 ± 0,39 Ba	1,94 ± 0,14 Ba	1,66 ± 0,18 Ba	2,45 ± 0,14 Ba	2,02 ± 0,23 Ba	4,24 ± 0,49 Aab	3,52 ± 0,32 Ab	5,14 ± 0,53 Aa	4,08 ± 0,16 Aab	4,51 ± 0,34 Aab
Stem DW (g)	**	0,60 ± 0,13 Ba	0,35 ± 0,04 Bab	0,31 ± 0,04 Bb	0,50 ± 0,05 Bab	0,44 ± 0,08 Bab	0,83 ± 0,12 Aab	0,69 ± 0,08 Ab	1,18 ± 0,15 Aa	0,92 ± 0,07 Aab	1,14 ± 0,13 Aab
Root DW (g)	**	1,21 ± 0,25 Aa	0,64 ± 0,07 Ba	0,64 ± 0,10 Ba	0,96 ± 0,11 Aa	0,71 ± 0,14 Ba	1,52 ± 0,22 Ab	1,35 ± 0,19 Ab	2,34 ± 0,33 Aa	1,45 ± 0,13 Ab	1,95 ± 0,20 Aab
Total DW (g)	**	4,54 ± 0,75 Ba	2,93 ± 0,20 Ba	2,61 ± 0,31 Ba	3,92 ± 0,26 Ba	3,17 ± 0,44 Ba	6,60 ± 0,82 Aab	5,56 ± 0,55 Ab	8,67 ± 0,10 Aa	6,45 ± 0,30 Aab	7,60 ± 0,57 Aab
Root DW/ Shoot DW	ns	0,346 ± 0,030 Aa	0,282 ± 0,024 Aa	0,318 ± 0,026 Aa	0,322 ± 0,027 Aa	0,278 ± 0,022 Aa	0,295 ± 0,016 Aa	0,317 ± 0,023 Aa	0,366 ± 0,018 Aa	0,288 ± 0,018 Aa	0,357 ± 0,046 Aa
RGR-h (cm.cm ⁻¹ day)	*	0,027 ± 0,003 Aa	0,028 ± 0,001 Ba	0,027 ± 0,001 Ba	0,029 ± 0,003 Ba	0,028 ± 0,002 Aa	0,033 ± 0,001 Abc	0,044 ± 0,003 Aa	0,044 ± 0,003 Aa	0,041 ± 0,003 Aab	0,032 ± 0,002 Ac
Height (cm)	*	23,2 ± 2,48 Aa	18,18 ± 1,82 Ba	16,96 ± 1,47 Ba	19,52 ± 1,20 Ba	20,88 ± 1,52 Aa	26,26 ± 1,93 Aa	24,7 ± 1,77 Aa	28,7 ± 2,27 Aa	30,04 ± 1,60 Aa	24,12 ± 1,32 Aa
RGR-d (cm.cm ⁻¹ day)	ns	0,019 ± 0,001 Aa	0,014 ± 0,001 Aa	0,017 ± 0,001 Aa	0,018 ± 0,002 Aa	0,016 ± 0,000 Aa	0,020 ± 0,001 Aa	0,019 ± 0,002 Aa	0,021 ± 0,001 Aa	0,018 ± 0,002 Aa	0,020 ± 0,001 Aa
Stem diameter (cm)	ns	5,80 ± 0,26 Aa	4,93 ± 0,11 Ab	5,15 ± 0,19 Aab	5,30 ± 0,18 Aab	5,16 ± 0,14 Aa	6,10 ± 0,15 Aa	5,28 ± 0,24 Ab	5,92 ± 0,19 Aab	5,62 ± 0,26 Aab	6,23 ± 0,22 Aa
Leaf venation (mm/mm ²)	ns	8,65 ± 0,57 Aa	8,30 ± 0,40 Aa	8,76 ± 1,77 Aa	8,37 ± 1,44 Aa	7,97 ± 1,75 Aa	7,39 ± 0,63 Aa	7,59 ± 0,55 Aa	8,81 ± 0,74 Aa	8,79 ± 0,38 Aa	7,77 ± 0,60 Aa
Leaf thickness (mm)	ns	0,522 ± 0,110 Aa	0,431 ± 0,101 Aa	0,535 ± 0,155 Aa	0,417 ± 0,102 Aa	0,434 ± 0,081 Aa	0,490 ± 0,123 Aa	0,410 ± 0,048 Aa	0,546 ± 0,042 Aa	0,562 ± 0,032 Aa	0,596 ± 0,157 Aa
PP thickness (mm)	*	0,196 ± 0,059 Aa	0,172 ± 0,041 Aa	0,194 ± 0,036 Aa	0,155 ± 0,039 Ba	0,174 ± 0,039 Ba	0,194 ± 0,048 Ab	0,160 ± 0,018 Ab	0,203 ± 0,017 Aab	0,222 ± 0,018 Aab	0,276 ± 0,073 Aa
SP thickness (mm)	ns	0,250 ± 0,045 Aa	0,189 ± 0,053 Aa	0,259 ± 0,110 Aa	0,194 ± 0,054 Aa	0,193 ± 0,038 Aa	0,217 ± 0,063 Aa	0,187 ± 0,038 Aa	0,261 ± 0,032 Aa	0,261 ± 0,023 Aa	0,239 ± 0,074 Aa
UE thickness (mm)	ns	0,047 ± 0,013 Aa	0,042 ± 0,008 Aa	0,048 ± 0,008 Aa	0,040 ± 0,005 Aa	0,041 ± 0,009 Aa	0,048 ± 0,012 Aa	0,038 ± 0,003 Aa	0,049 ± 0,004 Aa	0,045 ± 0,003 Aa	0,045 ± 0,008 Aa
LE thickness (mm)	ns	0,035 ± 0,005 Aa	0,030 ± 0,004 Aa	0,034 ± 0,007 Aa	0,030 ± 0,005 Aa	0,028 ± 0,003 Aa	0,033 ± 0,010 Aa	0,027 ± 0,004 Aa	0,032 ± 0,003 Aa	0,033 ± 0,003 Aa	0,034 ± 0,002 Aa

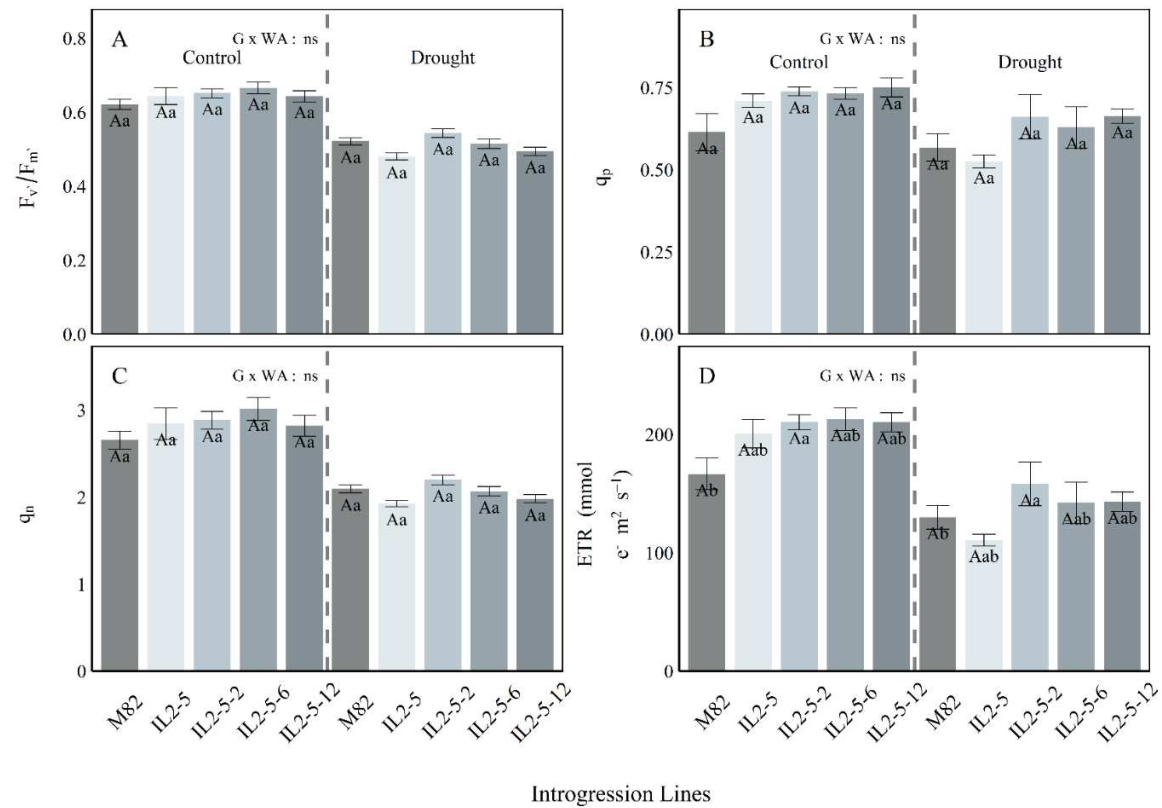
means that differ between the genotypes within a treatment ($P < 0,05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($P < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x (CO₂) atmosphere environment interaction. Non-significative (ns); significative at 5% (*); significative at 1% (**). Values are presented as means \pm SE (n = 5), except for DW, TLA, and SLA (n = 6).

Supplementary table 3: Photosynthesis related parameters of tomato plants treated with two different levels of water availability (control and four days under drought). The table displays data from the IL2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82). CO₂ assimilation rate (*A*); internal CO₂ concentration (*C_i*); stomatal conductance to water vapor (*g_s*); transpiration (*E*); intrinsic water-use efficiency (*WUE_i*); water-use efficiency (*WUE*); rates of dark respiration (*R_d*); the electron transport rate (*ETR*); PSII maximum efficiency (*F_v'/F_m'*); photochemical quenching (*q_p*); non-photochemical quenching (*q_n*); leaf water potential at pre-dawn (LWP_{pd}); leaf water potential at midday (LWP_m). Lowercase letters compare means that differ between the genotypes within a treatment ($P < 0,05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($P < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x (WA) water availability interaction. Non-significative (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE (n = 3), except for LWP (n = 5).

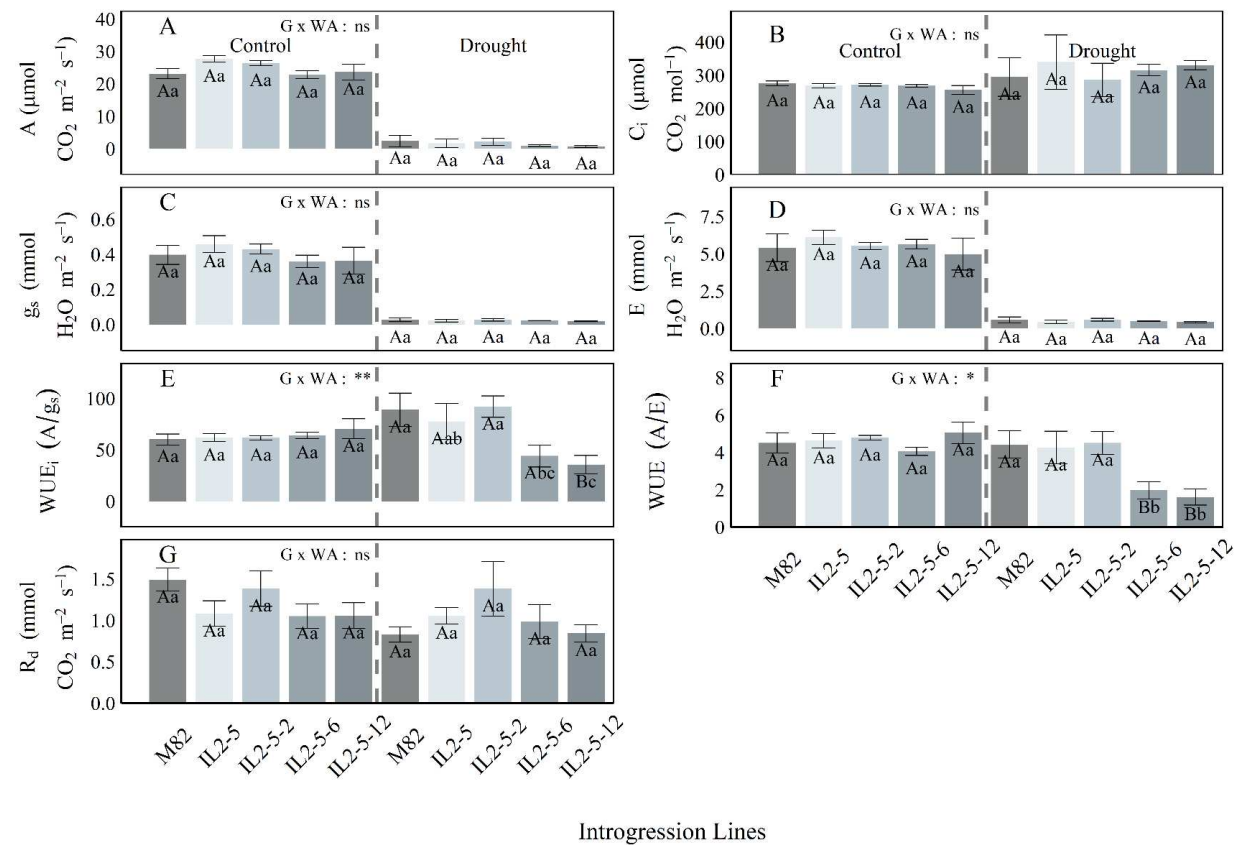
Trait	G x WA	Control					Four days drought				
		M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12	M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12
<i>A</i> ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	ns	22,96 \pm 0,82 Aa	23,98 \pm 0,84 Aa	25,53 \pm 1,39 Aa	25,32 \pm 1,73 Aa	24,77 \pm 0,81 Aa	16,60 \pm 0,98 Aa	14,37 \pm 1,83 Aa	18,78 \pm 1,31 Aa	14,50 \pm 0,30 Aa	13,31 \pm 0,89 Aa
<i>C_i</i> ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	ns	281,94 \pm 7,47 Aa	278,97 \pm 9,63 Aa	272,86 \pm 3,70 Aa	266,71 \pm 19,05 Aa	259,50 \pm 3,23 Aa	200,76 \pm 26,77 Aa	205,58 \pm 5,25 Aa	200,28 \pm 10,11 Aa	188,36 \pm 27,40 Aa	207,05 \pm 4,29 Aa
<i>g_s</i> ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	ns	0,420 \pm 0,039 Aa	0,442 \pm 0,077 Aa	0,425 \pm 0,030 Aa	0,452 \pm 0,107 Aa	0,357 \pm 0,021 Aa	0,161 \pm 0,025 Aa	0,134 \pm 0,018 Aa	0,173 \pm 0,011 Aa	0,130 \pm 0,017 Aa	0,125 \pm 0,012 Aa
<i>E</i> ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	ns	6,096 \pm 1,148 Aa	5,782 \pm 1,020 Aa	6,012 \pm 0,255 Aa	6,184 \pm 0,758 Aa	4,716 \pm 0,349 Aa	2,752 \pm 0,343 Aa	2,476 \pm 0,386 Aa	3,463 \pm 0,080 Aa	2,664 \pm 0,349 Aa	2,349 \pm 0,084 Aa
<i>WUE_i</i> (<i>A/g_s</i>)	ns	55,89 \pm 4,90 Aa	57,60 \pm 6,59 Aa	60,39 \pm 2,19 Aa	64,08 \pm 11,93 Aa	69,72 \pm 2,50 Aa	110,03 \pm 16,15 Aa	108,07 \pm 3,50 Aa	108,51 \pm 5,78 Aa	117,95 \pm 16,94 Aa	107,56 \pm 2,85 Aa
<i>WUE</i> (<i>A/E</i>)	ns	4,10 \pm 0,62 Aa	4,45 \pm 0,62 Aa	4,24 \pm 0,07 Aa	4,19 \pm 0,31 Aa	5,32 \pm 0,33 Aa	6,20 \pm 0,47 Aa	5,92 \pm 0,43 Aa	5,41 \pm 0,28 Aa	5,70 \pm 0,69 Aa	5,65 \pm 0,18 Aa
<i>ETR</i> ($\text{mmol e}^- \text{ m}^{-2} \text{ s}^{-1}$)	ns	194,30 \pm 10,70 Ab	221,29 \pm 5,53 Aab	228,46 \pm 7,94 Aab	215,61 \pm 9,30 Aab	221,91 \pm 7,47 Aa	167,00 \pm 11,98 Ab	188,12 \pm 20,98 Aab	185,78 \pm 8,38 Aab	158,99 \pm 9,96 Aab	207,96 \pm 7,56 Aa
<i>F_v'/F_m'</i>	ns	0,621 \pm 0,014 Aa	0,656 \pm 0,007 Aa	0,664 \pm 0,010 Aa	0,645 \pm 0,016 Aa	0,660 \pm 0,008 Aa	0,579 \pm 0,016 Aa	0,588 \pm 0,028 Aa	0,590 \pm 0,013 Aa	0,557 \pm 0,052 Aa	0,606 \pm 0,010 Aa
<i>q_p</i>	ns	0,717 \pm 0,028 Ab	0,776 \pm 0,020 Aab	0,793 \pm 0,017 Aab	0,768 \pm 0,017 Aab	0,771 \pm 0,020 Aa	0,660 \pm 0,048 Ab	0,729 \pm 0,054 Aab	0,722 \pm 0,024 Aab	0,655 \pm 0,044 Aab	0,790 \pm 0,017 Aa
<i>q_n</i>	ns	2,65 \pm 0,10 Aa	2,91 \pm 0,06 Aa	2,98 \pm 0,08 Aa	2,83 \pm 0,12 Aa	2,95 \pm 0,07 Aa	2,39 \pm 0,09 Aa	2,46 \pm 0,17 Aa	2,45 \pm 0,08 Aa	2,26 \pm 0,03 Aa	2,54 \pm 0,07 Aa
LWP _{pd}	*	-0,223 \pm 0,020 Aa	-0,279 \pm 0,029 Aa	-0,249 \pm 0,025 Aa	-0,277 \pm 0,012 Aa	-0,217 \pm 0,034 Aa	-0,940 \pm 0,029 Bb	-0,805 \pm 0,052 Bab	-0,752 \pm 0,031 Ba	-0,939 \pm 0,070 Bb	-0,861 \pm 0,038 Bab
LWP _m	ns	-1,097 \pm 0,067 Aab	-1,082 \pm 0,035 Aab	-0,924 \pm 0,025 Aa	-1,092 \pm 0,046 Aa	-1,220 \pm 0,060 Ab	-1,576 \pm 0,087 Aab	-1,504 \pm 0,073 Aab	-1,406 \pm 0,055 Aa	-1,262 \pm 0,082 Aa	-1,800 \pm 0,159 Ab



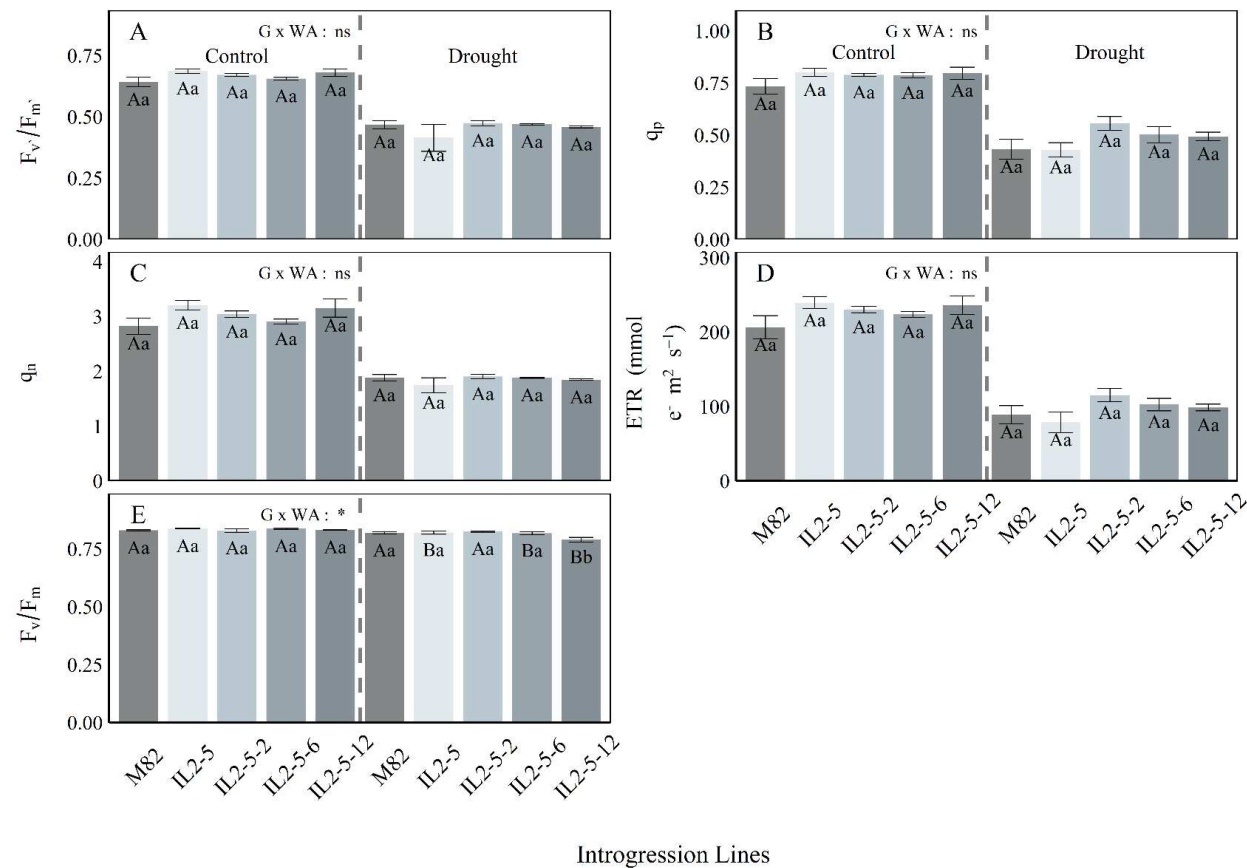
Supplementary figure 3: Gas exchange of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - eight days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO₂ assimilation rate (**A**); internal CO₂ concentration (**B**); stomatal conductance to water vapor (**C**); transpiration (**D**); intrinsic water-use efficiency (**E**); water-use efficiency (**F**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).



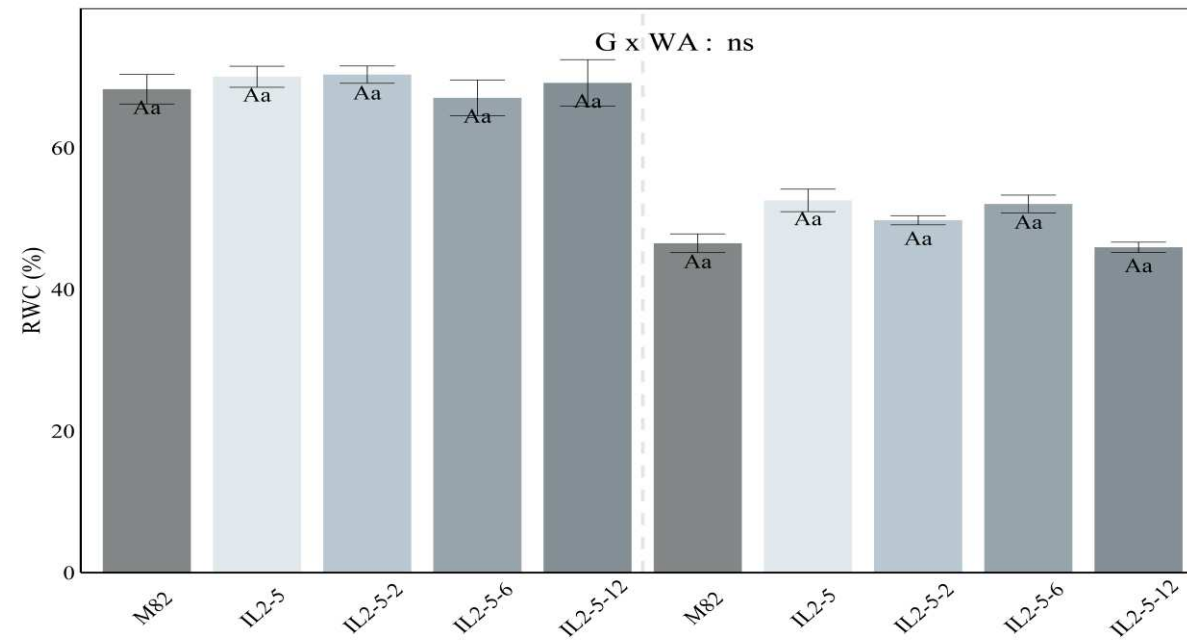
Supplementary figure 4: Fluorescence and *ETR* of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - eight days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Aiseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. PSII maximum efficiency (**A**); photochemical quenching (**B**); non-photochemical quenching (**C**); the electron transport rate (**D**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).



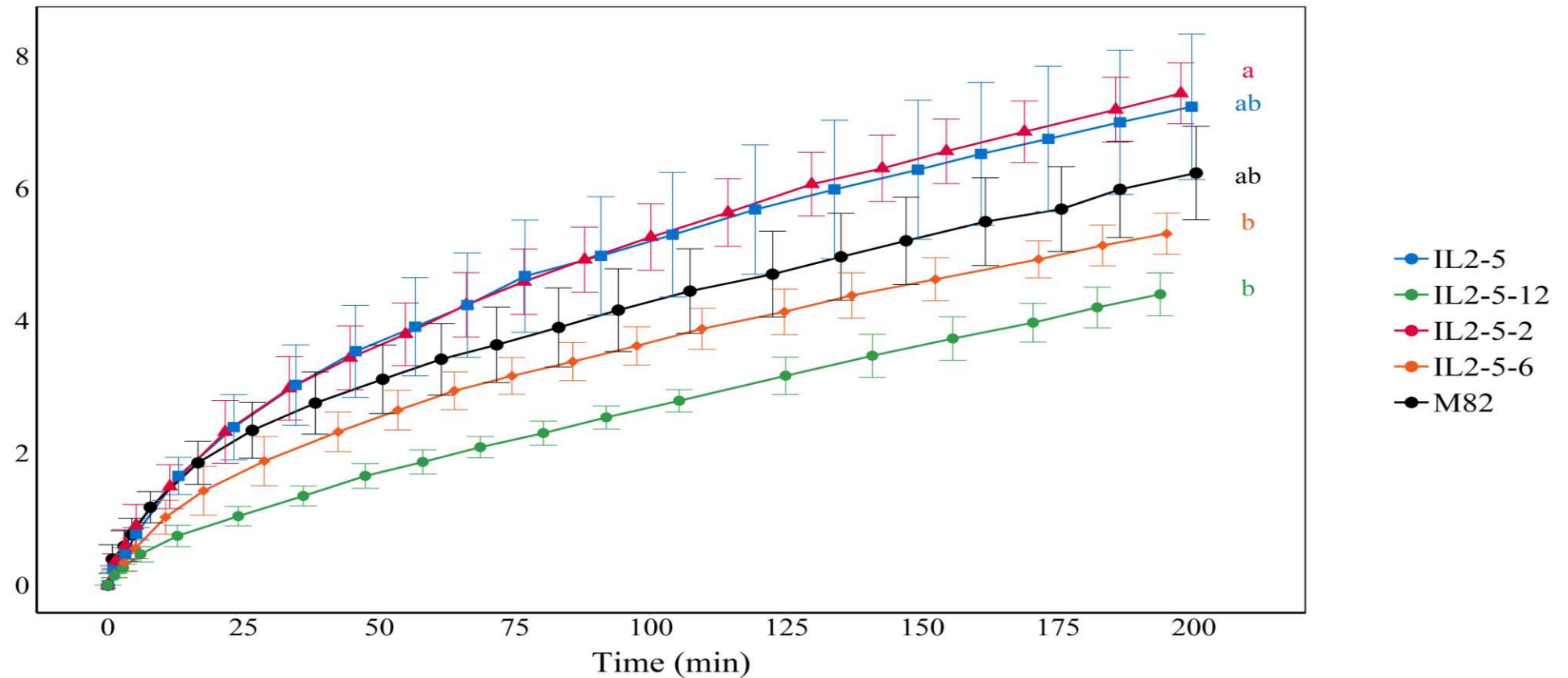
Supplementary figure 5: Gas exchange of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - 11 days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. CO_2 assimilation rate (**A**); internal CO_2 concentration (**B**); stomatal conductance to water vapor (**C**); transpiration (**D**); intrinsic water-use efficiency (**E**); water-use efficiency (**F**); rates of dark respiration (**G**). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).



Supplementary figure 6: Fluorescence and *ETR* of tomato plants treated with two different water availabilities (control - daily-irrigated plants; and drought - 11 days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. PSII maximum efficiency (A); photochemical quenching (B); non-photochemical quenching (C); the electron transport rate (D); Maximum quantum efficiency of PSII photochemistry (E). Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE ($n = 4$).



Supplementary figure 7: Relative water content (RWC) of tomato plants treated with two different water availabilities (control - daily irrigated plants; and drought - 11 days under stress). The IL 2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the grey bars. Lowercase letters compare means that differ between the genotypes within a treatment ($p < 0.05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($p < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x water availability (WA) interaction. Non-significant (ns); significant at 5% (*); significant at 1% (**). Values are presented as mean \pm SE (n = 6).



Supplementary figure 8: Leaf water loss of daily irrigated tomato plants grown in a $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ atmosphere. The IL2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseikh et al., 2013), and the parental *Solanum lycopersicum* (M82) are illustrated by the colored lines. Letters compare means that differ between the genotypes ($p < 0.05$) by the Tukey test. Values are presented as means \pm SE ($n = 5$).

Supplementary table 4: Photosynthesis related parameters of tomato plants treated with two different levels of water availability (control and eight days under drought). The table displays data from the IL2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82). CO₂ assimilation rate (*A*); internal CO₂ concentration (*C_i*); stomatal conductance to water vapor (*g_s*); transpiration (*E*); intrinsic water-use efficiency (*WUE_i*); water-use efficiency (*WUE*); rates of dark respiration (*R_d*); the electron transport rate (*ETR*); PSII maximum efficiency (*F_v/F_m'*); photochemical quenching (*q_p*); non-photochemical quenching (*q_v*); leaf water potential at pre-dawn (LWP_{pd}); leaf water potential at midday (LWP_m). Lowercase letters compare means that differ between the genotypes within a treatment ($P < 0,05$) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments ($P < 0.05$) by the Tukey test. This test was performed assuming random effects for genotype (G) x (WA) water availability interaction. Non-significative (ns); significant at 5% (*); significant at 1% (**). Values are presented as means \pm SE ($n = 3$), except for LWP ($n = 5$).

Trait	G x WA	Control					Eight days drought				
		M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12	M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12
<i>A</i> ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	ns	10,07 \pm 0,75 Aa	11,94 \pm 1,09 Aa	12,36 \pm 1,01 Aa	11,88 \pm 1,04 Aa	11,06 \pm 1,15 Aa	3,18 \pm 0,43 Aa	1,53 \pm 0,44 Aa	4,89 \pm 1,24 Aa	2,37 \pm 0,67 Aa	1,59 \pm 0,38 Aa
<i>C_i</i> ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	ns	299,20 \pm 17,00 Aa	278,14 \pm 15,91 Aa	280,61 \pm 12,71 Aa	274,40 \pm 10,70 Aa	268,54 \pm 17,53 Aa	205,66 \pm 10,17 Aa	240,66 \pm 33,80 Aa	213,13 \pm 4,96 Aa	238,48 \pm 7,58 Aa	237,52 \pm 14,18 Aa
<i>g_s</i> ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	ns	0,213 \pm 0,038 Aa	0,201 \pm 0,038 Aa	0,213 \pm 0,043 Aa	0,205 \pm 0,036 Aa	0,178 \pm 0,045 Aa	0,028 \pm 0,003 Aa	0,017 \pm 0,003 Aa	0,044 \pm 0,011 Aa	0,025 \pm 0,006 Aa	0,017 \pm 0,004 Aa
<i>E</i> ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	ns	2,721 \pm 0,412 Aa	2,664 \pm 0,473 Aa	2,921 \pm 0,493 Aa	2,958 \pm 0,432 Aa	2,692 \pm 0,555 Aa	0,501 \pm 0,030 Aa	0,312 \pm 0,005 Aa	0,788 \pm 0,155 Aa	0,518 \pm 0,122 Aa	0,389 \pm 0,085 Aa
<i>WUE_i</i> (<i>A/g_s</i>)	ns	52,74 \pm 10,62 Aa	64,84 \pm 10,30 Aa	62,88 \pm 8,25 Aa	61,93 \pm 7,53 Aa	70,65 \pm 11,43 Aa	114,19 \pm 6,38 Aa	92,70 \pm 21,51 Aa	108,50 \pm 2,73 Aa	92,97 \pm 4,27 Aa	93,13 \pm 9,02 Aa
<i>WUE</i> (<i>A/E</i>)	ns	3,90 \pm 0,51 Aa	4,75 \pm 0,54 Aa	4,46 \pm 0,54 Aa	4,14 \pm 0,38 Aa	4,43 \pm 0,57 Aa	6,28 \pm 0,54 Aa	5,07 \pm 1,22 Aa	5,97 \pm 0,45 Aa	4,47 \pm 0,21 Aa	4,05 \pm 0,49 Aa
<i>ETR</i> ($\text{mmol e m}^{-2} \text{ s}^{-1}$)	ns	166,68 \pm 13,39 Ab	200,80 \pm 12,11 Aab	210,51 \pm 6,38 Aa	213,01 \pm 9,66 Aab	210,36 \pm 7,94 Aab	130,16 \pm 10,01 Ab	110,86 \pm 5,13 Aub	158,21 \pm 18,36 Aa	142,42 \pm 17,33 Aab	143,29 \pm 8,24 Aab
<i>F_v/F_m'</i>	ns	0,622 \pm 0,014 Aa	0,644 \pm 0,023 Aa	0,652 \pm 0,012 Aa	0,666 \pm 0,016 Aa	0,644 \pm 0,015 Aa	0,522 \pm 0,010 Aa	0,481 \pm 0,010 Aa	0,544 \pm 0,012 Aa	0,516 \pm 0,013 Aa	0,495 \pm 0,011 Aa
<i>q_p</i>	ns	0,615 \pm 0,057 Aa	0,710 \pm 0,021 Aa	0,739 \pm 0,014 Aa	0,733 \pm 0,018 Aa	0,751 \pm 0,029 Aa	0,568 \pm 0,042 Aa	0,526 \pm 0,019 Aa	0,662 \pm 0,068 Aa	0,629 \pm 0,063 Aa	0,664 \pm 0,023 Aa
<i>q_v</i>	ns	2,66 \pm 0,10 Aa	2,85 \pm 0,18 Aa	2,89 \pm 0,10 Aa	3,01 \pm 0,13 Aa	2,82 \pm 0,12 Aa	2,10 \pm 0,04 Aa	1,93 \pm 0,04 Aa	2,20 \pm 0,06 Aa	2,07 \pm 0,05 Aa	1,98 \pm 0,05 Aa
LWP _{pd}	*	-0,253 \pm 0,029 Aa	-0,290 \pm 0,018 Aa	-0,265 \pm 0,036 Aa	-0,268 \pm 0,028 Aa	-0,189 \pm 0,012 Aa	-1,398 \pm 0,060 Bab	-1,307 \pm 0,103 Bab	-1,172 \pm 0,078 Ba	-1,312 \pm 0,102 Bab	-1,547 \pm 0,083 Bb
LWP _m	ns	-0,769 \pm 0,072 Aa	-0,952 \pm 0,085 Aa	-0,890 \pm 0,075 Aa	-0,772 \pm 0,088 Aa	-0,791 \pm 0,095 Aa	-1,706 \pm 0,145 Aa	-1,886 \pm 0,068 Aa	-1,538 \pm 0,221 Aa	-1,285 \pm 0,202 Aa	-1,659 \pm 0,187 Aa

Supplementary table 5: Photosynthesis related parameters of tomato plants treated with two different levels of water availability (control - daily irrigated; drought - 11 days without irrigation). The table displays data from the IL2-5 (Eshed and Zamir, 1995), three subILs (IL2-5-2; IL2-5-6; IL2-5-12) (Alseekh et al., 2013), and the parental *Solanum lycopersicum* (M82). CO₂ assimilation rate (*A*); internal CO₂ concentration (*C_i*); stomatal conductance to water vapor (*g_s*); transpiration (*E*); intrinsic water-use efficiency (*WUE_i*); water-use efficiency (*WUE*); rates of dark respiration (*R_d*); the electron transport rate (*ETR*); PSII maximum efficiency (*F_v'/F_m'*); Maximum quantum efficiency of PSII photochemistry (*F_v/F_m*) photochemical quenching (*q_P*); non-photochemical quenching (*q_N*); total leaf area (TLA); specific leaf area (SLA); dry weight (DW); relative growth rate in height (RGR-h); relative growth rate in stem diameter (RGR-d). Lowercase letters compare means that differ between the genotypes within a treatment (*P* < 0,05) by the Tukey test. Capital letters compare means that differ in a single genotype between the two treatments (*P* < 0.05) by the Tukey test. This test was performed assuming random effects for genotype (G) x (WA) water availability interaction. Non-significative (ns); significant at 5% (*); significant at 1% (**). Values are presented as means ±SE (n = 5).

Trait	G x WA	Control - 400 μmol CO ₂ mol ⁻¹					11 days drought - 400 μmol CO ₂ mol ⁻¹				
		M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12	M82	IL2-5	IL2-5-2	IL2-5-6	IL2-5-12
<i>A</i> (μmol CO ₂ m ⁻² s ⁻¹)	ns	23,15 ± 1,58 Aa	27,77 ± 0,96 An	26,45 ± 0,78 Aa	22,87 ± 1,15 An	23,66 ± 2,49 An	2,43 ± 1,76 Aa	1,68 ± 1,36 Aa	2,21 ± 1,12 Aa	0,99 ± 0,27 Aa	0,77 ± 0,30 Aa
<i>C_i</i> (μmol CO ₂ mol ⁻¹)	ns	275,50 ± 7,14 Aa	268,89 ± 6,36 Aa	271,01 ± 3,51 Aa	268,23 ± 4,52 Aa	255,68 ± 13,72 Aa	294,92 ± 57,73 Aa	340,00 ± 81,37 Aa	286,54 ± 50,21 Aa	315,61 ± 17,14 Aa	330,97 ± 13,84 Aa
<i>g_s</i> (μmol H ₂ O m ⁻² s ⁻¹)	ns	0,398 ± 0,055 Aa	0,458 ± 0,048 Aa	0,431 ± 0,028 Aa	0,361 ± 0,036 Aa	0,365 ± 0,076 Aa	0,027 ± 0,010 Aa	0,023 ± 0,008 Aa	0,027 ± 0,007 Aa	0,022 ± 0,002 Aa	0,019 ± 0,003 Aa
<i>E</i> (mmol H ₂ O m ⁻² s ⁻¹)	ns	5,41 ± 0,92 Aa	6,11 ± 0,49 Aa	5,54 ± 0,24 Aa	5,66 ± 0,33 Aa	4,99 ± 1,07 Aa	0,59 ± 0,20 Aa	0,45 ± 0,13 Aa	0,60 ± 0,09 Aa	0,49 ± 0,02 Aa	0,44 ± 0,06 Aa
<i>WUE_i</i> (<i>A/g_s</i>)	**	60,31 ± 5,28 Aa	61,85 ± 3,88 Aa	61,78 ± 2,26 Aa	64,30 ± 2,99 Aa	70,56 ± 9,79 Aa	89,08 ± 16,24 Aa	77,68 ± 17,42 Aab	92,17 ± 10,46 Aa	44,21 ± 10,72 Abc	35,77 ± 8,88 Bc
<i>WUE</i> (<i>A/E</i>)	*	4,52 ± 0,54 Aa	4,63 ± 0,39 Aa	4,79 ± 0,13 Aa	4,06 ± 0,22 Aa	5,06 ± 0,57 Aa	4,43 ± 0,73 Aa	4,26 ± 0,88 Aa	4,51 ± 0,62 Aa	1,98 ± 0,46 Bb	1,61 ± 0,43 Bb
<i>R_d</i> (mmol CO ₂ m ⁻² s ⁻¹)	ns	1,49 ± 0,14 Aa	1,08 ± 0,15 Aa	1,38 ± 0,21 Aa	1,05 ± 0,15 Aa	1,06 ± 0,15 Aa	0,83 ± 0,09 Aa	1,06 ± 0,10 Aa	1,38 ± 0,33 Aa	0,99 ± 0,20 Aa	0,85 ± 0,11 Aa
<i>ETR</i> (mmol e ⁻ m ⁻² s ⁻¹)	ns	206,72 ± 15,66 Aa	239,70 ± 7,83 An	230,45 ± 4,17 An	223,97 ± 3,81 An	236,51 ± 12,57 An	89,20 ± 12,13 An	78,80 ± 13,94 An	115,42 ± 9,09 An	102,86 ± 8,42 An	98,82 ± 4,54 An
<i>F_v'/F_m'</i>	ns	0,643 ± 0,019 Aa	0,687 ± 0,009 An	0,671 ± 0,006 An	0,656 ± 0,005 An	0,680 ± 0,016 An	0,468 ± 0,016 An	0,415 ± 0,055 An	0,475 ± 0,011 An	0,469 ± 0,003 An	0,460 ± 0,005 An
<i>F_v/F_m</i>	*	0,830 ± 0,002 Aa	0,839 ± 0,002 An	0,830 ± 0,007 An	0,838 ± 0,002 An	0,833 ± 0,001 An	0,820 ± 0,005 An	0,821 ± 0,007 Ba	0,826 ± 0,003 An	0,818 ± 0,007 Ba	0,791 ± 0,010 Bb
<i>q_P</i>	ns	0,734 ± 0,037 Aa	0,801 ± 0,020 An	0,789 ± 0,008 An	0,788 ± 0,013 An	0,797 ± 0,029 An	0,433 ± 0,047 An	0,430 ± 0,035 An	0,557 ± 0,032 An	0,503 ± 0,039 An	0,494 ± 0,020 An
<i>q_N</i>	ns	2,82 ± 0,15 Aa	3,20 ± 0,09 An	3,04 ± 0,06 An	2,91 ± 0,05 An	3,16 ± 0,02 An	1,89 ± 0,06 An	1,75 ± 0,14 An	1,91 ± 0,04 An	1,88 ± 0,01 An	1,85 ± 0,02 An
RWC	ns	68,29 ± 2,08 Aa	70,05 ± 1,48 An	70,35 ± 1,22 An	67,06 ± 2,54 An	69,19 ± 3,29 An	46,53 ± 1,32 An	52,59 ± 1,58 An	49,79 ± 0,65 An	52,08 ± 1,25 An	45,96 ± 0,73 An
TLA (m ²)	ns	0,310 ± 0,063 Aa	0,312 ± 0,016 An	0,300 ± 0,040 An	0,291 ± 0,032 An	0,320 ± 0,055 An	0,124 ± 0,008 An	0,111 ± 0,008 An	0,101 ± 0,013 An	0,111 ± 0,015 An	0,122 ± 0,015 An
SLA (m ² g ⁻¹)	ns	0,0412 ± 0,004 Aa	0,036 ± 0,004 Ab	0,035 ± 0,003 Ab	0,034 ± 0,002 Ab	0,034 ± 0,005 Ab	0,028 ± 0,003 An	0,026 ± 0,002 Ab	0,024 ± 0,003 Ab	0,024 ± 0,002 Ab	0,027 ± 0,002 Ab
Leaf DW (g)	ns	7,54 ± 1,77 Aa	8,80 ± 0,85 An	8,69 ± 1,65 An	8,62 ± 0,79 An	9,30 ± 0,78 An	4,49 ± 0,50 An	4,28 ± 0,14 An	4,24 ± 0,26 An	4,55 ± 0,43 An	4,48 ± 0,40 An
Stem DW (g)	ns	1,57 ± 0,42 Ab	2,26 ± 0,30 An	2,19 ± 0,26 An	2,33 ± 0,40 An	2,37 ± 0,34 An	1,29 ± 0,21 Ab	1,68 ± 0,10 An	1,47 ± 0,13 An	1,59 ± 0,25 An	1,58 ± 0,19 An
Root DW (g)	ns	2,51 ± 0,74 Aa	2,48 ± 0,51 An	3,18 ± 0,80 An	2,63 ± 0,53 An	2,40 ± 0,82 An	1,56 ± 0,49 An	1,65 ± 0,58 An	1,41 ± 0,17 An	1,50 ± 0,18 An	1,44 ± 0,29 An
Total DW (g)	ns	11,61 ± 2,66 Aa	13,54 ± 1,37 An	14,07 ± 2,29 An	13,58 ± 1,57 An	14,07 ± 1,44 An	7,35 ± 1,06 An	7,61 ± 0,55 An	7,12 ± 0,42 An	7,64 ± 0,68 An	7,51 ± 0,87 An
Root DW/ Shoot DW	ns	0,280 ± 0,066 Aa	0,225 ± 0,043 An	0,294 ± 0,065 An	0,239 ± 0,029 An	0,206 ± 0,064 An	0,268 ± 0,065 An	0,278 ± 0,098 An	0,247 ± 0,027 An	0,245 ± 0,024 An	0,236 ± 0,025 An
RGR-h (cm.cm ⁻¹ day)	ns	0,019 ± 0,005 Aa	0,029 ± 0,011 An	0,026 ± 0,017 An	0,028 ± 0,018 An	0,021 ± 0,013 An	0,000 ± 0,016 An	0,001 ± 0,005 An	0,006 ± 0,012 An	0,006 ± 0,004 An	-0,006 ± 0,006 An
Height (cm)	ns	28,58 ± 6,95 Ab	38,18 ± 6,44 An	38,62 ± 7,41 An	39,72 ± 7,80 An	32,11 ± 4,19 Ab	26,87 ± 5,37 Ab	34,98 ± 3,17 An	34,7 ± 5,67 An	32,92 ± 5,87 An	26,62 ± 4,36 Ab
RGR-d (cm.cm ⁻¹ day)	ns	0,165 ± 0,005 Ab	0,168 ± 0,006 Aab	0,171 ± 0,006 Aa	0,170 ± 0,005 Anb	0,173 ± 0,003 Anb	0,163 ± 0,005 Ab	0,165 ± 0,003 Aab	0,169 ± 0,005 An	0,165 ± 0,005 Aab	0,166 ± 0,004 Anb
Stem diameter (cm)	ns	8,04 ± 0,63 Aa	7,89 ± 0,51 Aa	7,93 ± 0,46 Aa	8,05 ± 0,35 Aa	7,45 ± 0,97 Aa	6,30 ± 0,71 An	6,24 ± 0,42 An	6,32 ± 0,71 An	5,86 ± 0,25 An	6,06 ± 0,45 An

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