ETHYLENE MODULATES TOMATO GROWTH DURING DROUGHT STRESS

ETILENO CONTROLA O CRESCIMENTO DO TOMATEIRO EM CONDIÇÃO DE DÉFICIT HÍDRICO

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ABSTRACT

The aim of this study was to investigate the involvement of ethylene in signaling between root and shoot under drought stress. Thus, it were used the tomato mutants *Never ripe* (*Nr*), with low sensitivity to ethylene, and the 'Micro-Tom' (MT). 15 days-old plants from both genotypes were combined (MT/MT; *Nr*/*Nr*; MT/*Nr*; *Nr*/MT) by cleft grafting. Drought stress was generated withholding the irrigation for seven days while the control plants were continuously irrigated. Dry weight, leaf and root area, root parameters, pigments contents, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and leaf temperature were assessed. Drought stress strongly reduced shoot growth in all plants, as well increased leaf temperature. Moreover, *Nr* used as rootstock or scion improved plant growth in both well-watered and drought stress conditions, indicating that ethylene sensitivity is a key factor for drought tolerance.

Keywords: Abiotic stress. Grafting. 'Micro-Tom'. *Never ripe*. Rootstock.

RESUMO

O objetivo do presente trabalho foi investigar o envolvimento do etileno na sinalização entre raiz e parte aérea durante o déficit hídrico. Para tanto, foram utilizados os mutantes de tomateiro *Never ripe* (*Nr*), com baixa sensibilidade ao etileno, e o 'Micro-Tom' (MT). Plantas com 15 dias foram enxertadas (MT/MT; *Nr*/*Nr*; MT/*Nr*; *Nr*/MT) por fenda cheia. A seca foi gerada pela suspensão da irrigação por sete dias, enquanto as plantas controle foram irrigadas continuamente. Foram avaliados a massa seca das plantas, área foliar, parâmetros radiculares, pigmentos, peróxido de hidrogênio (H_2O_2), malondialdeído (MDA) e temperatura foliar. O déficit hídrico causou forte redução do crescimento da parte aérea em todas as plantas, bem como aumentou a temperatura foliar. Além disso, quando *Nr* foi utilizado como porta-enxerto ou enxerto, melhorou o crescimento das plantas irrigadas e não irrigadas, indicando que a sensibilidade ao etileno é um fator chave para a tolerância à seca.

Palavras-chaves: Enxertia. Estresse abiótico. 'Micro-Tom'. *Never ripe*. Porta-enxerto.

Drought stress is one of the most limiting environmental factors for crop production worldwide, and this situation might get more severe due to climate change (DAI, 2011; SARDANS; PEÑUELAS, 2013). In addition, from root sensitivity to leaf modifications, several signaling molecules have to be transported through conducting tissues to coordinate responses against drought stress (HOLBROOK et al., 2002; MARTORELL et al., 2015). This way, to reduce water loss for example, plants can control stomatal movements even before the shoot tissues are dehydrated (HOLBROOK et al., 2002). This process is regulated by the production of abscisic acid (ABA) in leaves, independently from the production of this hormone in roots, indicating the existence of other signals from roots in response to low water potential (HOLBROOK et al., 2002; KOROVETSKA et al., 2014). Actually, almost all the hormone classes are involved in drought stress responses (VIJAYALAKSHMI et al., 2014; OLLAS; DODD, 2016). Among them, ethylene (ET) has been receiving special attention for its involvement in long distance root-to-shoot signaling during environmental adversities. Indeed, it is known that plants grown under abiotic stresses have their ET levels modified (MORGAN, 1990; SOBEIH et al., 2004), negatively affecting growth and development, inducting foliar abscission and plant senescence (SHARP; LENOBLE, 2002).

Furthermore, several studies indicate that the use of inhibitors of ET biosynthesis or perception can ameliorate deleterious effects of abiotic stresses on plants (KAWAKAMI; OOSTERHUIS; SNIDER, 2010; HUBERMAN et al., 2014). For example, transgenic maize plants, with lower ET biosynthesis, have shown higher grain yield when compared to conventional plants, when grown under drought stress conditions (HABBEN et al., 2014; SHI et al., 2015). Additionally, grafting is a valuable tool to comprehend the role of several molecules on long distance communication among tissues, allowing the combination of scions and rootstocks of different genetic constitutions (TURNBULL; LOPEZ-COBOLLO, 2013). This way, tomato mutant with low sensitivity to ET was combined with its wild-type through grafting and grown under water deficit, aiming to elucidate the possible role of ET on long distance signaling during drought stress.

Seeds of the tomato (*Solanum lycopersicon* L.) mutant *Never ripe* (*Nr*), with low sensitivity to ET due to defective gene for *LeETR3* receptor biosynthesis (WILKINSON et al., 1995) and its wild-type, Micro-Tom (MT), were germinated in boxes containing a mixture of 1:1 (by volume) commercial pot mix (BioPlant) and vermiculite. Fifteen days after sowing, plants were grafted by cleft method, combining MT and *Nr* in reciprocal grafting (MT/MT; *Nr*/*Nr*; MT/*Nr*; *Nr*/MT, with the first genotype indicating the scion and the second the rootstock). Immediately, grafted plants were placed in a moist chamber and remained there until complete healing (*c.* 15 days), then transferred to glasshouse. To establish the stress treatment, the irrigation was suspended for part of the plants for a seven-day period. On the other hand, control plants continued with daily irrigation, maintaining the substrate moisture close to its maximum holding capacity. After seven days under the respective growth conditions, well-watered and drought stress, the growth and biochemical analyzes were performed.

Leaf area was measured using an Image Analysis System (Delta-T Devices, Cambridge, UK), whereas root area, diameter, length and density were measured using a Hewlett Packard 125C scanner (Delta-T Scan software). After this, they were oven-dried at 60^{o}C for 72 h, and then dry weight was determined using an analytical balance (Denver Instrument Company AA-200). In addition, leaf temperature readings were performed at 13:00 h using an infrared thermometer (Fluke 59 Max®), in the terminal leaflet of the third fully expanded leaf.

The pigments content was determined spectrophotometrically using Lichtenthaler (1987) method. Circular pieces were cut from the third leaf of each plant and placed in 2 mL tubes filled with acetone (80%). The reading of the extract was performed at 661.6 nm (chlorophyll *a*); 644.8 nm (chlorophyll *b*); and 470 nm (carotenoids). Lipid peroxidation was estimated by assessment of malondialdehyde (MDA) levels through the content of thiobarbituric acid reactive substances (TBARS) (GRATÃO et al., 2012). The content of hydrogen peroxide (H_2O_2) was determined by reaction with potassium iodide (KI) as described by Alexieva et al. (2001).

The experimental design was completely randomized, in a 4x2 factorial scheme with three replicates, considering four combinations of grafting (MT/MT; *Nr*/*Nr*; MT/*Nr*; *Nr*/MT) and two growth conditions (well-watered and drought stress). Data were subjected to analysis of variance (ANOVA), and means were compared using Tukey test (at $P \leq 0.05$).

We observed that, after a period of stress, plants exhibited a strong reduction of leaf area, but it was noticeable that self-grafted (*Nr*/*Nr*) and reciprocal-grafted plants with *Nr* (MT/*Nr*; *Nr*/MT) had bigger leaf area than MT/MT treatment, in both growth conditions (Figure 1A). On the other hand, it was verified no difference with respect to the root area. Similarly to leaf area, when *Nr* was used as scion or rootstock shoot dry weight accumulation was higher than MT/MT, regardless of the growing condition (Figure 1B). Whereas *Nr*/MT exhibited the higher root dry weight compared to MT/MT and MT/*Nr* (Figure 1B). In short, drought stress severely decreased leaf area, and root and shoot dry weight (Figure 1). However, plants grafted with *Nr* remained with higher leaf area and shoot dry weight in comparison to MT/MT (Figure 1).

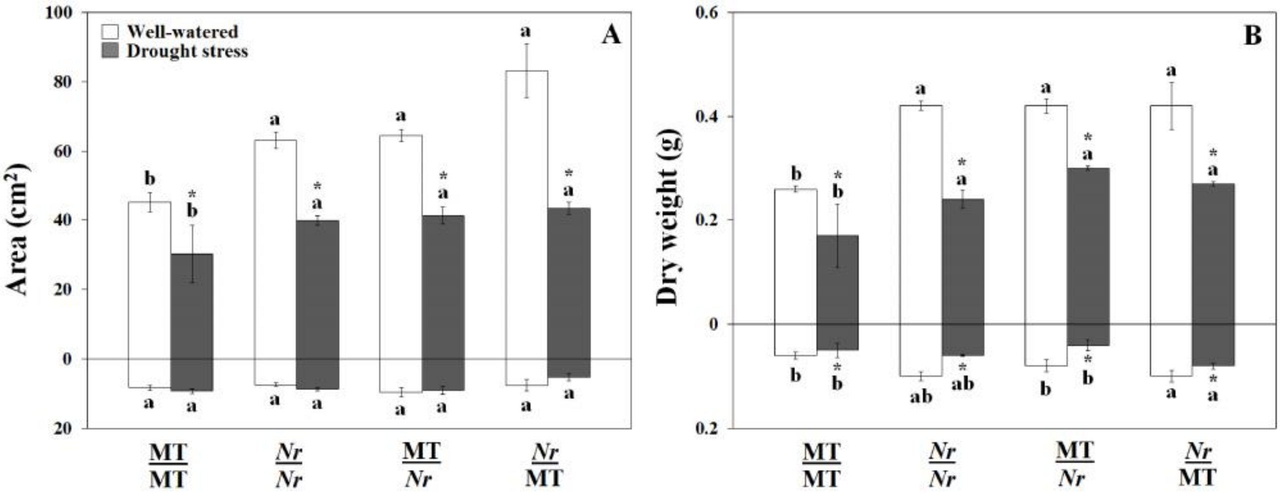

Figure 1. Area **(A)** and dry weight **(B)** assessments of self- and reciprocal-grafted Micro-Tom (MT) and *Never ripe* (*Nr*) tomato mutants under well-watered or drought stress conditions. In the coordinate axis, values above and below 0 correspond to leaf and root values, respectively. Values are the means of each treatment (n = 3) and bars represent the standard error. Letters above the bars represent the differences in the means among the grafting treatments within each hydric condition, and asterisks mark differences between hydric conditions within the same grafting combination, calculated by Tukey test at 5%.

These results show that lower sensitivity to ethylene enhances plant growth in both in watered and stressed conditions. Moreover, *Nr* as rootstock was able to positively affect shoot growth, indicating the existence of long distance signaling between roots and shoot. This means that the use of *Nr* as rootstock can be a valuable tool to promote plant growth under adverse conditions; however, its use as scion can be problematic, as low sensitivity to ET negatively affects fruit ripening and leading to obvious limitations in the production of climacteric fruits (LELIÈVRE et al., 1997; LIU et al., 2015).

There were no significant differences between treatments despite of the growth conditions, as observed for root diameter, length and density (Figures 2A, 2B and 2C, respectively). Drought stress implied in higher leaf temperature, with the slighter increase in plants with *Nr* rootstock

(Table 1). Increased leaf temperature is one of the most important signals of water deficit in plants, as it is related to impaired transpiration frequently caused by stomatal closure. Therefore, lower values observed in plants with *Nr* rootstock can be related to improved water absorption or lower stomatal closure, illustrating the essential role of ET perception in leaf drought responses. Other studies have been highlighting the involvement of ET in stomatal movement and transpiration regulation (DESIKAN et al., 2006; XIA et al., 2015).

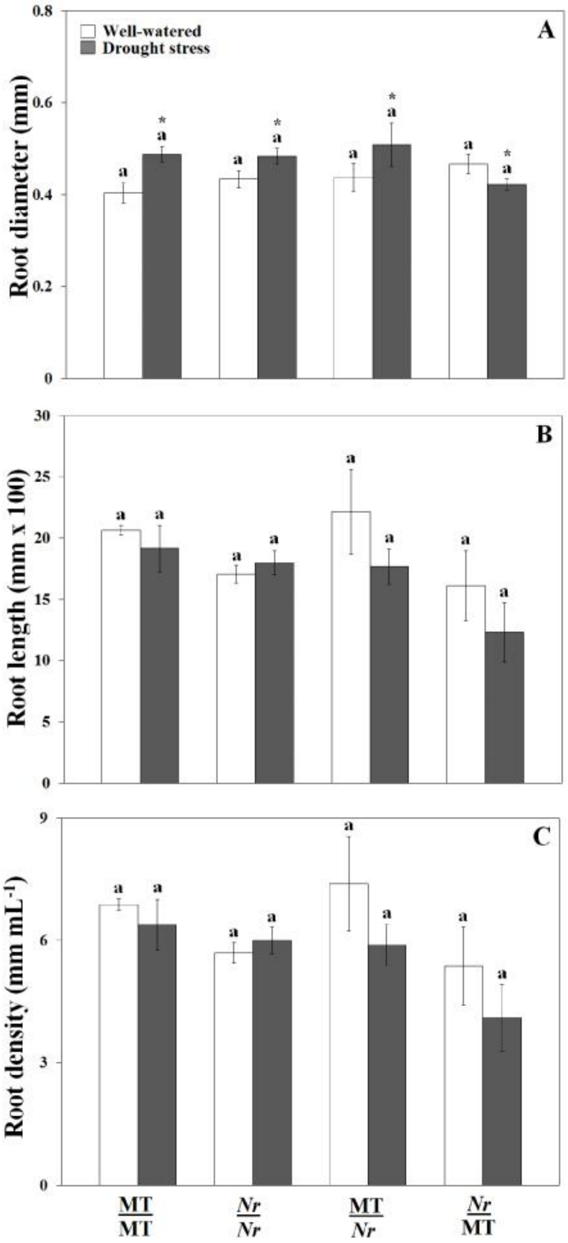

Figure 2. Growth analyzes of self- and reciprocal-grafted tomato Micro-Tom (MT) and *Never ripe* (*Nr*) under well-watered or drought stress conditions. **(A)** Root diameter. **(B)** Root length. **(C)** Root density.

Values are the means of each treatment (n = 3) and bars represent the standard error. Letters above the bars represent the differences in the means among the grafting treatments within each hydric condition, and asterisks mark differences between hydric conditions within the same grafting combination, calculated by Tukey test at 5%.

Curiously, photosynthetic pigments contents were higher in plants under drought stress, except for *Nr*/*Nr* (Table 1). Additionally, MT/MT plants exhibited the higher chlorophyll values in both well-watered and drought conditions. Probably, the increment of pigment contents is a consequence of the substantial impairment of leaf area (Figure 1A). On the other hand, based on MDA and H_2O_2 assessments of leaves and roots, it was not observed an increase of oxidative stress due to water deprivation, independent from the selected genotype combination (Table 1).

Table 1. Leaf temperature (LT), total chlorophyll content (Chl), carotenoids content (Car), shoot and root malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents of self- and reciprocal-grafted tomato mutants Micro-Tom (MT) and *Never ripe* (*Nr*). The first genotype of each combination of grafting indicates the scion, and the second the rootstock. Letters indicate differences in the means among the grafting treatments within each hydric condition, and asterisks mark differences between hydric conditions within the same grafting combination, calculated by Tukey test at 5%.

Grafting	**Conditions**	**LT (°C)**	**Chl (µg cm^{-2})**	**Car (µg cm^{-2})**	**Shoot MDA (nmol g^{-1} FW)**	**Root MDA (nmol g^{-1} FW)**	**Shoot H_2O_2 (nmol g^{-1} FW)**	**Root H_2O_2 (nmol g^{-1} FW)**
MT/MT	**Control**	30.6±0.12 b	0.70±0.01 a	0.45±0.01 a	2.44±0.30 a	0.23±0.06 a	70.12±7.31 a	2.18±1.24 a
	Drought	43.3±0.72 a*	0.93±0.02 a*	0.53±0.01 a	2.46±0.16 a	0.33±0.01 a	72.75±8.50 a	4.61±0.73 a
Nr*/*Nr	**Control**	29.0±0.17 ab	0.49±0.06 b	0.37±0.01 ab	2.08±0.30 a	0.26±0.04 a	41.58±4.23 a	2.27±0.25 a
	Drought	35.4±0.38 c*	0.26±0.02 c*	0.18±0.02 b*	2.71±0.43 a	0.25±0.02 a	65.98±9.85 a	4.35±2.54 a
MT/*Nr*	**Control**	30.9±0.81 a	0.31±0.01 c	0.21±0.01 b	2.04±0.06 a	0.22±0.03 a	59.68±4.81 a	2.27±0.53 a
	Drought	34.8±0.74 c*	0.88±0.01 a*	0.53±0.01 a*	2.17±0.21 a	0.31±0.04 a	48.96±8.63 a	2.81±0.66 a
***Nr*/MT**	**Control**	28.0±0.27 b	0.39±0.02 bc	0.25±0.01 ab	2.11±0.19 a	0.26±0.04 a	42.30±2.77 a	3.77±1.86 a
	Drought	38.9±0.48 b*	0.71±0.02 b*	0.42±0.01 a*	1.72±0.24 a	0.23±0.02 a	43.85±4.56 a	1.86±0.24 a

The obtained results demonstrated an important role of ET on plant development in both well-watered and water-limited conditions. Under drought stress, lower sensitivity to ET improved tomato shoot growth as well maintained lower leaf temperature in comparison to self-grafted MT/MT plants. Furthermore, ET perception in roots seems to be a key factor in the control of shoot responses to drought stress. Therefore, the use of rootstock with low sensitivity to ET could be an interesting alternative to improve drought stress tolerance without, however, affecting the fruit quality yield.

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