# Drought stress in tomato increase the performance of adapted and non-adapted strains of *Tetranychus urticae*

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## Abstract

The performance of the two-spotted spider mite, *Tetranychus urticae* Koch, on plants depends on the rate of adaptation of mite populations to each particular host and can be influenced by environmental conditions. We have tested the effects of drought stress, caused by water deficiency, in the interaction of tomato plants with tomato adapted (TA) and tomato non-adapted (TNA) strains of *T. urticae*. Our data revealed that mite performance was enhanced for the TA strain when reared on drought-stressed tomato plants, rising population growth and leaf damage. Population growth in the case of the TNA strain was negative, but they laid more eggs and the number of mobile forms was higher on drought stressed tomato plants than on control plants. Water stress resulted in tomato plants with increased concentrations of essential amino acids and free sugars, improving the nutritional value of drought-stressed tomato plants for *T. urticae*. Mite infestation alone had almost no effect on the nutritional composition of tomato leaves, with the exception of an increase of free sugars. Tomato plant defense proteins were induced by both drought stress and mite infestation. However, the induction of protease inhibitors was higher in tomatoes exposed to mites from the TNA strain than in tomatoes that were fed upon by mites from the TA strain. The better performance of the TA strain could be associated to both changes in the digestive (cysteine and aspartyl protease and α-amylase activities) and detoxification (esterase activity) physiology of the mites and the attenuation of some of the plant defenses (protease inhibitors). Taken together, our results suggest that drought stress might favor outbreaks of *T. urticae* on tomato, by enhancing population growth of adapted populations and increasing the suitability of tomato as a host for non-adapted ones.

**Key words:** Spider mite, climate change, plant-mite interaction, nutrition, digestion, detoxification

## 1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, is a polyphagous pest with a worldwide distribution. While this species has been reported feeding on more than 1,000 plant species (Migeon and Dorkeld, 2006-2015), local mite populations do not perform equally well on all potential hosts (Gotoh et al., 1993; Kant et al., 2008). The capability of *T. urticae* to cope with the varying nutritional value and defense compounds of different host plants has been explained by the proliferation of gene families involved in digestion and detoxification processes (Grbic et al., 2011; Van Leeuwen and Dermauw, 2016) and by its transcriptional plasticity in response to host plant shifts (Dermauw et al., 2013; Wybouw et al., 2015). Moreover, it has been demonstrated that horizontally transferred genes from microorganisms facilitate the metabolism of toxic xenobiotics (Ahn et al., 2014).

*T. urticae* populations initially show low acceptance for tomato (*Solanum lycopersicum* L.) as a host, but they can rapidly became adapted (Fry, 1989; Agrawal et al., 2002), causing crop losses worldwide (Jayasinghe and Mallik, 2010; Meck et al., 2013). Studies on tomato–*T. urticae* interactions highlight the importance of secondary metabolites (e.g., phenylpropanoids, flavonoids and terpenoids) and anti-digestive proteins (e.g., protease inhibitors, polyphenol oxidases, amino acid catabolizing enzymes and peroxidases) as prominent components of tomato-induced defenses elicited by spider mite feeding (Li et al., 2002; Kant et al., 2004; Martel et al., 2015). Induction of these defenses is especially relevant, since the ingestion of protease inhibitors has been shown to be harmful to *T. urticae* (Carrillo et al., 2011; Santamaría et al., 2012). However, some genotypes of *T. urticae* are able to manipulate the main defense pathways in tomato, resulting in an attenuated induction of defense genes to levels at which they are less detrimental (Kant et al., 2008; Alba et al., 2015). Interestingly, Wybouw et al. (2015) demonstrated that during their adaptation to tomato, *T. urticae* extensively rearranged their xenobiotic metabolism through differential expression of genes that code for detoxifying enzymes and xenobiotic transporters, and acquired the ability to interfere with plant defenses. Candidate effector-proteins in *T. urticae* that attenuate plant defenses have been identified in their saliva (Villarroel et al., 2016).

Because of their tight relationship with host plants, *T. urticae* has been reported to be differentially affected by water deficit. For example, *T. urticae* performance has been shown to increase (Hollingsworth and Berry, 1982; Nansen et al., 2010), decrease

(Gould, 1978; Sadras et al., 1998) or vary (English-Loeb, 1990) depending on the plant species and the intensity of the water stress. Plants under drought stress are potentially more suitable as food because of increased nutrient availability (Brodbeck and Strong, 1987; Showler, 2013), and can therefore contribute to improve herbivore performance (Huberty and Denno, 2004; Showler, 2013). A positive correlation has been reported between *T. urticae* fecundity and amino acid and sugar content on plants (Dabrowski and Bielak, 1978; Wermelinger et al., 1991). Aside from acting as direct sources of available nutrients, free sugars and amino acids have also been reported to be feeding stimulants for many phytophagous species (Showler, 2013). On the other hand, drought stress has been also associated with an increase in the production of plant defense compounds making stressed plants potentially less suitable for herbivores (Gould, 1978; Mattson and Haack, 1987). The resulting performance of *T. urticae* on drought-stressed plants will then depend on the balance of induced nutrients and chemical defenses in the plant, and how the mites adapt to these changes. Drought stress triggers both the mobilization of plant nutritional compounds (Bauer et al., 1997) and the induction of plant defenses (English-Loeb et al., 1997; Inbar et al., 2001) in tomato. Recently, it has been reported that the tomato red spider mite *T. evansi*, an oligophagous mite feeding mainly on solanaceous plants, takes advantage of the changes in the nutritional quality of drought-stressed tomato plants and it is capable of circumventing the potential adverse effects of tomato plant defenses (Ximénez-Embún et al., 2016).

In the present study, we tested the effect of drought stressed tomato plants in both, tomato adapted and tomato non-adapted *T. urticae* strains, since its performance might vary depending on the rate of adaptation of mite populations to this host (Kant et al., 2008). Furthermore, changes in plant nutritional composition and defense proteins, as well as mite enzymatic (hydrolytic and detoxification) activities, were analyzed to understand how mite and plant physiological responses interact against each other under drought stress, which might provide insights for mite outbreaks under future deficit irrigation scenarios.

# 2. Materials and Methods

## 2.1 Plant material and mites rearing

Tomato (cv. Moneymaker) plants were grown from seeds in 40 wells trays. Plants with three expanded leaves were transferred to 2.5 liters pots (diameter: 16 cm, height: 15 cm) (Maceflor, Valencia, Spain) filled with 600 g of Compo Sana®

106 Universal potting soil (Compo GmbH, Münster, Germany) and watered to saturation.
107 Bean (*Phaseolus vulgaris* L. cv California Red Kidney Beans) seeds were germinated
108 and transferred to 1.6 liters pot (diameter: 15 cm, height: 13 cm) (Plásticos Alber,
109 Granada, Spain) filled with 500 g of the same growing medium. All plants were
110 maintained in a rearing room at 25 ºC±1 ºC, 50±5% relative humidity and a 16 h light/8
111 h dark photoperiod.
112 Two colonies of *T. urticae* derived from the London strain that was originated in
113 Ontario (Canada) and kept in laboratory conditions for over 100 generations were used:
114 tomato adapted (TA) and tomato non-adapted (TNA). The TA colony was maintained
115 on detached tomato leaves for about 30 generations. The petiole of the leaves was in
116 contact with a thin layer of water in the bottom of ventilated plastic cages (22x30x15
117 cm) to contain the mites and to maintain the leaf turgor. The TNA colony was
118 maintained on potted bean plants for the same period of time. Both colonies were kept
119 in two separate growth chambers (Sanyo MLR-350-H, Sanyo, Japan) at 25 ºC ± 1 ºC,
120 70 ± 5% relative humidity and a 16 h light/8 h dark photoperiod.
121
122 **2.2 Drought stress regime**
123 Drought stress was attained by water deficiency as described by Ximénez-
124 Embún et al. (2016). In brief, tomato plants were well-watered until they developed
125 four-five expanded leaves, then we imposed three watering treatments, defined as
126 control, and mild and moderate drought stress, in order to establish three irrigation
127 regimes. Control plants were watered every two-three days to maintain the soil
128 volumetric water content ($\theta$) up to 74%. For mild and moderate drought stress,
129 watering was stopped for four and seven days, respectively, and thereafter plants were
130 watered to maintain $\theta$ between 36 and 50% in case of the mild stress and between 21
131 and 30% in case of the moderate stress. The $\theta$ was determined gravimetrically by
132 recording single plant pot weight (balance BSH 6000, PCE Iberica, Tobarra, Spain).
133 Steady stress conditions were reached at about seven-nine days after ceasing irrigation.
134 Both water stress regimes were over the wilting point associated with severe drought
135 stress that was established at $\theta$ = 16% for Moneymaker in our experimental conditions
136 (Ximénez-Embún et al., 2016).
137 The severity of drought stress was assessed by measuring the following
138 parameters on the sub-terminal leaflet of the 4$^{th}$ leaf: a) stomatal conductance (gs) using
139 a leaf porometer (SC-1 Decagon-T, Pullman, USA); and b) variations in maximum

quantum yield of photosystem II photochemistry (Fv/Fm), using a FluorPen FP 100 (PSI, Drasov, Czech Republic). Plant growth was estimated by measuring the stem length (distance between the soil and the terminal bud) and by weighting the aerial part of the plant (transformed to dry weight by using the water content data calculated as referred below).

### 2.3. Bioassays to test drought stress effects on mite performance and plant nutritional composition.

A factorial design with two levels of water stress (control or drought), two levels of *T. urticae* infestation (infested or non-infested) and two sampling times (4 or 10 days post infestation = dpi) was conducted. Mild and moderate drought stressed plants were coupled with well-watered (control) plants in independent experiments. When drought stress conditions were reached a subset of tomato plants were infested. Mite females, of random age, were collected from the laboratory colony using a vacuum pump D-95 (Dinko S.A., Barcelona, Spain) with a sucking power of 10-50 mmHg connected to a modified Eppendorf. They were placed on the two sub-terminal leaflets of tomato leaves three, four, and five (eight mites per leaflet, each plant receiving 48 mites in total). All plants (infested and non-infested) were confined with a ventilated metacrylate cylinder fitting the pot and set up in a rearing chamber in a complete randomized block design (each experiment was repeated two-three times). Temperature and humidity inside the cylinders was recorded introducing USB dataloggers Log 32 (Dostmann electronic GmbH, Wertheim, Germany), on average the relative humidity was 79±2% inside the well-watered cylinders, and 71±2%, and 58±2% for mild and moderate drought stress conditions, respectively. The average temperature was 24±1º C in all cases. In each experiment, a subset of plants was used to assess mite performance and other subset for plant nutritional composition.

Mite performance was assessed at 4 and 10 dpi for the two levels of drought stress (moderate and mild) when infested with the TA strain, whereas only moderate drought stress was tested with the TNA strain. These sampling times were chosen to study the effect of drought stress on two consecutive generations of spider mites. At each time, 15 infested plants per treatment (control or drought) were analyzed in the moderate stress experiments, and nine infested plants per treatment in the mild stress one. All leaves were detached from the plants, and the number of eggs and mobile mite stages (larvae, nymphs and adults) counted under a stereomicroscope M125 (Leica

Mycrosystem, Wetzlar, Germany). The leaf damaged area ($mm^2$ of chlorotic lesions) was determined as described by Ximénez-Embún et al. (2016) by scanning the damaged leaflets using hp scanjet (HP Scanjet 5590 Digital Flatbed Scanner series, USA) and analyzing the scanned leaflets with the program GIMP 2.8 (www.gimp.org).

The nutritional composition of plant material was analyzed in control and moderate drought stressed plants, infested and non-infested (six plants per treatment). The samples were obtained at 4 and 10 dpi with the TA strain, but only at 4 dpi with the TNA strain since most mites from the TNA strain have already moved away from the plant at 10 dpi (see results section). The left leaflets from leaves three, four, and five were pooled and grounded in liquid nitrogen to a fine powder and stored for free amino acids and protein analysis. The right leaflets from the same leaves were dried together in an oven at 70 ºC for 3 days, weighed before and after drying to assess the percentage of water and ground using a mortar and pestle to obtain a fine powder and stored for C, N and free sugar analysis.

**2.4. Bioassays to test drought stress effects on plant defense proteins and mite enzymatic activities**

A factorial design with two levels of water stress (control or moderate drought) and three levels of *T. urticae* infestation (infested with TA, infested with TNA or non-infested) was conducted. When drought stress conditions were reached, a subset of tomato plants were infested by placing 150 adult female mites on leaves three, four, and five (450 mites per plant), laying a thin barrier of Lanolin (Manuel Riesgo S.A., Madrid, Spain) on the petiole to contain the mites. Plants (infested and non-infested) were placed in a rearing chamber in a complete randomized block design (the experiment was repeated two times in time) and maintained at 25 ºC±1 ºC, 50±5% relative humidity and a 16 h light/8 h dark photoperiod. The experiment was terminated at 4 dpi.

Plant material (six per treatment) was collected by sampling together infested leaflets from leaves three, four and five. Mites were collected from a different subset of plants (six per treatment) by using the pump system previously described. Plant and mite material was immediately frozen in liquid nitrogen and stored at -80 ºC. Mites from the TNA strain reared on bean plants were also collected for comparison.

**2.5 Chemical and biochemical analysis**

**2.5.1 Chemicals and Equipment**

Unless otherwise specified, all chemical compounds used were from Sigma-Aldricht (St Luis, USA). Fluorimetric measurements were made using a Varioskan Flash reader (ThermoFisher Scientific, Willmington, USA), and spectrophotometric measurements with a VERSAmax microplate reader (Molecular Devices Corp., Sunnyvale, USA).

### 2.5.2 Plant nutritional composition analysis

**Total C and N composition.** Samples of 1 mg of dried leaf powder were analyzed to determine total nitrogen and carbon concentration at the Elemental Microelement Center of Complutense University (Madrid, Spain) by using a microelement analyzer LECO CHNS-932 (LECO, St Joseph, MI, USA).

**Free sugars.** Samples of 3 mg of dried leaf powder were homogenized in 650 µl of ethanol 95% (v/v), heated at 80 ºC for 20 min, centrifuged at 10,000 rpm for 10 min, and the supernatant collected. The process was repeated two more times and the three supernatants were pooled. A volume of 750 µl of the mixture was dried on a SpeedVac Concentrator Savant SVC-100H (ThermoFisher scientific, Willmington, DE, USA) and redissolved in 500 µl of water. Soluble carbohydrate concentration was estimated by the anthrone method (Maness, 2010) using glucose as standard. In brief, 1 ml of anthrone reagent (0.2% v/v anthrone on 95% sulfuric acid) was added to the extract, heated at 90 ºC for 15 min, and the absorbance measured at 630 nm

**Free amino acids.** The extraction of the free amino acids was done as described by Hacham et al. (2002). Samples of 50 mg of leaf frozen powder were homogenized with 600 µl of water:chloroform:methanol (3:5:12 v/v/v). After centrifugation at 12,000 rpm for 2 min, the supernatant was collected and the residue was re-extracted with 600 µl of the same mixture, pooling the two supernatants. A mixture of 300 µl of chloroform and 450 µl of water were added to the supernatants, and after centrifugation the upper water:methanol phase was collected and dried in the SpeedVac. The samples were dissolved on 100 µl of sodium citrate loading buffer pH 2.2 (Biochrom, USA) and 10 µl were injected on a Biochrom 30 Amino Acid Analyser (Biochrom, USA) at the Protein Chemistry Service at CIB (CSIC, Madrid, Spain).

**Soluble Protein.** Samples of 100 mg of leaf frozen powder were homogenized in 500 µl of 0.15 M NaCl, ground with fine sand. The homogenate was centrifuged at 12,000 rpm for 5 min at 4 ºC, and the soluble protein quantified by absorbance at 280 nm on a Nanodrop 1000 spectophotometer (ThermoFisher scientific, Willmington, USA).

### 2.5.3 Plant defense proteins

Samples of 100 mg of leaf frozen powder were homogenized with 500 µl of extraction buffer (0.15 M NaCl for protease inhibitors, and 0.1 M phosphate buffer, pH 7.0; 5% w:v polyvinylpolypyrrolidine for oxidative enzymes) and soluble protein quantified as explained above.

The inhibitory activity of plant protein extracts was tested against commercial enzymes: papain (EC 3.4.22.2), cathepsin B from bovine spleen (EC 3.4.22.1), trypsin from bovine pancreas (EC 3.4.21.4), α-chymotrypsin from bovine pancreas (EC 3.4.21.1), cathepsin D from bovine spleen (EC 3.4.23.5) and leucine aminopeptidase from porcine pancreas (EC 3.4.11.1); as described by Ximénez-Embún et al. (2016). Reaction conditions are summarized in Table S1. Results were expressed as a percentage of protease activity inhibited.

Polyphenol oxidase (PPO) and Peroxidase (POD) activities of plant extracts were analyzed as described by Ximénez-Embún et al. (2016). Reaction conditions are summarized in Table S2.

**2.5.4 Mite enzymatic activities**

Samples of 1 mg of mites were homogenized in 100 µl of 0.15M NaCl, centrifuged at 12,000 rpm for 5 min and the supernatant was collected. Total protein content was determined according to the method of Bradford (1976).

Hydrolytic enzymes (cathepsin B-, cathepsin L-, cathepsin D- and legumain-like) activities were assayed as described by Santamaría et al. (2015a), and leucine aminopeptidase- and α-amylase-like activities and detoxification enzymes (esterase, glutathione S-transferase and P450) activities as described by Ximénez-Embún et al. (2016). Reaction conditions are summarized in Table S3.

**2.6 Statistical analysis**

All plant and mite parameters analyzed were checked for the assumptions of normality and heteroscedasticity, and transformed if necessary. Stem length and stomatal conductance were $\log_{10}(x)$ transformed, Fv/Fm was $\log_{10}(x+1)$ transformed; and these three parameters and stem dry weight data were statistically analyzed using a three-way ANOVA (using as fixed factors drought stress treatment, *T. urticae* infestation and time). The number of *T. urticae* eggs and mobile forms and leaf damaged area were $\log_{10}(x)$ transformed and analyzed by a two way ANOVA (drought stress treatment, time). For stem length, plant aerial dry weight, stomatal conductance, Fv/Fm, mite eggs and mobile forms, and leaf damage a Bonferroni *post hoc* test was performed to compare drought-stress treatments within each time. Percentage of water,

nitrogen, protein, free amino acids and free sugars, as well as C:N ratio and protease inhibition were arcsen(squareroot(x)) transformed. These data and the oxidative enzyme activities were analyzed using a two-way ANOVA for each time separately using as fixed factors drought stress treatment and *T. urticae* infestation. A Newman-Keuls *post hoc* test was performed to see differences of mean between treatments, except for essential and non-essential amino acids that were analyzed by a Dunnet *post hoc* test to compare the different treatments with respect to control non-infested plants. Spider mite enzymatic activity data were $\log_{10}(x)$ transformed and analyzed by a one-way ANOVA, using Newman-Keuls as *post hoc* test.

# 3. Results

## 3.1 Effects of drought stress on stomatal conductance, photosynthetic efficiency and tomato plant growth.

Drought stress significantly affect leaf stomatal conductance, which was reduced between five and seven times in the case of moderate drought stress and between 1.3 and 1.8 times in the case of mild drought stress when compared to control plants (Fig. S1 a). The photosynthetic efficiency determined as Fv/Fm was also significantly lower on drought stressed plants in all experiments at 10 dpi (Fig. S1 b). These results corroborated that the severity of the stress was different for the two water stress regimes imposed (moderate and mild), but severe drought stress conditions were never reached, since Fv/Fm values were never below 0.7 (Richie, 2006). Moderate drought stress slowed the plant growth, as both stem length and aerial dry weight were significantly smaller compared to control plants in most measured points, whereas mild drought stress only produced a significant reduction on aerial dry weight at 10 dpi (Fig. S2 a, b). The infestation by *T. urticae* (TA or TNA) did not affect stomatal conductance, photosynthetic efficiency, and tomato plant growth. Thus, data from infested and non-infested plants were pooled within each experiment.

## 3.2 Effects of drought stress on *T. urticae* (TA and TNA strains) population growth and leaf damage

The performance of mites from the TA strain was enhanced when reared on both moderate and mild drought-stressed tomato plants. Females laid a significantly higher number of eggs on moderately stressed plants than on control plants at 4 dpi (2.4 fold) and 10 dpi (1.5 fold) (Figure 1d). The F1 generation (mobile forms) was significantly

more abundant on moderately stressed plants at 4 and 10 dpi (1.4 and 2 fold respectively) (Figure 1e). As a result, damaged leaf area doubled in moderately stressed plants compared to control tomatoes (Fig. 1f). The same trend was observed under mild drought stress, but the differences among treatments were less pronounced and only significant in the case of number of eggs laid (1.5 fold) at 4 dpi, and mobile forms (1.4 fold) and leaf damage (1.5 fold) at 10 dpi (Fig. 1a, b and c). Population growth in the case of the TNA strain was negative, with lower number of mobile forms at 4 and 10 dpi than at the beginning of the assay. Only a few dead mites were found on the tomato leaves, indicating that mites are most likely dispersing from the plant. Nevertheless, they laid more eggs (about 2 fold) and the number of mobile forms was higher (about 1.5 fold) on drought stressed tomato plants than on control plants (Fig 1g and h). Damaged leaf area as a result of TNA infestation was markedly lower than that produced by TA infestation and could not be reliably measured.

### 3.3 Changes in plant nutritional composition induced by drought stress and *T. urticae* (TA and TNA)

Drought stress was the most significant factor in plant nutritional composition (Table 1), resulting in a decrease in the relative amount of water (all cases) and protein (TA at 10 dpi), and an increase on total free amino acids (TA at 10 dpi and TNA at 4 dpi) and sugars (all cases) and C:N ratio (TA at 4 dpi). The effect of drought stress on nitrogen was not consistent, as it decreased in the TA experiment at 4 dpi and increased in the TNA experiment. Concentrations of free sugars increased in plants exposed to both mite strains at 4 dpi, while total protein quantities increased in tomatoes exposed to mites from the TA strain and decreased when tomatoes were fed upon by mites from the TNA strain.

The levels of specific amino acids, classified as essential or non-essential for *T. urticae* according to Rodriguez and Hampton (1966), were also analyzed (Fig. 2). Proline was the amino acid most highly induced by drought stress alone or in combination with mites (about 10-16 times depending on the experiment). Likewise, most of the essential amino acids for this species (valine, isoleucine, leucine, tyeosine, phenylalanine, histidine, lysine and arginine) and the non-essential amino acid serine were also induced by drought stress alone or in combination with TA or TNA mite infestation. However, none of the amino acids analyzed were significantly affected when plants were stressed only by mite infestation.

### 3.4 Effect of drought stress and *T. urticae* (TA and TNA) on plant defense proteins

Tomato plant defense proteins were affected by both drought stress and mite infestation. The response was generally higher to the TNA strain than to the TA strain (Table 2). The inhibitory activity against chymotrypsin was significantly induced by both TA and TNA strains and the inhibitory activity against trypsin by TNA, independently of the watering conditions. The infestation with TA only induced trypsin inhibitory activity when combined with drought stress, and aminopeptidase inhibitory activity was only induced by TNA under control conditions. Drought stress induced peroxidase activity, but it was only significant when combined with TA mite infestation, whereas the interaction drought stress-infestation was significant for poliphenol oxidase activity. No significant effect was found on the levels of cathepsin B, D, and papain inhibition.

### 3.5 Physiological response of *T. urticae* (TA and TNA) to drought-stressed tomato plants

None of the hydrolytic enzymes activities analyzed in mites of the TA and TNA strain was affected by the water status of the tomato plant host, whereas there were significant differences between mite strains (Table 3). When feeding on tomato, the TNA strain had lower activity than the TA strain for cathepsin B- and legumain-like proteases, in both drought-stressed and well watered plants, and for cathepsin L- and D-like proteases and α-amylase activities in well watered plants. In contrast, aminopeptidase activity was higher on the TNA strain. When the TNA strain was fed on bean (the habitual host plant for this strain), all the hydrolytic activities, except cathepsin D-like and aminopeptidase, were significantly higher than when fed on tomato, and even higher than for TA in the case of cathepsin B-, L-like proteases and aminopeptidase.

Among the detoxification enzymes studied, we only found significant differences between the strains in the case of esterase activity (Table 3). The TA strain showed the highest activity, the TNA strain reared on bean the lowest, and the TNA strain feeding on tomato an intermediate level.

## 4. Discussion

Performance of *T. urticae* on tomato depends on the rate of adaptation of mite populations to this particular host (Agrawal et al., 2002; Kant et al., 2008). Moreover, some of the changes induced by drought stress on tomato nutritional composition and plant defenses (Bauer et al., 1997; English-Loeb et al., 1997; Inbar et al., 2001) has been identified as key factors affecting mite host preferences and performance (Kant et al., 2008; Wybouw et al., 2015). Our data revealed that drought stressed tomato plants increased the performance of *T. urticae*, enhancing population growth of a tomato adapted TA strain and being more suitable as a host for a non-adapted TNA strain. These findings could have significant implications for mite outbreaks under future climate change scenarios, when longer periods of drought and less water availability are expected for irrigated crops like tomato in semi-arid environments (IPCC, 2013). Moreover, *T. urticae* is expected to have more generations per year as a direct effect of rising temperature on mite developmental rates, increasing mite pressure (Ludeling et al., 2011), which might contribute to exacerbate mite damage.

We have found that the increases of available free sugars and essential amino acids, which are limiting nutrients for mite growth and reproduction, seemed to improve the nutritional value of drought-stressed tomato plants for *T. urticae*. Plants under drought stress mobilize existing proteins and complex carbohydrates into amino acids and simple sugars, respectively, for osmotic adjustments and for the transference of available plant nitrogen and carbon to younger leaves and reproductive organs (Hummel et al., 2010; Showler, 2013). Drought-stressed tomato leaves showed an increase on the concentration of total free amino acids and free sugars and a decrease of total protein, as already reported for tomato (Bauer et al., 1997; Ximénez-Embún et al., 2016). However, the effect of drought stress on nitrogen was not consistent, as it decreased in the assay performed with the TA strain but increased in the assay performed with the TNA strain. Likewise, nitrogen content of tomato plants has been reported to increase under drought stress in some cases (English-Loeb et al., 1997) and decrease in others (Inbar et al., 2001). Interestingly, when particular amino acids were analyzed, most of the considered as essential for *T. urticae* (Rodriguez and Hampton, 1966) rose in drought-stressed tomato leaves. Some of these essential amino acids has been reported to stimulate *T. urticae* feeding (Tulisalo, 1971; Dabrowski and Bielak, 1978). Nonetheless, the amino acid most clearly induced by drought stress was proline, which is not an essential nutrient. Proline has been shown to stimulate feeding in many different phytophagous arthropods (Showler, 2013; Ximénez-Embún et al., 2016). In addition, proline is unique

among the amino acids because can be used by arthropods as a direct energy substrate for glycolysis and the production of ATP (Scaraffia and Wells, 2003). A similar role as sources of available nutrients can be proposed for the increase in free sugars, since it has been reported that fecundity of *T. urticae* correlated with sugar content in plants (Wermelinger et al., 1991).

Herbivores attack can also induce changes in the primary metabolism of plants, including the reallocation of carbon and nitrogen resources (Zhou et al., 2015, Martel et al., 2015). We observed that the levels of free sugars were increased in tomato leaves infested with mites from both the TA and the TNA strains. However, mite infestation had different effects on total protein concentration, as was reduced by TNA while increased after 4 dpi with TA, though no differences in the levels of total or specific free amino acids were observed. Likewise, Ximénez-Embún et al. (2016) reported that a tomato adapted strain of *T. evansi* also triggered the levels of free sugars in tomato leaves. As a result, more edible carbohydrates are apparently available in mite-exposed tomato plants. Our results do not allow distinguish whether these effects correspond to a plant defensive response, which may result in subsequent carbon remobilization to other tissues, or that the mites are manipulating plant primary metabolism for their own benefit.

The induction levels of several tomato defense genes were found to correlate negatively with *T. urticae* performance (Kant et al., 2008; Wybouw et al., 2015). Our data revealed that tomato plants responded to mite infestation by the induction of serine (trypsin and chymotrypsin) and aminopeptidase protease inhibitors (PIs), resulting the inhibitory activity higher in response to the TNA than to the TA mites, despite the damage inferred by the adapted strain was higher. These results are in line with those previously reported, in which serine PIs has been shown to be consistently induced upon *T. urticae* attack, but this response is attenuated by tomato adapted strains (Li et al. 2002; Kant et al. 2004, 2008; Alba et al., 2015). However, we did not observe mite induction of tomato polyphenol oxidase (PPO) and peroxidase (POD) activities, as found in some of these studies (Alba et al., 2015; Martel et al. 2015). This could be related to the fact that these genes are induced in tomato as an early response to *T. urticae* infestation, occurring maximum induction within 24h post infestation, whereas our experiment run for at least 4 days. Regardless, our results appear to be species specific since contrast with those reported for tomato-*T. evansi* interactions, in which this mite infestation induce tomato cysteine PI activity and the specific activities of both

PPO and POD, but has no effect on the plant inhibitory activity against serine proteases (Ximénez-Embún et al. 2016).

Proteolitic digestion in *T. urticae* relies mostly on cysteine (cathepsin B-, cathepsin-L and legumain-like) and aspartyl (cathepsin D-like) proteases and aminopeptidases, whereas serine proteases do not appear to be directly involved in the hydrolysis of dietary proteins (Carrillo et al. 2011; Santamaría et al. 2015a). Thus, tomato serine PIs may be probably targeting other physiological processes in mites, such as the regulation of growth and development (Santamaría et al., 2012). Indeed, both cysteine and serine PIs can be harmful when ingested by *T. urticae* (Carrillo et al., 2001; Santamaría et al., 2012, 2015b). Interestingly, we have found that when feeding on tomato, cysteine and aspartyl protease and α-amylase activities were higher in *T. urticae* mites of the TA strain, whereas aminopeptidase activity was higher in the TNA strain. The increase of the aminopeptidase activity in the TNA strain can be associated to the induction of aminopeptidase PIs in tomatoes infested with mites from this strain. However, the rise of the other hydrolytic activities in the TA strain seems to be related to its higher feeding rate, since tomato cysteine and aspartyl PIs were not affected by *T. urticae* feeding. Moreover, cathepsin B- and L-like protease and α-amylase activities increased significantly in TNA when feeding on bean, its preferred host, resulting even higher than for TA in tomato. Nonetheless, we can not discard that the higher activity of these digestive proteases in the TA strain may have an adaptive value, since gut proteases have the potential to inactivate plant defense proteins targeting the digestive system or other tissues (Ortego, 2012). In this sense, it has been shown that the expression of cysteine and aspartyl protease genes increased in *T. urticae* after feeding on plants over-expressing the HvCPI-6 cystatin, that specifically targets cathepsins B and L, but also when feeding on plants over-expressing the CMe trypsin inhibitor that targets serine proteases (Santamaría et al., 2015a). However, we did not find differences for any of the *T. urticae* hydrolytic enzyme activities analyzed when fed on drought-stressed plants, despite drought stress also induced serine PIs and POD when combined with mite infestation.

*T. urticae* produces large amount of detoxifying enzymes to circumvent plant secondary metabolites (Grbic et al., 2011; Van Leeuwen and Dermauw, 2016), and transcriptional changes in genes coding for P450s, GSTs, and esterases has been reported to occur during the adaptation process of *T. urticae* to tomato (Dermauw et al., 2013; Wybouw et al., 2015). We have found that esterase activity was significantly

higher in mites of the TA strain, which can be related to their adaptation to tomato plants. An induction of the esterase activity was also observed when mites of the TNA strain maintained on beans were transferred to tomato, probably in response to some of the xenobionts present in tomato. However the level of activity was lower than that of the TA strain on tomato, which may partially explain their lower performance.

## 5. Conclusions

Our data indicate that tomato adapted and non-adapted *T. urticae* benefit from the improved nutritional value of tomato plants induced by drought stress (increased concentrations of essential amino acids and free sugars). Yet, the improved performance of the tomato adapted strain could be associated to changes on the digestive (higher cysteine and aspartyl protease and α-amylase activities) and detoxification (higher esterase activity) physiology of the mites and the attenuation of some of the defense responses (PIs) of the plant. Adaptation to tomato and drought stress favored mite performance, having an additive effect on most of the physiological parameters analyzed. However, assessing the relative impacts of plant nutrients versus defenses will require further research. As drought events are expected to be more frequent and prolonged due to climate change (IPCC 2013), our results imply an increase in the risk of outbreaks of *T. urticae* on tomato by significantly enhancing population growth of adapted populations and increasing the suitability of tomato as a host for non-adapted ones. Moreover, breeding programs aimed at improving drought tolerance in tomato might take into consideration varieties whose nutritional composition is not enhanced by drought stress for avoiding or mitigating spider mites damage.

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## Figure legends

**Fig. 1** Performance of *T. urticae* tomato adapted strain (TA) on mild (a, b and c) and moderate (d, e and f) drought stressed tomato plants and of the non-adapted strain (TNA) on moderate stressed plants (g and h). Plants were infested with 48 adult females (day 0) and eggs laid on plants (a, d and g), total mobile forms (b, e and h) and leaf damaged area (c and f) assessed at 4 and 10 days post infestation (dpi). Leaf damage was negligible in TNA. Data are mean ±SE. * indicates statistically significant differences between drought stress treatments within each time (Two-way ANOVA, Bonferroni *post hoc* test, $p<0.05$).

**Fig. 2** Levels of free amino acids in tomato leaves from control plants and plants under moderate drought stress and/or infested with *T. urticae* tomato adapted (TA) and non-adapted (TNA) strains at 10 and 4 days post infestation (dpi), respectively. Data are mean ± SE of amino acid contents (% dry weight) represented at logarithmic scale. The division between essential and non-essential amino acids for *T. urticae* is based on Rodriguez and Hampton (1966). * Indicate significant difference of the treatment with respect to control plants (Two-way ANOVA, Dunnet *post hoc* test, $p<0.05$).

671 **Table. 1**. Effect of moderate drought stress and infestation by *T. urticae* tomato adapted
672 (TA) and non-adapted (TNA) strains on nutritional composition of tomato leaves.

673

| | Non-infested | | Infested | | ANOVA ($p<0.05$) |
|---|---|---|---|---|---|
| | **Control** | **Drought** | **Control** | **Drought** | |
| **TA strain** | | | | | |
| **4 Days Post Infestation** | | | | | |
| **Water**[1] | 93.0±0.4 a | 91.0±0.6 b | 93.0±0.4 a | 91.0±0.7 b | D |
| **Nitrogen**[2] | 6.7±0.3 | 5.9±0.3 | 6.8±0.2 | 6.0±0.3 | D |
| **Protein**[2] | 21.7±1.8 | 18.5±2.1 | 24.0±1.2 | 25.8±2.7 | I |
| **Free aa**[2] | 1.1±0.2 | 1.3±0.2 | 1.1±0.1 | 1.2±0.3 | - |
| **C:N**[3] | 5.8±0.1 | 6.6±0.3 | 5.8±0.1 | 6.3±0.3 | D |
| **Free Sugars**[2] | 3.3±0.1 a | 3.6±0.1 a | 4.5±0.1 b | 4.9±0.2 b | D; I |
| **10 Days Post Infestation** | | | | | |
| **Water**[1] | 93.0±0.7 a | 91.0±0.5 b | 93.0±0.3 a | 92.0±0.4 ab | D |
| **Nitrogen**[2] | 6.2±0.1 | 6.1±0.3 | 6.0±0.3 | 5.9±0.2 | - |
| **Protein**[2] | 17.7±1.3 ab | 14.5±1.5 b | 25.7±2.6 a | 13.6±1.9 b | D |
| **Free aa**[2] | 0.9±0.2 | 1.2±0.1 | 0.9±0.1 | 1.4±0.2 | D |
| **C:N**[3] | 6.3±0.1 | 6.3±0.2 | 6.1±0.1 | 6.3±0.1 | - |
| **Free Sugars**[2] | 3.5±0.1 | 4.2±0.1 | 3.8±0.2 | 4.2±0.4 | D |
| **TNA strain** | | | | | |
| **4 Days Post Infestation** | | | | | |
| **Water**[1] | 94.0±0.0 a | 89.0±1.0 ab | 91.0±2.0 ab | 87.0±2.0 b | D |
| **Nitrogen**[2] | 5.7±0,1 | 6.1±0.2 | 5.7±0.1 | 6.1±0.1 | D |
| **Protein**[2] | 28.0±6.0 | 23.0±2.0 | 18.0±4.0 | 14.0±3.0 | I |
| **Free aa**[2] | 0.6±0.1 ab | 1.0±0.1 b | 0.5±0.1 a | 1.0±0.2 b | D |
| **C:N**[3] | 6.2±0.2 | 6.1±0.2 | 6.1±0.1 | 6.0±0.1 | - |
| **Free Sugars**[2] | 3.6±0.2 a | 4.3±0.2 a | 4.1±0.1 a | 5.7±0.6 b | D; I |

Data are mean ± SE: (1) % fresh weight (2) % dry weight (3) ratio. D (drought stress) and I (Mites infestation) indicates significant factor in a two-way ANOVA. Different lower case letters within rows indicates significant differences (Newman-Keuls *post hoc* at $p<0.05$)

674

676 **Table. 2** Effect of moderate drought stress and infestation with *T. urticae* tomato
677 adapted (TA) and non-adapted (TNA) strains on tomato plant defense proteins at 4 days
678 post infestation.

| | Non-infested | | Infested with TA | | Infested with TNA | | ANOVA |
|---|---|---|---|---|---|---|---|
| | Control | Drought | Control | Drought | Control | Drought | (p<0.05) |
| **Protease inhibitors (% inhibition)** | | | | | | | |
| **Cathepsin B** | 38±7 | 27±3 | 36±6 | 35±5 | 45±9 | 29±5 | - |
| **Papain** | 54±6 | 39±7 | 45±2 | 44±5 | 50±7 | 45±9 | - |
| **Cathepsin D** | 52±4 | 53±2 | 51±3 | 56±3 | 52±4 | 52±2 | - |
| **Trypsin** | 40±3 a | 33±5 a | 41±7 a | 61±7 b | 64±8 b | 84±5 c | D, I |
| **Chymotrypsin** | 47±4 a | 46±6 a | 71±10 b | 91±2 c | 86±4 c | 97±1 c | D,I |
| **Aminopeptidase** | 32±5 ab | 23±1 a | 25±3 a | 27±4 a | 43±4 b | 33±2 ab | D,I |
| **Oxidative enzymes (specific activity)** | | | | | | | |
| **PPO**[1] | 4.2±0.3 | 3.3±0.3 | 3.3±0.5 | 4.3±0.2 | 4.0±0.5 | 3.2±0.4 | D*I |
| **POD**[2] | 1.9±0.2 ab | 3.0±0.5 ab | 1.6±0.3 a | 3.4±0.5 b | 2.2±0.4 ab | 2.7±0.5 ab | D |

Data are mean ± SE. D (drought stress) and I (mite infestation) indicate significant factors in a two-way ANOVA. Different lower case letters within rows indicates significant differences (Newman-Keuls *post hoc* test at p<0.05).
(1) PPO (poliphenol oxidases): nmol metabolized Cathecol/ mg Protein*min
(2) POD (peroxidases): nmol metabolized Guaiacol/ mg Protein*min.

679

681 **Table. 3** Enzymatic activities of mites from *T. urticae* tomato adapted (TA) and non-
682 adapted (TNA) strains when feeding for 4 days on control or moderate drought stressed
683 tomato plants. TNA mites feeding on beans were also analysed.

| | TA strain | | TNA strain | | |
|---|---|---|---|---|---|
| | **Tomato control** | **Tomato drought** | **Tomato control** | **Tomato drought** | **Bean** |
| **Protein**[1] | 43±3 | 46±3 | 48±4 | 50±5 | 44±2 |
| **Hydrolytic enzymes**[2] | | | | | |
| **Cathepsin B** | 4.9±0.2 b | 3.9±0.3 b | 0.6±0.1 c | 0.7±0.1 c | 9.0±1.0 a |
| **Cathepsin L** | 26±2 b | 21±1 bc | 14±2 c | 21±4 bc | 39±3 a |
| **Cathepsin D** | 23±2 a | 20±3 ab | 14±2 b | 13±1 b | 18±2 ab |
| **Legumain** | 1.4±0.1 a | 1.2±0.1 a | 0.3±0.1 b | 0.6±0.1 b | 1.1±0.1 a |
| **Aminopeptidase** | 5.5±0.4 b | 4.7±0.2 b | 8.5±0.3 a | 7.8±0.7 a | 8.7±0.7 a |
| **α-Amylase** | 62±7 ab | 59±7 ab | 26±5 c | 40±8 bc | 79±8 a |
| **Detoxification enzymes**[2] | | | | | |
| **Esterase** | 263±35 a | 181±22 ab | 99±34 bc | 75±35 bc | 22±8 c |
| **GST** | 904±211 | 1126±298 | 667±168 | 743±210 | 1347±350 |
| **P450** | 0.75±0.09 | 0.74±0.06 | 0.60±0.08 | 0.49±0.11 | 0.63±0.10 |

Data are mean ± SE. Different lower case letters within rows indicates significant differences (One-way ANOVA, Newman-Keuls *post hoc* test at $p<0.05$).
(1) µg/mg fresh weight
(2) Specific activity as nmol substrate hydrolyzed per min and mg of protein for Cathepsin B, L, D, legumain, aminopeptidase, α-amylase and esterase. nmol CDNB conjugated per min and mg of protein for GST and nmol cytochrome c reduced per min and mg of protein for P450.

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Figure 1

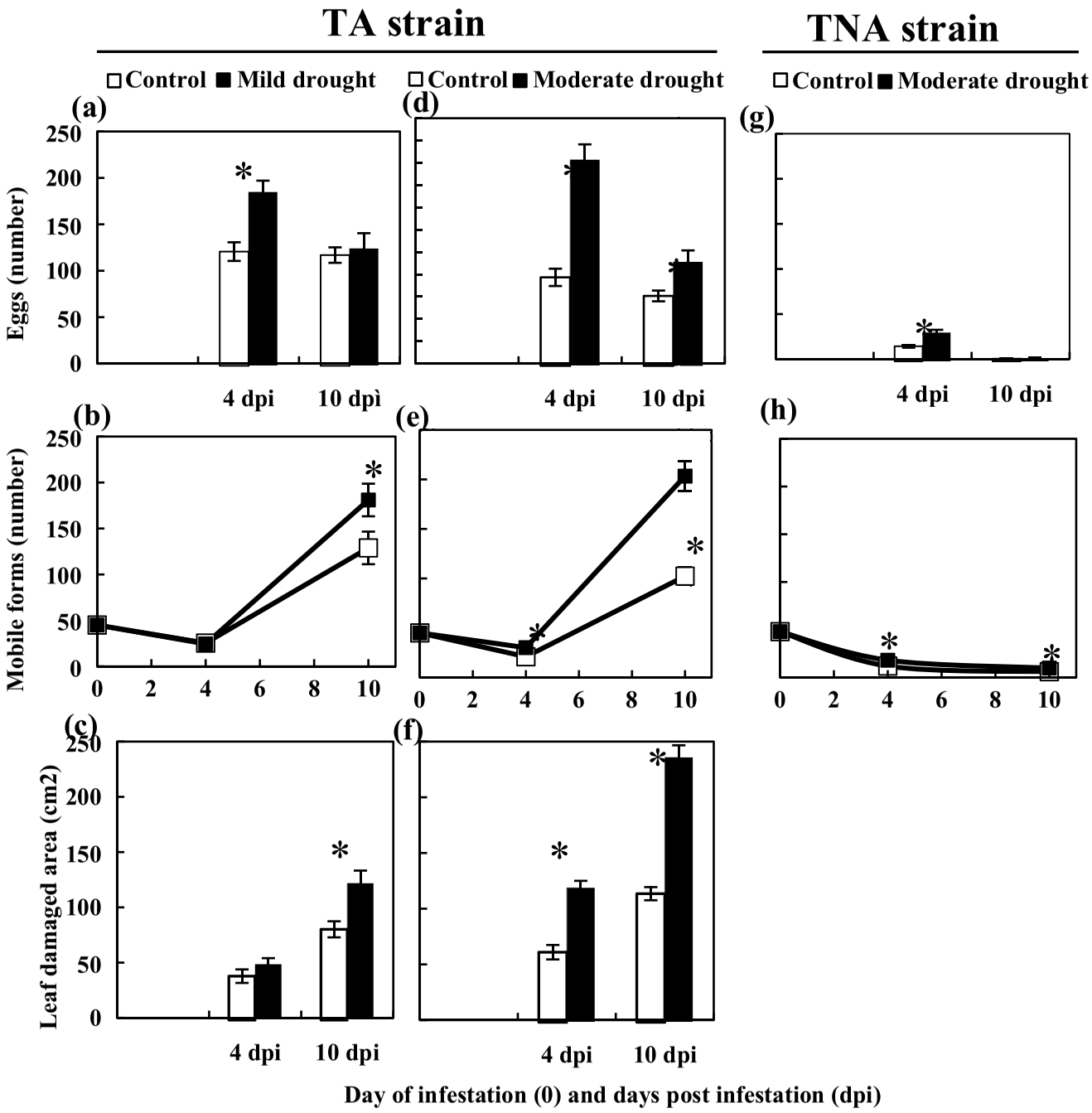
TA strain
TNA strain
Control
Mild drought
Moderate drought
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Eggs (number)
Mobile forms (number)
Leaf damaged area (cm2)
4 dpi
10 dpi
Day of infestation (0) and days post infestation (dpi)

Figure 2

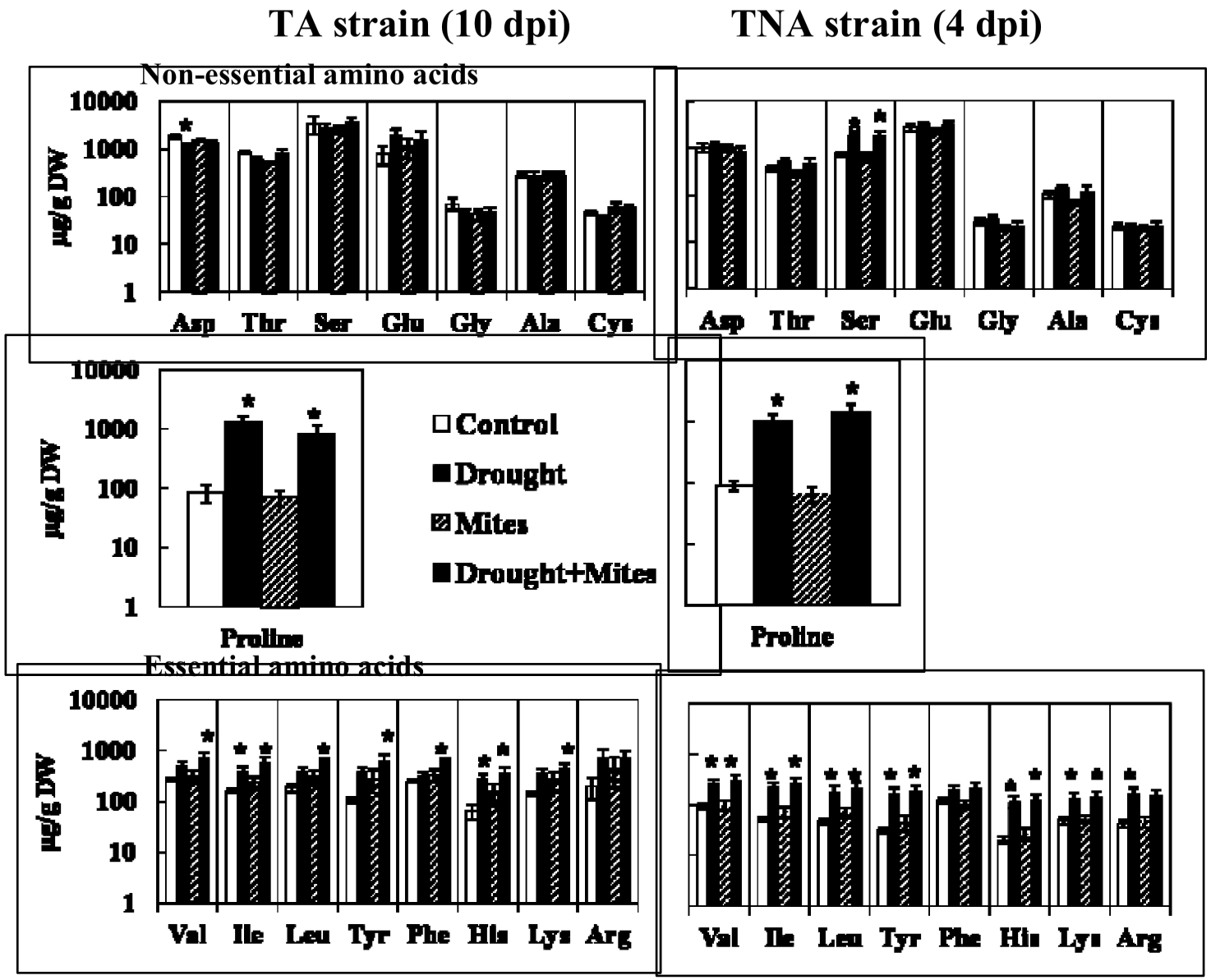
TA strain (10 dpi)
TNA strain (4 dpi)
Non-essential amino acids
μg/g DW
10000
1000
100
10
1
Asp
Thr
Ser
Glu
Gly
Ala
Cys
Proline
Control
Drought
Mites
Drought+Mites
Essential amino acids
Val
Ile
Leu
Tyr
Phe
His
Lys
Arg

**Fig. S1** Effect of drought stress (moderate and mild) on A) tomato stomatal conductance (gs) and B) maximum quantum yield of PSII photochemistry (Fv/Fm) at mite infestation and at 4 and 10 days post infestation (dpi). Data (mean ± SE) shown are average of the values on infested and non infested plants on experiments with *T. urticae* adapted (TA) and no adapted (NTA) strains, as mite infestation didn´t show a significant effect. * indicates a statistically significant differences between drought strain treatments within each time (Three-way ANOVA, Bonferroni *post hoc* test, $p<0.05$).

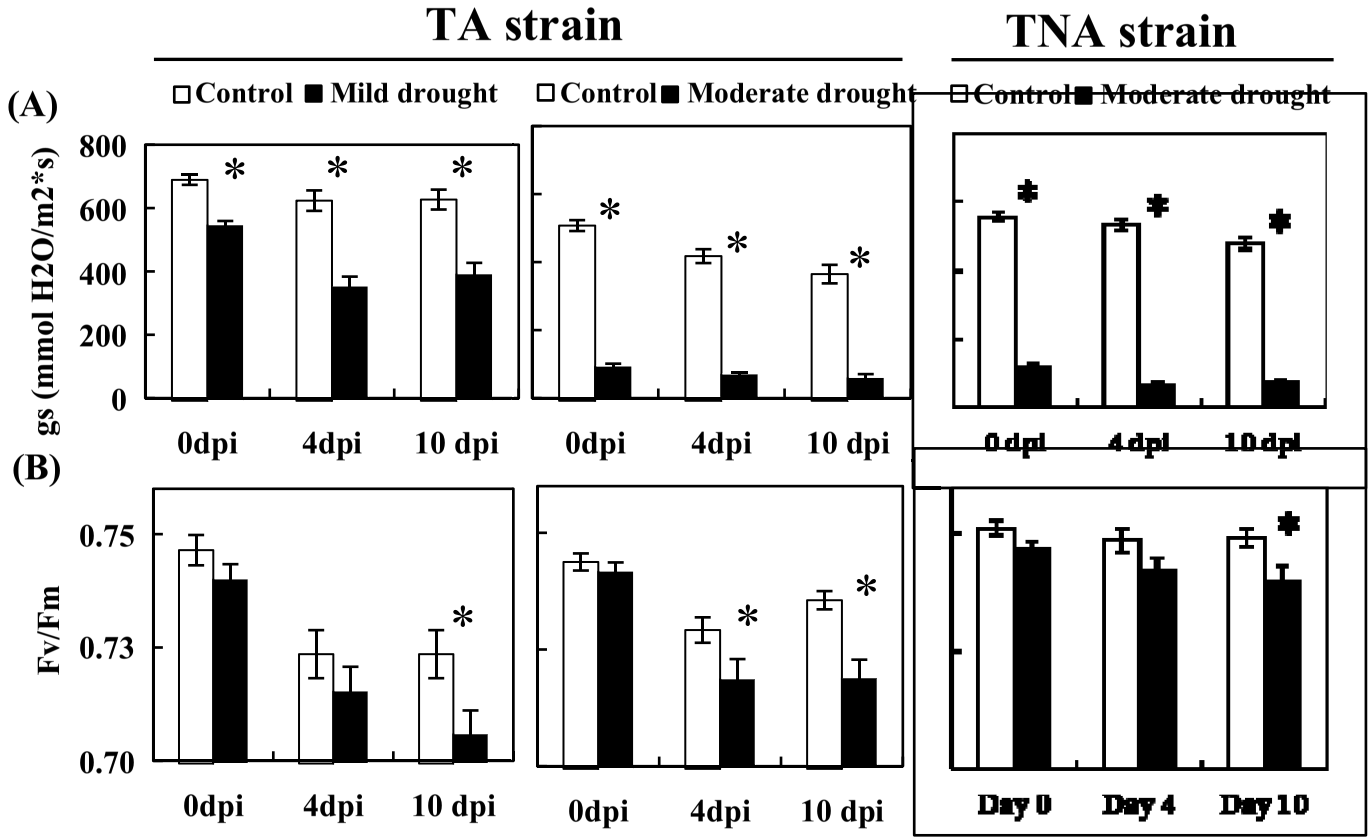

**Fig. S2** Effect of drought stress (moderate and mild) on tomato plant growth, expressed as A) aerial dry weight at 4 and 10 days post infestation (dpi) and B) stem length trough the experiment. Data (mean ± SE) shown are average of infested and non infested plants on experiments with *T. urticae* adapted (TA) and no adapted (NTA), as mite infestation didn´t have a significant effect. * indicates a statistically significant difference between drought treatments within each time (Three-way ANOVA, Bonferroni *post hoc* test, $p<0.05$).

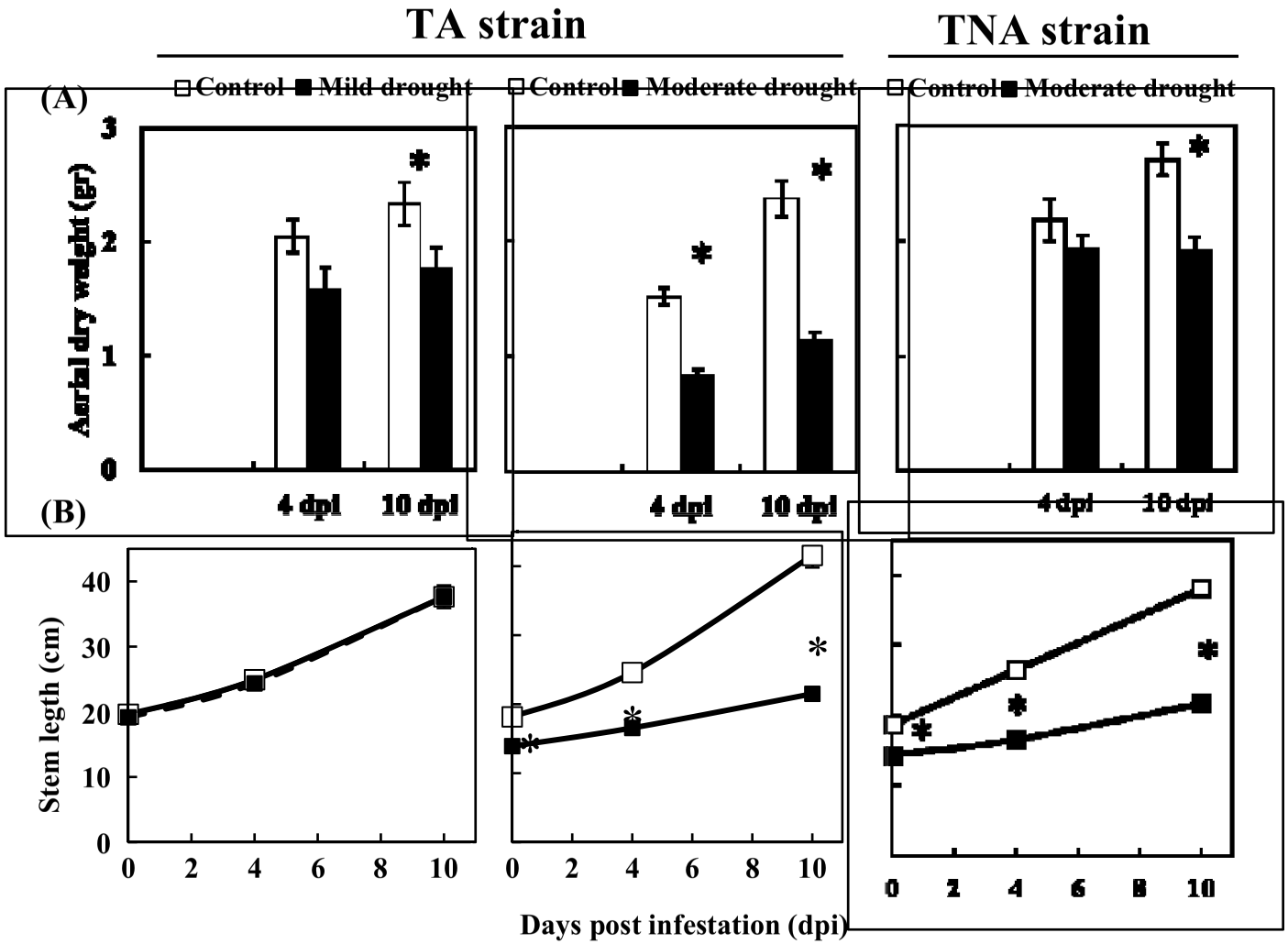

**Table S1.** Summary of analytical methods to assess the inhibitory activity of plant protein extracts [1]

| Commercial enzyme [2] | Substrate [3] | Buffer [4] | Incubation | Measurement [5] |
|---|---|---|---|---|
| Cathepsin B from bovine spleen (EC 3.4.22.1) | Z-RR-AMC | 100 mM NA phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35) | 1 h at 28 ºC | excitation filter 350 nm emission filter 465 nm |
| Papain (EC 3.4.22.2) | Z-FR-AMC | 100 mM Na phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35) | 1 h at 28 ºC | excitation filter 350 nm emission filter 465 nm |
| Cathepsin D from bovine spleen (EC 3.4.23.5) | MocAc-GKPILFFRLK (Dnp)-D-R-NH2 | 100 mM sodium citrate, pH 3.5 (0.15M NaCl, 5 mM MgCl2) | 10 min at 30 ºC | excitation filter 328 nm emission filter 393 nm |
| Trypsin from bovine pancreas (EC 3.4.21.4), | Z-LA-AMC | 100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2) | 1 h at 35 ºC | excitation filter 350 nm emission filter 465 nm |
| α-Chymotrypsin from bovine pancreas (EC 3.4.21.1), | SucAAPF-AMC | 100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2) | 1 h at 35 ºC | excitation filter 350 nm emission filter 465 nm |
| Leucine aminopeptidase from porcine pancreas (EC 3.4.11.1). | LpNa | 100 mM Tris-HCl, pH 8 (0.15M NaCl, 5 mM MgCl2) | 1 h at 30 ºC | absorbance at 410 nm |

[1] Procedures adapted from Ximénez-Embún et al. (2016). Samples of 20 µg of plant protein extracts (40 µg in case of leucine aminopeptidase) were preincubated for 10 min with 100 ng of the commercial enzyme.

[2] All purchased from Sigma-Aldricht (St Luis, USA).

[3] The substrates were added at a final concentration of 20 µM. Z-RR-AMC (N-carbobenzoxyloxy-Arg-Arg-7-amido-4-methylcoumarin) for cathepsin B, Z-FR-AMC (N-carbobenzoxyloxy-Phe-Arg-7-amido-4-methylcoumarin) for papain, Z-LA-AMC (Z-L-Arg-7-amido-4-methylcoumarin) for trypsin, SucAAPF-AMC (Suc-Ala-Ala-Pro-Phe-7-amido-4-methylcoumarin) for chymotrypsin, all purchased from Calbiochem (MerkMilipore, Billerica, USA), MocAc-GKPILFFRLK(Dnp)-D-R-NH2 from Peptanova (Germany) for cathepsin D, and LpNa (L-leucine p-nitroanilide) from Sigma-Aldricht (St Luis, USA) for leucine aminopeptidase.

[4] Concentrations are expressed at molarity in the reaction mixture.

[5] AMC (7-amino-4-methylcoumarin) (Bachem, Swizerland) as standard for all fluorescent substrates, except MCA (MoCAC-Pro-Leu-Gly) (Peptanova GmbH, Germany) for cathepsin D. Double blanks were used to account for spontaneous breakdown of substrates and the plant protease activity, and all assays were done in duplicate.

**Table S2.** Summary of analytical methods to assess the enzymatic activity of plant protein extracts

| **Plant Oxidative Enzymes** [1] | **Substrate** [2] | **Buffer** [3] | **Incubation** | **Measurement** |
|---|---|---|---|---|
| Polyphenol oxidase (PPO) | cathecol (40 mM) | Tris-HCl pH 8.5 | 1 h at 30 ºC | absorbance at 420 nm |
| Peroxidase (POD) | guaiacol (5 mM) | Potassium phosphate pH 6, with 2.5 mM $H_2O_2$ | 10 min at 30 ºC | absorbance at 470 nm |

[1] Procedures adapted from Ximénez-Embún et al. (2016). Assays were performed with 20 µl (PPO activity) and 20 µl of a 1:10 dilution (POD activity) of the plant protein extract

[2] The substrates were added at a final concentration of 40 mM (PBO) and 5 mM (POD). Both from Sigma-Aldricht (St Luis, USA).

[3] Concentrations are expressed at molarity in the reaction mixture.

**Table S3.** Summary of analytical methods to assess the enzymatic activity of mite extracts

| | Substrate [2] | Buffer [3] | Incubation | Measurement [4] |
|---|---|---|---|---|
| **Hydrolytic Enzymes [1]** | | | | |
| Cathepsin B-like | Z-RR-AMC | 100 mM sodium citrate, pH 5.5 (0.15 M NaCl, 1 mM DTT) | 15 min at 30 ºC | excitation filter 350 nm emission filter 465 nm |
| Cathepsin L-like | Z-FR-AMC | 100 mM sodium citrate, pH 5.5 (0.15 M NaCl, 1 mM DTT) | 15 min at 30 ºC | excitation filter 350 nm emission filter 465 nm |
| Cathepsin D-like | MocAc-GKPILFFRLK (Dnp)-D-R-NH2 | 100 mM sodium citrate, pH 3.5 (0.15 M NaCl, 1 mM DTT, 10µM E-64) | 15 min at 30 ºC | excitation filter 328 nm emission filter 393 nm |
| Legumain-like | Z-VAN-AMC | 100 mM sodium citrate, pH 4.5 (0.15 M NaCl, 1 mM DTT) | 15 min at 30 ºC | excitation filter 350 nm emission filter 465 nm |
| Leucine aminopeptidase-like | LpNa | 100mM Tris-HCl, pH 7.5 (0.15M NaCl, 5mM MgCl2) | 4 h at 30 ºC | absorbance at 410 nm |
| α-Amylase-like | Starch | 100 mM Tris-HCl, pH 6.0 (40 mM CaCl2, 20 mM NaCl) | 4 h at 30 ºC | absorbance at 580 nm |
| **Detoxification Enzymes [1]** | | | | |
| Esterase | 1-NA | 100 mM Tris-HCl, pH 7.0 (0.15M NaCl). | 1 h at 30 ºC | absorbance at 600 nm |
| Glutathione S-transferase | CDNB | 100 mM Tris-HCl, pH 8.0 (0.15M NaCl, 5 mM reduced glutathione) | 15 min at 30 ºC | absorbance at 340 nm |
| P450 | cytochrome c | 100 mM Tris-HCl, pH 7.0 (0.15M NaCl, containing the NADPH generating system [5] | 4 h at 30 ºC | absorbance at 550 nm |

[1] Procedures for cathepsin B-, L- and D- and legumain-like activities adapted from Santamaría et al. (2015a); and for leucine aminipeptidase- and α-amylase-like activities and esterase, glutathione S-transferase and P450 activities from Ximénez -Embún et al. (2016). Assays were performed with 60 µl (P450), 40 µl (glutathione S-transferase), 20 µl (α-amylase-like), 10 µl (cathepsin B- and L-, legumain- and leucine aminopeptidase-like activities ), 5 µl (cathepsin D-like activity) and 0.25 µl (esterase) of the mite protein extract.

[2] The substrates were added at a final concentration of 20 µM (cathepsin B- L-, D- and legumain-like activities), 1mM (LpNa), 0.25% starch, 0.25 mM (1-NA), (0.4 mM CDNB) and 50 µM (cytochrome c). Z-RR-AMC, Z-FR-AMC, MocAc-GKPILFFRLK(Dnp)-D-R-NH2 and LpNa as in Table S; Z-VAN-AMC (N-carbobenzoxyloxy-Val-Ala-Asn-7-amido-4-methylcoumarin) from Bachem (Bubendorf, Switzerland); starch, 1-NA (1-naphthyl acetate), CDNB (1-chloro-2,4-dinitrobenzene) and cytochrome c from Sigma-Aldricht (St Luis, USA).

[3] Concentrations are expressed at molarity in the reaction mixture.

[4] AMC and MCA were used as standards for fluorescent substrates as in Table S1; starch and 1 naphthol were used as standars for α-amylase-like and esterase activities

[5] The NADPH generating system consisted of 0.5 mM NADP, 2.5 mM glucose 6-phosphate and 0.3 units of glucose 6-phosphate dehydrogenase

**Highlights**

► Drought stressed tomato plants are nutritiously more suitable for *Tetranychus urticae*

► The adaptation of *T. urticae* to tomato is associated with mite physiological changes

► Drought stress increased the suitability of tomato as a host for non-adapted *T. urticae*