



## Article

# Impact of *Trichoderma asperellum* on Chilling and Drought Stress in Tomato (*Solanum lycopersicum*)

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**Abstract:** The acceleration of climate change is necessitating the adoption of shifts in farming practices and technology to ensure the sustainability of agricultural production and food security. Because abiotic stresses such as drought and chilling represent major constraints on agricultural productivity worldwide, in this study, the mitigation of such stresses by the fungus *Trichoderma asperellum* HK703 was evaluated. The fungus was grown on whole grain oats, kaolin and vermiculite for 5 days and then the formulation was mixed with the potting soil to colonize the roots of the plants. The effect of the bioinoculant on tomato under drought or chilling was analyzed in tomato (*Solanum lycopersicum*) plants. Leaf, stem and root succulence, electrolyte leakage, the relative growth rate of plant height, stem thickness and leaf area, as well new leaf emergence and chlorophyll content were determined. The results showed that drought or chilling increased electrolyte leakage and reduced plant growth and development traits and chlorophyll (*a,b*) content. However, inoculation with *T. asperellum* eliminated or reduced most of the negative impacts of drought compared to the non-stressed plants, with the exception of chlorophyll *b* content. Furthermore, inoculation with *T. asperellum* improved some of the evaluated features in chilling stressed plants but had no effect on plant height or chlorophyll (*a,b*) content. The results of this study indicate that *T. asperellum* was more effective in alleviating drought than chilling stress in tomato plants.

**Keywords:** *Trichoderma asperellum*; plant priming; drought; chilling; stress tolerance

## 1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) published in 2016 that agricultural production will have to increase by about 60% by 2050 in order to feed the growing global human population [1]. However, climate change is having negative impacts on agricultural productivity that threatens food security. If the current situation of climate change continues until 2100, there may be a decline in maize yields by 20–45%, wheat yields by 5–50%, rice yields by 20–30% and soybean yields by 30–60% [1]. Parallel to these drastic impacts, climate change would have negative effects on food quality and access, affecting public health [2].

From previous studies, it is recognized that crop production is extremely sensitive to current climate change, especially in subtropical and tropical regions [3,4]. Among factors

affecting agricultural yields and food security are extreme weather, changing precipitation regimes, drought and increases in atmospheric carbon dioxide levels [3,5].

Drought stress in plants results from rising summer temperatures and reduced precipitation caused by climate change [3]. It is expected that air temperature may increase by about 2 °C above present levels by the end of this century [6] and that drought may cause serious plant growth problems for more than 50% of arable lands by 2050 [7]. Drought stress is considered to be one of the most important agricultural yield-reducing factors in the world [8]. Typically, drought stress is characterized by a reduction in water content, leaf water potential, turgor loss, closure of stomata, cell enlargement and diminished growth [9]. The decline in photosynthesis and respiration during intense drought periods may lead to the death of the entire plant [10].

In addition, chilling (exposure to low but non-freezing temperatures in the range of 1–10 °C) has been recognized as a stress that leads to numerous physiological disturbances in sensitive plants, including water regime, mineral nutrition, photosynthesis, respiration and metabolism that may also ultimately lead to plant death [11]. Chilling injury is considered to be one of the abiotic stresses that constrain the productivity of crops and is especially important for tropical and subtropical plants such as cotton, cowpea, groundnut, maize, rice and tomato [12,13].

Plants have developed several mechanisms to perceive and respond to stress in order to combat these conditions and maintain the appropriate physiology for their survival and adaptation [14]. Signal transduction is essential for enhancing these responses against abiotic stress. During stress responses, the defense is orchestrated by a diverse range of molecules, including stress hormones, reactive oxygen species (ROS), Ca<sup>2+</sup> and protein kinases such as mitogen-activated protein kinases (MAPKs) that activate downstream transcription factors to enhance stress-responsive gene expression [15].

Additionally, plants can be 'prepared' to be more tolerant to future biotic and abiotic stress conditions through priming or pre-conditioning [16]. Priming is a low-cost defensive measure [17] and can be considered as the sensitized state in which the plants respond more rapidly and/or more robustly if subsequently re-exposed to biotic and abiotic stress [14]. The pre-conditioning phase is initiated by a triggering stimulus that can deploy a systemic defense which persists until the plant is exposed to a challenging stress [18].

Defense elicitation can be induced by chemical compounds such as β-aminobutyric acid (BABA) [19–21], salicylic acid (SA) [22,23], jasmonic acid (JA), volatile compounds [24] or by pathogens [25] and insect herbivores [26]. However, priming can also be the result of associations between plants and beneficial soil organisms. The colonization of plant roots by non-pathogenic bacteria such as rhizobacteria [7,27] and some species of *Pseudomonas* [28], *Bacillus* [29] or *Bradyrhizobium* [30] can induce tolerance against biotic and abiotic stressors through induced resistance (IR) [28]. This type of resistance primarily requires JA and ethylene (ET) signaling. Additionally, there are some interactions between plants and beneficial fungi that also result in systemic IR. These include the endophytic fungi *Serendipita indica* [31], *Trichoderma* spp. [32,33] and *Glomus* spp. [34]. *Trichoderma* spp. are soil-borne filamentous fungi that play an important role in the control of phytopathogens by the activation of local and systemic responses in plants [35]. The systemic immune response induced by *Trichoderma* spp. has been recognized as a form of long-lasting defence since it activates plant basal resistance that may improve tolerance against different types of stress [36,37].

There is an increasing interest in some *Trichoderma* species that improve the survival of plants in hostile environments, including soil heavy metal contamination, salinity, drought and extreme temperature [38–41]. Studies on the use of *T. harzanium* to mitigate the effects caused by abiotic stress in tomato seeds and plants [42–44] have been previously published. However, the evidence on whether *T. asperellum* has the ability to induce stress tolerance in tomato plants is yet unclear.

*T. asperellum* is able to increase certain fitness characteristics such as growth in tomato plants and therefore represents an organic low-cost fertilizer alternative and enhances the

induction of defense responses against biotic stress [33]. Recently it has been published that *T. asperellum* induces tolerance to drought in sugarcane plants by increasing the photosynthetic rate, stomatal conductance and water use efficiency [45].

In the current study, we analyzed the ability of *T. asperellum* to alleviate symptoms caused by drought and chilling stresses in tomato plants by assessing electrolyte leakage, the rate of growth in the height, stem diameter and leaf area, the number of green new leaves developed and the chlorophyll (*a*, *b*) content.

## 2. Materials and Methods

### 2.1. Experimental Site

The experiments were conducted from May 2019 to April 2021 at the Laboratory of Phytopathology and glasshouses located at the Instituto de Ciencias Agropecuarias, UAEH, in Tulancingo, Hidalgo (latitude 20°03'40" N, longitude 98°23'00" O, and altitude of 2163.1 m).

### 2.2. Plant Material and Growth Conditions

For the experiments, seeds of *Solanum lycopersicum* cultivar Vita were used. Seeds were germinated on 200-cavity trays with sterile peat moss (Sunshine, Sun Gro Horticulture; Agawam city, Massachusetts USA) and perlite (agrolite) (Dicalite de México S.A. de C.V.; Tlalnepantla, Mexico State). After 14 days from germination, the seedlings were transplanted (see below) and maintained in the glasshouse (24–30 °C/18 °C day/night, 60–70% relative humidity, 16-h day/8-h night, natural light). Irrigation with tap water or Hoagland's nutrient solution was applied on alternate days until stress treatment application. Plants were used at 35 days of age for all experiments.

### 2.3. Inoculation with *Trichoderma*

*T. asperellum* (HK703, NRRL50191) was obtained from Dr. Hernández at Centro de Biotecnología Genómica (IPN), México. To prepare the bioinoculant, 20 g of oat, 50 mL of kaolin and 100 mL of vermiculite were mixed and 60 mL of water added, mixed and stored in sealed polythene bags. The polythene bags were sterilized by autoclaving and aseptically inoculated with *T. asperellum* ( $1 \times 10^5$  spores mL<sup>-1</sup>). The sterilized bags were incubated for 5 days at 25 °C and shaken daily [33].

The contents of the bags were added into 6-inch pots, which were filled with peat moss and vermiculite (7:3 *v/v*) mixture and then mixed until homogenous. The pH value recorded for the mixture was 5.9. Elicitation of plants was achieved by transplanting the two-week-old seedlings into independent pots containing either the inoculated growing mixture or a non-inoculated control mixture of identical composition. Pots containing inoculated and non-inoculated plants were transferred in two separate glasshouses (one for inoculated plants and one for non-inoculated plants).

### 2.4. Abiotic Stress Treatment

Three weeks after transplanting (35 days after germination), non-inoculated plants, as well as those that were inoculated, were divided into three groups to assess tomato tolerance in response to drought or chilling. One third of the non- and inoculated plants were kept in the glasshouse under normal conditions (Control group).

For drought stress application, irrigation was interrupted for another third of non-inoculated and inoculated plants (Drought group). Every third day, soil relative water contents (SRWC) were estimated by using the following equation:

$$\text{SRWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where FW is fresh weight, DW is the dry weight and TW is the saturated soil weight [46].

For the chilling stress application, the remaining non-inoculated and inoculated plants (one third of the total) were transferred to a growth chamber at a constant temperature of 4 °C (Chilling group).

After 9 days of the abiotic stress application, the succulence, electrolyte leakage, plant height, stem diameter and number of new leaves were measured. Leaf size as well as chlorophyll content were measured 6 days after stress application because of the high level of leaf rolling caused by the abiotic stress treatment.

### 2.5. Succulence

At the end of each experiment, the fresh leaves were removed from whole plants (6 replicates per treatment). The leaves were weighed to obtain the fresh weight (FW). Then the samples were placed in a dryer (80 °C, 48 h) to obtain the dry matter (DW). Succulence was calculated according to the ratio of fresh weight/dry weight [47].

### 2.6. Assessment of Drought and Chilling Stress Tolerance of Tomato Plants

Plant tolerance to stress was evaluated using visible symptoms. Photographs were taken immediately after 9 days of stress.

Additionally, to evaluate the stress tolerance of the tomato leaves, 1 fully developed leaf from each plant was collected 9 days after stress. Twenty-five 0.5-cm diameter discs were punched from each leaf with a cork borer and immersed in 15 mL of deionized water in 50-mL plastic tubes, and the electrical conductivity (EC) of the samples was measured using a conductivity meter (CON2700, Oakton, Illinois USA) and was considered as EC<sub>0</sub>. Then, the samples were washed slowly (150 rpm) for three hours on an orbital shaker (Orbit™ 1000, Labnet, New Jersey USA) at room temperature. The extent of electrolyte leakage in the incubation solution was determined and designed as EC<sub>3</sub>. Tubes containing the tomato leaf discs were then placed in an 80 °C hot water bath for 30 min followed by shaking for 30 min at room temperature on the orbital shaker. Then, electrolyte leakage (EL) was determined and was considered as the total electrolyte leakage or EC<sub>T</sub> [48]. The cell membrane injury was determined according to the following equation:

$$\% \text{ EL} = \frac{\text{EC}_3 - \text{EC}_0}{\text{EC}_T} \times 100$$

and expressed as a percentage of electrolyte leakage (% EL).

### 2.7. Measurements of Plant Traits

To evaluate the performance of the plants subjected to abiotic stress, the Absolute Growth Rate (AGR) of plant height, stem diameter and leaf size were calculated. AGR represents the change in size of a plant trait per unit of time. In this study, the AGR was expressed as cm day<sup>-1</sup> for plant height and stem diameter and as cm<sup>2</sup> day<sup>-1</sup> for leaf area.

The absolute growth rate growth of plant height, stem diameter and leaf area were calculated according to the formula described by Carmassi [49]:

$$\text{AGR} = \left( \frac{\Delta L}{\Delta T} \right)$$

ΔL: Difference between the first and the final measurements;

ΔT: Interval of time between subsequent measurements.

Plant height was measured from the soil surface up to the last leaf tip. Stem diameter was determined at the midpoint between the second basal nodes using Vernier calipers.

Leaf area was calculated from non-destructive measures of leaf dimensions as follows [50]:

$$\text{Leaf area (cm}^2\text{)} = 0.5 \times \text{leaf length} \times \text{leaf width}$$

The number of new leaves emerged per plant during 9 days of stress was also counted.

For the measurements of the variables of plant traits, six biological replicates were analyzed.

### 2.8. Determination of Chlorophyll (a, b) Content of Tomato Leaves

Chlorophyll *a* and *b* from the third fully expanded leaves were extracted in 80% acetone, and the contents were analyzed according to the method of Arnon [51]. First, 100 mg of total leaf sample was ground and mixed with 7 mL of 80% acetone in a porcelain mortar. The extract obtained was filtered through Whatman No. 1 filter paper and the volume of solvent adjusted to give a final volume of 10 mL of 80% acetone. Chlorophyll content was analysed by absorption measurements at 663- and 645-nm wavelengths on a spectrophotometer (T60 UV/VIS, PG instruments, Leicestershire United Kingdom) and calculated according to the following equations:

$$\text{chl } a \text{ (mg/g F.W.)} = \frac{[(12.7 \times \text{OD}_{663}) - (2.69 \times \text{OD}_{645})] \times V}{1000 \times W}$$

$$\text{chl } b \text{ (mg/g F.W.)} = \frac{[22.9 \times \text{OD}_{645} - (4.68 \times \text{OD}_{663})] \times V}{1000 \times W} \quad (1)$$

where OD = optical density (nm), V = final volume made (mL) and F.W.= fresh weight of leaves (g).

### 2.9. Statistical Analysis

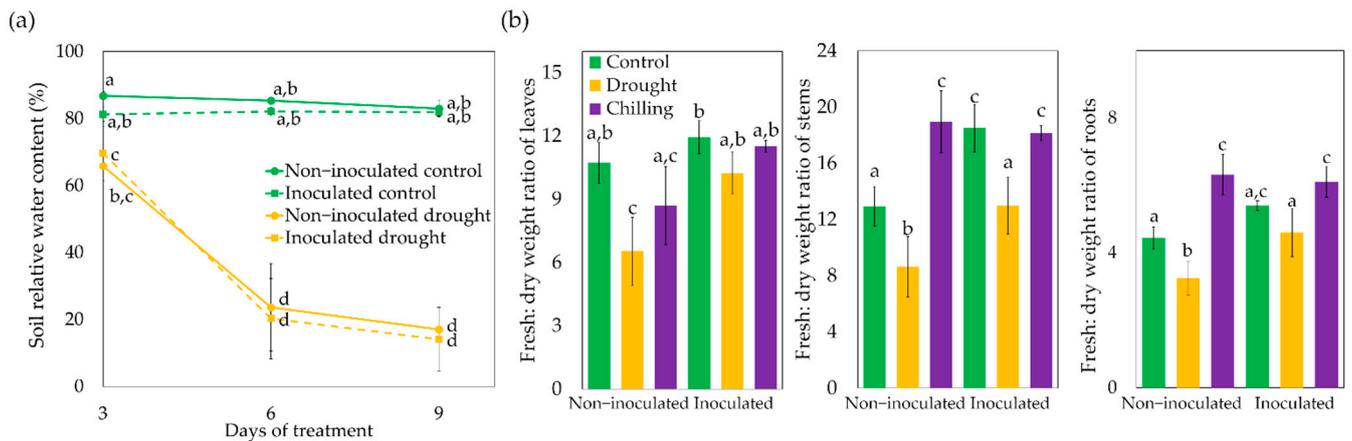
The variables evaluated in this report were processed using Excel 2010 (Microsoft Inc., Redmond, WA, USA). The data were expressed as mean  $\pm$  standard deviation (SD) for six replicates in each group. Differences in mean values among the treatments were determined by using the one-way ANOVA method with Tukey HSD tests at a significance level of  $p < 0.05$  [52]. The analyses were performed by using *Minitab*<sup>®</sup>14 statistical software for windows [53].

## 3. Results

### 3.1. Effect of *Trichoderma asperellum* on Loss of Water Storage Capacity under Drought and Chilling Stresses

To validate the effects of water deficits on plants during drought treatment, the soil water status was monitored. The soil relative water contents (SRWC) over time are shown in Figure 1a. The soil moisture of non-inoculated and inoculated pots with daily irrigation was in the range of 81 to 87%. In contrast, the water-withholding treatment reduced the SRWC over time. The soil water content was in the range of 66–70% after 3 days of water deprivation, while the water content was in the range of 20–24 and 14–17% after 6 and 9 days, respectively. No significant difference was observed between the soil moisture from non-inoculated and inoculated pots under drought conditions over time, indicating the bioinoculant did not affect the soil moisture.

Next, this study evaluated whether the tomato plants were experiencing the effects of drought by assessing the succulence of leaves, stems and roots according to the ratio of fresh: dry leaf weight (Figure 1b). The most severely affected fresh/dry weight ratios were observed in the leaves, stems and roots from the non-inoculated plants under drought stress, where they decreased by 39%, 33% and 27% below the control, respectively. Interestingly, the succulence of drought-stressed inoculated plants remained at the same level as the well-watered inoculated control plants, indicating that *T. asperellum* is protecting the plants from a loss of water storage capacity.

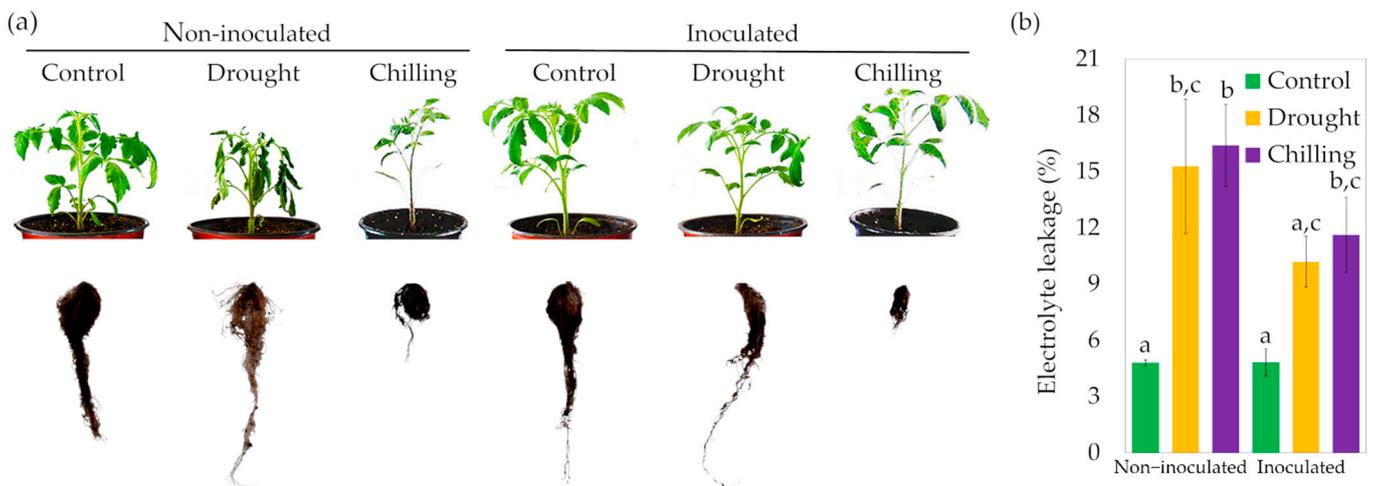


**Figure 1.** Changes in (a) soil relative water content (SRWC) during water deprivation treatment and (b) succulence according to the fresh weight: dry weight ratio for each treatment at 9 days after stress application. Results are the mean ± SD from six replicates. Bars with different letters indicate significant differences by Tukey’s test at  $p < 0.05$ .

Since chilling in sensitive plants, such as tomato, induces a loss of water, we also explored the succulence in plants under this abiotic stress. There were no significant changes in the fresh/dry weight ratio of the leaves from plants stressed with chilling compared with plants grown in normal conditions (Figure 1b). However, the FW/DW ratio of stems and roots increased in the non-inoculated plants under chilling stress. In all conditions, *T. asperellum* increased the FW/DW ratio, except for stems and roots under chilling conditions.

### 3.2. Effect of *Trichoderma asperellum* on Electrolyte Leakage, Growth and Chlorophyll Content under Drought and Chilling Stress

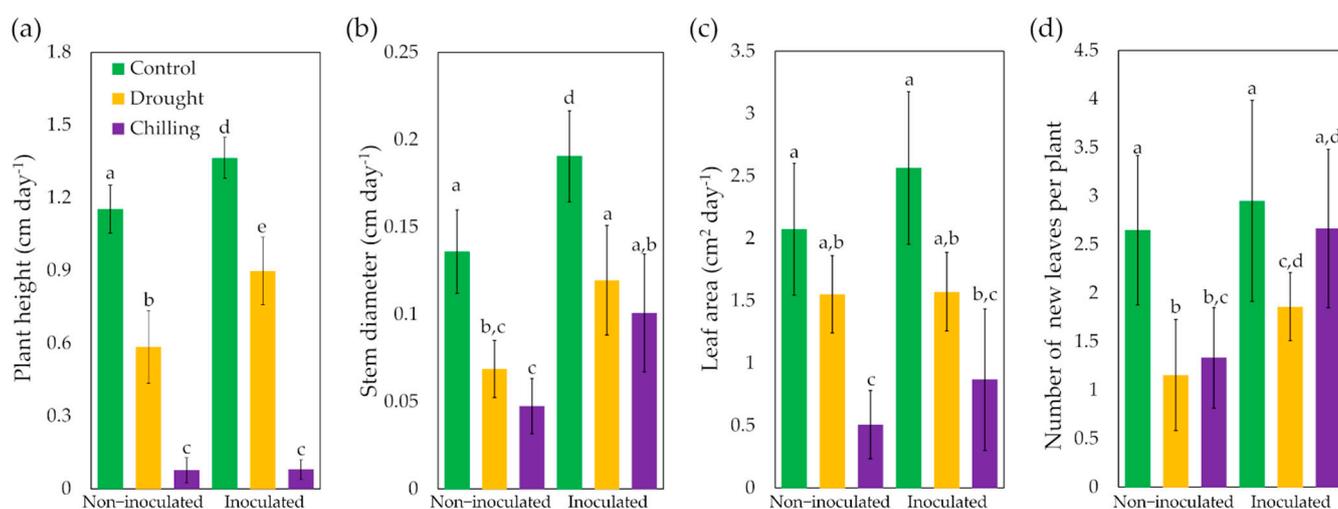
The current study investigated the effect of *T. asperellum* in the attenuation of injury severity caused by drought or chilling for 9 days (Figure 2a). Water withdrawal resulted in a severe wilting and inhibition of root growth of non-inoculant plants, an effect which was mitigated by *T. asperellum*. Chilling stress led to a limitation of shoot and root growth. However, the impact of *T. asperellum* on shoot and root growth was almost imperceptible in chilling-stressed plants.



**Figure 2.** Tolerance to drought and chilling is induced by *T. asperellum* in tomato plants. The tolerance to stressors was assessed in non-inoculated and inoculated plants using (a) phenotypic and (b) electrolyte leakage analysis. Photographs from selected and representative plants were taken on the ninth day of drought and chilling treatments from three independent experiments with six replicates each. Data in (b) represents the means ± SD ( $n = 6$ ). Different letters denote significantly differences among treatments based on Tukey’s test ( $p < 0.05$ ).

The relative tolerance induced by *T. asperellum* to drought and chilling stress was also assessed by comparing electrolyte leakage from the leaf tissues (Figure 2b). This technique has been widely used as an indicator of stress-induced injury in plants. As expected, the electrolyte leakage increased in both stress conditions for the non-inoculated plants. However, the electrolyte leakage from tomato inoculated with *T. asperellum* stressed with drought and chilling showed intermediate values between non-stressed and stressed conditions from the plants free of bioinoculant.

Additionally, the influence of *T. asperellum* on the AGRs of plant height, stem diameter and leaf area were evaluated (Figure 3a–c). At 9 days after drought stress, the AGR of the height and stem diameter from non-inoculated plants was significantly inhibited by up to 49%. This effect was less marked in inoculated drought-challenged plants, where the inhibition was 34% (height) and 37% (stem diameter) in relation to the control. Notably, the inoculated drought-stressed plants had similar AGRs for the stem diameter to well-watered, non-inoculated plants.



**Figure 3.** Agronomic traits of tomato plants under drought stress. AGR of (a) Plant height, (b) Stem diameter and (c) Leaf area; (d) Number of new emerged leaves per plant. Plant height, stem diameter and number of leaves were measured 9 days after treatment. For leaf area, data were taken at 6 days. Data expressed as mean  $\pm$  SD from three independent experiments with six replicates each. Different letters denote significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

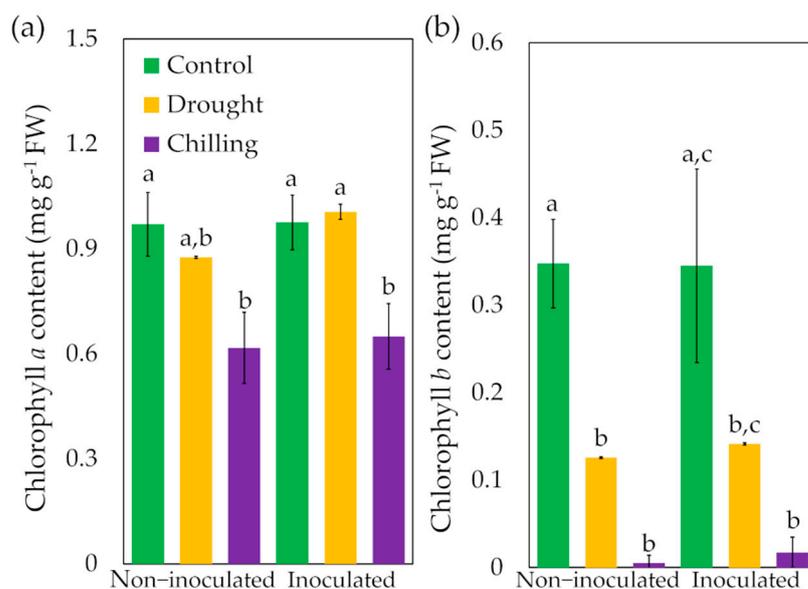
On the other hand, chilling caused severe inhibition of the AGR of plant height (over 93%), which was not alleviated by *T. asperellum* (non-inoculated = 0.076 vs. inoculated = 0.079 cm day<sup>-1</sup>). Regarding the AGR for stem diameter, the inhibition was less severe (by 63% and 47% compared to their controls for non-inoculated and inoculated plants, respectively). Once again, the AGR of stem diameter was statically similar between non-inoculated control plants and inoculated chilling-stressed plants (0.136 vs. 0.101 cm day<sup>-1</sup>, respectively).

The measurements of the leaf area had to be carried out at 6 days after application of the stress due to the high leaf rolling observed at day 9. No significant differences were found in the AGR of leaf areas under drought stress (non-inoculated = 1.552; inoculated = 1.566 cm day<sup>-1</sup>) in comparison to their respective controls (non-inoculated = 2.074; inoculated = 2.566 cm day<sup>-1</sup>), however, the AGR of the leaf area was more severely affected by chilling stress, showing a percent inhibition of 75% (non-inoculated) and 66% (inoculated) compared to the controls.

Under drought or chilling, the leaf appearance rate was also measured (Figure 3d). After 9 days, the formation of leaves in the non-inoculated plants was reduced by more than 50% when they were stressed. In inoculated plants, drought statistically reduced leaf

formation by 37%, while no difference was observed in leaf production after chilling stress (Figure 3d).

Chlorophyll from tomato leaves was extracted and measured to investigate the changes after exposure to drought or chilling stress (Figure 4). The leaf chlorophyll *a* concentration of tomato plants exposed to drought did not differ significantly from non-stressed plants, irrespective of whether *T. asperellum* was inoculated or not. Nonetheless, chilling stress significantly reduced the chlorophyll *a* content in both non-inoculated and inoculated plants by 36% and 33%, respectively.



**Figure 4.** Changes in chlorophyll content in leaves of tomato plants inoculated with *T. asperellum* under drought or chilling exposure. Leaves of 3-week-old tomato plants were dipped in *T. asperellum* and then exposed to drought or chilling for six days: (a) Chlorophyll *a*; (b) Chlorophyll *b*. Data were expressed as mean  $\pm$  SD ( $n = 6$ ). Bars with different superscripts differ from each other significantly at  $p < 0.05$  (Tukey's test).

As shown in Figure 4b, both drought and chilling stress markedly decreased the chlorophyll *b* content. These decreases were not significantly affected by the interaction between the tomato and *T. asperellum* (reduced vs. control by 64% and 59% for non-inoculated and inoculated drought-stressed plants, respectively, and by 98% and 95% for non-inoculated and inoculated chilling-stressed plants, respectively). Altogether, these results indicate that the negative effect of chilling on chlorophyll content was more accentuated than that observed for drought and that the depressive effect in chlorophyll (*a*, *b*) content could not be alleviated by *T. asperellum*.

#### 4. Discussion

Drought and chilling are two major abiotic stresses that adversely affect the growth and productivity of field crops, and ultimately the food security. Since tomato is very sensitive to water and chilling stress [13,54], this study evaluated the effect of *T. asperellum* to mitigate the effects of both stresses in this crop.

Plants may exhibit common molecular and physiological responses upon exposure to chilling and drought [55]. One of the most noticeable symptoms in herbaceous plants exposed to these abiotic stress conditions is leaf and hypocotyl wilting [11], which is used as an indicator of insufficient moisture. In the present study, drought stress resulted in whole plant wilting in non-inoculated but not in *T. asperellum*-inoculated plants, which exhibited a mitigation of the wilting response to drought, indicating such conditioning helps maintain leaf turgor in the plant.

To further explore the tolerance of tomato to drought and chilling, the current study evaluated leaf electrolyte leakage, since this technique provides information on the integrity of cell membranes [48]. As shown by previous reports [56,57], drought and chilling stress damaged the cell membranes, resulting in ion leakage in the tomato plants. Nevertheless, the present study found an ameliorative effect of *T. asperellum* on electrolyte leakage after drought or chilling stress, suggesting the electrolyte leakage may be used as an indicator of plant stress tolerance, as has previously been proposed [58]. The mechanism for disturbances in membranes may involve the activation of lipid peroxidation induced by high levels of reactive oxygen species (ROS) produced during drought or chilling stress [59,60]. The efficient detoxification of reactive oxygen species is thought to play a key role in enhancing the tolerance of plants to abiotic stresses [59,61], and recently, it has been reported that *Trichoderma* spp. imparts tolerance against biotic and abiotic stress through the modulation of ROS [33,39,41].

Limitations of water availability in plants can occur by either a rapid decline in the absorption of water by the roots or the inability to close stomata to reduce transpiration [11]. To improve tolerance of drought or chilling, plants have developed several physiological and molecular mechanisms including the disturbance of the water regime [11,62]. Succulence and stem diameter reflect plant water status and they are an important basis for drought tolerance [63,64]. The present study detected that succulence and stem thickness of non-inoculated plants under drought stress exhibited a significant decrease, contrary to what was observed in inoculated plants by comparing with control and non-inoculated conditions. After chilling, no changes were detected in succulence of the leaves, stems and roots from both non-inoculated and inoculated-plants. These findings were consistent with the turgor maintenance observed in the plants at low temperatures, indicating the ability to store water remained unaltered. Altogether, the data indicated maintenance of high water content was an important marker of drought stress tolerance in plants.

Plant stress tolerance is often related to their morphological traits. In response to drought and chilling stress, plants commonly reduce their growth [62,65,66] by inhibiting cell division in the meristems [67,68] or by suppressing cell elongation and expansion due to low turgor pressure [62,69]. In this study, water scarcity and suboptimal temperature in tomato plants evoked significant changes in the AGR of a number of plant traits, namely: height, stem diameter and leaf area, which could be associated with a decline in cell enlargement or with a decrease in the number of dividing cells or rate of division. The association with *T. asperellum* improved the growth in tomato plants exposed to drought. Such results are in line with those of Khadka et al. [70], Rawat et al. [71], Mona [40], Zhao [72] and Scudeletti [45], who found that *Trichoderma* spp. enhanced the growth of rice, wheat, tomato, cucumber and sugarcane, respectively, after drought exposure.

Water scarcity can affect photosynthesis via the closure of stomata to prevent the loss of water through transpiration. One stress tolerance mechanism to ensure the maintenance of photosynthesis under such conditions consists of reducing the plant leaf area and restricting the expansion of new leaves [6]. Both drought and chilling inhibit cell division in the meristem of plants, contributing to this reduced leaf growth. Although in the current study we did not find a significant reduction in leaf area, drought stress reduced new leaf emergence. The reduction in the formation of new leaves has also been reported for *Conocarpus erectus* [73] and marigold [74] under drought stress, suggesting that this response is a mechanism to preserve plant water content [75].

Photosynthetic pigments are important stress markers [76]. Drought and chilling are able to inhibit photosynthesis by affecting chlorophyll content [77,78]. The effect of drought stress on chlorophyll depends on plant genotype and environmental conditions. For example, in severe drought stress conditions, chlorophyllase levels increase, resulting in a decrease in chlorophyll content. On the contrary, it also has been reported that the chlorophyll content of leaves of resistant cultivars increases under this stress condition [74]. When drought stress was applied in the current study, only chlorophyll *b* was reduced.

This result agrees with a number of previous studies, which indicated that chlorophyll *b* is more sensitive than chlorophyll *a* in response to drought [74,79–81].

Despite *T. asperellum* moderately improving stem diameter, the formation of new leaves and electrolyte leakage from chilling stressed plants, it could not counteract decreases in root size, plant height and chlorophyll *a,b* content caused by this stress, indicating that chilling represents a severe stress for the tomato plants. The degradation of photosynthetic pigments could be a mechanism to prevent an excessive generation of ROS driven by excess energy absorption in the photosynthetic apparatus during drought or chilling stress [82,83]. Nevertheless, the observed decreases in content of both chlorophylls may also be the result of synthesis impairment caused by a decrease in the content of magnesium, an increase in membrane permeability and lipid peroxidation or by a reduction in other nutrients such as calcium, iron or manganese that are essential in chlorophyll synthesis or electron transport [84]. The reduction in chlorophyll content might affect the photosynthetic efficiency, which would partly explain the decrease in plant growth shown in this study.

There are some species of *Trichoderma* that are capable of growing at 5 °C; however most of them are mesophilic [85]. Studies have found that temperature affects the spore germination and germ-tube growth of *Trichoderma* spp. [86]. In the current study, the efficiency of *T. asperellum* to induce tolerance against chilling stress could be affected by low temperatures by reducing fungal growth and survival. Information about the influence of temperature on growth, colonization and survival of *T. asperellum* is necessary to elucidate whether its efficacy is dependent on temperature to mitigate the effect of chilling stress.

## 5. Conclusions

Both climate change and the need to increase agricultural production are two of the biggest challenges humans are facing to prevent a more conflictive near future. Climate change negatively affects agricultural production, especially for those species that are considered as tropical and subtropical crops. One of these species is *Solanum lycopersicum*, which is mainly cultivated in tropical and subtropical areas, considered to be the most susceptible regions to the effects of climate change. In this study, the results showed that the priming of tomato plants with *T. asperellum* provided distinct benefits under drought and chilling stress conditions, helping to alleviate physiological and agronomic symptoms of these stresses in this crop. Although future studies are required in order to discern the mechanisms by which *T. asperellum* evokes this stress tolerance, it is evident that this fungus-plant relationship, as shown in this report, promoted sustainable protection against abiotic stresses in tomato plants. This represents an organic alternative of low cost to enhance the induction of crop defenses against abiotic stress, which due to physiological cross talk in plant environmental responses, could also provide benefits against biotic stresses, as previously suggested (Herrera et al., 2019).

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