

Faculté des bioingénieurs

**Impacts of heat and water stresses on the cultivated tomato *Solanum lycopersicum* and its wild relative *Solanum chilense***

Author: Anaïs CAUCHIE

Promoter: Muriel QUINET

Readers: Stanley LUTTS, Rémy SCHOPPACH

Academic year 2021-2022

Master's Thesis submitted for the obtention of the degree of  
Agricultural Bioengineering



## Acknowledgments

First of all, I would like to warmly thank Muriel Quinet, my promoter, for her availability, her encouragement in the face of all the problems that marked my experiments and the time she took to give me precious feedbacks throughout the 6 months that I spent in the laboratory.

Then, a big thank you to Servane Bigot, who supervised me on a daily basis, and who didn't hesitate for a second to help me in my work and to answer all my questions. It was a pleasure to work with you.

Thanks to my readers, Stanley Lutts and Rémy Schoppach.

Of course, I can't hand in this work without thanking all the members of the laboratory that I came into contact with and who lent me a helping hand: Adrien, the king of statistics, Paul who dared to try the PEG dosage protocol, Marie-Eve and Brigitte.

Overall, I felt very welcome in the laboratory and that was really heart-warming.

Finally, thanks to everyone who supported me when stress or discouragement set in: my family, of course, but also my friends and my boyfriend.

Thank you all!



## Abstract

Climate change is a very topical issue with well-known causes and varying consequences. Global increases in temperature and imbalances in precipitation patterns threaten crop productivity, putting an increasing proportion of the world's population at risk of food insecurity. Water stress and heat stress are among the most common and impactful stresses on plant growth and development, and the combination of the two can have drastic effects.

As one of the world's major vegetable crops and a plant with a high transpiration flux, the tomato *Solanum lycopersicum* L. is threatened by climate change. Introgression of traits from wild relatives is a technique used to improve the tolerance of cultivated species to environmental stresses, both biotic and abiotic. In the case of *S. lycopersicum*, one species in particular has an interesting potential for tolerance to water and heat stress. It is the species *Solanum chilense* Dun., a wild tomato native to the Atacama Desert in Chile, and a species with great genetic diversity and adapted to drought, heat and salinity.

Individuals of both species were subjected to heat stress (21°C vs. 28°C) as well as water stress (well-watered conditions vs. water stress) implemented by using 10% polyethylene glycol in hydroponics. Morphological parameters and biomass were measured on shoots and roots. Physiological parameters were measured, such as water content, osmolality, malondialdehyde content, sugar content and photosynthesis-linked parameters.

Heat stress did not produce a lot of results and very few differences between the two investigated species have emerged. These differences were essentially about morphological impacts : both species had a lower leaf area and *S. chilense* had a higher stomatal density under heat stress than temperate conditions while *S. lycopersicum* did not seem to adapt.

Water stress results were more informative and highlighted that even though both species were negatively affected, *S. chilense* was impacted less severely than *S. lycopersicum* on most indicators. This confirmed the results of previous studies suggesting that *S. chilense* was more tolerant to water stress than its cultivated relative. Moreover, differences in the response of aerial and root systems were observed.

The conclusion of this work is that *S. chilense* is a promising crop wild relative to *S. lycopersicum* for trait introgression in a context of water stress tolerance. The study of the root system of *S. chilense* as well as the comparison of aboveground and belowground responses to stresses are an open field to be explored.



## Table of contents

LIST OF FIGURES.....	9
LIST OF TABLES .....	10
1. INTRODUCTION.....	11
2. STATE OF THE ART .....	13
2.1 Tomato.....	13
2.1.1 Production .....	13
2.1.2 The <i>Solanum</i> genus, sect. <i>Lycopersicon</i> .....	13
2.1.3 <i>Solanum lycopersicum</i> .....	14
2.1.4 <i>Solanum chilense</i> .....	14
2.2 Climate change.....	16
2.2.1 Generalities.....	16
2.2.2 Temperatures .....	16
2.2.3 Drought.....	18
2.3 Food security.....	19
2.4 Adaptation.....	20
2.5 Abiotic stresses and plants.....	21
2.5.1 Impact of abiotic stresses on plants .....	21
2.5.2 Stress tolerance .....	24
2.5.3 Impact of abiotic stresses on <i>Solanum</i> sect. <i>Lycopersicon</i> .....	24
3. OBJECTIVES.....	25
4. MATERIAL AND METHODS.....	25
4.1 Plant material and growing conditions .....	25
4.2 Experimental design .....	27
4.3 First experiment: impact of water and heat stress.....	28
4.4 Second experiment: impact of water stress.....	29
4.5 Analysed parameters.....	30
4.5.1 Morphological parameters.....	30
4.5.2 Physiological parameters.....	31
4.6 Development of a protocol for the determination of polyethylene glycol intake.....	35
4.7 Data management and statistical analysis .....	35
5. RESULTS.....	37

<b>5.1</b>	<b>First experiment: heat and water stress</b> .....	<b>37</b>
5.1.1	Morphological parameters .....	37
5.1.2	Physiological parameters .....	41
5.1.3	Determination of PEG content .....	48
<b>5.2</b>	<b>Second experiment: water stress</b> .....	<b>51</b>
5.2.1	Morphological parameters .....	51
5.2.2	Physiological parameters .....	56
<b>6.</b>	<b>DISCUSSION</b> .....	<b>59</b>
6.1	Impact of heat stress .....	59
6.2	Impact of water stress.....	61
6.3	Impact of combined heat and water stress .....	64
6.4	Methodological critique.....	65
<b>7.</b>	<b>CONCLUSION AND PERSPECTIVES</b> .....	<b>67</b>
<b>8.</b>	<b>BIBLIOGRAPHY</b> .....	<b>69</b>

## List of figures

Figure 1 – (a) full <i>S. lycopersicum</i> plant, aged 14 days; (b) leaf; (c) inflorescence; (d) fruit.....	14
Figure 2 – (a) full <i>S. chilense</i> plant, aged 14 days; (b) leaf; (c) inflorescence; (d) fruit.....	15
Figure 3 – Projected global mean warming of different scenarios .....	17
Figure 4 – Trends in the frequency of meteorological droughts between 1950 and 2015 across Europe.....	19
Figure 5 – Aerial view of tank number 13, in 28°C WW conditions, as an example for the placing of plants in the tanks .....	27
Figure 6 – Custom system for root observation made of polystyrene: (a) aerial view; (b) side view .....	28
Figure 7 – Experimental design for the first experiment .....	29
Figure 8 – Experimental design for the second experiment .....	29
Figure 9 – Measurement of one of the largest xylem element on a micrograph.....	31
Figure 10 – Experimental device for the measurement of photosynthesis parameters and fluorescence .....	33
Figure 11 – Picture of tanks at 28°C, (a) well-watered, (b) water-stressed, taken at day 10 of water stress...	38
Figure 12 – Dry weight of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress .....	38
Figure 13 – Leaf production of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions.....	39
Figure 14 - Leaf area of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress.....	40
Figure 15 – Stomatal density on the inferior side of the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	40
Figure 16 – Length of the three longest roots of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions, after 22 days of stress .....	41
Figure 17 – Water content of the shoots and roots of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress .....	41
Figure 18 – Chlorophyll content index on the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	42
Figure 19 – Osmotic potential of the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	43
Figure 20 – Malondialdehyde (MDA) leaf content in mmol/g fresh weight of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 18 days of stress .	43
Figure 21 – Total soluble sugars leaf content of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 18 days of stress .....	44
Figure 22 – Photosynthesis rate as a response to PAR on the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	45
Figure 23 – Electron transport rate (ETR) as a response to PAR on the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	47
Figure 24 – Electron transport rate at saturating irradiance (ETR <sub>max</sub> ) on the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS .....	47
Figure 25 – Absorbance spectrum (280 to 700 nm) of processed PEG ranging in concentrations from 0 to 0.15625 mg/ml, according to expected quantities in fresh samples (Lawlor, 1970). .....	49

Figure 27 – Absorbance of processed PEG between 280 and 300 nm.....	49
Figure 28 – Calibration lines for the processed PEG range .....	50
Figure 29 – PEG concentrations in <i>S. lycopersicum</i> and <i>S. chilense</i> growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS.....	50
Figure 30 – Dry weight of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.....	51
Figure 31 – Leaf production of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions .....	52
Figure 32 – Leaf area of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.....	53
Figure 33 – Stomatal density on the inferior side of the leaves of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of WS.....	53
Figure 34 – Length of the three longest roots of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.....	54
Figure 35 – Mean diameter of segments of the main root of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress .....	54
Figure 36 – Mean diameter of the two largest xylem elements in segments of the main root of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.....	55
Figure 37 – Ratio between the diameter of the xylem elements and the diameter of the root of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.....	56
Figure 38 – Water content of the leaves, stems and roots of <i>S. lycopersicum</i> and <i>S. chilense</i> growing under well-watered (WW) and water stress (WS) conditions after 17 days of stress .....	57
Figure 39 – Chlorophyll content index of the leaves, stems and roots of <i>S. lycopersicum</i> and <i>S. chilense</i> growing under well-watered (WW) and water stress (WS) conditions after 17 days of stress .....	57
Figure 40 –MDA content of the shoot and root of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p<0.05$ ) between treatments or species. ....	58
Figure 41 – Total soluble sugars content of the shoot and root of <i>S. lycopersicum</i> and <i>S. chilense</i> growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p<0.05$ ) between treatments, species or organs.....	59

## List of tables

Table 1 – Composition of the Hoagland solution used in the experiment. ....	26
Table 2 – Experimental setup of the two experiments showing the conditions, number of tanks and number of plants per treatment.....	27
Table 3 – Percentage of dead individuals per species in each treatment through time.....	37
Table 4 – Photosynthesis parameters extracted from the A-Q curves.....	46
Table 5 – Minimum saturating irradiance $E_k$ [ $\mu\text{mol}/\text{m}^2.\text{s}$ ]. ....	48
Table 6 – Parameters extracted from A-Q curve raw data. ....	48

## 1. Introduction

Climate change is a reality that recurrent reports from the Intergovernmental Panel on Climate Change (IPCC) do not allow to ignore. Global warming is a part of the issue: the global temperatures have already risen over 1°C since the pre-industrial levels and are expected to rise at least by 1.5°C by 2035. After that, many paths are possible, depending on international policy effort (IPCC et al., 2022). It is still likely that the global temperatures will remain higher than the current levels. Since this is a global average, it means that in some parts of the world the temperatures could decrease while in some others, they could increase drastically (IPCC, 2018a); in Europe, local temperatures are rising faster than the global average (European Environment Agency, 2022). The change of temperatures is impacting rainfall patterns, leading to extreme climate events such as heat waves, droughts or floods (IPCC, 2018a).

A consequence of the high temperatures and shifting rainfall patterns is meteorological drought, a period of time during which the precipitations are below normal to the point where the low availability of water impacts negatively vegetation development in natural and agricultural systems (Krishna et al., 2019). Europe is witnessing a rise in the frequency of drought events in most of its parts (European Environment Agency, 2021a), and the forecasts predict a worsening in the water shortages in spring and summer, two crucial periods for the crops (Iglesias & Garrote, 2015).

The cultivated tomato (*Solanum lycopersicum* L.) is one of the most prominent vegetable crops in the world, the second most consumed vegetable after the potato. Its huge production makes it the most economically important species in the *Solanum* genus (Bergougnoux, 2014; Knapp & Peralta, 2016). However, this species is sensible to water stress due to its high transpiration rate, but also to heat stress and saline stress (Hernandez-Espinoza & Barrios-Masias, 2020; Jangid & Dwivedi, 2016; Rick, 1978).

One way to address the problem of a threatened crop production due to biotic and abiotic stresses is the use of wild relatives. By studying their responses to the stresses, scientists can identify the traits responsible for stress tolerance and later introgress them into the cultivated crop, in this case *S. lycopersicum*.

One good candidate for gene introgression in the cultivated tomato is *Solanum chilense* Dun., a relative species to *S. lycopersicum* originated from the Atacama desert in Chile. This species is divided into populations adapted to a range of conditions from sea coasts to high altitudes and aridity (Blanchard-Gros et al., 2021; Böndel et al., 2018; Tapia et al., 2016). It is one of the most diverse tomato species, genetically speaking (Bretó et al., 1993). However, the mechanisms underlying stress resistance in this species remain largely unknown.

The objective of this Master's thesis is to improve our understanding of the response of *S. chilense* to abiotic stress linked to climate changes and compare it to the cultivated tomato (*S. lycopersicum*). The experimental setup aims to show the differences in the physiology of the two tomato species under different conditions of heat stress and water stress. The effects of the stresses will be observed on the aerial and root systems for the vegetative phase.

## 2. State of the art

### 2.1 Tomato

#### 2.1.1 Production

Tomato is the second most consumed vegetable in the world after the potato. It is sold all over the world not only fresh but also in a wide range of preparations, from paste to juice or ketchup (Bergougnoux, 2014). The worldwide production increases each year, reaching 186 million tons in 2020 (FAOSTAT, 2021). It is therefore of major economical interest.

The largest producer is China, which produced, in 2020, 64.8 million tons (35% of the worldwide production). However, the share of China has decreased since 2011 when it was responsible for 57% of the worldwide tomato production (Bergougnoux, 2014). In 2020, the best yield was achieved in Belgium with a production of over 502 tons per hectare (FAOSTAT, 2021).

However looking widely diverse in appearance, colour, shape and size, the essential of the worldwide production is based on the *Solanum lycopersicum* species (Gharbi, 2017).

#### 2.1.2 The *Solanum* genus, sect. *Lycopersicon*

Tomato (*Solanum lycopersicum* L.) belongs to the *Solanum* genus, the most economically important genus of the Solanaceae family, which includes potato and eggplant as well. *Solanum* species can be found in all temperate and tropical continents and have a remarkable morphological and ecological diversity (Bergougnoux, 2014; Knapp & Peralta, 2016).

The *Lycopersicon* section of the *Solanum* genus is constituted of the cultivated tomato (*S. lycopersicum*) along with 13 related species. All of them are diploid ( $2n=24$ ) (Moyle, 2008). Tomato and its close relatives can be distinguished from all the other groups of *Solanum* species thanks to their bright yellow flowers and their pinnatifid, non-spiny leaves (Knapp & Peralta, 2016).

All species of the genus are hermaphrodite. Some species are autogamous, such as *Solanum lycopersicum*, while most of the wild types, such as *Solanum chilense*, are self-incompatible and therefore allogamous (Chetelat et al., 2009; Gharbi, 2017). The pollination is done by insects and wind in the wild, but the dehiscence of the stamens can ensure fertilization within the same flower in autogamous species (Picken, 1984). *Solanum lycopersicum*, the cultivated tomato, can be crossed with all but two wild relatives, with variable degrees of difficulty (Knapp & Peralta, 2016).

Tomato is easy to cultivate because of its great adaptiveness to a wide range of environments and the high yield of seeds per pollinated flower (Rick, 1978).

### 2.1.3 *Solanum lycopersicum*

*Solanum lycopersicum* L. (Figure 1) is the only cultivated species of tomato. It is perennial in its natural state, but it is cultivated as an annual, among other reasons because heavy fruit production drains the plant's reserves (Rick, 1978). Its cycle is quite short: from 90 to 150 days only. The plant usually produces 6 to 11 leaves before entering the reproductive phase (Gharbi, 2017) and the complete reproductive phase can last from 40 to 60 days from flowering until full ripeness of the fruit (Rick, 1978).

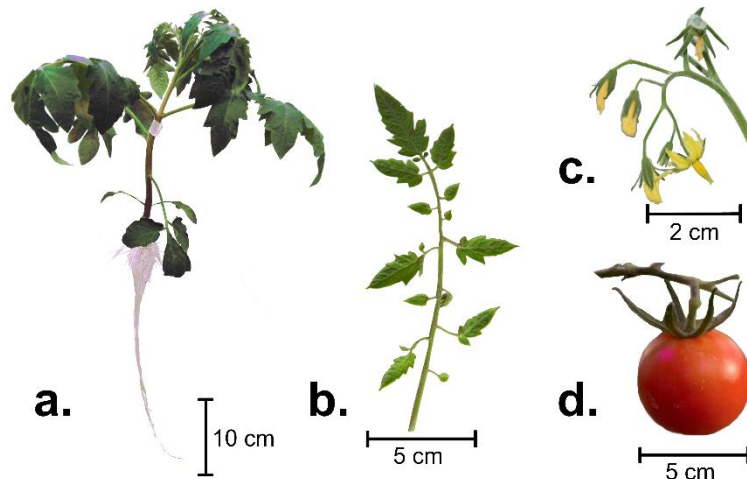


Figure 1 – (a) Full *S. lycopersicum* plant, aged 14 days; (b) leaf; (c) inflorescence; (d) fruit. Sources: plant, leaf and inflorescence, personal pictures; fruit, courtesy of Ms. Aurélie Grosjean.

Although there seems to be a wide variability within the species, in shape, size and colour, the genetic base has been narrowed because of selection of specific traits such as plant height, earliness, fruit morphology and fruit colour, in a phenomenon known as 'domestication syndrome'. The number of single-nucleotide polymorphisms (an indicator of genetic diversity) was shown to be twenty times lower in *S. lycopersicum* than in its wild species. This low genetic diversity is now an obstacle to tomato improvement towards biotic and abiotic resistance (The 100 Tomato Genome Sequencing Consortium et al., 2014).

### 2.1.4 *Solanum chilense*

*Solanum chilense* Dun. (Figure 2) is a relative species of *Solanum lycopersicum*. This wild tomato originates from the Atacama Desert in South America, known for its extreme aridity. The species is actually acclimated to a set of conditions ranging from sea coasts to high altitudes. It is able to thrive on stony and rocky soils (Tapia et al., 2016).

*Solanum chilense* can be distinguished from *S. lycopersicum* by morphological characteristics on the architecture, the leaves, the flowers and the fruit. The leaves of *S. chilense* are more indented, smaller and have a more greyish colour (Grandillo et al.,

2011). The anthers are smaller than the stigma, which seems, in tomato, linked to allogamy. The fruit are small and show a variety of colours when mature (Chetelat et al., 2009).

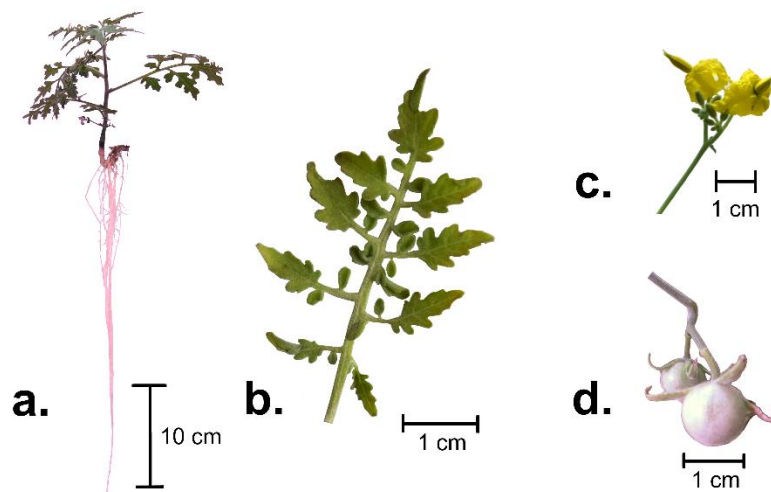


Figure 2 – (a) Full *S. chilense* plant, aged 14 days; (b) leaf; (c) inflorescence; (d) fruit.  
Source: plant, leaf and inflorescence, personal pictures; fruit, courtesy of Ms. Servane Bigot.

*Solanum chilense* is one of the tomato species with the widest genetic diversity (Bretó et al., 1993). The species is divided into populations with various characteristics of tolerance to biotic and abiotic stresses (Blanchard-Gros et al., 2021; Böndel et al., 2018). While resistance to biotic stresses has been investigated and *S. chilense* traits have already been undergoing introgression into the cultivated tomato against diseases such as tomato yellow leaf curl virus (Ji et al., 2009; Pérez de Castro et al., 2013) and cucumber mosaic virus (Stamova & Chetelat, 2000), the species has only recently been under investigation for its resistance to abiotic stresses such as salinity (Bigot et al., 2022; Gharbi et al., 2016; Martínez et al., 2012) or heat and drought (Blanchard-Gros et al., 2021; Tapia et al., 2016), even though *S. chilense* shows a promising response to drought and salt stress compared to *S. lycopersicum* (Kashyap et al., 2020). Böndel et al. (2018) showed that *S. chilense* as a species showed a great nucleotide diversity in abiotic tolerance genes, across the numerous endogenous populations found in Chile, making it a good candidate for gene introgression into the cultivated tomato.

Tapia et al. (2016) showed that, compared to *S. lycopersicum*, *S. chilense* was less affected by reduced watering conditions: the internode length did not change in *S. chilense* while it reduced by 63% in *S. lycopersicum*, and the osmotic potential of *S. chilense* was lower than that of *S. lycopersicum* thanks to a higher proline content.

## **2.2 Climate change**

### **2.2.1 Generalities**

Climate change is a wide topic that is being discussed more and more every year. The first warning came as soon as 1896 when the Swedish chemist Svante Arrhenius exposed the first theory about greenhouse warming due to burning of fossil fuels, but it was not until 1979 that a report stated that rising anthropogenic carbon dioxide emissions would lead to a change in climatic conditions. This scientific issue became a policy issue in the second half of the 1980s, and the international community agreed on a globalized counteraction in 1992 at the Rio de Janeiro summit, with the creation of the United Nations Framework Convention of Climate Change (UNFCCC) (Luterbacher & Sprinz, 2001).

The consequences of climate change are assessed and predicted by the Intergovernmental Panel on Climate Change (IPCC) that has issued reports since 1990 (IPCC, 2022). Climate change is a global issue. Since the emissions and politics of a country can have indirect effects on regions on the other side of the world, the responsibilities are hard to attribute. This is why international cooperation is critical for fighting against climate change (Luterbacher & Sprinz, 2001).

Nowadays, everyone has heard about climate change. Conferences of the Parties (COP) involved into the UNFCCC occur on a regular basis with the aim to reach more and more ambitious climate pacts. The most famous one is the Paris Agreement, signed in 2015, being the first legally-binding agreement (UNFCCC, n.d.) and replacing the unsuccessful Kyoto protocol (Keohane & Oppenheimer, 2016).

### **2.2.2 Temperatures**

The IPCC special report on 1.5°C global warming states that, between 2006 and 2015, many regions of the world have recorded a rise in temperature above average and higher than 1.5°C above the pre-industrial level (IPCC, 2018a), while the average change of temperature on Earth was of +0.87°C on this period (IPCC, 2018b). Since this is an average, it means that certain regions have experienced heat periods over 1.5°C hotter than the normal, with possibly drastic effects (IPCC, 2018a). Based on current greenhouse gases (GHG) emissions trends, it is likely that the temperature rise will exceed 1.5°C during this century; the pathways that allow to return to this threshold before 2100 imply that it is not exceeded by more than 0.15 to 0.3°C. Figure 3 shows that in 2022 the global warming is already over 1°C higher than the 1850-1900 level, that it will continue to rise at least until around 2035 depending on the scenario and could continue to rise steadily until 2100 at least (IPCC, 2022).

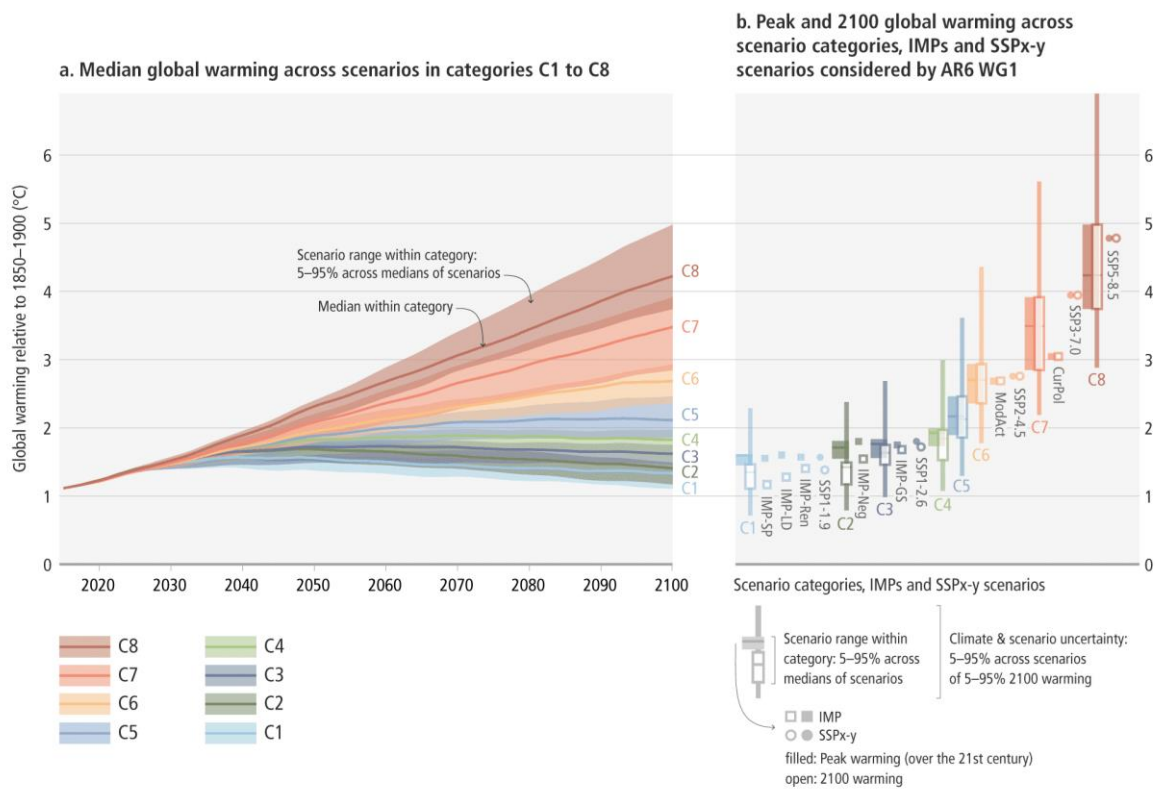


Figure 3 – Projected global mean warming of different scenarios. C1 to C8 relate to different categories of projections: C1 = limitation to 1.5°C in 2100 with no or limited overshoot; C2 = limitation to 1.5°C in 2100 with high overshoot; C3 = peak warming to 2°C over the century; C4, C5, C6 and C7 = warming limited to 2°C, 2.5°C, 3°C, 4°C, respectively, over the century; C8 = warming exceeding 4°C over the century. In some scenarios in C4 and many scenarios in C5–C8, warming continues beyond the 21st century. (a) projected median warming across modelled pathways within a category, with the p5–p95 range; (b) peak and 2100 expected temperatures for categories C1 to C8 as well as illustrative mitigation pathways (IMP) and illustrative scenarios (SSP). Source: IPCC Summary for Policymakers (2022).

The impact of a continued rise in average temperatures will differ from one region to another, and some of them are under threat of being exposed to always more extreme climate events, such as heat waves, droughts or floods due to shifting rainfall patterns (IPCC, 2018a). The 2018 IPCC special report on 1.5°C global warming stressed that these were not assumptions anymore, but predictions (IPCC, 2018a).

In Europe, temperatures are rising faster than the global average: the anomaly ranged from 2.51°C to 2.74°C above the pre-industrial levels in 2020. The rise is projected to be especially noticeable in north-eastern Europe, northern Scandinavia and inland of Mediterranean countries, while western Europe should be less affected (European Environment Agency, 2022).

It is crucial to note that effects of temperature rise is not linear: the consequences of a 1.5°C and a 2°C rise could be completely different, and rising above 1.5°C would be dramatic on a global scale (IPCC, 2018a).

### **2.2.3 Drought**

Drought is defined as a period of time during which the available water quantity is below seasonal norms and affects vegetation development in agricultural and natural systems. It is the most harmful and widespread source of abiotic environmental stress (Krishna et al., 2019). It declines in four types: meteorological drought (rainfall deficit), agricultural drought (deficit in soil moisture – currently worsening across Europe), hydrological and ground-water drought (deficit in surface and subsurface water), and socio-economic drought (imbalance between availability and demand), in timeframes going from weeks to years (European Environment Agency, 2021b).

Climate change has a huge impact on the water cycle, but the changes are difficult to assess with certainty (IPCC, 2018b), because the impacts can be direct or indirect and will vary with regard to the region (IPCC, 2018a). In some regions, the number of rainy days decreases while the intensity of the rain events increases; in some regions, the frequency of droughts increases while it decreases in others. Altogether, the water available for agriculture is likely to become scarce in many developing regions of the world, while the global population and the needs are growing (IPCC, 2018b).

In Europe, as shown on Figure 4, the frequency of drought events has risen significantly over the period 1950 to 2015 across all regions, especially in the Mediterranean, and has decreased in some parts of the British isles, Scandinavia and Iceland (European Environment Agency, 2021a). The current water management patterns will not be able to cope with the forecasted conditions that include water shortages in spring and summer, decrease of water quality due to the higher temperatures and slower runoff and higher risk of flooding in winter, among others (Iglesias & Garrote, 2015).

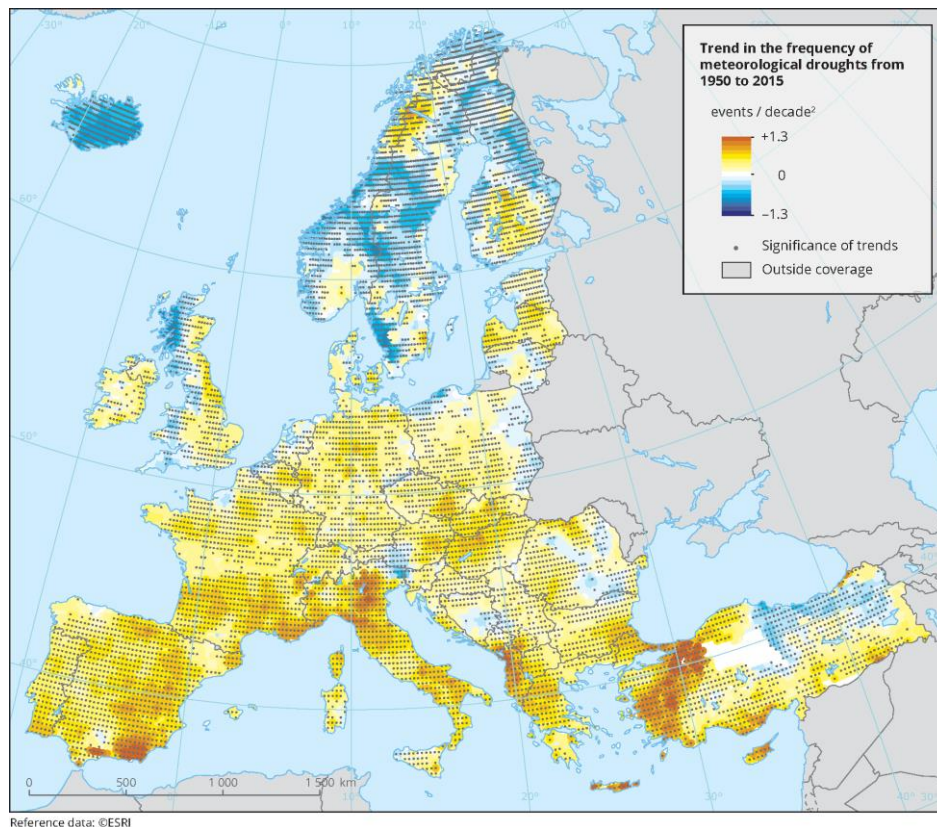


Figure 4 – Trends in the frequency of meteorological droughts between 1950 and 2015 across Europe. The hatching indicates areas where the trends are statistically significant at the 0.95 level. Source: European Environment Agency (2021).

Drought is linked to temperatures. The rise in temperature around the globe causes droughts in regions where there is a strong coupling between air temperature and soil moisture. An increased dryness will prevent a cooling by evaporation, and will therefore lead to an increased dryness due to higher temperature, in a vicious circle (IPCC, 2018b).

### 2.3 Food security

Crop suitability has already been affected by the changes in the environmental conditions, leading to changes in the levels of production of some of the main crops all over the world. The most impacted crops are typical local species growing in specific climate conditions (IPCC, 2018b). As an example, over 65% of historical maize-growing areas from Africa would experience yield losses for 1°C warming under optimal watering conditions; a proportion that would rise to 100% under drought conditions (Lobell et al., 2011).

According to the definition of the Food and Agriculture Organization (FAO, 2002), which was refined through the years, “food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life.”

Food security has been an increasing issue in the last few years. Even though the number of undernourished people decreased by 230 million between 2004 and 2014, it has been increasing ever since and reached an estimated 768 million undernourished people in 2021.

The number of people affected by moderate or severe food insecurity has been increasing ever since the first data available on the FAOSTAT website, in 2014, and reached 2.3 billion people in 2021 (FAOSTAT, 2022). This number is expected to rise alongside the world population, expected to reach 9 billion in 2050

As shown in the definition, food security is not only about food production but covers numerous variables, which means that crop models are not enough to predict the effects of climate change on food security (Devereux & Edwards, 2004; Ingram et al., 2008; IPCC, 2018b). According to Schmidhuber & Tubiello (2007), the crucial aspect of food security is not whether food exists, but if monetary and non-monetary resources are sufficient to allow each individual to access to adequate quantities of food. A country can be self-sufficient and its inhabitants submitted to food insecurity, and vice-versa (Devereux & Edwards, 2004; Ingram et al., 2008; Schmidhuber & Tubiello, 2007).

In many regions where food security is lacking, local communities rely heavily on small-scale production; the products are consumed and sold on the local markets. If the climatic conditions change, it is then not only the quantity of food that will decrease, but the income as well, while the costs of maintaining a farm are constant (Brown & Funk, 2008).

Agronomy then faces two major challenges: developing food production systems that can improve food security in a context of climate change (adaptation) and reducing the causes of climate forcing (mitigation) linked to agricultural practises (Ingram et al., 2008). Indeed, agriculture has a significant responsibility in the share of global GHG emissions, producing in majority CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. Altogether, it is obvious that agricultural and food systems will face some of the biggest impacts of climate change over the next few decades (Brown & Funk, 2008).

## **2.4 Adaptation**

Adaptation is crucial since the climate has already changed and is expected to continue to do so in the next decades, even with the strictest mitigation measures.

The increase in global population and the adverse effects of climate change on production bring the need for higher yields. Effort must be put into intensification because extensification is not possible due to increased competition for agricultural inputs such as land, water, energy and other inputs (Garnett et al., 2013). Increasing demand is expected to reinforce these emissions (Garnett et al., 2013; Kang & Banga, 2013). There are cheap adaptation options such as changing planting dates or switching to existing crop varieties that will enable to moderate the negative impacts, but the biggest benefits would probably result from more costly efforts such as the development of new crop varieties and expansion of irrigation. These adaptations will require investments at all levels, from farmers to states, and will therefore require a work of prioritization for these actors whose resources are already under demand (Lobell et al., 2008).

The changes in crops are a way to adapt on the long term, with the creation of new varieties with improved performance in the environmental conditions that are expected in the future. This requires, at first, an assessment of the genetic diversity, a selection and a recombination of genetic resources into new varieties (Chapman et al., 2012). These new varieties will need not only to have high yields under abiotic stresses, but also good nutritional values and good resistance to pests and diseases (Smirnov, 2014). A good way to identify stress-tolerance traits in plants is investigating their wild relatives to understand local adaptations that can potentially improve crops (Böndel et al., 2018; Tapia et al., 2016). The critical point will be the compromise between stress tolerance and yield. Indeed, species adapted to low-resource environments have slow growth rates and do not respond positively to better conditions. The introduction of wild traits in crops could lead to a decreased yield (Hajjar & Hodgkin, 2007; Smirnov, 2014).

Further on, once the new varieties are fixed, management will have to be adapted to these new crops (Chapman et al., 2012).

The role of crop scientists and agronomists is to participate, in an inclusive and cooperative manner, in the development of new, more resilient cropping systems (Ingram et al., 2008) by identifying physiological traits or genes that will be useful in breeding programmes (Smirnov, 2014), while limiting the environmental consequences of agriculture (Ingram et al., 2008).

## **2.5 Abiotic stresses and plants**

### **2.5.1 Impact of abiotic stresses on plants**

Stress can be defined as the occurrence of an external factor that affects the plant in a negative way or a negative deviation from the plant's optimal environmental conditions (Gerszberg & Hnatuszko-Konka, 2017). It can be biotic (induced by living organisms such as predators, parasites and diseases) or abiotic (induced by environmental conditions).

The impact of abiotic stresses on plants vary with the intensity and length of the stress. The effects of different stresses such as temperature and drought can be common or specific to each stress (Descamps et al., 2018). The impact may also differ between plant species and between populations inside a same species, as seen on *S. chilense* populations (Blanchard-Gros et al., 2021), but also depending the development stage of the plant and the duration of the stress (Krishna et al., 2019). Stresses lead to short-term and long-term responses in the plants (Descamps et al., 2018; Tapia et al., 2016).

The timing of the stress in the plant's phenology is also important: vegetative stages, although sensible to stresses, often have the ability to recover at least partially. Reproductive stages on the other hand, can be impacted irreversibly, for example if there was a seed abortion (Cohen et al., 2021).

The combination of stresses can lead to a synergy, where the effects of the combined stresses will be different than an addition of the impacts of the different stresses taken individually (Cohen et al., 2021; Descamps et al., 2018).

### **Stress sensing in plants**

The first step of stress sensing is the perception of stress signals by the receptors of the cell wall, which leads to the activation of signal transduction events involving different types of intermediate stress genes. These belong to one of three categories: gene-encoding proteins with known functions, transcription factors and regulatory proteins or proteins with (yet) unknown functions (Gerszberg & Hnatuszko-Konka, 2017).

### **Temperatures**

High temperatures and subsequent heat stress on plants have shown to impact negatively the yields of a lot of commodity crops around the world, from rice in China to wheat in Eastern Europe or maize in Africa (Haider et al., 2021; Hassan et al., 2021; Lobell et al., 2011).

Heat stress inhibits photosynthesis, among others, because of its impact on the photosystems II (PSII), but also because it increases the fluidity of membranes, affecting membrane functions involved in photosynthesis and respiration (Descamps et al., 2018; Haider et al., 2021; Hassan et al., 2021). It also activates the production of reactive oxygen species (ROS), compromises protein folding and disrupts the enzymes involved in nutrient metabolism (Haider et al., 2021; Hassan et al., 2021). Heat stress impacts most of the plant's stages of life: seed germination, growth rate, biomass production, grain filling, fruit survival... Eventually, even moderate heat stress, on the long run, can lead to the death of the plant. However, this always depend on the species (Haider et al., 2021; Hassan et al., 2021).

In order to limit the leaf temperature rise and subsequent damage, the usual avoidance strategy is to curl the leaves and boost intense transpiration, but also modify the lipidic membrane composition to decrease its fluidity. On a longer term, stomatal and hair density can be increased and the vessels can become larger. Speeding up the reproductive stages is also an escape from extended hot periods, but at the cost of part of the yield (Hassan et al., 2021)

In order to limit the heat and the damage, the usual mechanism of tolerance against heat stress when water is available in sufficient quantities is the increase of the transpiration rate by increasing the leaf number and area, while the height of the plant can be reduced (Descamps et al., 2018).

### **Drought**

Water stress (WS) affects most of the plant's stages of life: seed germination, growth rate, biomass production, grain filling, fruit survival... Eventually, even moderate water stress, on the long run, can lead to the death of the plant (Anjum et al., 2017).

Water stress leads, at first, to short-term impacts on photosynthesis: the plant will close its stomata (Ahluwalia et al., 2021; Descamps et al., 2018; Tapia et al., 2016) in order to limit the loss of water vapour to the atmosphere and preserve its water status, which leads to a decrease in CO<sub>2</sub> intake and therefore in photosynthesis (Anjum et al., 2017). They will also produce ROS and stress hormones, adapt their root and shoot morphology to take more water in and let less water out... (Ahluwalia et al., 2021) among others. Exposed to drought, young roots tend to mature closer to the root tip: the suberin and lignin depositions will form barriers to the apoplastic pathway of water and promote the cell-to-cell pathway (Hernandez-Espinoza & Barrios-Masias, 2020).

Some plant species are adapted to drought and use different pathways to assimilate CO<sub>2</sub> while limiting water losses: the C<sub>4</sub> and CAM pathways, in opposition to the 'classic' C<sub>3</sub> pathway (which is used by the main crops as well as tomato). The C<sub>4</sub> pathway consists in avoiding photorespiration thanks to a modified anatomy, allowing to use CO<sub>2</sub> even in small concentrations, for example when the stomata are closed (RIPE, 2020). The CAM pathway involves opening the stomata at night to absorb CO<sub>2</sub> and store it until using it by day (Yang et al., 2021).

### **Oxidative stress**

Almost all biotic and abiotic stresses cause oxidative stress in the plants. This generalized response consists of an over-production and accumulation of reactive oxygen species (ROS), which can damage cell components and cause dysfunction. The ROS are produced by the plant as part of the signalling system for stress, among other functions still under investigation (Demidchik, 2015).

The effects of oxidative stress include lipid oxidation, which is considered as the main indicator of oxidative stress (Farmer & Mueller, 2013). If it is severe, it leads to damage of the lipidic membranes and their barrier function, as well as the disintegration of organelles and dysfunction of proteins, RNA and DNA. The products of the oxidation of lipids include malondialdehyde, 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal and acrolein (Demidchik, 2015), which are markers of oxidative stress and easy to measure (Farmer & Mueller, 2013).

### **Combined stresses**

As said earlier, combined stresses have a bigger impact on the plants. In particular, the combination of heat and drought stresses could severely impact the plants in terms of stomatal conductance, photosynthesis and respiration (Cohen et al., 2021). The usual strategy of increasing area to enhance higher transpiration rate against heat stress will become inefficient. The effects on photosynthesis are deleterious and the reproductive success is threatened (Cohen et al., 2021; Descamps et al., 2018).

Environmental stresses often occur simultaneously, and this is especially true for heat and water stresses. However, little is yet known about their effects on plants when they are combined. While photosynthesis rate modification is a temporary physiological response

of the plant under stress due to the closing of stomata and subsequent insufficient CO<sub>2</sub> availability, a cooccurrence of heat and water stress could lead to impairment of the PSII, a decrease in Rubisco activity, stomatal conductance and photosynthetic pigments concentration, which would durably impair photosynthesis. The production of ROS would also be worse, with worse lipid oxidation as one of the results (Ahluwalia et al., 2021).

### **2.5.2 Stress tolerance**

Tolerance is an ability from the plant to limit the damage due to stress. It involves a plasticity in the metabolic reactions that allow the plant to function in an unfavourable environment by avoiding, tolerating or recovering from the stress conditions (Gerszberg & Hnatuszko-Konka, 2017).

Tolerance mechanisms allowing acceptable performances under stress conditions find their source in the genes. Breeding is a way to select these traits but it is a slow process, which is why a branch of research is now focusing on identifying the effects of different genes involved in tolerance mechanisms (Tardieu & Tuberosa, 2010). One source of introgression of such traits is in the crop wild relatives: crops' parent species adapted to wild conditions, often subjected to more stresses than the cultivated species. They are mostly used for resistance to abiotic stress, less against biotic stress, and very rarely to enhance yield, since wild species often perform poorly (Hajjar & Hodgkin, 2007).

It was shown that some woody species see a decrease in their xylem vessels as a response to drought, limiting cavitation but also impairing water conductivity. Little is known about the effect of drought on non-woody species (Hernandez-Espinoza & Barrios-Masias, 2020).

### **2.5.3 Impact of abiotic stresses on *Solanum* sect. *Lycopersicon***

Abiotic stresses caused by environmental factors lead to up to 70% of yield loss in cultivated tomato, depending on development stage and duration of stress (Blanchard-Gros et al., 2021). The main limit to the growth of *S. lycopersicum* is the lack of water (Jangid & Dwivedi, 2016; Rick, 1978) due to high transpiration rates (Hernandez-Espinoza & Barrios-Masias, 2020), but extreme temperatures and salinity also threaten its growth and development, and ultimately the yield (Jangid & Dwivedi, 2016; Rick, 1978). *S. chilense* is more tolerant (Blanchard-Gros et al., 2021).

Tomato root hydraulic conductivity was shown to be affected by salinity, temperature, drought and flooding. Hernandez-Spinosa and Barrios-Masias (2020) showed that the xylem vessels diameter and total xylem area decreased under water stress, limiting water conductivity. However, tomato shows high plasticity to water deficit thanks to the partial root drying technique and increases this way its water use efficiency (Hernandez-Espinoza & Barrios-Masias, 2020).

### 3. Objectives

Under the hypothesis that *S. chilense* is more tolerant to environmental stresses than *S. lycopersicum*, the present work initially aimed to investigate how *S. lycopersicum* (♀) x *S. chilense* (♂) hybrids, issued from manual crosses, would perform in comparison to the parent species, under sole and combined conditions of heat stress and water stress. A genetic identification of the hybrids (Annex 1) revealed that the alleged hybrids were in fact genetically identical to the *S. lycopersicum* parent due to a fail of the cross ; moreover, there was a high mortality rate. Due to these reasons, the initial objectives could not be met.

The revised objectives were to compare the response of hydroponic *S. lycopersicum* and *S. chilense* to heat stress and, separately, to water stress, in terms of morphology and physiology during the vegetative stages, and differentiating aerial and shoot systems. Morphological parameters included growth parameters and architectural parameters, and physiological parameters included photosynthesis parameters, water content and content of some stress-related metabolites.

A secondary objective was to establish a protocol for the determination of PEG intake by the plants.

### 4. Material and methods

#### 4.1 Plant material and growing conditions

The species under investigation are the cultivated tomato *Solanum lycopersicum*, cv. Ailsa Craig (AC) (accession number LA2838A) and *Solanum chilense* (SC) (accession number LA4107). Seeds of *S. lycopersicum* and *S. chilense* were firstly obtained from respectively the Tomato Genetics Resource Center (TGRC, University of California, Davis, CA, USA) and INIA-La Cruz (La Cruz, Chile), before multiplication in the UCLouvain greenhouses.

One hundred *S. chilense* seeds were submitted to a disinfection protocol before sowing due to their low germination rate. This treatment consisted on washing the seeds with a 0.735% chlorine bleach solution for 10 minutes in Eppendorf tubes, then rinsing them 3 to 5 times with demineralized water. The seeds were put to germinate on two layers of sterile filter paper moistened with sterile water at a rate of  $\approx$  30 seeds per Petri dish. The dishes were sealed with parafilm and placed in a culture chamber under a photoperiod of 16 hours at 24°C.

The *S. chilense* seedlings were transplanted in germination trays 6-7 days after the germination protocol. The 6-cm-deep plastic germination trays were filled with potting soil and covered with a glass pane after watering to maintain humidity. Germination trays were put in temperate greenhouse conditions with a photoperiod of 16h. The glass pane was

removed once the seedlings grew to touch it. Seedlings were watered three times a week after removing of the glass pane.

100 *S. lycopersicum* seeds were sown 7 days after the transplanting of the *S. chilense* in the same germination trays and potting soil under greenhouse conditions. They were also covered with a glass pane and watered three times a week after removing of the glass pane. The delay of sowing between both species was intended to obtain plants about the same size at stress imposition as *S. chilense* grows slower than *S. lycopersicum* at seedling stage.

Seedlings of both species were transplanted in hydroponics at the same time, 12 days after the *S. lycopersicum* sowing. They were transplanted in 10L tanks, containing 9L of Hoagland nutritive solution, the composition of which can be found in Table 1 (Hoagland & Arnon, 1950). There were 5 tanks per condition. Each tank hosted 3 replicates of each species, placed randomly (example with *S. lycopersicum*, *S. chilense* and hybrids shown in Figure 5). The total replicates per species and condition was of 15 individuals.

Table 1 – composition of the Hoagland solution used in the experiment.

Molecule	Concentration [mg.L <sup>-1</sup> ]
NH <sub>4</sub> NO <sub>3</sub>	4
Ca(NO <sub>3</sub> ) <sub>2</sub> .4 H <sub>2</sub> O	416
KNO <sub>3</sub>	203.5
KH <sub>2</sub> PO <sub>4</sub>	137
MgSO <sub>4</sub> .7H <sub>2</sub> O	123
MnSO <sub>4</sub> .5H <sub>2</sub> O	0.265
H <sub>3</sub> BO <sub>3</sub>	0.7
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.075
(NH <sub>4</sub> )Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0.04
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.3
Fe EDDHA	10



Figure 5 – Aerial view of tank number 13, in 28°C WW conditions, as an example for the placing of plants in the tanks. In each tank, three individuals of each species (*S. lycopersicum*, *S. chilense* and the hybrid) were placed randomly in holes in the polystyrene support and annotated: AC = *S. lycopersicum* (cv. Ailsa Craig); SC = *S. chilense*; Hyb = hybrid. Each individual plant has its own number for easier referencing.

## 4.2 Experimental design

Two separate experiments were conducted for testing, respectively, the effects of heat stress and water stress on the investigated species. The detail of the conditions can be found in Table 2.

Table 2 – Experimental setup of the two experiments showing the conditions, number of tanks and number of plants per treatment (WW = well-watered; WS = water stress).

Experiment 1				
Tanks per treatment		Species	Replicates per treatment	
21°C	WW	<i>S. lycopersicum</i>	15	
	WS		15	
28°C	5	<i>S. chilense</i>	15	
	5	Hybrids	15	
Experiment 2				
Tanks per treatment		Species	Replicates per treatment	
5	WW	<i>S. lycopersicum</i>	15	
	WS	<i>S. chilense</i>	15	

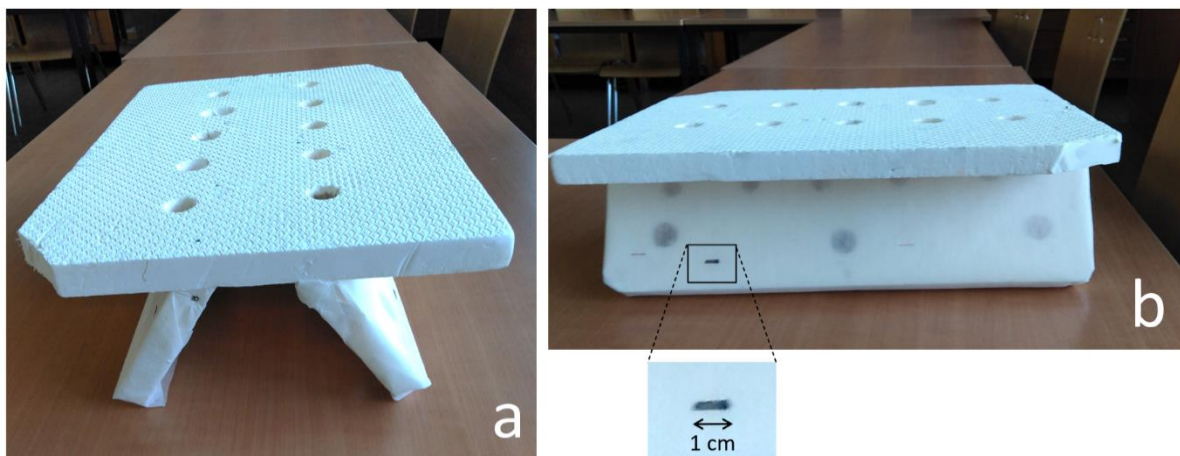
Polyethylene glycol (PEG) is a neutral polymer used in aqueous solutions to adjust osmotic potential ( $\psi_s$ ) (Lawlor, 1970; Michel & Kaufmann, 1973); it can be used in nutritive solutions to induce water stress in a controlled manner for experimental purposes (Lagerwerff et al., 1961). Water stress (WS) was simulated as an osmotic stress in both experiments 4 days

after transplanting by a 10% PEG (molecular weight 6000, Sigma-Aldrich) solution with the same Hoagland concentration as the WW conditions, adjusted to 9L. The pH was brought to 5.5 to 6.5. In experiment 2, water-stressed tanks were oxygenated twice a week using a syringe.

On experiment 2, 1 tank per treatment was equipped with a custom system for root observation (Figure 6) adapted from Chen et al. (2011). The device consisted of a horizontal panels pierced with holes and two oblique panels attached under the horizontal panel and aligned with the holes in it. The inferior panels were covered with Calbiochem® Miracloth (filtration material) for root adhesion and were immersed into the nutritive solution. One-centimetre-long bars were drawn on the side panels for subsequent picture analysis.

The plants were installed in the hole on the superior side so that the root system would develop on the sloped pane underneath. Pictures of the root system were taken three times a week, on the days of leaf counting.

This device did not allow the extraction of usable data.



*Figure 6 – Custom system for root observation made of polystyrene: (a) aerial view; (b) side view. The inferior panels were covered with Calbiochem® Miracloth (filtration material) for root adhesion and were immersed into the nutritive solution. One-centimetre-long bars were drawn on the side panels for subsequent picture analysis.*

### **4.3 First experiment: impact of water and heat stress**

The temperature conditions were implemented from the moment of transplanting in hydroponics, by placing the tanks in two different greenhouses, one at  $21.61 \pm 0.03^\circ\text{C}$  with a relative humidity (RH) of  $46.8 \pm 0.2\%$  and the other one at  $27.33 \pm 0.02^\circ\text{C}$  with a RH of  $62.6 \pm 0.2\%$ . The photoperiod was maintained at 16h with supplemental lighting (LumiGrow Pro Series LED Lighting Systems).

The water conditions were well-watered (WW) and water-stressed (WS). The conductivity of the solutions was  $0.877 \pm 0.02$  mS/cm in WW tanks and  $0.663 \pm 0.04$  mS/cm in WS tanks. The osmotic potential of the solutions was  $-0.025 \pm 0.010$  MPa in WW tanks and  $-0.229 \pm 0.031$  MPa in WS tanks.

With 15 replicates for each species (*S. lycopersicum*, *S. chilense* and hybrids) and treatment (21°C WW, 21°C WS, 28°C WW, 28°C WS), there were 180 plants in total.

Figure 7 shows a graphic representation of the experimental design.

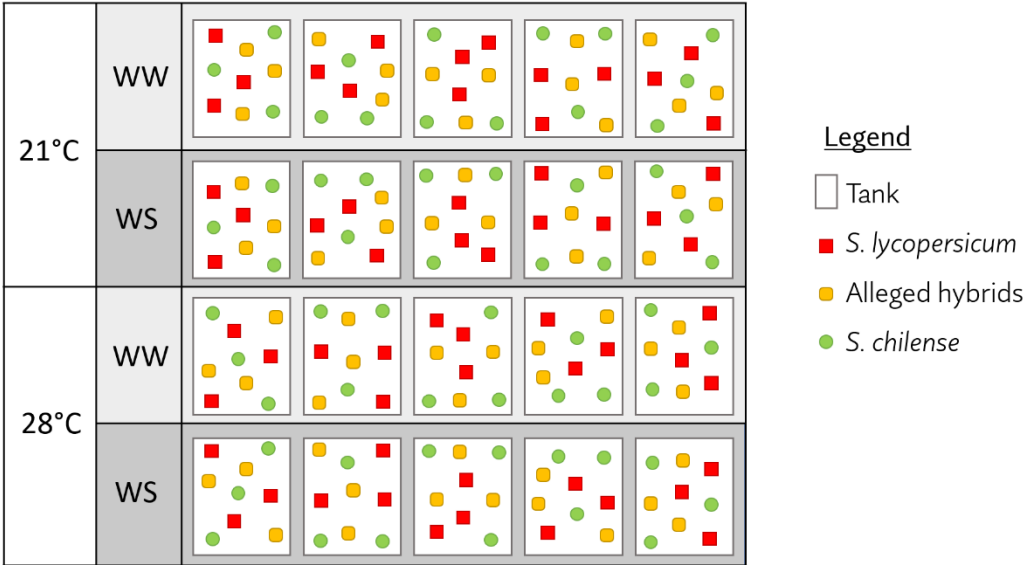


Figure 7 – Experimental design for the first experiment. WW = well-watered; WS = water stress.

#### 4.4 Second experiment: impact of water stress

All plants were in the same temperature conditions, in a temperate greenhouse at  $22.05 \pm 0.04^\circ\text{C}$ , with a RH of  $44.5 \pm 0.2\%$  and a photoperiod of 16h.

The water conditions were well-watered (WW) and water stress (WS). The conductivity of the solutions was  $0.882 \pm 0.029 \text{ mS/cm}$  in WW tanks and  $0.636 \pm 0.067 \text{ mS/cm}$  in WS tanks. The osmotic potential of the solutions was  $-0.049 \pm 0.016 \text{ MPa}$  in WW tanks and  $-0.221 \pm 0.032 \text{ MPa}$  in WS tanks.

With 15 replicates for each species (*S. lycopersicum* and *S. chilense*) and treatment (WW and WS), there were 60 plants in total.

Figure 8 shows a graphic representation of the experimental design.

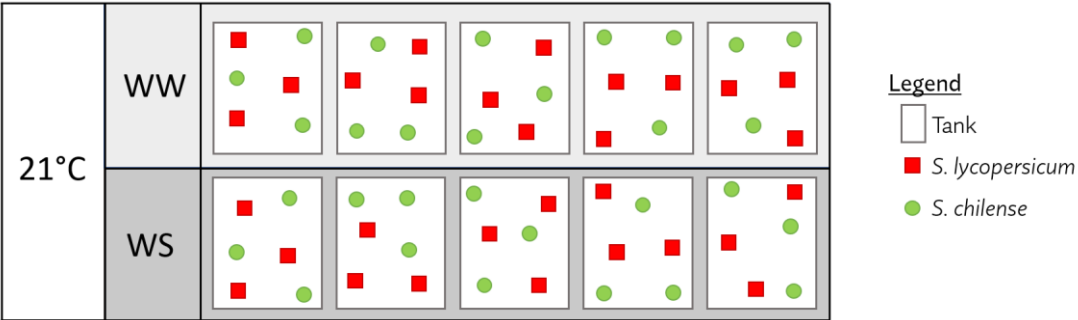


Figure 8 – Experimental design for the second experiment. WW = well-watered; WS = water stress.

## 4.5 Analysed parameters

Unless otherwise stated, all measurements were taken on 3 plants from each treatment. In experiment 1, default analysis was only performed on 21°C WW and 28°C WW treatments (heat treatments), unless otherwise stated.

### 4.5.1 Morphological parameters

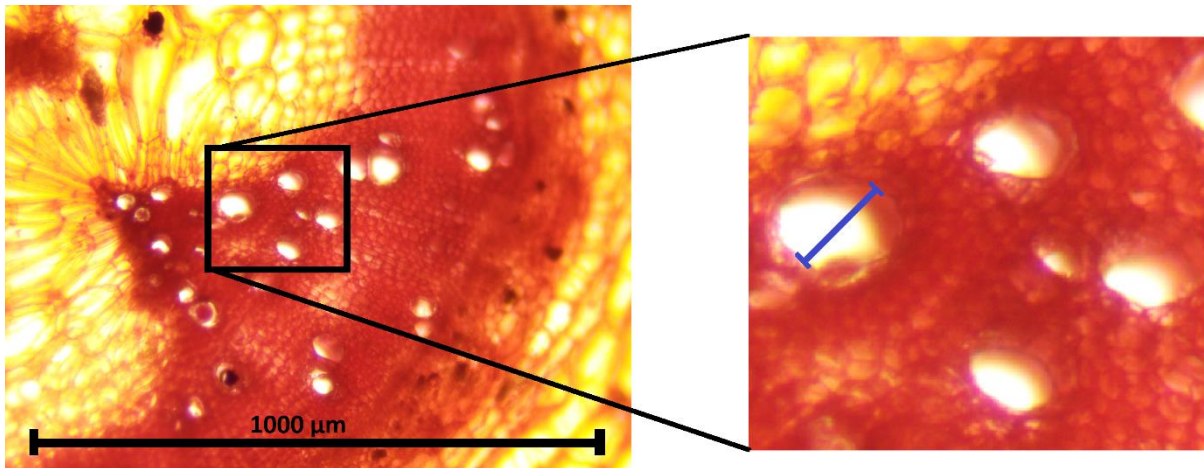
- a) **Mortality:** dead individuals were counted three times a week, during the leaf counting, for all individuals of each treatments and species, except from the hybrids.
- b) **Biomass production:** fresh and dry weight. At the end of the experiments, aerial and root systems were harvested, weighed fresh and put for a week at 70°C (experiment 1)/60°C (experiment 2) to dry before being weighed again. The shoot/root ratio was calculated as the ratio between the dry weight of the shoot and the dry weight of the root.
- c) **Leaf production:** From transplanting, leaf number was counted three times a week for all individuals of each treatments and species, except from the hybrids. Axillary leaves were counted as leaves as soon as they flattened.
- d) **Leaf area:** At the end of the experiment, the aerial systems of 5 individuals per species and treatment were scanned with a LI-3100 Leaf Area Meter (LI-COR) which returned immediate surface values, or photographed and analysed with ImageJ to extract leaf surfaces when the leaves were too small for the LI-3100 Leaf Area Meter.
- e) **Stomatal density:** After two weeks of cultivation it was measured by putting transparent nail polish on the epidermis of the inferior side of the distal leaflet of the biggest leaf of 3 individuals of every species and treatment (including the WS treatments in experiment 1), detaching it, putting it on a microscope slide and photographing it. The pictures were then analysed with ImageJ for counting the number of stomata on a measured surface.
- f) **Root length:** At the end of the experiment, the root systems of 5 individuals per species and treatment were scanned with an Epson Perfection V850 Pro scanner and analysed in ImageJ to measure the 3 longest roots.

In experiment 2, root development through time was measured by photographing the roots of the plants in the tanks equipped with a root observation system three times a week. The pictures were then analysed in the ImageJ software (Rasband, 1997) to observe the explored area and the length of the 3 longest roots at each date.

- g) **Root diameter:** In experiment 2, the roots were collected and conserved in FAA (Formalin: acetic acid: alcohol, 1:1:18 vol.), then cut in segments (first and second segments: 1 cm ; third segment and further: 1.5 cm). The segment were cut in cross-sections and observed under microscope or stereomicroscope and photographed. The

images were analysed with ImageJ to extract the diameter of the roots at the different segments.

- h) **Xylem elements diameter:** Diameter of the two largest xylem elements was measured as shown on Figure 9 on the same cross-section pictures as root diameter.
- i) **Ratio xylem/root diameters:** The ratio xylem diameter/root diameter was calculated as the ratio between the diameter of each xylem element and the diameter of the corresponding root.



*Figure 9 – Measurement of one of the largest xylem element on a micrograph. The scale was set then the xylem element diameter was measured. In order to obtain a rough average between the focal axis (longest ‘diameter’) and secondary axis (shortest ‘diameter’) of non-circular elements, the measurement was done on an intermediary length.*

#### **4.5.2 Physiological parameters**

##### **a) Water content (WC)**

The WC was calculated from fresh and dry weight using the formula (Equation 1):

$$WC [\%] = 100 \times \frac{FW - DW}{FW} \quad (1)$$

Where *FW* = fresh weight and *DW* = dry weight.

##### **b) Chlorophyll content index (CCI)**

The CCI was measured with a plant nutritional analyser (TYS-3N/4N) once on one random leaf of 5 plants per condition. In experiment 1 it was on day 17 of heat stress and in experiment 2 on day 9 of water stress. It is expressed in SPAD units, SPAD standing for ‘Soil Plant Analysis Development’.

##### **c) Osmotic potential of the leaf cells sap.**

Each leaf sample (several leaflets of one random leaf per individual) was submitted to three frost/defrost cycles in liquid nitrogen then centrifugated 8 min at 5867g, 4°C in a bottom-perforated Eppendorf tube placed in another non-perforated Eppendorf tube.

The collected sap was analysed with a VAPRO® Model 5600 osmometer (ELITechGroup).

**d) Malondialdehyde (MDA) content.**

The method used was this of Heath & Packer (1968). 250 mg fresh material (or, when insufficient material, as much as possible) was ground in liquid nitrogen and weighed; the powder was suspended in 5 mL trichloroacetic acid (TCA) 5% w/v with 1.25% glycerol, centrifuged at 12,000g for 10 minutes then filtered. 2 mL of the supernatant were added to 2 mL of thiobarbituric acid (TBA) 0.67%. Samples were heated for 30 min at 100°C in a water bath, cooled down on ice then centrifuged at 12,000g for 1 min. Absorbance (A) was measured at 532 and 600 nm with a Shimadzu UV-1800 spectrometer. The results were transformed as follow (equations 2, 3 and 4) to obtain the MDA content (C) of the fresh material [mmol/g].

$$A = A_{532} - A_{600} \text{ (elimination of non-specific absorbance due to turbidity)} \quad (2)$$

$$C_{\text{cuvette}} = A/\epsilon \quad (3)$$

$$C_{\text{fresh material}} = \frac{C_{\text{cuvette}} * \text{dilution} * V_0}{m_{\text{fresh material}}} \quad (4)$$

Where  $\epsilon$  is the molar extinction coefficient (155 mmol/L.cm), **dilution** is the dilution factor (2),  $V_0$  is the initial volume (5 mL) and  $m_{\text{fresh material}}$  is the weighed initial quantity of fresh material [g].

**e) Total soluble sugars content.**

Preparation of the samples: 250 mg (or, when insufficient material, as much as possible) fresh material was ground in liquid nitrogen and weighed, the powder was suspended in 4 mL ethanol 70% and let to react 5 min on ice; the solution was centrifuged 10 min at 5867g, 4°C, then filtered. The residue was re-suspended in 2 mL ethanol 70% and let to react 5 min on ice. The solution was centrifuged 10 min at 5867g, 4°C, then filtered. The supernatant was added to the previous one. These steps were repeated once more on the residue. The volume of the combined supernatants was brought to 7 mL with ethanol 70%.

Preparation of the solutions: an anthrone solution was prepared with 12.5 mL distilled water, 250 mL H<sub>2</sub>SO<sub>4</sub> and 500 mg anthrone, cooled down on ice and kept away from light. A D-glucose solution was prepared in ethanol 70%, with concentrations ranging from 0 to 500 mg.L<sup>-1</sup> by steps of 50 mg.L<sup>-1</sup> in a 200 µL volume, with 3 replicates.

Analysis: 3 replicates of 200 µL were collected for each sample. 1 mL anthrone was added to each sample and D-glucose range sample, which was then put in the water bath at 100°C for 10 min. After cooling down in ice, absorbance at 625 nm was measured with a Shimadzu UV-1800 spectrometer. The equation of the calibration line was extracted from the values of absorbance linked with the range of D-glucose and

the concentrations ( $C_{fresh\ sample}$  [mg/g<sub>fresh matter</sub>]) for the samples were calculated with equation 5.

$$C_{fresh\ sample} = \frac{C_{sample} * V_0}{m_{fresh\ sample}} \quad (5)$$

Where  $C_{sample}$  is the concentration of the ethanol extract [mg/L],  $V_0$  is the initial volume (7 mL) and  $m_{fresh\ material}$  is the weighed initial quantity of fresh material.

#### f) Photosynthesis parameters.

In experiment 1, photosynthesis parameters were measured on the youngest fully developed leaf of 3 individuals of each species under 21°C WW, 21°C WS and 28°C WW conditions with an integrated fluorometer and gas exchange system (iFL, ADC Bioscientific, Hoddesdon, UK). For each plant, a photosynthetic light response curve (A-Q curve) and a rapid light curve (RLC) combining measures of gas exchanges and fluorescence were performed. Ambient PAR was 60  $\mu\text{mol}/\text{m}^2\cdot\text{s}$  and absorptance was fixed at 0.84.

The machine (Figure 10) is constituted of an infrared gas analyser (IRGA) [1] and a fluorimeter [2] linked to a head [3, detail on picture b]. A probe [4] was drawn to the exterior to get the atmospheric CO<sub>2</sub> concentration (436 ppm). A leaf was then pinched in the head and air was brought into the leaf chamber [6] at a flow rate of 200  $\mu\text{mol}\cdot\text{s}^{-1}$ . H<sub>2</sub>O and CO<sub>2</sub> concentrations were measured before and after passing on the surface of the leaf, at different photosynthetically active radiation (PAR) intensities emitted by the light module [5].

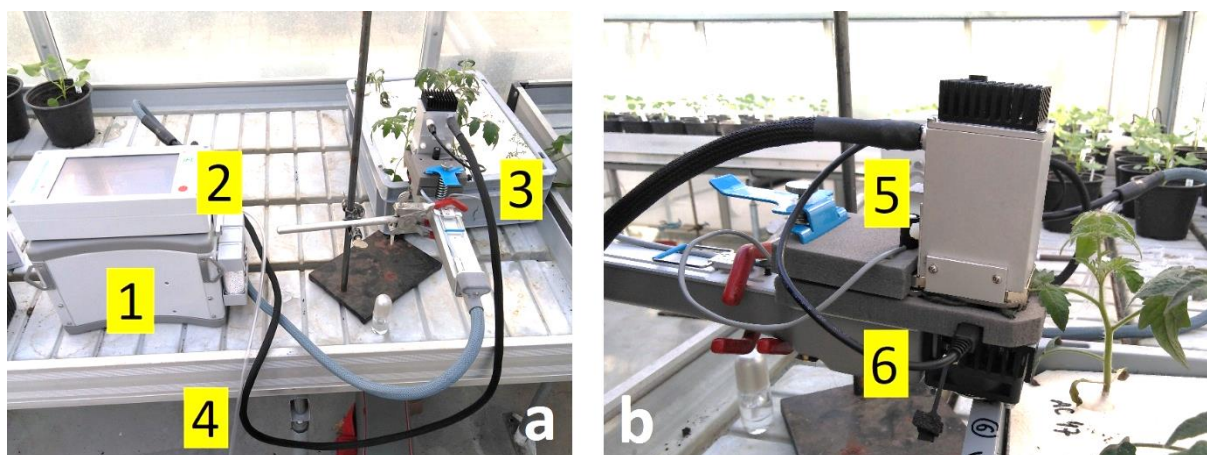


Figure 10 – Experimental device for the measurement of photosynthesis parameters and fluorescence. (a) complete iFL machine: [1] infrared gas analyser, [2] fluorimeter, [3] head, [4] ambient CO<sub>2</sub> probe; (b) head: [5] lamp, [6] leaf chamber. When the claw was pinched on a leaf, light was sent by the light module at desired intensity or intensity sequence, and air flow was sent through the gas chamber.

The A-Q curves (long curves) were measured with a light sequence from 0 to 1400  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ , in steps of 100  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ , 3 minutes under each intensity. The A-Q curves returned by the machine (asymptotic curves) were fitted using the following equation (equation 6), from de Lobo et al. (2013):

$$A = \frac{\Phi * PAR + A_{sat} - \sqrt{(\Phi * PAR + A_{sat})^2 - 4 * \theta * \Phi * PAR * A_{sat}}}{2 * \theta} - Rd \quad (6)$$

Where  $A$  is the photosynthesis rate,  $\Phi$  is the slope of the linear phase of the curve and represents the maximum apparent yield of photosynthesis,  $A_{sat}$  is the plateau of the curve and represents the photosynthesis at saturating irradiance,  $\theta$  is the convexity of the curve and represents the photosynthesis at saturating irradiance,  $Rd$  is the y-axis intercept and represents daytime respiration. The parameters mentioned above were extracted with a R script developed by Tomeo (2019), as well as the LCP which represents the light compensation point or the value of PAR at which the photosynthesis exactly balances the daytime respiration and  $Q_{sat\_75\%}$  which represents the PAR at 75% of saturation of photosynthesis.

The RLC (rapid curves) were performed with the same device as mentioned above (Figure 10). The PAR was defined as a sequence from 0 to 900  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ , in steps of 100  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ , 17 seconds at each intensity. The curves returned were asymptotes and were fitted using the following equation (equation 7), described by Platt et al. (1980) and Ralph & Gademann (2005):

$$ETR = ETR_{max} \left[ 1 - \exp\left(-\frac{\alpha * PAR}{ETR_{max}}\right) \right] \quad (7)$$

Where  $ETR_{max}$  is the electron transport rate at saturating irradiance and  $\alpha$  is the slope of the linear phase of the curve.

One of the parameters mentioned above,  $ETR_{max}$ , was extracted from the fitted curves with a R script using the Phytotools package, as well as  $E_k$ , the minimum saturating irradiance and limit between photochemical and nonphotochemical quenching.

#### g) Parameters extracted from A-Q curve raw data

The efficiency of photosystems II ( $\Phi_{PSII}$ ) was measured at the same time and under the same PAR intensity sequence as the photosynthesis parameters. The light module measured the fluorescence emitted by the leaf and calculated  $\Phi_{PSII}$  as follows (equation 8):

$$\Phi_{PSII} = \frac{F'_m - F_s}{F'_m} \quad (8)$$

Where  $F'_m$  is the maximum fluorescence of the system adapted to obscurity and  $F_s$  is the basic fluorescence under constant lighting.

The values of  $\Phi_{PSII}$  at different PAR intensities were then approximated with a second degree fitting curve and the values at ambient PAR were extracted and analysed.

Transpiration ( $E$ , [ $\mu\text{mol}/\text{m}^2.\text{s}$ ]) was extracted from the raw data at the PAR intensity closest to ambient value ( $100 \mu\text{mol}/\text{m}^2.\text{s}$ ). It was calculated by the machine as follows (equation 9):

$$E = \Delta H_2O * \frac{u_s}{p} \quad (9)$$

Where  $\Delta H_2O$  is the quantity of water evaporated [mbar],  $u_s$  is the air flow reported to the leaf surface in the leaf chamber [ $\mu\text{mol}/\text{m}^2.\text{s}$ ] and  $p$  is the atmospheric pressure (1012 mbar in that case).

The stomatal conductance  $g_s$  was also extracted at the PAR intensity closest to ambient value ( $100 \mu\text{mol}/\text{m}^2.\text{s}$ ).

#### **4.6 Development of a protocol for the determination of polyethylene glycol intake**

In the first experiment, a new protocol for determining the uptake of PEG by the plants was experimented based on Boulter & McMichael (1970) and Lawlor (1970). The following protocol was extracted and tested on fresh material from experiment 1. The final version of the deliverable is in Annex 2.

Preparation of the samples: around 500 mg fresh material (or, when insufficient material, as much as possible) was ground in liquid nitrogen and weighed, the powder was suspended in 8 mL distilled water, centrifugated 10 minutes at 500g and filtrated, then resuspended in 10 mL distilled water.

Preparation of the solutions. The following solutions were prepared prior to the experiment:  $\text{BaCl}_2$  10% (w/v),  $\text{Ba}(\text{OH})_2$   $0.15 \text{ mol.L}^{-1}$  and  $\text{ZnSO}_4$  5% (w/v) (extraction reagents), gum arabic  $36 \text{ mg.L}^{-1}$  and a mix of trichloroacetic acid (TCA) 30% and  $\text{BaCl}_2$  5%. On the day of the experiment, a TCA mix was prepared with 4 parts of the TCA- $\text{BaCl}_2$  solution and 1 part of the gum arabic solution. To prepare a standard curve, a range of PEG solutions was also prepared with concentrations ranging from 0 to  $0.16 \text{ mg.L}^{-1}$ .

Analysis: 0.6 mL  $\text{BaCl}_2$ , 1.2 mL  $\text{Ba}(\text{OH})_2$  and 1.2 mL  $\text{ZnSO}_4$  were added to 3 mL of the samples and to the range samples, both supplemented with 3 mL distilled water, then the tubes were centrifugated 10 min at 500g. 2 mL of the supernatant were collected and supplemented with 2 mL of the TCA mix. Absorbance at 420 and 635 nm was read with a Shimadzu UV-1800 spectrometer.

#### **4.7 Data management and statistical analysis**

Data was stored and manipulated in Excel sheets (Microsoft® Excel®, Microsoft 365 MSO, version 2205 for Windows) and statistical analyses and graphs were performed using RStudio (RStudio 2022.02.3 for Windows).

Every statistical analysis featured a Shapiro-Wilk test of normality and a Levene test of homoskedasticity. If those tests confirmed normality and homoskedasticity of the data, a linear model was made and an analysis of variance was performed. If normality and homoscedasticity were ruled out, the factors were compared separately with a Kruskal-Wallis test.

Default p-value was  $\alpha = 0.05$ ; it was raised to  $\alpha = 0.1$  in some cases. A complete table of the test statistics and corresponding p-values can be found in Annex 3 for experiment 1 and Annex 4 for experiment 2.

## 5. Results

### 5.1 First experiment: heat and water stress

All test statistics and p-values from the statistical analyses of experiment 1 results can be found in Annex 3.

When data was available, all 4 treatments (21°C WW, 21°C WS, 28°C WW and 25°C WS) were compared. Due to mortality, there was no data for most of the parameters under water stress. In these cases, only the heat levels of well-watered treatments were compared: 21°C WW and 28°C WW.

#### 5.1.1 Morphological parameters

##### a) Mortality

The mortality rate was large in *S. chilense* in both water stress conditions, rising up to 67% of the individuals at day 14. *S. lycopersicum* under water stress conditions was also impacted although less severely (47% under 28°C WS) (Table 3).

Table 3 – Percentage of dead individuals per species in each treatment through time.  
WW = well-watered; WS = water stress.

Days from start of stress	<i>S. lycopersicum</i>				<i>S. chilense</i>			
	21°C		28°C		21°C		28°C	
	WW	WS	WW	WS	WW	WS	WW	WS
3	0	0	0	0	0	0	0	6.7
5	0	0	0	6.7	0	6.7	0	33.3
7	0	0	0	6.7	0	20	0	40
10	0	0	0	33.3	0	33.3	0	46.7
12	0	6.7	0	40	0	46.7	0	53.3
14	0	13.3	0	46.7	0	60	0	66.7

The surviving plants in 28°C WS conditions were stunted and dehydrated (Figure 11).

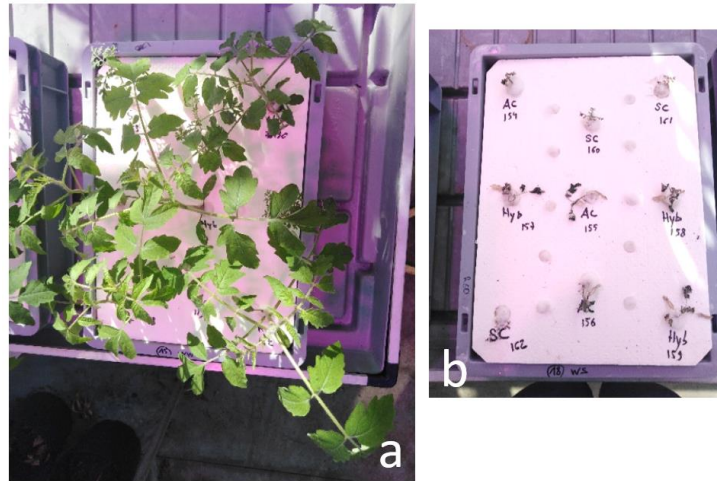


Figure 11 – Picture of tanks at 28°C, (a) well-watered, (b) water-stressed, taken at day 10 of water stress.

It must be noted that the *S. chilense* plantlets transplanted in the tanks were smaller than expected from plants of this species at this age.

This high mortality in water-stressed plants seriously limited the biological material quantity for destructive analyses, which were therefore only conducted on well-watered plants and allowed to observe the effect of heat stress only.

### b) Biomass production

*Solanum lycopersicum* showed a higher dry weight than *S. chilense*. Regardless of treatment, the shoot of *S. lycopersicum* weighed 82.6% more and its roots weighed 84.6% more than the corresponding organs of *S. chilense* (Figure 12, Annex 3).

In both species, root weight was lower than shoot weight (-84.9% in *S. lycopersicum* and -84.3% in *S. chilense*) under a same treatment (Annex 3).

There was no significant impact of temperature on the dry weight (Figure 12, Annex 3).

The shoot/root ratio did not highlight any significant difference between temperatures (Annex 3).

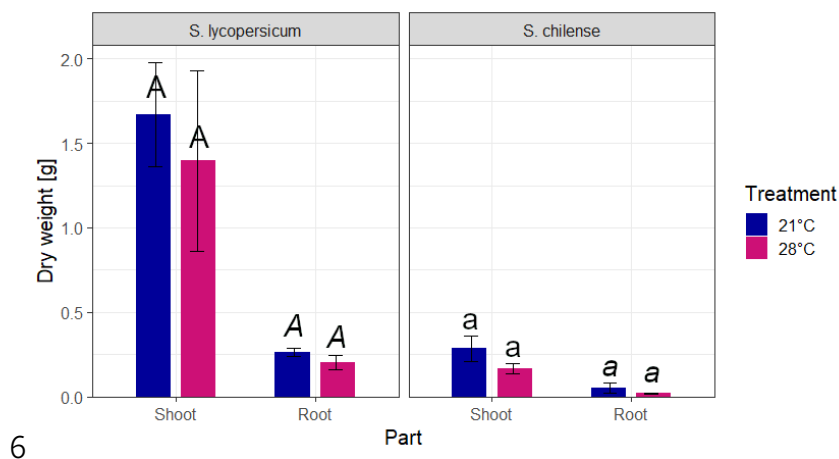


Figure 12 – Dry weight of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress. The standard deviation from the mean is shown on

top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in roman for the shoot and in italics for the root).

### c) Leaf production

Leaf production showed a linear rate in the 14 days of water stress (Figure 13, Annex 3).

The rate of leaf production was different between species: *S. chilense* reached a higher number of leaves at the end of the experiment but also had more leaves than *S. lycopersicum* at the time of transplanting. *Solanum lycopersicum* and *S. chilense* both showed an impact of the water treatment with more leaves under well-watered conditions (+27% for *S. lycopersicum* and +31% for *S. chilense*), independently of heat treatments (Annex 3).

*Solanum chilense* was not impacted by temperature. *Solanum lycopersicum*, on the other hand, showed a lower number of leaves at 28°C than at 21°C under water stress (-27%), but no significant difference due to temperature under well-watered conditions (Annex 3).

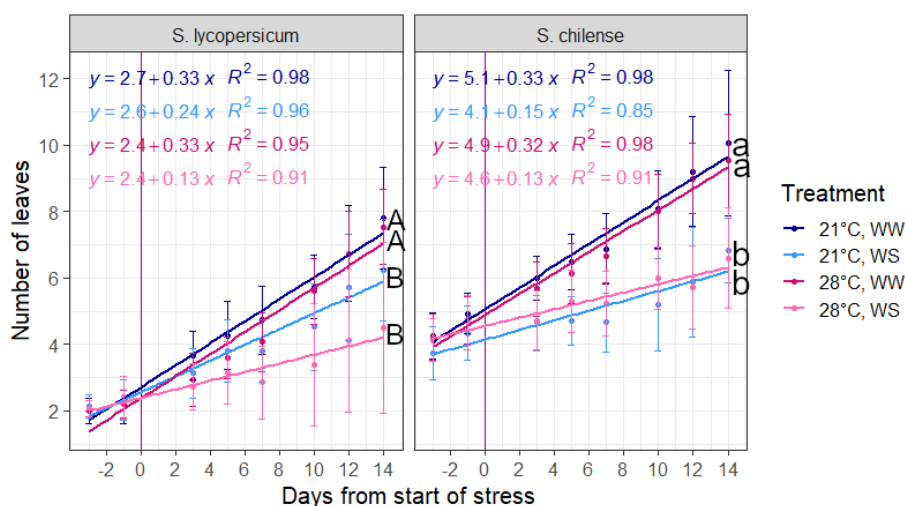


Figure 13 – Leaf production of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions. The equations and R<sup>2</sup> values of the linear regressions are shown, as well as the standard deviation from the mean. The letters indicate significant differences ( $p < 0.05$ ) in the number of leaves at the end of experiment (day 14), in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### d) Leaf area

*Solanum lycopersicum* showed, regardless of treatment, a leaf area 83% larger than *S. chilense* (Figure 14, Annex 3).

A visual evaluation of the results shown in Figure 14 seemed to show an effect of heat stress on *S. lycopersicum*, but this visual impression was not supported by statistical results due to the high variability among data. Only *S. chilense* showed a significant difference between

heat treatments with an area 44% smaller under heat stress than under control conditions (Annex 3).

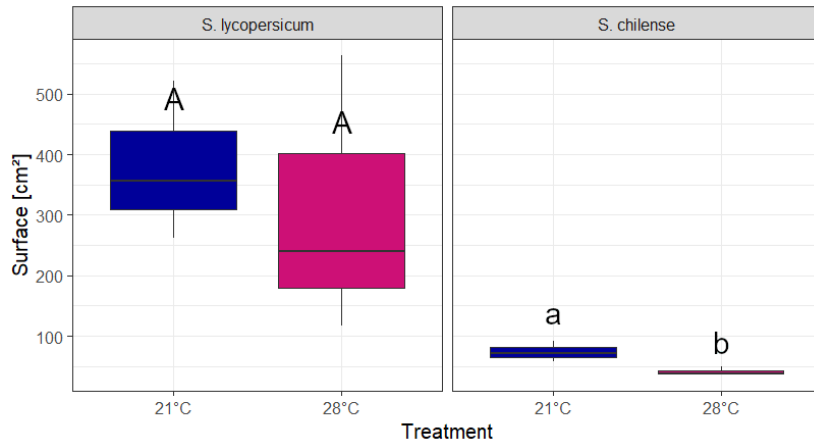


Figure 14 - Leaf area of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress. Different letters indicate significant differences ( $p < 0.05$ ) between treatments inside a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### e) Stomatal density

There was a significant difference in stomatal density between *S. lycopersicum* and *S. chilense* at 21°C with a higher density on *S. lycopersicum* leaves (+44%), regardless of treatment (Figure 15, Annex 3).

*Solanum chilense* showed a difference in stomatal density between heat treatments, with a higher number of stomata at 28°C under well-watered conditions (+30%) but there was no significant effect of the heat treatment on *S. lycopersicum* (Annex 3).

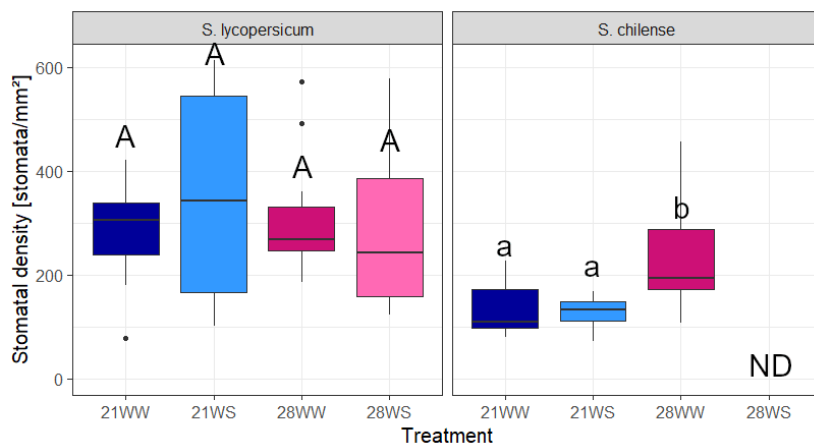


Figure 15 - Stomatal density on the inferior side of the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*. ND = no data.

There was no data for *S. chilense* at 28°C WS conditions due to the mortality rate.

### f) Root length

The length of the three longest roots was not significantly different between species at 21°C but was higher in *S. lycopersicum* than in *S. chilense* at 28°C (+37%, Figure 16, Annex 3).

*Solanum lycopersicum* did not show a significant reduction depending on heat stress but *S. chilense* did show reduced roots at 28°C compared to 21°C (-43%) (Annex 3).

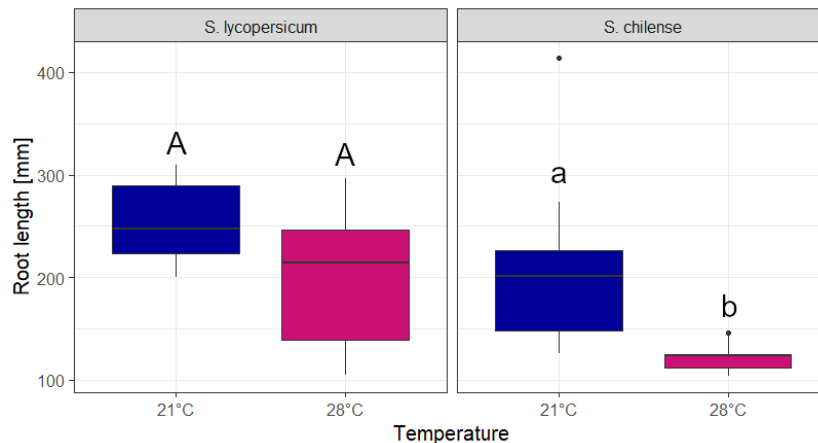


Figure 16 – Length of the three longest roots of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions, after 22 days of stress. Different letters indicate significant differences ( $p < 0.05$ ) between species or treatments, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

## 5.1.2 Physiological parameters

### a) Water content

There was no difference in the water content between species nor between heat treatments (Figure 17, Annex 3). However, the water content was 1.38% and 2.69% higher in the root than in the shoot for respectively *S. lycopersicum* and *S. chilense*.

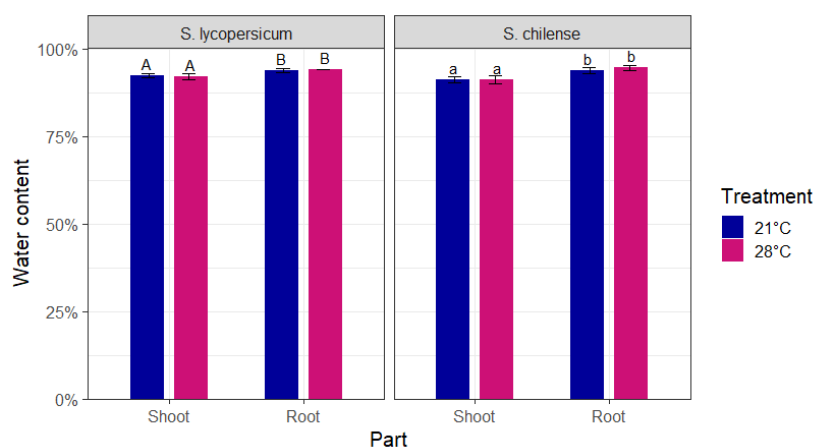


Figure 17 – Water content of the shoots and roots of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 22 days of stress. The standard deviation from the mean is shown on top of the bars. The letters show significant difference

( $p < 0.05$ ) between organs and treatments in each species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

## b) Chlorophyll content index

Leaves of water-stressed plants were often too small to offer a sufficient surface for the machine's sensor, especially in *S. chilense* whose leaves are usually more dentate and did not offer a sufficient plane surface for the sensor.

There was a significant difference in the chlorophyll content index (CCI) between *Solanum lycopersicum* and *S. chilense*, with 23% less chlorophyll in *S. chilense* (Figure 18, Annex 3).

Only *S. lycopersicum* showed to be impacted by water stress under both temperature treatments, with a rise of 13.5% under water stress compared to well-watered conditions independently of temperature (Annex 3).

Although *S. chilense* seemed, on the graph, to be impacted by heat under water stress, this was not statistically significant (Annex 3).

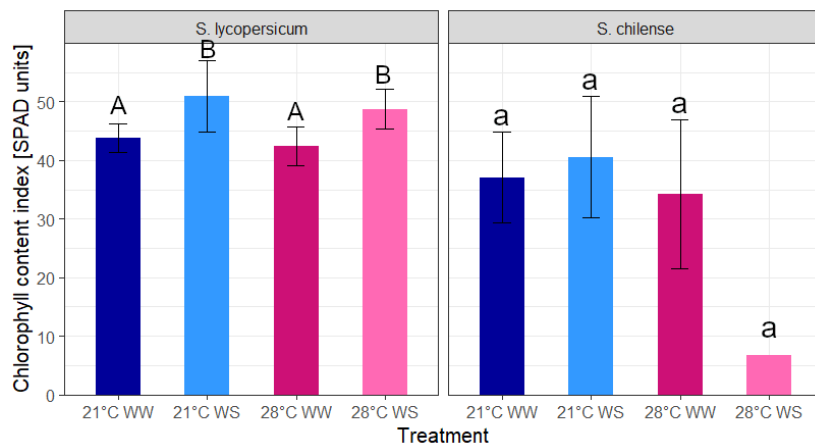


Figure 18 – Chlorophyll content index on the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between species and treatments, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

## c) Osmotic potential

Some of the water-stressed samples did not produce enough sap for analysis to carry the experiment on the required 3 repetitions, but each one allowed at least one measurement.

There was no significant difference between the two species (Figure 19, Annex 3).

In terms of water treatments, there was a significant difference between well-watered conditions and water stress in osmotic potential at 21°C but not at 28°C for both species. The potential was lower under water stress, by 65% in *S. lycopersicum* and 195% in *S. chilense*.

In terms of heat treatments, the osmotic potential was significantly lower at 28°C than at 21°C for both water treatments in *S. lycopersicum* (-12% under well-watered conditions

and -49% under water stress) and only under well-watered conditions in *S. chilense* (-24%) (Annex 3).

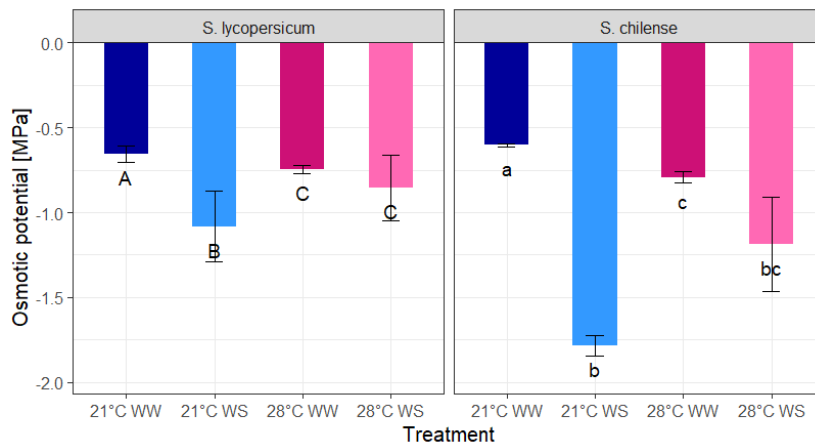


Figure 19 – Osmotic potential of the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

#### d) Malondialdehyde content

*Solanum lycopersicum* and *S. chilense* showed a significant difference in MDA content, with 37% more MDA per gram of fresh material in *S. lycopersicum* regardless of treatment (Figure 20, Annex 3).

No significant effect of heat treatment was apparent within the species.

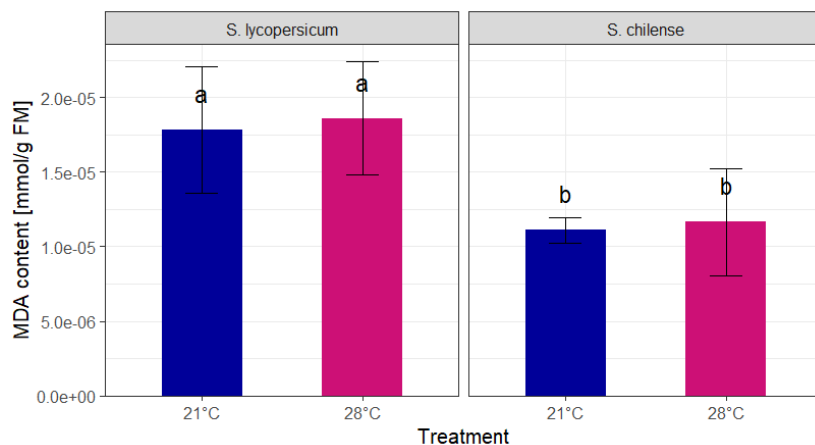


Figure 20 – Malondialdehyde (MDA) leaf content in mmol/g fresh weight of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 18 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between the species.

#### e) Total soluble sugars content

This analysis was done at a 0.90 level of confidence.

*Solanum chilense* showed a higher sugar content in the leaves at 21°C than at 28°C and a higher content at 21°C than *S. lycopersicum* under both temperature conditions (Figure 21, Annex 3).

No effect of the temperature on the total soluble sugars content was apparent in *S. lycopersicum* (Annex 3).

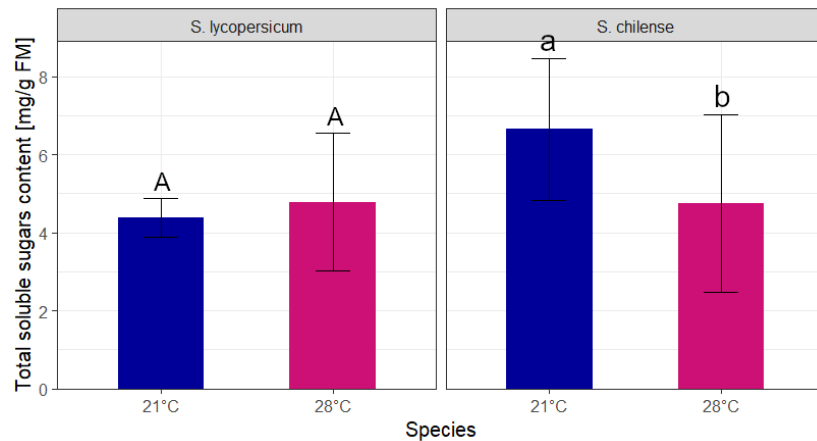


Figure 21 – Total soluble sugars leaf content of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered conditions after 18 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.1$ ) between species or treatments, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

#### f) Photosynthesis parameters

The values returned by the iFL were analysed with scripts that extracted parameters of a fitting curve (Figure 22). Photosynthetic light response curve (A-Q curve) enabled to extract  $A_{sat}$  (photosynthesis (A) at saturating irradiance),  $\Phi$  (maximum apparent yield of photosynthesis), LCP (light compensation point) and  $Q_{sat\_75\%}$  (PAR at 75% of  $A_{sat}$ ), and rapid light curves (RLC) enabled to extract  $ETR_{max}$  (electron transport rate (ETR) at saturating irradiance), and  $E_k$ .

No data was extracted from plants under 28°C WS conditions due to high mortality and because the surviving individuals were too stunted to allow the insertion of a flat leaf surface in the machine.

##### 1. A-Q curves

Some measurements were interrupted by a malfunction of the machine (see the lowest 21°C WS curve for *S. chilense* on Figure 22) and some measurements were impacted by the break of the petiole due to the tension caused by the difficulty of inserting leaves in the head (see the 21°C WS curve for *S. lycopersicum* on Figure 22). There was thus only one measurement for treatments 21°C WW and 21°C WS in both species. The acquisition time being too important (around 1h), there was not enough time to keep on the measurements.

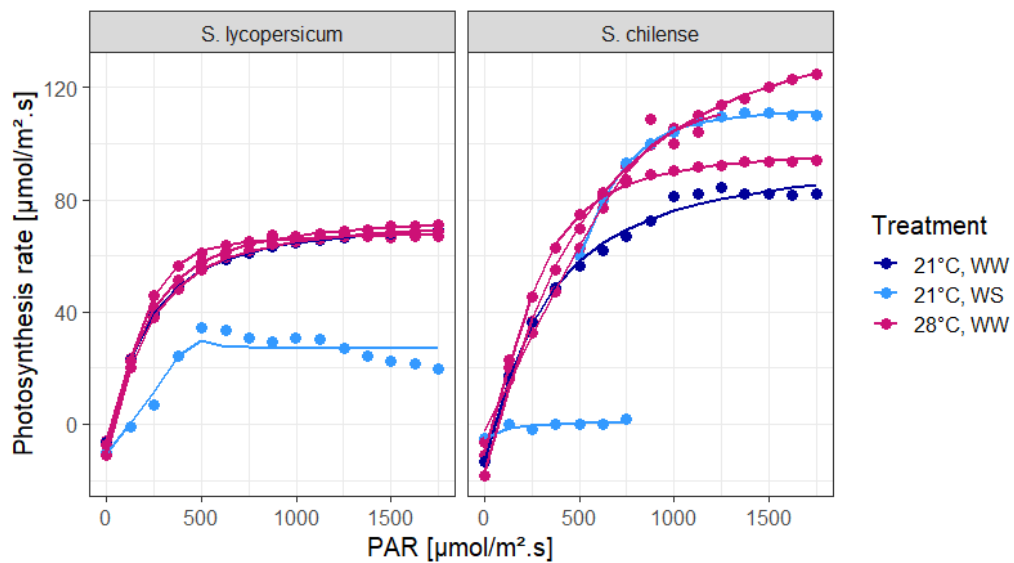


Figure 22 – Photosynthesis rate as a response to PAR on the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. The dots represent raw values and the lines the corresponding fitting curves.

$A_{sat}$  is the value of photosynthesis (A) at a saturated irradiance (depending of the species and conditions, around 1000 - 1500  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ).  $A_{sat}$  showed to be indifferent to heat and water treatments. It was different only between species, with 47% more carbon fixed at saturation in *S. chilense* than in *S. lycopersicum* (Table 4, Annex 3).

The maximum apparent yield of photosynthesis ( $\Phi$ ) represents the quantity of  $\text{CO}_2$  fixed per quantity of photons absorbed, in other words the efficiency of the conversion of light into fixed molecules of  $\text{CO}_2$ . No significant effect of species, heat treatments or water treatments appeared on the maximum apparent yield of photosynthesis (Table 4, Annex 3).

The light compensation point (LCP) represents the value of PAR at which the photosynthesis exactly balances the daytime respiration. The LCP showed to be depending on species and treatments (Table 4, Annex 3). LCP was 42% higher in *S. chilense* than in *S. lycopersicum*, regardless of treatment. It was 79% higher under water stress than under well-watered conditions at 21°C, regardless of species. It also showed a difference between temperatures under well-watered conditions (-3.8% at 28°C), regardless of species (Annex 3).

The PAR at 75% of saturation ( $Q_{sat\_75\%}$ ) of photosynthesis represents the PAR value when 75% of  $A_{sat}$  is reached. No significant effect of species, heat treatments or water treatments appeared on  $Q_{sat\_75\%}$  (Table 4, Annex 3).

Table 4 – Photosynthesis parameters extracted from the A-Q curves. There was no data for 28°C WS conditions for any species.

	<i>S. lycopersicum</i>			<i>S. chilense</i>		
	21°C		28°C	21°C		28°C
	WW	WS	WW	WW	WS	WW
A <sub>sat</sub>	81.79	37.39	81.32 ± 5.70	111.20	161.89	134.95 ± 35.46
Φ	0.2985	0.0867	0.2754 ± 0.0290	0.2718	0.2057	0.2235 ± 0.0754
LCP	23.16	119.71	30.63 ± 7.64	50.20	231.57	40.01 ± 25.55
Q <sub>sat_75%</sub>	759.9	529.7	657.7 ± 11.1	1538.3	239.7	1201.4 ± 874.7

## 2. Rapid light curves (RLC)

The fitting curves for the RLC were less well fitted to the data than they were for the A-Q curves (Figure 23). They were nonetheless analysed with a script to extract their parameters.

The electron transport rate is the quantity of electrons transported by the electron transport chain per quantity of intercepted PAR. The maximum electron transport rate (ETR<sub>max</sub>) is the maximum quantity of electrons that can be transported by the transport chain. ETR<sub>max</sub> showed no significant effect of species, heat treatments or water treatments (Figure 24, Annex 3). The number of repetitions was heterogenous due to malfunctions of the machine and the lack of time to perform more measurements. The number of repetitions varied from, at the most, 4 repetitions for *S. lycopersicum* at 21°C WW down to 2 repetitions for *S. chilense* 21°C WS and 1 repetition for *S. lycopersicum* 21°C WS.

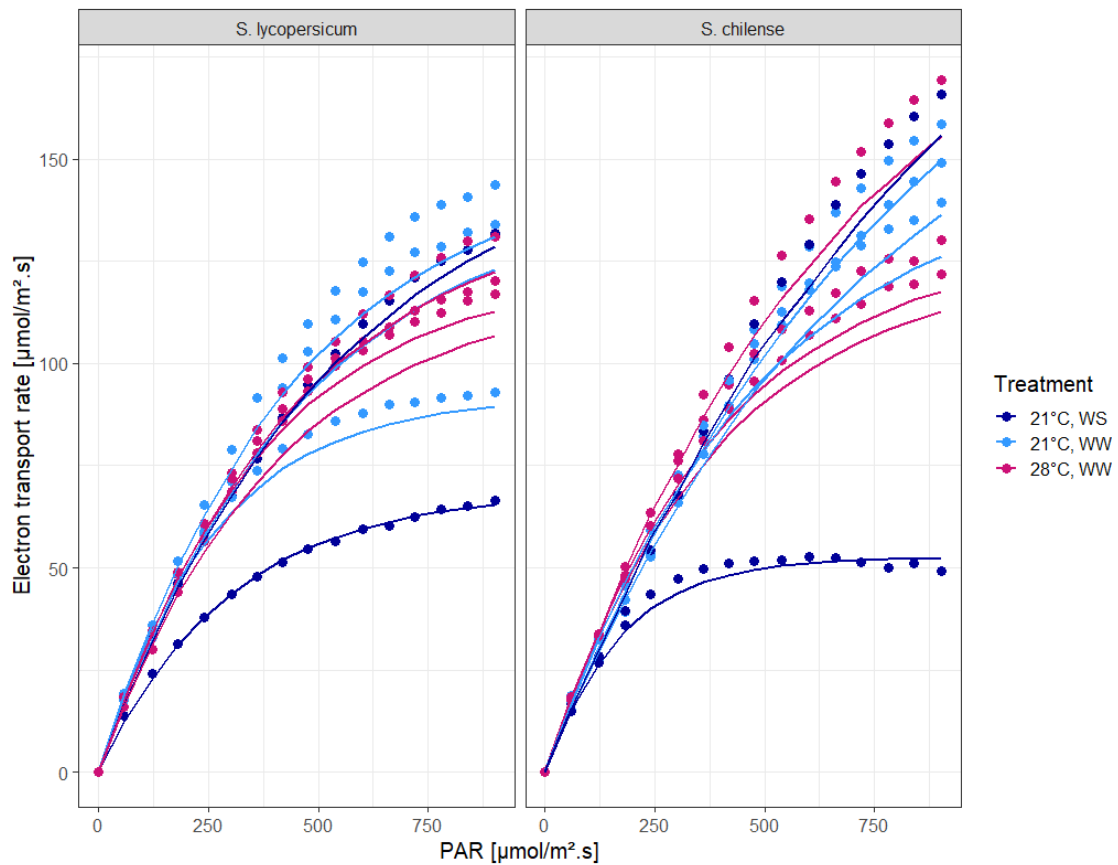


Figure 23 – Electron transport rate (ETR) as a response to PAR on the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. The dots represent raw values and the lines the corresponding fitting curves.

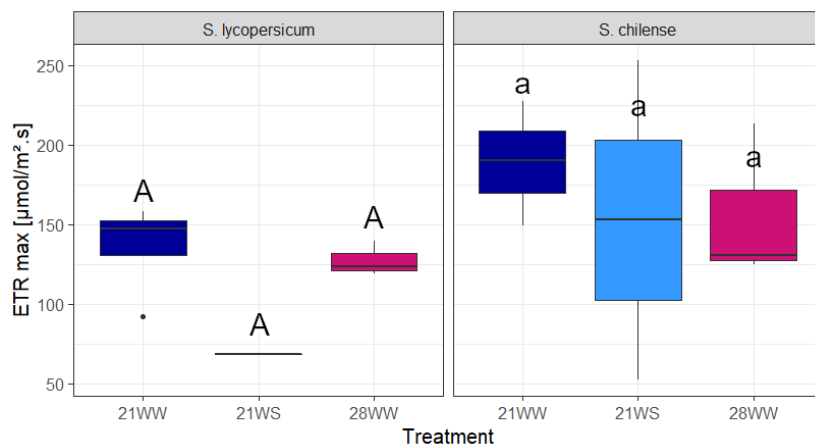


Figure 24 – Electron transport rate at saturating irradiance ( $ETR_{max}$ ) on the leaves of *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

The script also allowed to extract  $E_k$ , the minimum saturating irradiance.  $E_k$  was not impacted by heat stress nor water stress (Table 5, Annex 3).

Table 5 - Minimum saturating irradiance  $E_k$  [ $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ].

	<i>S. lycopersicum</i>			<i>S. chilense</i>		
	21°C		28°C	21°C		28°C
	WW	WS	WW	WW	WS	WW
$E_k$	426 ± 120	300	400 ± 34	681 ± 182	556 ± 548	489 ± 174

### g) Parameters extracted from A-Q curve raw data

The efficiency of the photosystems II ( $\Phi_{PSII}$ ) manifested no significant effect of species, heat treatments or water treatments (Table 6 – Parameters extracted from A-Q curve raw data Table 6, Annex 3).

The transpiration rate ( $E$ ) was only affected by water stress with a reduction of 72% in *S. lycopersicum* and 93% in *S. chilense* under water stress compared to well-watered conditions, at 21°C (Table 6, Annex 3).

The stomatal conductance ( $g_s$ ) was only affected by water stress as well, with a reduction of 96% in *S. lycopersicum* and 99% in *S. chilense* (Table 6, Annex 3).

Table 6 – Parameters extracted from A-Q curve raw data.  $\Phi_{PSII}$  = efficiency of photosystem II [-];  $E$  = transpiration rate [ $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ];  $g_s$  = stomatal conductance [ $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ].

	<i>S. lycopersicum</i>			<i>S. chilense</i>		
	21°C		28°C	21°C		28°C
	WW	WS	WW	WW	WS	WW
$\Phi_{PSII}$	0.668	0.631	0.646 ± 0.009	0.650	0.545	0.607 ± 0.039
$E$	6.84	1.9	7.85 ± 1.09	6.71	0.45	4.27 ± 2.01
$g_s$	1.98	0.08	6.95 ± 4.44	1.14	0.01	0.427 ± 0.251

### 5.1.3 Determination of PEG content

On a visual basis, it seemed that there was an intake of PEG because the water-stressed plant presented a white crystallization at wounds that was not apparent on well-watered individuals.

The recommended wavelengths for determining PEG concentrations were 420 nm (Boulter & McMichael, 1970) and 635 nm (Lawlor, 1970). We first created a spectrum from 280 nm to 700 nm with a range of PEG concentrations having undergone the whole protocol to see if these wavelengths matched peaks in absorbance (Figure 25).

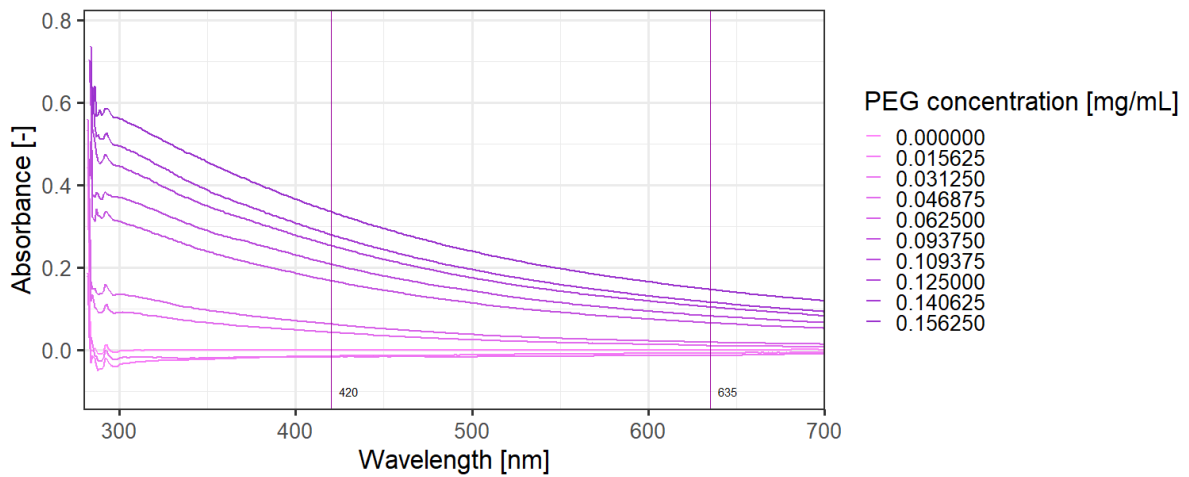


Figure 25 – Absorbance spectrum (280 to 700 nm) of processed PEG ranging in concentrations from 0 to 0.15625 mg/mL, according to expected quantities in fresh samples (Lawlor, 1970).

There was no peak at these wavelengths but a small peak at 292.5 nm (Figure 26).

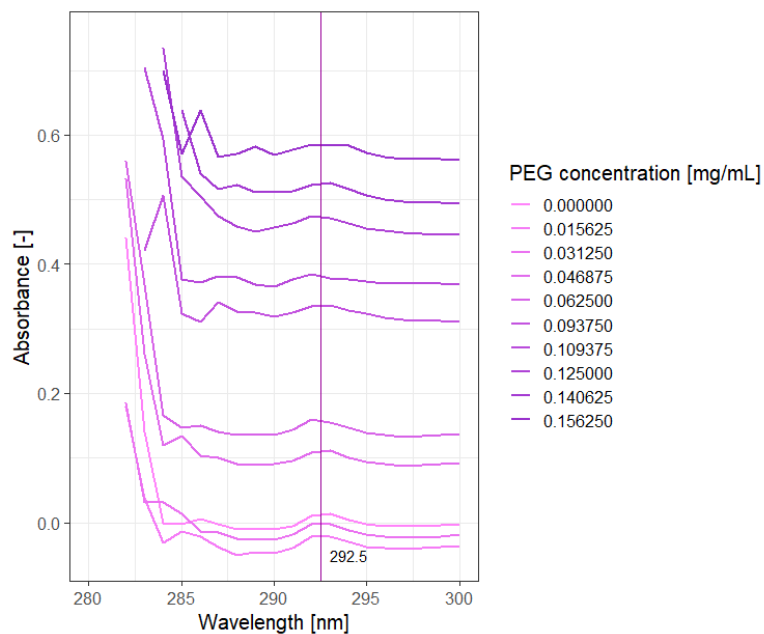


Figure 26 – Absorbance of processed PEG between 280 and 300 nm. The y-axis scale was limited to better show the peak at 292.5 nm, which interrupted the lines as soon as noise appeared.

This peak was very close to the wavelengths at which noise started, and it was decided not to use it but to use the wavelengths from literature: 420 nm and 635 nm. Indeed, even though these two wavelengths did not show a peak, there was a linear relationship between PEG concentration and absorbance that could be extrapolated (Figure 27). The lower concentrations (0.015625 and 0.03125 mg/mL) did not show this relationship and were not included in the making of the calibration lines.

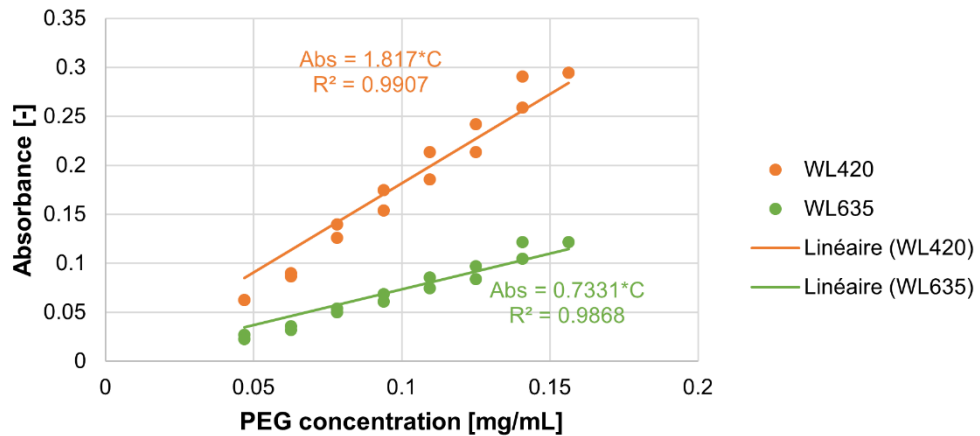


Figure 27 – Calibration lines for the processed PEG range. The dots represent the values obtained in the repetitions for the two wavelengths (WL420 = 420 nm (in orange), WL635 = 635 nm (in green)); the lines of corresponding colours (Linéaire (WL420) and Linéaire (WL635)) are the linear fitting curves forced to (0,0) intercept. The equations and R<sup>2</sup> of these calibration lines are shown on the figure in corresponding colours.

Both equations were used to determine PEG concentrations in processed plant samples (Figure 28).

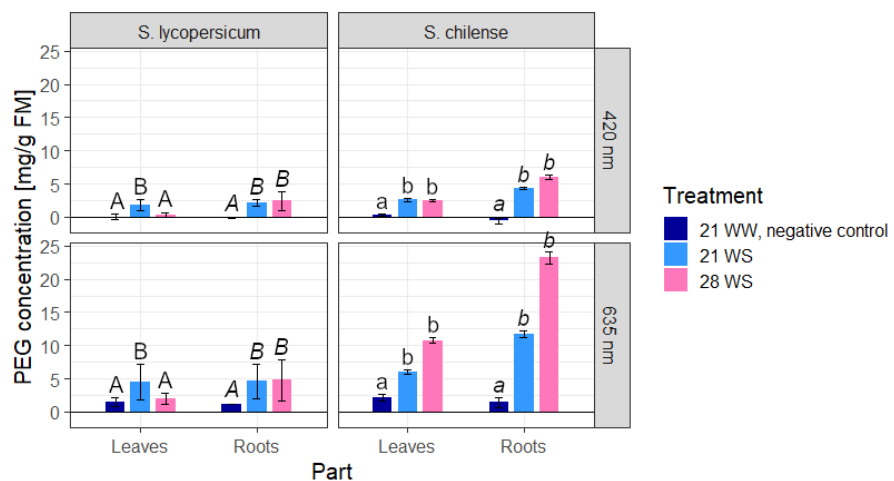


Figure 28 – PEG concentrations in *S. lycopersicum* and *S. chilense* growing at two temperatures (21°C vs. 28°C) under well-watered (WW) and water stress (WS) conditions after 18 days of heat stress and 14 days of WS. These concentrations (mg of PEG per g of fresh matter) were calculated from absorbance at 420 nm and 635 nm with the fitting curves equations. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in roman for the leaves and in italics for the roots). There was no sample for 28°C WW treatment.

Although being calibrated on the same PEG range and processed with the corresponding fitting curve equation, the calculated concentration of PEG in the samples was different depending on the wavelength at which the absorbance was read, with 69% higher calculated concentrations at 635 nm than at 420 nm, regardless of treatments, organs and species (Annex 3).

Even though the calculated concentrations differed quantitatively depending on the wavelength for the same organ of a species, the same trends were still qualitatively observable. For most combinations of species, organ and treatment, there was a significant

difference in PEG content between well-watered conditions and water stress. In the case of *S. lycopersicum* leaves, there was also a significant difference between 21°C WS and 28°C WS, the latter being similar to 21°C WW (Annex 3).

## 5.2 Second experiment: water stress

All test statistics and p-values from the statistical analyses of experiment 2 results can be found in Annex 4.

### 5.2.1 Morphological parameters

#### a) Mortality

There was no mortality, although some individuals became stunted towards the end of the experiment.

#### b) Biomass production

The most prominent difference appeared between water treatments with a much lower dry weight under water stress in both species and every organ: -93% in *S. lycopersicum* and -85% in *S. chilense*, independently of organ. (Figure 29, Annex 4).

*Solanum lycopersicum* had a 90% higher dry weight than *S. chilense*. Within a species, under well-watered conditions, the leaves were significantly heavier than the stems, and in *S. lycopersicum*, they were also heavier than the root. Under water stress, there was no significant difference between parts in *S. lycopersicum* but there was a difference between leaves and roots in *S. chilense*.

The shoot/root ratio did not highlight any significant difference between water treatments (Annex 4).

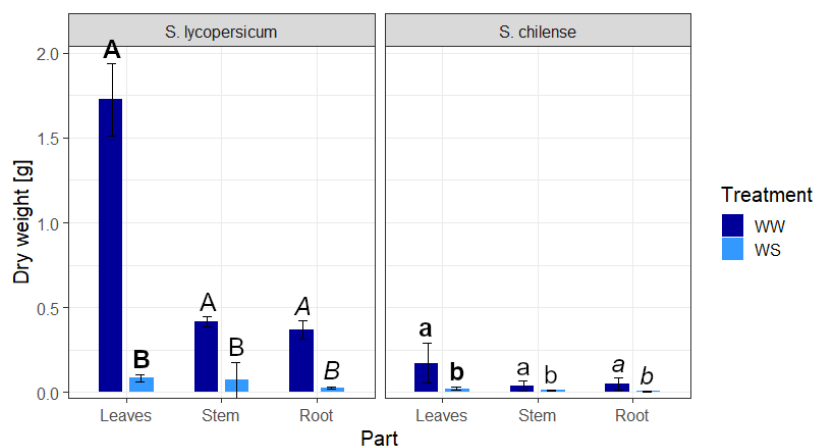


Figure 29 – Dry weight of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in bold for the leaves, in roman for the stem and in italics for the root).

### c) Leaf production

Leaf production showed a linear rate in the 13 days of water stress (Figure 30, Annex 4).

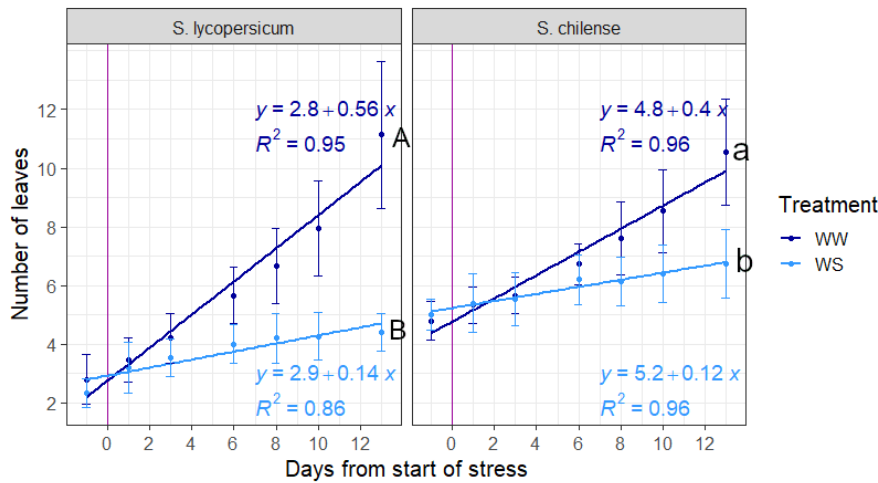


Figure 30 – Leaf production of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions. The equations and  $R^2$  values of the linear regressions are shown, as well as the standard deviation from the mean. Different letters indicate significant differences ( $p < 0.05$ ) in the number of leaves at the end of the experiment (day 13) between treatments within a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

When looking at the number of leaves at the end of experiment (after 13 days of stress), it is interesting to note that there was no difference between *S. lycopersicum* and *S. chilense* under well-watered conditions although they started with a different number of leaves at transplanting. Under water stress, however, there was a significant difference between the two species with *S. chilense* having more leaves (Annex 4).

In both species, water stress induced a decrease of the final number of leaves compared to well-watered plants (Annex 4).

Comparing the rates of leaf production, *S. lycopersicum* produced more leaves than *S. chilense* under well-watered conditions while the two species had a similar rate under water stress. If the changes in the slopes are compared, the decrease in leaf production rate between well-watered conditions and water stress was slightly higher for *S. lycopersicum* (-49%) than for *S. chilense* (-45%) (Annex 4).

### d) Leaf area

*Solanum lycopersicum* showed a leaf area 92% higher than *S. chilense* under well-watered conditions (Figure 31, Annex 4).

Water treatment influenced leaf area in *S. lycopersicum* only, with a decrease of 97% (Annex 4).

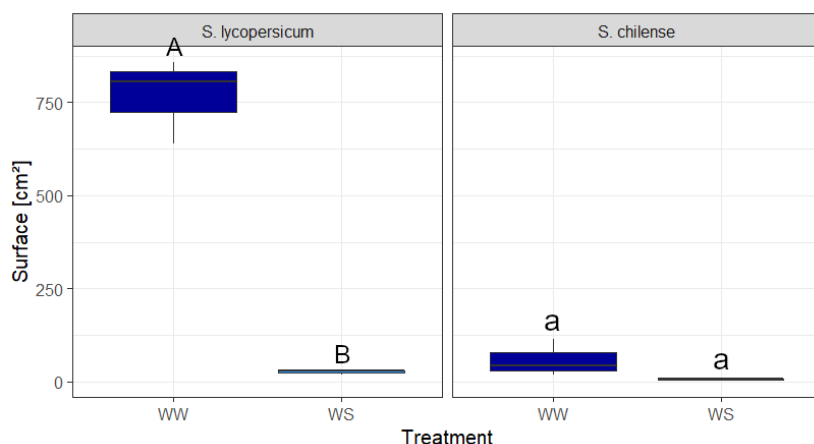


Figure 31 – Leaf area of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. Different letters indicate significant differences ( $p < 0.05$ ) between treatments or species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### e) Stomatal density

Under both water treatments, the stomatal density was significantly different between *S. lycopersicum* and *S. chilense* with a 50% higher density in *S. lycopersicum* (Figure 32, Annex 4).

No significant difference linked to the water treatment appeared within a species (Annex 4).

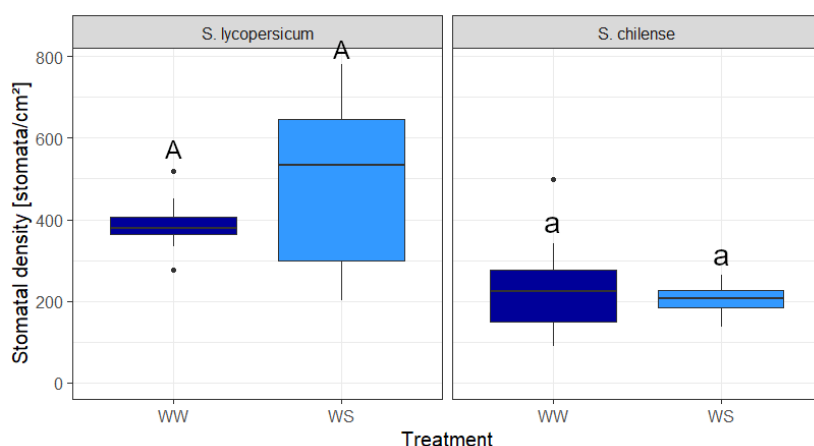


Figure 32 – Stomatal density on the inferior side of the leaves of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of WS. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### f) Root length

In *S. lycopersicum*, the three longest roots produced were longer than in *S. chilense* under both water treatments (+50%, Figure 33, Annex 4) but also of more homogenous lengths under well-watered conditions (the standard deviation from the mean was twice as large in *S. chilense* WW than in *S. lycopersicum* WW).

The water treatment impacted the length of these roots similarly in both species, with a decrease of 60% in *S. lycopersicum* and a decrease of 63% in *S. chilense* (Annex 4).

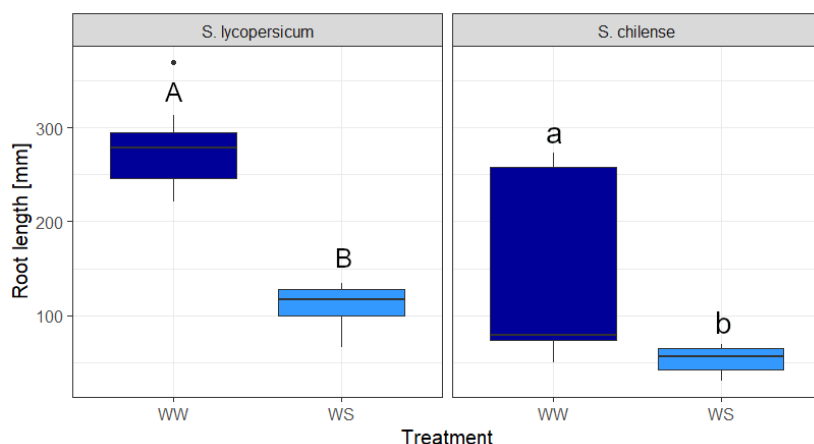


Figure 33 – Length of the three longest roots of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. Different letters indicate significant differences ( $p < 0.05$ ) between treatments and species, capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### g) Root diameter

Not all roots were the same length, therefore there were not as many segments on each root, segments being 1 to 1.5 cm each; well-watered *S. chilense* roots never exceeded 5 cm and there was no segment 4 and 5 on them. Roots of individuals submitted to WS never exceeded 5 cm and never had segments 4 and 5 in *S. lycopersicum*, never exceeded 2 cm and never had segments 3 and further in *S. chilense* (Figure 34, Annex 4).

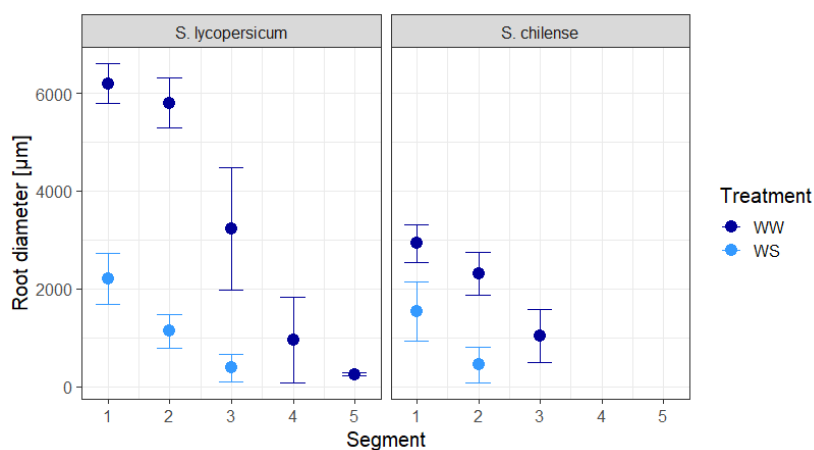


Figure 34 – Mean diameter of segments of the main root of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The lines represent the standard deviation from the mean.

*Solanum chilense* main roots were not only shorter but also half as wide (-51%) on average as *S. lycopersicum* main roots in all combinations of species and treatment where data was available (Annex 4).

The water treatment affected both species at all segments. *S. chilense* was less affected than *S. lycopersicum* with a decrease in root diameter of 50% while *S. lycopersicum* decrease was of 70% (Annex 4).

Regardless of species and treatment, the segments showed a large reduction in diameter between segments 2 and 3 and between segments 3 and 4. Segments 1 and 2, and 4 and 5 were of less different diameters between each other.

#### h) Xylem element diameter

The diameter of the two largest xylem elements showed a significant difference between species in all combinations of species, segment and treatment where data was available (Figure 35, Annex 4).

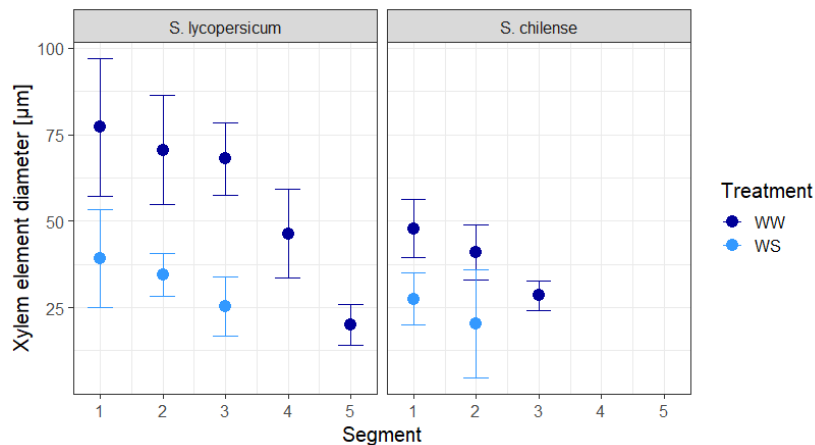


Figure 35 – Mean diameter of the two largest xylem elements in segments of the main root of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.

Water treatment affected the diameter of the xylem elements for all segments in both species (Annex 4).

A decrease in diameter between segments within a condition only appeared between segments 3, 4 and 5 in *S. lycopersicum* and between segments 2 and 3 in *S. chilense*.

The ratio xylem diameter/root diameter was significantly linked to the species under well-watered conditions in segments 1 and 2, being on average 36% larger in *S. lycopersicum* than in *S. chilense* (Figure 36).

The difference in the xylem/root diameters ratio between water treatments was significant in all segments where data were available except in segment 1 of *S. chilense*. The increase was 54% in *S. lycopersicum* on average on the 3 segments that could be compared, and it was lower with 50% in *S. chilense* in segment 2.

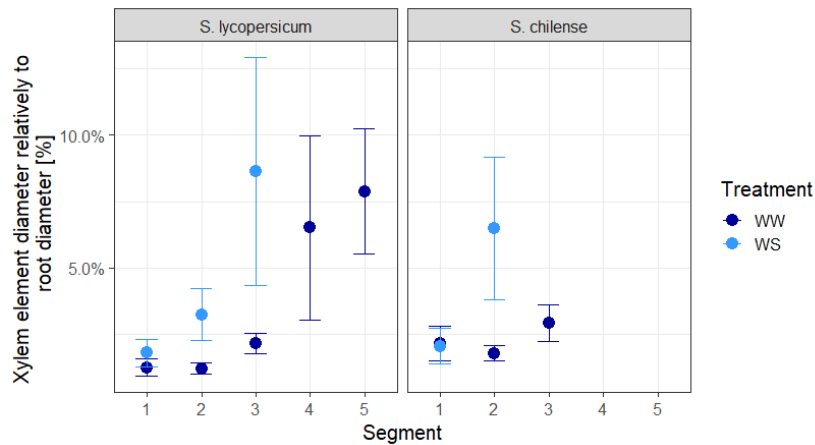


Figure 36 – Ratio between the diameter of the xylem elements and the diameter of the root of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress.

## 5.2.2 Physiological parameters

### a) Water content

The species had no effect on the water content of any organ of the plants regardless of the water treatment (Figure 37, Annex 4).

The water treatment impacted significantly the leaves and stems of both species: in *S. lycopersicum*, the leaves WC decreased by 16% and the stem WC decreased by 23%; in *S. chilense*, the leaves WC decreased by 36%, and the stem WC by 16% (Annex 4).

Finally, the different organs of the plants showed different water contents under identical water conditions: in *S. lycopersicum*, leaves WC was 17% lower than root WC under water stress and was 4% lower than stem content under well-watered conditions. In *S. chilense*, root water content was 38% higher than leaf water content and 16% higher than stem water content under water stress, and only 4% higher than leaf water content under well-watered conditions (Annex 4).

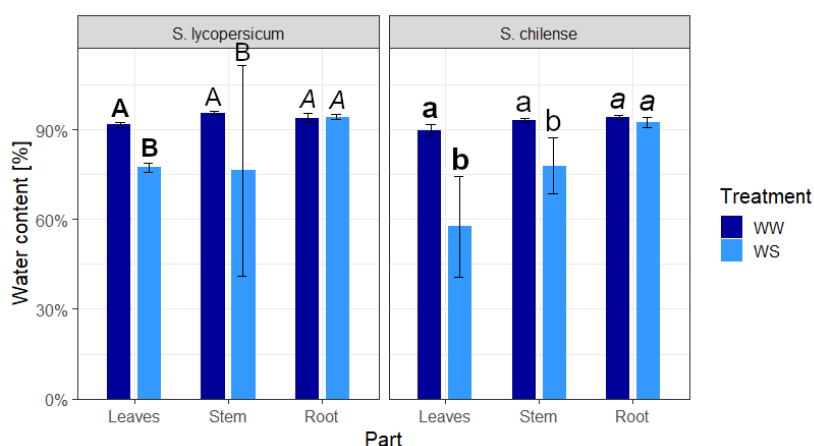


Figure 37 – Water content of the leaves, stems and roots of *S. lycopersicum* and *S. chilense* growing under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in bold for the leaves, in roman for the stem and in italics for the root).

### b) Chlorophyll content index

The chlorophyll content index (CCI) showed a significant difference between species under water stress and a strong interaction between species and treatment: the *S. lycopersicum* plants had a higher CCI under water stress (+37%) while there was a decrease from the well-watered conditions values in *S. chilense* (-41%) (Figure 38, Annex 4).

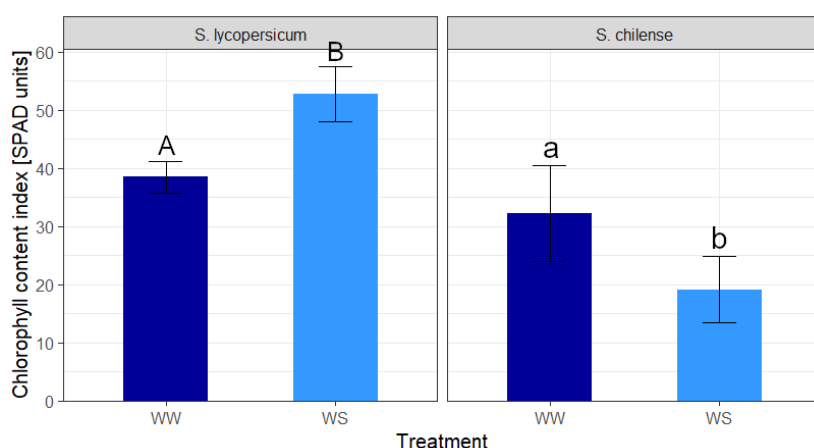


Figure 38 – Chlorophyll content index of the leaves, stems and roots of *S. lycopersicum* and *S. chilense* growing under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. The letters show significantly different groups, in capitals for *S. lycopersicum* and in lowercase for *S. chilense*.

### c) Osmotic potential

No water stress sample produced any sap, neither in *S. lycopersicum* nor *S. chilense*. The analysis was thus only performed to compare the two species under well-watered conditions, which were significantly different with a 20% lower osmotic potential in *S. lycopersicum* (osmotic potential of  $-0.786 \pm 0.034$  MPa) than in *S. chilense* (osmotic potential of  $-0.620 \pm 0.072$  MPa) (Annex 4).

#### d) Malondialdehyde content

The malondialdehyde (MDA) content was 60% higher in the shoot of *S. lycopersicum* than in its root under water stress (Figure 39, Annex 4).

In *S. chilense* shoots, the MDA content was not significantly different between water treatments. MDA content of both species was unaffected in roots (Annex 4).

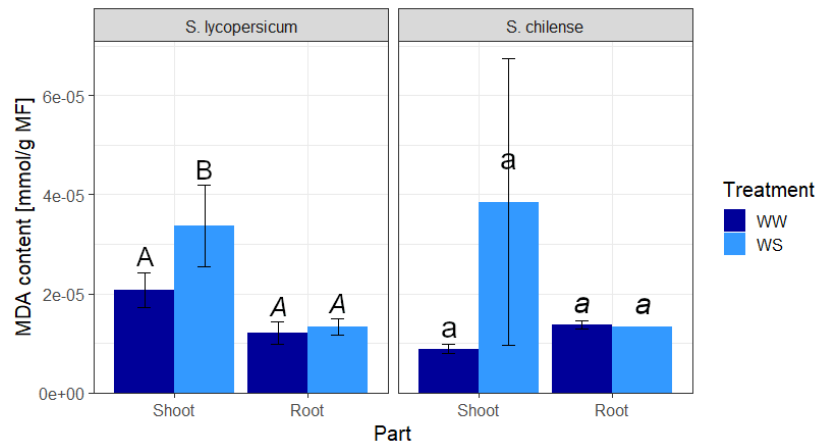


Figure 39 – MDA content of the shoot and root of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in roman for the shoot and in italics for the root).

#### e) Total soluble sugars content

Because of the lack of plant material, the analysis could not be performed on water-stressed *S. chilense* at all.

The total soluble sugars (TSS) content was not significantly different between species (Figure 40, Annex 4).

In *S. lycopersicum*, an effect of the treatment appeared, in interaction with the organ: TSS content rose in the shoot (+69%) with water stress but TSS content was not significantly affected by water stress in the roots (Annex 4).

Independently of treatment, for both species, the TSS content was higher in the shoot than in the root (+64% in *S. lycopersicum* and +56% in *S. chilense*) (Annex 4).

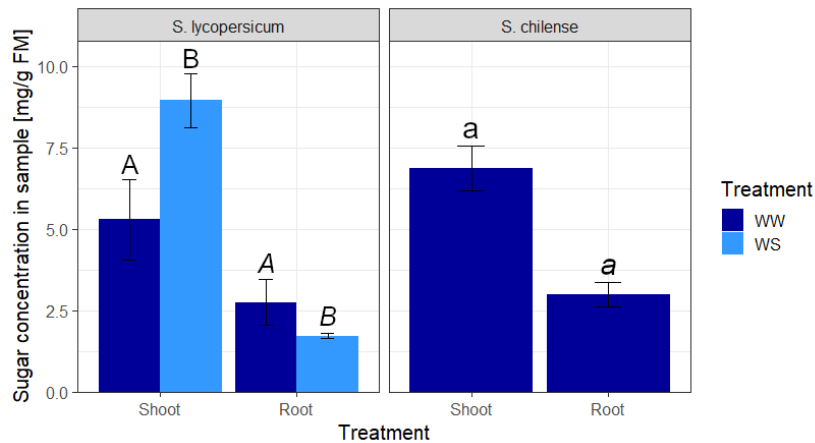


Figure 40 – Total soluble sugars content of the shoot and root of *S. lycopersicum* and *S. chilense* growing at 21°C under well-watered (WW) and water stress (WS) conditions after 17 days of stress. The standard deviation from the mean is shown on top of the bars. Different letters indicate significant differences ( $p < 0.05$ ) between treatments within a species (in capitals for *S. lycopersicum* and in lowercase for *S. chilense*) and organ (in roman for the shoot and in italics for the root).

## 6. Discussion

### 6.1 Impact of heat stress

Experiment 1 showed some impacts of heat stress on *Solanum lycopersicum* and *Solanum chilense* in the vegetative stage.

The ideal daytime temperature window for the growth of the cultivated tomato is between 20.5°C and 24.5°C, according to Burke (1990), with nights between 18°C and 25°C (J. Zhou et al., 2012). The growth of tomato is impacted by higher temperatures (Krishna et al., 2019; J. Zhou et al., 2012). In our experiments, there was a slight decrease in biomass production in both species but the water content remained constant, which is not always observed in heat stress (Mathieu et al., 2014). This could mean that the plants maintained their integrity and were able to take as much water as they needed for the growth they could achieve, with no excess losses due to transpiration. Indeed, heat stress induces a rise in leaf temperature (Hassan et al., 2021) and a strategy to decrease the leaf temperature is to increase the transpiration (Descamps et al., 2018); in some species, intensive transpiration can lower leaf temperature up to 6°C-10°C (Hassan et al., 2021). Our experiment did not highlight an impact of heat stress on the transpiration rate nor the stomatal conductance of any of the species. However, Blanchard-Gros (2021) showed that heat stress increased transpiration rate in *S. lycopersicum* but not in *S. chilense* using the same accessions that our study. Common strategies used by the plants to increase the transpiration and cope with heat stress are the change in leaf orientation, and, on a longer term, the increase of stomatal density (Hassan et al., 2021). An increase in stomatal density was observed in *S. chilense* but not in *S. lycopersicum* in response to heat in our study. It was also found that under higher temperatures, the leaf production rate increased, leading to a higher number of leaves produced on a shorter leaf-elongation duration (Prasad et al., 2008).

This increase in leaf production rate under higher temperatures was not observed in our experiment, possibly because the period of growth was very short (14 days) or because the temperature was too high. Indeed, an increase in leaf production was observed in *S. lycopersicum* but not in *S. chilense* in response to heat (28°C) on a long-term experiment (Blanchard-Gros, 2021). Knowing that the number of leaves did not change in any of the species in this experiment,, the fact that the leaf area decreased in *S. chilense* means that the leaves were individually smaller under heat stress, with a constant water content. Hughes et al. (1970) showed that, for several non-stressed dicotyledonous species, the relationship between leaf area and leaf absolute water content (difference between fresh and dry weight, or in other words total water quantity in the leaf) was linear, meaning that the more water present in the leaf, the larger the area. The fact that leaf area changed under heat stress in some cases but that the relative water content (difference between fresh and dry weight, divided by the fresh weight) remained stable suggests that this relationship would still be valid under heat stress.

A reduction in leaf area under heat stress has been observed in tomato (R. Zhou et al., 2020) and in other species such as buckwheat. It was then explained by a possible reduction of cellular elongation or a decrease in the number of cells (Aubert et al., 2020) which is contradiction with Prasad et al. (2008) stating that higher temperatures induce increased leaf elongation rates. This difference is likely linked to a consortium of influencing factors, in interaction with each other, probably including the temperature at which the stress was installed, the investigated species and the day/night temperature difference. This reduction could be linked to the fact that heat stress is known to damage biochemical functions of the plant, threatening the integrity of lipidic membranes and therefore their functions. Since these functions include photosynthesis functions such as the transport of electrons in the light phase of photosynthesis (Hassan et al., 2021), and less photosynthesis meaning less carbon assimilation, it could be imagined that less resources were available for the plant to grow, thus limiting leaf area.

However, this hypothesis was not supported by evidence in this experiment, since no measured photosynthesis parameter, nor the chlorophyll content was impacted by heat stress. However, the measurements of photosynthesis parameters could only be performed on one repetition of well-watered plants in both species, which decreases of course the significance of all statistical analyses on these parameters.

What is more, the concentration of malondialdehyde, a product of lipid peroxidation and main indicator of oxidative stress (Farmer & Mueller, 2013), did not increase under heat stress for any species. This suggests that the membranes were not more impacted by oxidative stress at 28°C than at 21°C, either because not many reactive oxygen species were produced, or because of an efficient antioxidant reaction. If the membranes were not impacted by heat stress, the risk of having damages impacting photosynthesis decreases (Hassan et al., 2021).

In terms of water management, *S. lycopersicum* did not show significant morphological or physiological changes, hinting that it did not adapt to its environment.

*Solanum chilense*, on the other hand, showed what could be indications of adaptation towards a higher transpiration rate and thus better temperature regulation. Indeed, the stomatal density increased on the surface of the leaves, allowing to evaporate more water even though the leaf area was impacted, potentially by a lack of assimilates for growth.

Surprisingly, the sugar content decreased under heat stress in our experiment, while sugars usually act as an osmoprotectant and help maintaining the cellular structure (Hassan et al., 2021). However, the osmotic potential decreased, meaning that this role of osmoprotection was probably performed by non-sugar osmolytes, maybe proline which is a common osmolyte used by the tomato (Claussen, 2005). Moreover, some osmolytes also have a role as antioxidants by inactivating ROS (Al Hassan et al., 2015), which would explain how the malondialdehyde content did not increase under heat stress in our experiments.

To sum up, it looks like the growth only was slightly affected. Other parameters seemed to show that *S. lycopersicum* did not adapt while *S. chilense* modified water flow parameters, enhancing higher transpiration rates on otherwise diminished leaf area by increasing its stomatal density.

The sample for every investigated parameter was small and interindividual heterogeneity could have masked the effects of heat stress, especially in *S. chilense* due to its status as a population and not as a variety (Chetelat et al., 2009), while *S. lycopersicum* cv. Aisla Craig plants are nearly genetically identical.

In both cases, no impact of heat stress on photosynthesis or physical integrity was found but it must be kept in mind that this analysis was based on heat stress as sole stress ; in field conditions, heat stress is often combined with other stresses such as water stress (Cohen et al., 2021). In the present case, the combination could only be analysed for a few parameters, and this analysis of heat stress and water stress will be developed further on.

Finally, these results came from constant heat conditions, with similar temperatures during the day and the night. In field conditions, heat stress can manifest very differently, with higher temperatures peaking by day (sometimes over 30°C or 40°C) and decreasing by night, with different levels of contrast.

## **6.2 Impact of water stress**

Experiment 2 allowed to highlight the effects of water stress on the vegetative phase of *Solanum lycopersicum* and *Solanum chilense*, and to see how the reactions sometimes differed between the two species. The effects can be divided into categories.

In terms of growth and development, both *S. lycopersicum* and *S. chilense* were impacted by water stress: biomass was reduced on all investigated organs (leaves, stem and roots), as well as the number of leaves produced, leaf area and length of the three longest roots,

which is in line with the expected results for biomass production and root length, as described in a meta analysis of plant response to water stress (Mundim & Pringle, 2018). A possible explanation for this reduced growth was that the primary metabolism, impacted by the decrease in photosynthesis, would prioritize the production of stress metabolites over growth (Mundim & Pringle, 2018). The water content did not change significantly, suggesting that plants developed adaptation to maintain water in the plant. Transpiration and stomatal conductance were more limited in *S. chilense* than *S. lycopersicum*, which tends to confirm this hypothesis. About the leaf area, these results correspond to the conclusions of Hughes et al. (1970) that leaf area and absolute water content were linked linearly by the water content, which suggests that this relationship would still be valid under water stress. The shoot/root ratio was not impacted by water stress, suggesting that the plants did not allocate more assimilates to the growth of one or the other organ. This could be surprising because *S. chilense* is known for having a developed root system (Chetelat et al., 2009).

*Solanum chilense* is described as a smaller plant than *S. lycopersicum* (Gharbi et al., 2016; Martínez et al., 2012). However, even if the values of the morphological parameters were lower for *S. chilense* under both water treatments than for *S. lycopersicum* under the same treatments, those parameters were actually less impacted by drought stress in *S. chilense* than in *S. lycopersicum*. Our results confirmed the drought-resistance character of *S. chilense* described in soil conditions by Blanchard-Gros et al. (2021) and Tapia et al. (2016) and under PEG-induced water stress by Shamim et al. (2016).

All the parameters related to root length and diameter were significantly affected by drought stress. Hernandez-Espinoza & Barrios-Masias (2020) also observed a decrease of the diameter of *S. lycopersicum* xylem vessels by 27% under water stress (with a soil water content of 11%, corresponding to a soil water potential of -1.02 MPa); they explained it by the need to limit transpiration rate but also to prevent embolism formation, a risk that is higher under drought but decreases with the diameter of the vessels. However, the ratio diameter of xylem vessels/diameter of root increased, relatively. Since the number of xylem elements should remain roughly equal (Hernandez-Espinoza & Barrios-Masias, 2020), this would mean that the total space allotted to the xylem would increase, relatively to the root diameter. In other words, the plants would be increasing the individual diameter of the xylem elements to counterbalance the decrease in root size, thus maintaining a water flow that would still be lower than normal and be an obstacle to water losses towards the atmosphere.

Cellular elongation is driven by cell turgescence. When the available water decreases, maintaining the growth while meeting transpiration demand requires to maintain the osmotic potential (Prasad et al., 2008), which is achieved by adjusting the concentrations of osmolytes. These compounds used for osmoregulation are of diverse natures. Sugar is one of them, along with, among others, some amino acids, proteins and organic acids (Farooq

et al., 2012). In tomato, the role of sugars in the osmotic adjustment is notable (Tapia et al., 2016). There was no data for *S. chilense* in our experiment due to low fresh material. However, in *S. lycopersicum*, the changes in soluble sugars content under water stress were linked to the organ: there was an increase in sugar content in the shoot and a decrease in the root, which increased the gap between the organs that was already apparent under well-watered conditions. The rise in the shoot could be a consequence of the fact that the shoot is in direct contact with the atmosphere and therefore prone to water losses by evapotranspiration. Decreasing the water potential of this part would limit the water movements towards the atmosphere. On the other hand, the decrease of sugar content in the root could be due to different causes. It could be a result of the limitation of available assimilates in a context of limited photosynthesis, where sugar had to be reallocated to the shoot that needs it the most to limit of transpiration thanks to its role in decreasing the leaf osmotic potential, while the losses in the roots would be lower since there is respiration but no transpiration. It could also be that the osmoprotection was performed, in the roots, by other components such as proline (an osmolyte frequent in tomato (Claussen, 2005)), that were not tested. Blanchard-Gros (2021) reported an increase in proline concentration due to water stress in both *S. lycopersicum* and *S. chilense* while Tapia et al. (2016) found that *S. chilense* had a higher proline accumulation than *S. lycopersicum* under drought conditions although these measurements were performed in the leaves but not in the roots.

The only parameter linked to photosynthesis that was measured in experiment 2 was the chlorophyll content index, which showed a difference between *S. lycopersicum* and *S. chilense*. In *S. lycopersicum*, the CCI increased under stress while it decreased in *S. chilense*. This contrasts with the results of Blanchard-Gros (2021) which showed an increase in CCI in both species under drought stress although higher in *S. lycopersicum* than in *S. chilense*. According to Yang et al. (2021), chlorophyll content usually decreases under water stress, probably because of the degradation on the molecule. Even if they also stated that the changes in chlorophyll content may differ between species or cultivars (some of them even seeing an increase), Zgallai et al. (2006) showed that the cultivated tomato chlorophyll levels decreased under water stress. Seeing that the present results suggested otherwise, the method of testing it with an optical device can be questioned. Firstly, because plants under water stress tend to have thicker leaves in order to preserve water content (de Oliveira Maia Júnior et al., 2019). The measured chlorophyll content seems therefore higher since it applies to a thicker layer of cells. Secondly, it is possible that the use of an optical device responding to some wavelengths may suffer interferences by other pigments present in the leaf; in this case, the plants were really dark, indicating potential nutrient deficiency. This possibility is supported by the fact that PEG is an obstacle to phosphorus intake (Emmert, 1974), that phosphorus content usually decrease in leaves under water stress (Mundim & Pringle, 2018) and that a phosphorus deficiency is signalled by the symptom of darkened to purplish coloration. A biochemical analysis could have allowed a more accurate measurement. However, it could not be excluded that the higher CCI in *S.*

lycopersicum is due to a lower leaf surface and reflects thus a concentration effect more than an increase in chlorophyll production, as it was observed in buckwheat (Aubert et al., 2022).

Oxidative stress was slightly higher in the shoots than in the roots under water stress, as indicated by the MDA content, its main indicator (Farmer & Mueller, 2013). This means either that aerial parts of the plants were more subjected to oxidative stress than the roots (being in contact with the atmosphere), or that antioxidant activity was more active in the roots than in the shoots. Moreover, water stress tended to increase MDA concentration in the shoot of both species but not in the root, although this increase was not always significant. Blanchard-Gros (2021) also observed an increase in MDA concentration in response to water stress in the leaves of both species, particularly in *S. chilense*. Antioxidant quantification would be required to investigate the oxidative stress management in these species under water stress. Martínez et al. (2014) reported that management of the oxidative status is a key mechanism allowing *S. chilense* (LA4107) to tolerate salinity. Similar mechanisms are maybe developed in response to water stress.

This experiment was based on a constant water stress throughout the experiment, which was actually an osmotic stress used as a proxy for water stress. There was a possible anoxia due to complete root immersion. In field conditions, water stress is expressed differently: first of all, during a season, stress usually occurs in periods and the soil partially or completely rewets before experiencing a new stress episode, offering a possibility to the plant to recover partially (if it wasn't too damaged) and adapt to prepare for a later return of the stress. Secondly, under severe water stress in the soil, the roots may dry whereas they always stay wet in hydroponics. In early stages of water stress, the drying roots produce abscisic acid (ABA) that will be transported to the leaves as a signal to reduce the losses of water through transpiration and therefore increase the water use efficiency (CGIAR Research Program on Water, Land and Ecosystems, 2015); this pathway could be impacted in hydroponic water stress.

### **6.3 Impact of combined heat and water stress**

Very little data was collected on the combination of heat and water stress due to the death rate in experiment 1, but some results were still usable. A combined stress is usually more deleterious than a single one, even if it is not always the case (Cohen et al., 2021).

The high mortality rate at 28°C under water stress was itself revealing of the deleterious effect of the combination of the two stresses and showed that the effects do not simply add up: heat stress by itself did not lead to any mortality, but together with water stress it increased the impact of water stress by 6.6% in *S. chilense* and 33.4% in *S. lycopersicum*. This is an example of how the response of the plants to a combination of stresses cannot be extrapolated from the effects of the stresses taken separately (Cohen et al., 2021).

In terms of leaf production, there was a notable difference between *S. lycopersicum* and *S. chilense* behaviour. In both cases, the number of leaves did not decrease significantly under heat stress. Under water stress, however, the trends differed: while *S. chilense* was equally affected by water stress under both temperatures, *S. lycopersicum* showed a significant difference between heat treatments under water stress. Indeed, the rate of leaf production was much lower at 28°C than 21°C, indicating that the combination of heat and water stress impacted the plants more than the stresses separately. On this parameter, *S. lycopersicum* was more sensible to stress combination than *S. chilense*.

## **6.4 Methodological critique**

### **Growing conditions**

The 9-liter tanks that were used provided a good surface and volume for the plantlets at first but the plants soon outgrew it in aboveground density. Still, the volume available for the root systems could not be considered as restricted compared to the volumes for testing rooting volume restriction conditions (Nishizawa & Saito, 1998). Since *S. lycopersicum* produced bigger leaves and longer stems, they induced a competition for light in which the *S. chilense* plants were disadvantaged. Given that the *S. chilense* were already smaller than expected at the time of transplantation, they could have been impacted by this competition for light.

In terms of root development, at the end of both experiments, under well-watered conditions, the roots of different individuals from both species were tangled. Separating them for further analysis damaged some of them and there could have been fragments of other individuals and/or species roots tangled in the roots that were used for the analyses.

Finally, nutritive solutions should usually be changed around once a week to prevent nutrient deficiencies. Given the short length of the experiments and the cost of the PEG, they were not changed during the present experiments. It is thus possible that plants underwent nutritive deficiencies.

### **The use of polyethylene glycol (PEG)**

Using PEG to simulate drought stress is an approximation since it actually induces osmotic stress. The benefits of using it are that it helps to standardize the level of stress perceived by the plants, to homogenize the water stress, but also to have an easier access to the root system: in hydroponics, the roots will develop without facing the physical constraints that appear in soil, especially when it is drying (Gaspar et al., 2013).

Using PEG for creating water-stress conditions has its downsides: it decreases the surface tension and increases solution viscosity (Lawlor, 1970), limits the solubility of oxygen and prevents the transport of phosphorus across the roots to the xylem (Emmert, 1974). In the present experiments, this led to a development of microorganisms on the inside surface of the tanks and the roots of the plants, especially at 28°C. In order to prevent this

proliferation, the solution was oxygenated twice a week during experiment 2 using a syringe, with little success. The harvested roots were coated in a gelatinous substance, that was removed as much as possible but given the state of the roots (necrosis), not all of it could be removed without destroying the roots. Injecting compressed air into the solution would improve root oxygenation especially under osmotic stress conditions. We did not use this type of system because our previous experiments in hydroponics showed that both species grew well without the need to oxygenate the solution (Gharbi, 2017) but the stresses applied were not with PEG. It would be interesting to repeat the experiment performed here with oxygenation of the solution.

Moreover, it was proven that there was an intake of PEG by the roots and translocation and accumulation in the whole plant, with higher concentrations in older leaves. The intake quantity and the impact of PEG on the plant are linked to its molecular weight and the length of exposure (Jacomini et al., 1988). PEG of molecular weight 6000 and over should theoretically not be taken in by the plants since the molecule's size should be bigger than the size of the pores (Carpita et al., 1979), but this was not coherent with our results.

When creating the protocol, the aim was to quantify the intake of PEG. As shown in the results, there was no specific absorbance peak for PEG and we tested it instead on two wavelengths found in literature (Boulter & McMichael, 1970; Lawlor, 1970), only to find different absolute quantifications, depending on the wavelength. This disqualifies the protocol for its first purpose of absolute quantification. However, when comparing the results at 420 nm and 635 nm, it seemed that the trends between treatments were similar, indicating that the protocol could be used for a qualitative and relative quantification between treatments.

This should still be done with caution since control treatments showed a non-zero absorbance when they should have, therefore it cannot be excluded that other components of the solution such as remaining plant compounds or extraction solutions were detected as well as processed PEG. Moreover, the proportional relationship between initial PEG concentration and absorbance was only valid for concentrations from 0,046875 mg/mL upwards (from the range), which means that results based on the calibration curve that are below this concentration are likely invalid and must be considered with caution.

## 7. Conclusion and perspectives

This study compared the response of *S. lycopersicum* and its wild relative *S. chilense* to heat and water stress during vegetative stage at shoot and root level. The root system of *S. chilense* remains largely unknown at the present time and this study is one of the firsts (along with Alaguero-Cordovilla et al. (2018)) to investigate the response of *S. chilense* root system to heat stress and water stress, and to compare the effects of heat and water stress on aboveground and belowground organs of *S. chilense*. Despite some plant mortality due to PEG accumulation, different conclusions could be drawn from this work.

Heat stress did not highlight many differences between *Solanum lycopersicum* and *Solanum chilense*, but a few parameters were still affected differently.

Both species showed a slight decrease in their biomass under heat stress, with a smaller leaf area while the leaf number and the water content remained constant. *Solanum chilense* counterbalanced the decrease in leaf area by an increase in stomatal density, as an adaptation allowing higher transpiration rates.

The integrity of the organs did not seem to be negatively impacted, as shown by the absence of significant difference in the malondialdehyde (MDA) and water content between 21°C and 28°C. This integrity was probably maintained by a good osmotic adjustment, as indicated by a lower osmotic potential in the leaves of both species under heat stress. This adjustment was probably not due to soluble sugars as it is common in tomato, given the lower total soluble sugars concentrations under heat stress. Other osmolytes could not be measured.

Water stress results were more informative and highlighted that even though both species were affected, *S. chilense* was impacted less severely than its relative species *S. lycopersicum* on most indicators, which confirmed the results of previous studies suggesting that *S. chilense* was more tolerant than its cultivated relative.

*Solanum chilense* had lower values than *S. lycopersicum* on most morphological parameters (biomass, leaf area, root diameter and xylem elements diameter) under both water treatments, being a smaller plant by nature. But relatively speaking, the decrease due to water stress was less large than it was on *S. lycopersicum*.

The roots of both species were reduced in length and diameter by water stress, and the diameter of xylem elements decreased as well. However, the ratio between root diameter and xylem elements diameter increased, significantly more in *S. lycopersicum* than in *S. chilense*.

The integrity of the organs was more impacted under water stress than it had been under heat stress. This time, the MDA content increased in the stress conditions, more in the shoots than in the roots. It affected both species similarly. Soluble sugars were allocated

differentially to the shoot and the root, with more sugars in the shoot than in the root. This tendency became even more drastic under water stress in *S. lycopersicum*.

Not as many sources observe the effects of the stresses on the root systems as they do on the shoot, and even fewer compare the resource allocation between above- and belowground organs under stress (Mundim & Pringle, 2018). Yet, it was apparent in our results that there was a differential response to stress between those organs, and further research would allow to better understand how they work together to provide a better tolerance to stress to the whole plant.

Stress studies are easy to find when looking for separate stresses. However, even though most of the time, there is a paragraph on combined stresses in said studies, thorough studies on combined stresses are rarer, especially in field conditions (Cohen et al., 2021). Given that combined stresses are very frequent in field conditions and are likely to happen more often in climate change conditions, this is a field of knowledge that should be investigated further, in a perspective of food security.

In conclusion, *S. chilense* is a promising crop wild relative to *S. lycopersicum* in a context of climate change, under water stress particularly. Tolerance traits observed in the present work were mostly morphological and aspects of metabolism and photosynthesis in particular would be an interesting field to investigate in further studies. Another step forward would also be the testing in field conditions of this species, and of course *S. lycopersicum* × *S. chilense* hybrids, which could not be observed in the present work.

## 8. Bibliography

- About the Secretariat / UNFCCC*. (n.d.). Retrieved 1 April 2022, from <https://unfccc.int/about-us/about-the-secretariat>
- Ahluwalia, O., Singh, P. C., & Bhatia, R. (2021). A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resources, Environment and Sustainability*, 5, 100032. <https://doi.org/10.1016/j.resenv.2021.100032>
- Al Hassan, M., Martínez Fuertes, M., Ramos Sánchez, F. J., Vicente, O., & Boscaiu, M. (2015). Effects of Salt and Water Stress on Plant Growth and on Accumulation of Osmolytes and Antioxidant Compounds in Cherry Tomato. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43(1), 1–11. <https://doi.org/10.15835/nbha4319793>
- Alaguero-Cordovilla, A., Gran-Gómez, F. J., Tormos-Moltó, S., & Pérez-Pérez, J. M. (2018). Morphological Characterization of Root System Architecture in Diverse Tomato Genotypes during Early Growth. *International Journal of Molecular Sciences*, 19(12), 3888. <https://doi.org/10.3390/ijms19123888>
- Anjum, S. A., Ashraf, U., Zohaib, A., Tanveer, M., Naeem, M., Ali, I., Tabassum, T., & Nazir, U. (2017). Growth and developmental responses of crop plants under drought stress: A review. *Zemdirbyste-Agriculture*, 104(3), 267–276. <https://doi.org/10.13080/z-a.2017.104.034>
- Aubert, L., Konrádová, D., Kebbas, S., Barris, S., & Quinet, M. (2020). Comparison of high temperature resistance in two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*. *Journal of Plant Physiology*, 251, 153222. <https://doi.org/10.1016/j.jplph.2020.153222>
- Bergougnoux, V. (2014). The history of tomato: From domestication to biopharming. *Biotechnology Advances*, 32(1), 170–189. <https://doi.org/10.1016/j.biotechadv.2013.11.003>
- Bigot, S., Pongrac, P., Šala, M., van Elteren, J. T., Martínez, J.-P., Lutts, S., & Quinet, M. (2022). The Halophyte Species *Solanum chilense* Dun. Maintains Its Reproduction despite Sodium Accumulation in Its Floral Organs. *Plants*, 11(5), 672. <https://doi.org/10.3390/plants11050672>
- Blanchard-Gros, R., Bigot, S., Martinez, J.-P., Lutts, S., Guerriero, G., & Quinet, M. (2021). Comparison of Drought and Heat Resistance Strategies among Six Populations of *Solanum chilense* and Two Cultivars of *Solanum lycopersicum*. *Plants*, 10(8), 1720. <https://doi.org/10.3390/plants10081720>
- Böndel, K. B., Nosenko, T., & Stephan, W. (2018). Signatures of natural selection in abiotic stress-responsive genes of *Solanum chilense*. *Royal Society Open Science*, 5(1), 171198. <https://doi.org/10.1098/rsos.171198>

- Boulter, J. M., & McMichael, H. B. (1970). Modification of polyethylene glycol estimation suitable for use with small mammals. *Gut*, *11*(3), 268–270. <https://doi.org/10.1136/gut.11.3.268>
- Bretó, M. P., Asins, M. J., & Carbonell, E. A. (1993). Genetic variability in *Lycopersicon* species and their genetic relationships. *Theoretical and Applied Genetics*, *86*(1), 113–120. <https://doi.org/10.1007/BF00223815>
- Brown, M. E., & Funk, C. C. (2008). Food Security Under Climate Change. *Science*, *319*, 3.
- Burke, J. J. (1990). Variation among Species in the Temperature Dependence of the Reappearance of Variable Fluorescence following Illumination. *Plant Physiology*, *93*(2), 652–656. <https://doi.org/10.1104/pp.93.2.652>
- Carpita, N., Sabulase, D., Montezinos, D., & Delmer, D. P. (1979). Determination of the Pore Size of Cell Walls of Living Plant Cells. *Science*, *205*(4411), 1144–1147. <https://doi.org/10.1126/science.205.4411.1144>
- CGIAR Research Program on Water, Land and Ecosystems. (2015, June 25). *Partial Root-Zone Drying Irrigation Technique*. Water, Land and Ecosystems. <https://wle.cgiar.org/content/partial-root-zone-drying-irrigation-technique>
- Chapman, S. C., Chakraborty, S., Dreccer, M. F., & Howden, S. M. (2012). Plant adaptation to climate change—Opportunities and priorities in breeding. *Crop and Pasture Science*, *63*(3), 251. <https://doi.org/10.1071/CP11303>
- Chen, Y. L., Dunbabin, V. M., Diggle, A. J., Siddique, K. H. M., & Rengel, Z. (2011). Development of a novel semi-hydroponic phenotyping system for studying root architecture. *Functional Plant Biology*, *38*(5), 355. <https://doi.org/10.1071/FP10241>
- Chetelat, R. T., Pertuzé, R. A., Faúndez, L., Graham, E. B., & Jones, C. M. (2009). Distribution, ecology and reproductive biology of wild tomatoes and related nightshades from the Atacama Desert region of northern Chile. *Euphytica*, *167*(1), 77–93. <https://doi.org/10.1007/s10681-008-9863-6>
- Claussen, W. (2005). Proline as a measure of stress in tomato plants. *Plant Science*, *168*(1), 241–248. <https://doi.org/10.1016/j.plantsci.2004.07.039>
- Cohen, I., Zandalinas, S. I., Huck, C., Fritschi, F. B., & Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum*, *171*(1), 66–76. <https://doi.org/10.1111/ppl.13203>
- de Lobo, F. A., de Barros, M. P., Dalmagro, H. J., Dalmolin, Â. C., Pereira, W. E., de Souza, É. C., Vourlitis, G. L., & Ortíz, C. E. (2013). Fitting net photosynthetic light-response curves with Microsoft Excel—A critical look at the models. *Photosynthetica*, *51*(3), 445–456. <https://doi.org/10.1007/s11099-013-0045-y>

de Oliveira Maia Júnior, S., Ribeiro de Andrade, J., Moura dos Santos, C., Carvalho Silva, J. A., Oliveira dos Santos, K. P., & Vieira Silva, J. (2019). Leaf thickness and gas exchange are indicators of drought stress tolerance of sugarcane. *Emirates Journal of Food and Agriculture*, 29–38. <https://doi.org/10.9755/ejfa.2019.v31.i1.1897>

Demidchik, V. (2015). Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. *Environmental and Experimental Botany*, 109, 212–228. <https://doi.org/10.1016/j.envexpbot.2014.06.021>

Descamps, C., Quinet, M., Baijot, A., & Jacquemart, A.-L. (2018). Temperature and water stress affect plant-pollinator interactions in *Borago officinalis* (Boraginaceae). *Ecology and Evolution*, 8(6), 3443–3456. <https://doi.org/10.1002/ece3.3914>

Devereux, S., & Edwards, J. (2004). Climate Change and Food Security. *IDS Bulletin*, 35(3), 22–30. <https://doi.org/10.1111/j.1759-5436.2004.tb00130.x>

Emmert, F. H. (1974). Inhibition of Phosphorus and Water Passage Across Intact Roots by Polyethylene Glycol and Phenylmercuric Acetate. *Plant Physiology*, 53, 663–665.

European Environment Agency. (2021a, November 2). *Trend in the frequency of meteorological droughts in Europe (1950-2015)*—European Environment Agency. European Environment Agency. <https://www.eea.europa.eu/data-and-maps/figures/trend-in-the-frequency-of>

European Environment Agency. (2021b, November 19). *Wet and dry—Drought*—European Environment Agency [Page]. European Environment Agency. <https://www.eea.europa.eu/publications/europes-changing-climate-hazards-1/wet-and-dry-1/wet-and-dry-drought>

European Environment Agency. (2022, June 22). *Global and European temperatures*. European Environment Agency. <https://www.eea.europa.eu/ims/global-and-european-temperatures>

FAO. (2002). *Chapter 2. Food security: Concepts and measurement*. Fao.Org. <https://www.fao.org/3/y4671e/y4671e06.htm>

FAOSTAT. (2021). *FAOSTAT - Crops and Livestock Products*. Fao.Org. <https://www.fao.org/faostat/en/#data/QCL>

FAOSTAT. (2022). *FAOSTAT - Suite of Food Security Indicators*. Fao.Org. <https://www.fao.org/faostat/en/#data/FS>

Farmer, E., & Mueller, M. (2013). ROS-Mediated Lipid Peroxidation and RES-Activated Signaling. *Annual Review of Plant Biology*, 64, 429–450.

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought Stress in Plants: An Overview. In R. Aroca (Ed.), *Plant Responses to Drought Stress: From Morphological to Molecular Features* (pp. 1–33). Springer. [https://doi.org/10.1007/978-3-642-32653-0\\_1](https://doi.org/10.1007/978-3-642-32653-0_1)

- Garnett, T., Appleby, M. C., Balmford, A., Bateman, I. J., Benton, T. G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P. K., Toulmin, C., Vermeulen, S. J., & Godfray, H. C. J. (2013). Sustainable Intensification in Agriculture: Premises and Policies. *Science*, *341*(6141), 33–34. <https://doi.org/10.1126/science.1234485>
- Gaspar, M. J., Velasco, T., Feito, I., Alía, R., & Majada, J. (2013). Genetic Variation of Drought Tolerance in *Pinus pinaster* at Three Hierarchical Levels: A Comparison of Induced Osmotic Stress and Field Testing. *PLOS ONE*, *8*(11), e79094. <https://doi.org/10.1371/journal.pone.0079094>
- Gerszberg, A., & Hnatuszko-Konka, K. (2017). Tomato tolerance to abiotic stress: A review of most often engineered target sequences. *Plant Growth Regulation*, *83*(2), 175–198. <https://doi.org/10.1007/s10725-017-0251-x>
- Gharbi, E. (2017). *Interactions entre l'acide salicylique, les polyamines et l'éthylène chez Solanum lycopersicum et sa parente sauvage Solanum chilense exposées à la toxicité saline*. UCLouvain, FST.
- Gharbi, E., Martínez, J.-P., Benahmed, H., Fauconnier, M.-L., Lutts, S., & Quinet, M. (2016). Salicylic acid differently impacts ethylene and polyamine synthesis in the glycophyte *Solanum lycopersicum* and the wild-related halophyte *Solanum chilense* exposed to mild salt stress. *Physiologia Plantarum*, *158*(2), 152–167. <https://doi.org/10.1111/ppl.12458>
- Grandillo, S., Chetelat, R., Knapp, S., Spooner, D., Peralta, I., Cammareri, M., Perez, O., Termolino, P., Tripodi, P., Chiusano, M. L., Ercolano, M. R., Frusciante, L., Monti, L., & Pignone, D. (2011). *Solanum* sect. *Lycopersicon*. In C. Kole (Ed.), *Wild Crop Relatives: Genomic and Breeding Resources: Vegetables* (pp. 129–215). Springer Berlin Heidelberg. [https://doi.org/10.1007/978-3-642-20450-0\\_9](https://doi.org/10.1007/978-3-642-20450-0_9)
- Haider, S., Iqbal, J., Naseer, S., Yaseen, T., Shaukat, M., Bibi, H., Ahmad, Y., Daud, H., Abbasi, N. L., & Mahmood, T. (2021). Molecular mechanisms of plant tolerance to heat stress: Current landscape and future perspectives. *Plant Cell Reports*, *40*(12), 2247–2271. <https://doi.org/10.1007/s00299-021-02696-3>
- Hajjar, R., & Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica*, *156*(1), 1–13. <https://doi.org/10.1007/s10681-007-9363-0>
- Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Barbanti, L., Aamer, M., Iqbal, M. M., Nawaz, M., Mahmood, A., Ali, A., & Aslam, M. T. (2021). Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—a review. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, *155*(2), 211–234. <https://doi.org/10.1080/11263504.2020.1727987>

- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*(1), 189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Hernandez-Espinoza, L. H., & Barrios-Masias, F. H. (2020). Physiological and anatomical changes in tomato roots in response to low water stress. *Scientia Horticulturae*, *265*, 109208. <https://doi.org/10.1016/j.scienta.2020.109208>
- Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Circular*, *347* (2nd edit).
- Hughes, A. P., Cockshull, K. E., & Heath, O. V. S. (1970). Leaf Area and Absolute Water Content. *Annals of Botany*, *34*(135), 259–266. <https://doi.org/10.1093/oxfordjournals.aob.a084366>
- Iglesias, A., & Garrote, L. (2015). Adaptation strategies for agricultural water management under climate change in Europe. *Agricultural Water Management*, *155*, 113–124. <https://doi.org/10.1016/j.agwat.2015.03.014>
- Ingram, J. S. I., Gregory, P. J., & Izac, A.-M. (2008). The role of agronomic research in climate change and food security policy. *Agriculture, Ecosystems & Environment*, *126*(1–2), 4–12. <https://doi.org/10.1016/j.agee.2008.01.009>
- IPCC. (2018a). Framing and Concepts. In *Global Warming of 1.5°C: an IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*.
- IPCC. (2018b). Impacts of 1.5°C of Global Warming on Natural and Human Systems. In *Global Warming of 1.5°C: an IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*.
- IPCC. (2022). *About—IPCC*. IPCC.Ch. <https://www.ipcc.ch/about/>
- IPCC, Shukla, P. R., Skea, J., Slade, R., Al Khourdajie, A., van Diemen, R., McCollum, D., Pathak, M., Some, S., Vyas, P., Fradera, R., Belkacemi, M., Hasija, A., Lisboa, G., Luz, S., & Malley, J. (2022). Summary for Policymakers. In *Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. [https://www.ipcc.ch/report/ar6/wg3/downloads/report/IPCC\\_AR6\\_WGIII\\_SPM.pdf](https://www.ipcc.ch/report/ar6/wg3/downloads/report/IPCC_AR6_WGIII_SPM.pdf)
- Jacomini, E., Bertani, A., & Mapelli, S. (1988). Accumulation of polyethylene glycol 6000 and its effects on water content and carbohydrate level in water-stressed tomato plants. *Canadian Journal of Botany*, *66*(5), 970–973. <https://doi.org/10.1139/b88-140>

- Jangid, K. K., & Dwivedi, P. (2016). Physiological Responses of Drought stress in Tomato: A Review. *International Journal of Agriculture, Environment and Biotechnology*, *9*(1), 53–61. <https://doi.org/10.5958/2230-732X.2016.00009.7>
- Ji, Y., Scott, J. W., Schuster, D. J., & Maxwell, D. P. (2009). Molecular Mapping of Ty-4, a New Tomato Yellow Leaf Curl Virus Resistance Locus on Chromosome 3 of Tomato. *Journal of the American Society for Horticultural Science*, *134*(2), 281–288. <https://doi.org/10.21273/JASHS.134.2.281>
- Kang, M. S., & Banga, S. S. (2013). Global Agriculture and Climate Change. *Journal of Crop Improvement*, *27*(6), 667–692. <https://doi.org/10.1080/15427528.2013.845051>
- Kashyap, S. P., Prasanna, H. C., Kumari, N., Mishra, P., & Singh, B. (2020). Understanding salt tolerance mechanism using transcriptome profiling and de novo assembly of wild tomato *Solanum chilense*. *Scientific Reports*, *10*(1), 15835. <https://doi.org/10.1038/s41598-020-72474-w>
- Keohane, R. O., & Oppenheimer, M. (2016). Paris: Beyond the Climate Dead End through Pledge and Review? *Politics and Governance*, *4*(3), 142–151. <https://doi.org/10.17645/pag.v4i3.634>
- Knapp, S., & Peralta, I. E. (2016). The Tomato (*Solanum lycopersicum* L., Solanaceae) and Its Botanical Relatives. In M. Causse, J. Giovannoni, M. Bouzayen, & M. Zouine (Eds.), *The Tomato Genome* (pp. 7–21). Springer Berlin Heidelberg. [https://doi.org/10.1007/978-3-662-53389-5\\_2](https://doi.org/10.1007/978-3-662-53389-5_2)
- Krishna, R., Karkute, S. G., Ansari, W. A., Jaiswal, D. K., Verma, J. P., & Singh, M. (2019). Transgenic tomatoes for abiotic stress tolerance: Status and way ahead. *3 Biotech*, *9*(4), 143. <https://doi.org/10.1007/s13205-019-1665-0>
- Lagerwerff, J. V., Ogata, G., & Eagle, H. E. (1961). Control of Osmotic Pressure of Culture Solutions with Polyethylene Glycol. *Science*, *133*(3463), 1486–1487. <https://doi.org/10.1126/science.133.3463.1486>
- Lawlor, D. W. (1970). Absorption of Polyethylene Glycols by Plants and Their Effects on Plant Growth. *New Phytologist*, *69*(2), 501–513. <https://doi.org/10.1111/j.1469-8137.1970.tb02446.x>
- Lobell, D. B., Bänziger, M., Magorokosho, C., & Vivek, B. (2011). Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nature Climate Change*, *1*(1), 42–45. <https://doi.org/10.1038/nclimate1043>
- Lobell, D. B., Burke, M. B., Tebaldi, C., Mastrandrea, M. D., Falcon, W. P., & Naylor, R. L. (2008). Prioritizing Climate Change Adaptation Needs for Food Security in 2030. *Science*, *319*(5863), 607–610. <https://doi.org/10.1126/science.1152339>

- Luterbacher, U., & Sprinz, D. F. (2001). *International Relations and Global Climate Change*. MIT Press.
- Martínez, J. P., Antúnez, A., Araya, H., Pertuzé, R., Fuentes, L., Lizana, X. C., Lutts, S., Martínez, J. P., Antúnez, A., Araya, H., Pertuzé, R., Fuentes, L., Lizana, X. C., & Lutts, S. (2014). Salt stress differently affects growth, water status and antioxidant enzyme activities in *Solanum lycopersicum* and its wild relative *Solanum chilense*. *Australian Journal of Botany*, *62*(5), 359–368. <https://doi.org/10.1071/BT14102>
- Martínez, J. P., Antúnez, A., Pertuzé, R., Acosta, M. D. P., Palma, X., Fuentes, L., Ayala, A., Araya, H., & Lutts, S. (2012). Effects of Saline Water on Water Status, Yield and Fruit Quality of Wild (*Solanum chilense*) and Domesticated (*Solanum lycopersicum* var. *Cerasiforme*) Tomatoes. *Experimental Agriculture*, *48*(4), 573–586. <https://doi.org/10.1017/S001447971200066X>
- Mathieu, A.-S., Lutts, S., Vandoorne, B., Descamps, C., Périlleux, C., Dielen, V., Van Herck, J.-C., & Quinet, M. (2014). High temperatures limit plant growth but hasten flowering in root chicory (*Cichorium intybus*) independently of vernalisation. *Journal of Plant Physiology*, *171*(2), 109–118. <https://doi.org/10.1016/j.jplph.2013.09.011>
- Michel, B. E., & Kaufmann, M. R. (1973). The Osmotic Potential of Polyethylene Glycol 6000. *Plant Physiology*, *51*(5), 914–916. <https://doi.org/10.1104/pp.51.5.914>
- Moyle, L. C. (2008). Ecological and Evolutionary Genomics in the Wild Tomatoes (*Solanum* Sect. *Lycopersicon*). *Evolution*, *62*(12), 2995–3013. <https://doi.org/10.1111/j.1558-5646.2008.00487.x>
- Mundim, F. M., & Pringle, E. G. (2018). Whole-Plant Metabolic Allocation Under Water Stress. *Frontiers in Plant Science*, *9*. <https://www.frontiersin.org/articles/10.3389/fpls.2018.00852>
- Nishizawa, T., & Saito, K. (1998). Effects of Rooting Volume Restriction on the Growth and Carbohydrate Concentration in Tomato Plants. *Journal of the American Society for Horticultural Science*, *123*(4), 581–585. <https://doi.org/10.21273/JASHS.123.4.581>
- Pérez de Castro, A., Julián, O., & Díez, M. J. (2013). Genetic control and mapping of *Solanum chilense* LA1932, LA1960 and LA1971-derived resistance to Tomato yellow leaf curl disease. *Euphytica*, *190*(2), 203–214. <https://doi.org/10.1007/s10681-012-0792-z>
- Picken, A. J. F. (1984). A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *Journal of Horticultural Science*, *59*(1), 1–13. <https://doi.org/10.1080/00221589.1984.11515163>
- Platt, T., Gallegos, C. L., & Harrison, W. G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research*, *38*. <https://repositorio.imarpe.gob.pe/bitstream/20.500.12958/1357/1/BOL%20EXTR.%20Inv%20estigaci%C3%B3n%20....-18.pdf>

- Prasad, P. V. V., Staggenborg, S. A., & Ristic, Z. (2008). Impacts of Drought and/or Heat Stress on Physiological, Developmental, Growth, and Yield Processes of Crop Plants. In *Response of Crops to Limited Water* (pp. 301–355). John Wiley & Sons, Ltd. <https://doi.org/10.2134/advagricssystemmodel1.c11>
- Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, *82*(3), 222–237. <https://doi.org/10.1016/j.aquabot.2005.02.006>
- Rasband, W. (1997). *ImageJ: Image Processing and Analysis in Java* (1.8.0\_172) [Java; Windows]. Research Services Branch, National Institute of Mental Health. <https://imagej.nih.gov/ij/index.html>
- Rick, C. M. (1978). The Tomato. *Scientific American*, *239*(2), 76–89.
- RIPE. (2020, March 18). *The difference between C3 and C4 plants / RIPE*. RIPE - Realizing Increased Photosynthetic Efficiency for Sustainable Increases in Crop Yield. <https://ripe.illinois.edu/blog/difference-between-c3-and-c4-plants>
- Schmidhuber, J., & Tubiello, F. N. (2007). Global food security under climate change. *Proceedings of the National Academy of Sciences*, *104*(50), 19703–19708. <https://doi.org/10.1073/pnas.0701976104>
- Shamim, F., Khan, K., & Khalid, S. (2016). Comparison among twelve exotic accessions of tomato (*Solanum Lycopersicum* L.) for root and shoot development under polyethylene glycol induced water stress. *Pakistan Journal of Phytopathology*, *28*(02), 161–171.
- Smirnoff, N. (2014). Plant Stress Physiology. In John Wiley & Sons, Ltd (Ed.), *ELS* (1st ed.). Wiley. <https://doi.org/10.1002/9780470015902.a0001297.pub2>
- Stamova, B. S., & Chetelat, R. T. (2000). Inheritance and genetic mapping of cucumber mosaic virus resistance introgressed from *Lycopersicon chilense* into tomato. *Theoretical and Applied Genetics*, *101*(4), 527–537. <https://doi.org/10.1007/s001220051512>
- Tapia, G., Méndez, J., & Inostroza, L. (2016). Different combinations of morpho-physiological traits are responsible for tolerance to drought in wild tomatoes *Solanum chilense* and *Solanum peruvianum*. *Plant Biology*, *18*(3), 406–416. <https://doi.org/10.1111/plb.12409>
- Tardieu, F., & Tuberosa, R. (2010). Dissection and modelling of abiotic stress tolerance in plants. *Current Opinion in Plant Biology*, *13*(2), 206–212. <https://doi.org/10.1016/j.pbi.2009.12.012>
- The 100 Tomato Genome Sequencing Consortium, Aflitos, S., Schijlen, E., de Jong, H., de Ridder, D., Smit, S., Finkers, R., Wang, J., Zhang, G., Li, N., Mao, L., Bakker, F., Dirks, R., Breit, T., Gravendeel, B., Huits, H., Struss, D., Swanson-Wagner, R., van Leeuwen, H., ... Peters, S. (2014). Exploring genetic variation in the tomato (*Solanum* section *Lycopersicon*) clade by

whole-genome sequencing. *The Plant Journal*, *80*(1), 136–148.  
<https://doi.org/10.1111/tpj.12616>

Tomeo, N. (2019). *Tomeopaste/AQ\_curves: AQ\_curve fitting release 1* (v1.0.0). Zenodo.  
<https://doi.org/10.5281/ZENODO.3497557>

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response Mechanism of Plants to Drought Stress. *Horticulturae*, *7*(3), 50. <https://doi.org/10.3390/horticulturae7030050>

Zgallai, H., Steppe, K., & Lemeur, R. (2006). Effects of Different Levels of Water Stress on Leaf Water Potential, Stomatal Resistance, Protein and Chlorophyll Content and Certain Anti-oxidative Enzymes in Tomato Plants. *Journal of Integrative Plant Biology*, *48*(6), 679–685. <https://doi.org/10.1111/j.1744-7909.2006.00272.x>

Zhou, J., Reddy, S., Zhou, S., Sauv e, R. J., Bhatti, S., Fish, T., & Thannhauser, T. W. (2012). Effect of Heat Stress on Leaf Proteome and Enzyme Activity in *Solanum chilense*. *Plant Stress*, *6*(1), 8–13.

Zhou, R., Yu, X., Li, X., Mendanha dos Santos, T., Rosenqvist, E., & Ottosen, C.-O. (2020). Combined high light and heat stress induced complex response in tomato with better leaf cooling after heat priming. *Plant Physiology and Biochemistry*, *151*, 1–9.  
<https://doi.org/10.1016/j.plaphy.2020.03.011>

# Impacts of heat and water stresses on the cultivated tomato *Solanum lycopersicum* and its wild relative *Solanum chilense*

Anaïs CAUCHIE

Climate change causes an increase in global temperatures and drought occurrence. Water stress and heat stress are among the most common and impactful environmental stresses on plant growth and development, and the combination of the two can have drastic effects.

The cultivated tomato *Solanum lycopersicum* is threatened by climate change. One of its wild relative species has an interesting potential for introgression of traits linked to tolerance to water and heat stress: *Solanum chilense*, a wild tomato native to the Atacama Desert in Chile.

Individuals of both species were subjected to heat stress (21°C vs. 28°C) as well as water stress (well-watered conditions vs. water stress) implemented by using 10% polyethylene glycol in hydroponics. Morphological parameters and biomass were measured on shoots and roots. Physiological parameters were measured, such as water content, osmolality, malondialdehyde content, sugar content and photosynthesis-linked parameters.

Under heat stress, very few differences between the two investigated species have emerged. These differences were essentially about morphological impacts: both species had a lower leaf area and *S. chilense* had a higher stomatal density under heat stress than temperate conditions while *S. lycopersicum* did not seem to adapt.

Water stress results were more informative and highlighted that even though both species were negatively affected, *S. chilense* was impacted less severely than *S. lycopersicum* on most indicators. Moreover, differences in the response of aerial and root systems were observed.

The conclusion of this work is that *S. chilense* is a promising crop wild relative to *S. lycopersicum* for trait introgression in a context of water stress tolerance. The study of the root system of *S. chilense* as well as the comparison of aboveground and belowground responses to stresses are an open field to be explored.