PHOTOSYNTHETIC RESPONSE OF TWO MANGO CULTIVARS SUBMITTED TO SALT STRESS AND INFECTED WITH Ceratocystis fimbriata

Resposta fotosintética de duas cultivares de manga submetidas ao estresse salino e infectadas por Ceratocystis fimbriata

Juliana Cristina Vieccelli1; Carlos Eduardo Aucique-Pérez2; Carla Silva Dias3; Dalmo Lopes Siqueira4; Fabrício Ávila Rodrigues5

1Estudante, Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV); jcviecelli@gmail.com
2Estudante, Departamento de Fitopatologia, UFV; carlosaucique@gmail.com
3Estudante, Departamento de Biologia Vegetal, UFV; carla.silva.dias.physiologist@gmail.com
4Professor, Departamento de Fitotecnia, UFV; sIQUEIRA@UFV.br
5Professor, Departamento de Fitopatologia, UFV; *autor para correspondência: fabrício@ufv.br

Abstract - This study aimed to investigate the alterations on the photosynthetic performance of mango plants from cultivars Tommy Atkins and Ubá when exposed to salt stress and infected with Ceratocystis fimbriata. Plants from these two cutivars were grown in plastic pots receiving nutrient solution with 0 and 90 mM NaCl for 50 days. At 42 days after fungal inoculation, the leaf gas exchange parameters net CO2 assimilation rate [A], stomatal conductance to water vapor [g], internal CO2 concentration [C], and transpiration rate [E] as well as the lesion length, the upward and the downward relative lesion length and the radial fungal colonization were evaluated. Based on the disease variables evaluated, plants from cultivar Ubá were more resistant to infection by C. fimbriata in comparison to plants from cultivar Tommy Atkins. Lower values of A were obtained for plants from cultivar Tommy Atkins submitted to salt stress and infected with C. fimbriata resulting, therefore, in reduced values of g and E. In general, plants from cultivar Tommy Atkins were more affected at the photosynthetic level in comparison to plants from cultivar Ubá under salt stress and infected with C. fimbriata. Under salt stress, stomatal closure reduced the C2 values especially on plants from cultivar Tommy Atkins. Plants from cultivar Tommy Atkins were more susceptible to infection by C. fimbriata even when exposed to salt stress.

Keywords - Mangifera indica; Photosynthesis; Leaf gas exchange

Resumo - Este estudo investigou as alterações na performance fotossintética de plantas de manga das cultivares Tommy Atkins e Ubá quando expostas ao estresse salino e infectadas por Ceratocystis fimbriata. As plantas dessas duas cultivares foram crescidas em vasos plásticos contendo solução nutritiva com 0 e 90 mM de NaCl durante 50 dias. Aos 42 dias após inoculação, avaliou-se os parâmetros de trocas gasosas taxa de assimilação líquida de CO2 [A], condutância estomática [g], concentração interna de CO2 [C] e taxa de transpiração [E], bem como o comprimento da lesão no caule, o comprimento relativo da lesão abaixo e acima do ponto de inoculação e a colonização radial do fungo. Baseado nos valores das variáveis relacionadas com a doença, as plantas da cultivar Ubá foram mais resistentes à infecção por C. fimbriata do que as plantas da cultivar Tommy Atkins. Menores valores de A ocorreram para as plantas da cultivar Tommy Atkins submetidas ao estresse salino e infectadas com C. fimbriata resultando, portanto, em reduzidos valores de g e E. Em geral, plantas da cultivar Tommy Atkins foram mais afetadas a nível fotossintético em comparação com as plantas da cultivar Ubá submetidas ao estresse salino e infectadas com C. fimbriata. Sob estresse salino, o fechamento estomatal reduziu os valores de C2 especialmente nas plantas da cultivar Tommy Atkins. As plantas da cultivar Tommy Atkins foram mais suscetíveis à infecção por C. fimbriata quando expostas ao estresse salino.

Palavras-chave: Mangifera indica, Fotossíntese; Trocas gasosas
INTRODUCTION

Mango (Mangifera indica) is one of the most important tropical fruit cultivated worldwide. Asia is the major mango producer and responsible for 76.4% of the world production while the remaining 23.6% is shared by Latin America, Africa countries and Oceania (FAO, 2014). Brazil, with an annual production of about 823,000 tons, is the 9th producer with a share of 3.4% in the total volume offered (FAO, 2014). The cultivar Tommy Atkins occupies approximately 90% of the area cultivated with mango in Brazil mainly due to the attractive color and good postharvest characteristics of the fruits. On the other hand, cultivar Ubá is cultivated only in some regions of Brazil, especially in the state of Minas Gerais, and is preferred by the juice industry due to some qualities of the fruits such as high-yield pulp, high soluble-solids, little fiber and maintenance of light yellow color and aroma after processing (ALMEIDA et al., 2001; BENEVIDES et al., 2008; FARAO; RAMOS; STRINGHETA, 2009).

Mango orchards are often affected by both abiotic and biotic types of stresses and the occurrence of mango wilt, caused by the fungus Ceratocystis fimbriata Ellis & Halst., and salt stress have greatly affected mango yield and the duration of the orchards (VAN WYK et al., 2011; ZUAZO; RAYA; RUIZ, 2003). Soil salinity has become a serious factor limiting the productivity and quality of several agricultural crops mainly because it affects plant development by reducing plant water potential, altering nutrients uptake, and increasing the accumulation of toxic ions (HASEGAWA et al., 2000; ZHU, 2001). High soil salinity damages approximately 20% of total irrigated lands worldwide and takes 1.5 million ha out of production each year (MUNNS and TESTER, 2008).

Mango wilt has been reported to occur in many states in Brazil from north to south and constitutes a serious problem for the new mango orchards (ROSSETTO et al., 1996; OLIVEIRA et al., 2016). The fungus C. fimbriata extensively limiting the productivity and quality of several agricultural crops mainly because it affects plant development by reducing plant water potential, altering nutrients uptake, and increasing the accumulation of toxic ions (HASEGAWA et al., 2000; ZHU, 2001). High soil salinity damages approximately 20% of total irrigated lands worldwide and takes 1.5 million ha out of production each year (MUNNS and TESTER, 2008).

Mango plants (∼2 years old) from cultivars Tommy Atkins and Ubá, moderately resistant and resistant, respectively, to C. fimbriata (ARAUJO et al., 2014) were grown in plastic pots containing 20 L of washed sand under greenhouse conditions (temperature of 30 ± 2°C and relative humidity of 70 ± 5%). Plants received nutrient solution (3 L per plastic pot) daily prepared as described by HOAGLAND and ARNON (1950) with some modifications as follow: 1.0 mM KNO3, 0.25 mM NH4H2PO4, 0.1 mM NH4Cl, 0.5 mM MgSO4 • 7H2O, 1.0 mM Ca(NO3)2, 0.30 mM CuSO4 • 5H2O, 0.33 mM ZnSO4 • 7H2O, 11.5 mM H3BO3, 3.5 mM MnCl2 • 4H2O, 0.1 mM (NH4)2MoO4• 2H2O, 25 mM FeSO4 • 7H2O and 25 mM EDTA disodium. After 40 days of plants adaptation to the nutrient solution mentioned above, sodium chloride (NaCl) was added to the nutrient solution to obtain the concentration of 90 mM NaCl. Plants supplied only with nutrient solution served as the control treatment. Plants were grown in the plastic pots receiving nutrient solution with 0 and 90 mM NaCl for 50 days. In order to maintain the nutrient
solution stable and to ensure that plants were under salt stress, an initial reading of the electrical conductivity (EC) was taken by using a portable conductivity meter. This initial reading served as a reference for the subsequent readings. The EC was checked weekly and whenever there was depletion equal to or greater than 20% of the initial electric conductivity, the pH of the nutrient solution was adjusted to 5.5 by using HNO₃ or KOH, both at 0.1 M.

Inoculation procedure

At 50 days after being supplied with nutrient solution containing either 0 or 90 mM NaCl, plants were inoculated with the isolate CEBS15 of C. fimбриata. This isolate was obtained from mango plants collected in the city of Brejo Santo, Ceará State (07°29'34″S, 38°59'06″W), Brazil, exhibiting mango wilt symptoms. The isolate was preserved according to the Castellani’s method (DHINGRA and SINCLAIR, 1995). Plugs of malt extract agar medium containing fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA) medium. After 3 days, the PDA plugs containing fungal mycelia were transferred to new Petri dishes containing the same culture medium and maintained in an incubator (temperature of 25°C and 12-h photoperiod) for 14 days. Plants were inoculated according to AL-SADI et al. (2010) with a few modifications (ARAUJO et al., 2014). Stem disks (10 mm diameter, approximately 2 mm in depth) were removed from the stems with the aid of a punch at approximately 5 cm above the graft scar. A PDA plug (10 mm diameter) obtained from a 14-day-old fungal colony was then placed into the punch hole. Each hole containing a PDA plug with fungal mycelia was covered with a piece of moistened cotton and wrapped with parafilm to maintain adequate moisture for fungal infection. The disks used to inoculate each plant were taken from the middle portion of each fungal colony to ensure that inoculation was as homogeneous as possible. The insertion of plugs of PDA into the holes in the stem of plant served as the control treatment.

Relative lesion indices

Disease progress was evaluated at 42 days after inoculation (dai). The upward, downward and radial colonization of the stem tissues by fungal hyphae was evaluated by measuring the length (in mm) of the internal necrotic tissue using a digital caliper. The upward relative lesion length (URLL) and the downward relative lesion length (DRLL) were determined as the ratio between the length from the graft scar to the top of the stem (LGST) and the lesion length (LL) in the same interval (upward and downward) from the inoculation point according to the following formula: URLL or DRLL = LL / LGST. The radial fungal colonization (RFC) was determined as the length of the necrotic tissue in relation to the total stem diameter × 100.

Photosynthetic measurements

Gas exchange parameters were measured on the first fully expanded leaf, from the base to the top, of each plant of the replication of each treatment at 42 dai. Measurements were conducted under ambient CO₂ (390 ± 10 µmol mol⁻¹) and temperature conditions with artificial light (1200 µmol photons m⁻² s⁻¹ at the leaf level). Net carbon assimilation rate (µmol CO₂ m⁻² s⁻¹), stomatal conductance to water vapour (gₛ), internal CO₂ concentration (Cᵢ) and transpiration rate (E) were measured throughout the morning (0730 to 1200 h) using a portable open-system infrared gas analyzer (LCpro model +, Portable Photosynthesis System ADC BioScientific Limited, UK). All of the measurements were performed at 25°C and the vapor pressure deficit was maintained at approximately 1.0 kPa while the amount of blue light was set to 10% of the photosynthetic photon flux density to optimize stomatal aperture.

Statistical analysis

Two 2 × 2 × 2 factorial experiments (denominated as Experiments 1 and 2), consisting of the factors cultivars (Tommy Atkins and Ubá), plant inoculation (non-inoculated and inoculated plants) and NaCl doses (0 or 90 mM) were arranged in a completely randomized design with five replications. Each experimental unit consisted of one mango plant per plastic pot. Data from all variables were subjected to analysis of variance (ANOVA). Data from RFC, URLL, DRLL and LL as well as from the four leaf gas exchange parameters from Experiments 1 and 2 were analyzed using the MIXED procedure of the SAS software (Release 8.02 Level 02M0 for Windows, SAS Institute, Inc., 1989, Cary, NC, USA) to determine if data from Experiments 1 and 2 could be combined (MOORE and DIXON, 2015). Data from each variable and parameter were submitted to an analysis of variance (ANOVA) and the treatment means were compared based on the F-test using SAS (version 9.0, SAS Institute Inc., Cary, NC, USA). Pearson correlation was used to determine the relationships among the gas exchange parameters, the NaCl doses and plant inoculation for each cultivar separately.

RESULTS

Disease variables

At least one of the factors cultivars and NaCl doses was significant for the RFC, URLL and DRLL. The interaction between these factors was significant only for LL. At the dose of 0 mM of NaCl, the RFC, URLL, DRLL and LL significantly increased by 63, 46, 70 and 60%, respectively, for plants from cultivar...
Tommy Atkins in comparison to plants from cultivar Ubá. At the dose of 90 mM of NaCl, the RFC and DRLL significantly increased by 58 and 60%, respectively, for plants from cultivar Tommy Atkins in comparison to plants from cultivar Ubá. The URLL, DRLL and LL significantly increased by 68, 55 and 60% for plants from cultivar Tommy Atkins grown at the dose of 0 mM NaCl in comparison to the dose of 90 mM NaCl (Fig. 1A-D).

Leaf gas exchange parameters

At least one of the factors cultivars, NaCl doses and plant inoculation as well as some interactions between these factors were significant for $A$, $g_s$, $C_i$ and $E$. For plants from cultivar Tommy Atkins at the dose of 0 mM NaCl, $A$, $g_s$ and $E$ significantly decreased by 38, 47 and 36%, respectively, for inoculated plants in comparison to non-inoculated ones (Fig. 2A, C and G). For plants from cultivar Ubá at the dose of 0 mM NaCl, $g_s$ significantly decreased by 27% for inoculated plants in comparison to non-inoculated ones (Fig. 2D). There were significant increases of 92, 47 and 29% for $A$, $g_s$ and $E$, respectively, for plants from cultivar Tommy Atkins supplied with 0 mM NaCl in comparison to the dose of 90 mM NaCl (Fig. 2A, C and G). For cultivar Tommy Atkins, $C_i$ significantly increased by 19% for plants supplied with 90 mM NaCl in comparison to the dose of 0 mM NaCl (Fig. 2E). For plants from cultivar Ubá supplied with the dose of 0 mM NaCl, $A$ and $C_i$ were significantly higher by 37 and 6%, respectively, in comparison to the dose of 90 mM NaCl (Fig. 2B and F). At the dose of 0 mM NaCl, $A$, $g_s$, $C_i$ and $E$ significantly decreased by 29, 48, 15 and 19%, respectively, for plants from cultivar Tommy Atkins in comparison to plants from cultivar Ubá at the dose of 90 mM NaCl (Fig. 2E and F).

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Figure 1- Radial fungal colonization (RFC) (A), upward relative lesion length (URLL) (B), downward relative lesion length (DRLL) (C) and lesion length (LL) (D) determined on the stem tissues of mango plants from cultivars Tommy Atkins and Ubá grown in nutrient solution containing 0 and 90 mM of NaCl at 42 days after inoculation with Ceratocystis fimbriata. Means of the cultivars Tommy Atkins and Ubá at each NaCl rate followed by an asterisk (*) are significantly different according to F-test ($P \leq 0.05$). The symbol ▼ indicates significant difference between the doses of 0 and 90 mM of NaCl for cultivar Tommy Atkins according to F-test ($P \leq 0.05$). Bars represent the standard deviation of means. $n = 10$. 
Figure 2- Leaf gas exchange parameters: net CO₂ assimilation rate (A) (A and B), stomatal conductance to water vapor (gₛ) (C and D), internal CO₂ concentration (Cᵢ) (E and F) and transpiration rate (E) (G and H) determined on the leaves of mango plants from cultivars Tommy Atkins (A, C, E and H) and Ubá (B, D, F and I) grown in nutrient solution containing 0 and 90 mM of NaCl and non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. For each cultivar and NaCl dose, means of the NI and I treatments indicated by an asterisk (*) are significantly different according to F-test ($P \leq 0.05$). The symbols ▲ and ▼ indicate significant difference between the doses of 0 and 90 mM of NaCl and between the cultivars Tommy Atkins and Ubá, respectively, according to F-test ($P \leq 0.05$). Bars represent the standard deviation of means. $n = 10$. 
DISCUSSION

The present study brings novel information regarding the photosynthetic performance, herein investigated by examining key parameters related to leaf gas exchange, of mango plants from two cultivars previously exposed to salt stress and challenged with *C. fimbriata*.

Based on the variables RFC, URLL, DRLL and LL, evaluated, plants from cultivar Uba were more resistant to mango wilt than plants from cultivar Tommy Atkins. According to Araujo et al. (2014, 2015), hypheae of *C. fimbriata* massively colonized the stem tissues of plants from cultivar Tommy Atkins in comparison to cultivar Uba resulting, therefore, in high disease severity and earlier plant wilt. Indeed, on stem tissues of plants from cultivar Uba, most of the cells reacted to *C. fimbriata* infection by accumulating phenolic-like material and higher levels of insoluble sulfur and calcium (Araujo et al., 2014). Hypothetically, this effect may be associated to a high salt concentration in the stem tissues which may have affected the colonization by *C. fimbriata*. Additionally, the lower transport in the xylem sap flow may reduce a possible dispersion of the pathogen’s structures through the vascular tissues and reducing, therefore, the available of water and nutrients necessary for fungal growth. Interestingly, plants from cultivar Tommy Atkins did not have their level of resistance to mango wilt decreased when exposed to salt stress. In the meantime, plants from cultivar Uba were resistant to mango wilt regardless of being submitted to salt stress. According to Zuaço, Raya e Ruiz (2003), plants from cultivar Tommy Atkins submitted to salt stress showed intense necrosis at both apex and margins of the leaves.

 Alterations on the photosynthesis has been reported to occur in several hosts affected by vascular pathogens such as *Verticillium dahlia* on pepper, *Fusarium oxysporum f. sp. zeae* on tomato, lethal yellowing phytoplasma on coconut and *C. fimbriata* on mango (Nogue & al., 2002; Mau et al., 2003; Pascual et al., 2010; Bispo et al., 2016a,b). In general, uninfected plants from cultivar Tommy Atkins infected with *C. fimbriata* showed lower values for $A$, $g$, and $E$ and unchanged values for $C$ in comparison to non-infected plants. In the meantime, changes in the values for the four leaf gas exchange parameters were minimal when comparing stressed plants non-infected and plants infected with *C. fimbriata*. Overall, $A$ was dramatically reduced for stressed plants regardless of inoculation with $C. fimbriata$ while the inverse was obtained for $C$. For non-infected and uninfected plants, the values for $g$, and $E$ were greater in comparison to the stressed ones. By contrast, changes on $g$ and $E$ were almost imperceptible. Generally, both uninfected and stressed plants from cultivar Ubá experienced less changes on the values of $A$, $g$, $C$, and $E$ regardless of whether they were challenged with *C. fimbriata*. However, stressed plants showed greater values for $A$ and $C$, regardless of being inoculated with *C. fimbriata*. By contrast, $g$ values were greater for stressed inoculated plants in contrast to the non-stressed and non-inoculated ones. Changes on $E$ between non-stressed and stressed plants were imperceptible. Unexpectedly, the stressed non-infected plants from cultivar Tommy Atkins in contrast to the stressed infected counterparts were not favored in terms of stomatal opening that possibly resulted in increases in both $A$ and $E$. There were progressive decreases in $A$, $g$, and $E$ for the noninfected plants from cultivar Tommy Atkins being the magnitude of the reduction quite imperceptible for stressed infected plants. However, despite the reduced stomatal aperture, which should reduce the $CO_2$ influx, the $C_4$ values did not change for nonstressed or stressed plants from cultivar Tommy Atkins regardless of inoculation with *C. fimbriata*. Reductions in $E$ for the nonstressed plants from cultivar Tommy Atkins infected with *C. fimbriata* can be linked to both reductions in $g$, and stomatal closure. In the present study, plants from cultivar Uba showed higher values of $E$ than plants from cultivar Tommy Atkins suggesting, therefore, a better efficiency of using water these plants that suffer water deprivation. Concomitant reductions in both $E$ and $g$ have been also reported to occur for the the mango-*C. fimbriata* interaction (Bispo et al., 2016a,b). Plants submitted to a high salt concentration had their stomatal opening altered due to an increase in the resistance to $CO_2$ diffusion and interference in the leaf tissue hydration as well as on the dissipation of light energy necessary for $CO_2$ fixation (Flexas et al., 2008). Plants from the mango cultivars Haden and Palmer showed a linear decrease in the $g$ values when exposed to an increase in NaCl concentration in contrast to plants from cultivars Tommy Atkins and Uba (Lucena, 2009).

It is known that vascular pathogens, as for example *C. fimbriata*, by massively colonizing the vascular vessels end up disrupting the transport of water, solutes and minerals which culminated in wilted leaves, intense necrosis of the stem tissue and alterations in photosynthesis as the result of a state of induction of water stress or stomatal closure (Araujo) is plausible to be associated with their physiological variability to these parameters. According to Iyer and Degani (1997), there is a great genetic variability among mango cultivars regarding their agronomic and physiological traits. It is important to point out that the reduced mango wilt symptoms, based on the lower values for RFC, URLL, DRLL and LL, for stressed
plants from cultivar Tommy Atkin suggest that factors associated with damage to the stem tissue caused by the excess of chloride ion indirectly affected the colonization of the stem tissue by *C. fimbriata*. It is known that plants submitted to conditions of salt stress show changes in the structural properties of their cell wall and have water permeability of the plasma membrane lowered besides alterations on the stomatal opening that lead to an increase in the resistance to CO₂ diffusion (IRAKJI et al., 1989; FLEXAS et al., 2008; CHAVES; FLEXAS; PINHEIRO, 2009).

CONCLUSIONS

Plants from cultivar Ubá were more resistant to mango wilt in comparison to plants from cultivar Tommy Atkins. In general, plants from cultivar Tommy Atkins were more affected at the photosynthetic level in comparison to plants from cultivar Ubá under both salt stress and fungal infection. Plants from cultivar Tommy Atkins were more susceptible to mango wilt even when exposed to salt stress. Under salt stress, stomatal closure limited carbon fixation especially on plants from cultivar Tommy Atkins. For instance, the findings from the present study may be of direct relevance to regions where *C. fimbriata* is endemic and salt concentration in the soil is quite high.

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