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Development of mango wilt in mango cultivars submitted to salt stress

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ABSTRACT: Mango wilt, caused by *Ceratocystis fimbriata*, is one of the most important diseases affecting mango yield worldwide. Salt stress can affect host defense responses against pathogens infection. The aim of this study was to evaluate the development of mango wilt in 2 mango cultivars submitted to salt stress. Mango plants from cultivars Tommy Atkins and Ubá, considered to be moderately resistant and resistant to mango wilt, respectively, were grown in plastic pots which contained 20 kg of washed sand and daily irrigated with 3 L of a modified Hoagland solution during 40 days before being submitted to salinization. For this process of salinization, the plants received nutrient solution containing 0, 30, 60, and 90 mmol·L^{-1} of sodium chloride (NaCl) during 50 days. At the 50th day, the plants were inoculated with *C. fimbriata*, and disease development was evaluated at 42 days after inoculation. During this period, the plants were also submitted to salinization. After disease evaluation, the stems of plants from each treatment were collected to determine the concentrations of chlorine (Cl) and sodium (Na). Plants from the 2 cultivars showed reduced mango wilt symptoms as the NaCl doses increased from 0 to 90 mmol·L^{-1}. Plants submitted to the highest NaCl doses showed greater Cl and Na concentrations on the stem. In conclusion, the resistance of plants against *C. fimbriata* infection can be potentiated when submitted to salt stress regardless of their basal level of resistance to mango wilt.

Key words: *Ceratocystis fimbriata*, *Mangifera indica*, resistance, salinity, vascular pathogen.

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Mango wilt, caused by the fungus *Ceratocystis fimbriata* Ellis & Halst., is one of the most important diseases affecting mango production worldwide, especially in Brazil (Ribeiro 2005; Viégas 1960). Mango wilt has caused significant decrease in yield in several mango growing areas because *C. fimbriata* can lead to the death of the entire tree in a few months upon roots infection or more slowly if the penetration occurs in wounded branches on the plant canopy caused mainly by beetles (Ribeiro 2005; Viégas 1960).

The use of certified seedlings free of *C. fimbriata* and the eradication of mango trees exhibiting disease symptoms are some of the major control strategies used by the growers to reduce the yield losses caused by mango wilt (Ribeiro 2005; Rossetto et al. 1996; Viégas 1960). In Brazil, the use of mango cultivars showing high level of basal resistance against *C. fimbriata* infection has been the most effective control strategy mainly because of a failure in the use of pesticides (Ribeiro 2005; Rossetto et al. 1996; Viégas 1960). Some mango cultivars showing greater resistance to mango wilt are at the same time very sensitive to salt stress (Lucena et al. 2012). For example, Tommy Atkins and Ubá cultivars, considered to be resistant to mango wilt (Araujo et al. 2014; Rossetto et al. 1996), are tolerant and sensitive, respectively, to salt stress (Lucena et al. 2012).

The number of cultivated areas suffering from salinity around the world has dramatically increased nowadays (Lucena et al. 2012; Zuazo et al. 2003; Zuazo et al. 2004; Zuazo et al. 2006) and it affects many important physiological processes on plants such as photosynthesis, synthesis of protein, and lipid metabolism (Carillo et al. 2011). According to Zuazo et al. (2003), Zuazo et al. (2004), and Zuazo et al. (2006), many mango cultivars sensitive to salinity when grown in saline soils show the apex or the edges of the leaves burned, reduction in growth, leaf abscission, and further plant death, and, for some pathosystems, the host defense responses against pathogens infection are negatively impaired (Bartels and Sunkar 2005; Dileo et al. 2010; Maurya and Gothandam 2014).

Considering the importance of mango wilt to decrease mango yield and the effect of salinity on plant performance, this study aimed to evaluate the development of mango wilt in 2 mango cultivars differing in their basal level of resistance to mango wilt when exposed to salt stress.

One-year-old plants were transferred into plastic pots filled with 20 kg of washed sand each. Plant in each pot was daily irrigated with 3 L of a modified nutrient solution (Hoagland and Arnon 1950) that consisted of: 4 $mmol \cdot L^{-1}$ KNO_3, 1 $mmol \cdot L^{-1}$ $NH_4H_2PO_4$, 13 $mmol \cdot L^{-1}$ NH_4Cl; 2 $mmol \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$; 5 $mmol \cdot L^{-1}$ $Ca(NO_3)_2$; 2 $mmol \cdot L^{-1}$ $S\text{-}SO_4^{2-}$; 0.50 $\mu mol \cdot L^{-1}$ $CuSO_4 \cdot 5H_2O$; 2 $\mu mol \cdot L^{-1}$ $ZnSO_4 \cdot 7H_2O$, 25 $\mu mol \cdot L^{-1}$ H_3BO_3; 2 $\mu mol \cdot L^{-1}$ $MnCl_2 \cdot 4H_2O$; 0.5 $\mu mol \cdot L^{-1}$ $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ and 80 $\mu mol \cdot L^{-1}$ $FeSO_4 \cdot 7H_2O$. Plants were grown in this nutrient solution for 40 days before being submitted to salt stress. For salinization, plants received nutrient solution containing 0, 30, 60, and 90 $mmol \cdot L^{-1}$ of sodium chloride (NaCl) during 50 days. In order to maintain the nutrient solution stable and to make sure that plants were under salt stress, an initial reading of the electrical conductivity (EC) was performed with the aid of a portable conductivity meter. This first reading served as a reference for the subsequent ones. The EC was checked weekly and, when the depletion was equal to or greater than 20% of the initial EC reading, the pH of the NaCl solutions was adjusted to 5.5 by using solutions of nitric acid or potassium hydroxide, both at 0.1 $mol \cdot L^{-1}$.

At the 50th day, the plants were inoculated with *C. fimbriata* according to Araujo et al. (2014). The isolate CEBS15 of *C. fimbriata*, used to inoculate the plants, was obtained from symptomatic mango plants collected in Brejo Santo, Ceará State, Brazil. The isolate was preserved using Castellani's method. Plugs of a malt extract agar medium containing fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA). After 3 days, the PDA plugs containing fungal mycelia were transferred to Petri dishes containing the same culture medium and maintained in an incubator (temperature of 25 °C and 12-h photoperiod) for 14 days. Bark disks (10 mm diameter and 2 mm height) were removed from the stems of plants from both cultivars using a punch. The bark disks were removed approximately 5 cm above the graft scar. A plug (10 mm diameter), removed from the middle portion of each PDA plate obtained from 14-day-old colonies of *C. fimbriata*, was placed in the wound. Each wound containing the fungal mycelia PDA plug was carefully covered with a piece of moistened cotton and enclosed with parafilm to maintain adequate moisture allowing fungal infection. Wounds that only received plugs of PDA medium served as the controls. Disease development was evaluated →

at 42 days after inoculation (dai). During this period, the plants were also submitted to salinization. The total relative lesion length (TRLL) was obtained by measuring the length (in cm) of internal necrotic tissue using an electronic digital caliper (Neiko 01407A, Stainless Steel, Mandaluyong, Philippines). The TRLL was determined as the ratio between the length from the graft scar to the top of the stem (LGST) and the lesion length (LL) at the same interval (upward and downward) from the inoculation point according to the following formula: TRLL = LL × 100/LGST. The plants were standardized to a length of 50 cm (distance from the graft scar to the top of the stem) (Araujo et al. 2014).

After disease evaluation, longitudinal stem sections containing the inoculation point at the center were collected from plants from the replications of each treatment to determine the concentrations of sodium (Na) and chlorine (Cl) according to Noguchi and Kamiya (1963) and Lucena et al. (2012). The stems were dried at 65 °C for 72 h and grounded in a Thomas-Wiley mill with a 20-mesh sieve. The Na was extracted by nitropercloric digestion and analyzed by atomic absorption spectrophotometry (Lucena et al. 2012). The Cl concentration was determined using hot water and titration with silver nitrate in the presence of potassium chromate (Noguchi and Kamiya 1963).

A 4 × 2 factorial experiment consisting of 4 NaCl doses (0, 30, 60, and 90 mmol·L^{-1} NaCl) and 2 mango cultivars (Tommy Atkins and Ubá) was arranged in a completely randomized design with 4 replications. Each replication consisted of a plastic pot containing 1 plant. The experiment was repeated once. The data from TRLL as well as from the concentrations of Na and Cl in the stem from the 2 experiments 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 both experiments could be combined (Moore and Dixon 2015) and then were submitted to an analysis of variance using the SAS software (v. 8.02 Level 02M0 for Windows, SAS Institute, Inc.).

The cultivars Tommy Atkins and Ubá were coded as 0 and 1, respectively. The largest model coefficients were analyzed by the Student's t-test ($p < 0.05$) by adjusting the best model with all significant coefficients:

$$Y_i = \beta_0 + \beta_1 R_i + \beta_2 R_i^2 + \beta_3 C_i + \beta_4 R_i C_i + e_i$$

where: Y_i represents the observed value of the variable in the observation i (i = 1, 2, 3, …, 64); R_i means the NaCl dose in observation i (0, 30, 60, and 90 mmol NaCl·L^{-1}); C_i is the cultivar in the observation i (0 = Tommy Atkins; 1 = Ubá); β_0 is the constant regression; β_1, β_2, β_3, and β_4 are the regression coefficients; i is the error associated with regression in the observed value y_i.

The TRLL was significantly higher for plants from cultivar Tommy Atkins in comparison with plants from cultivar Ubá at the doses of 0, 30, and 90 mmol·L^{-1} NaCl (Figure 1). The highest and lowest values for TRLL occurred for the doses of 0 and 60 mmol·L^{-1} NaCl, respectively, for the Tommy Atkins cultivar (Figure 1). In contrast, for the Ubá cultivar, the lowest and highest values for TRLL occurred for the doses of 0 and 30 mmol·L^{-1} NaCl, respectively (Figure 1). However, from the concentration 30 mmol·L^{-1} NaCl, the TRLL values were reduced constantly for the Ubá cultivar (Figure 1).

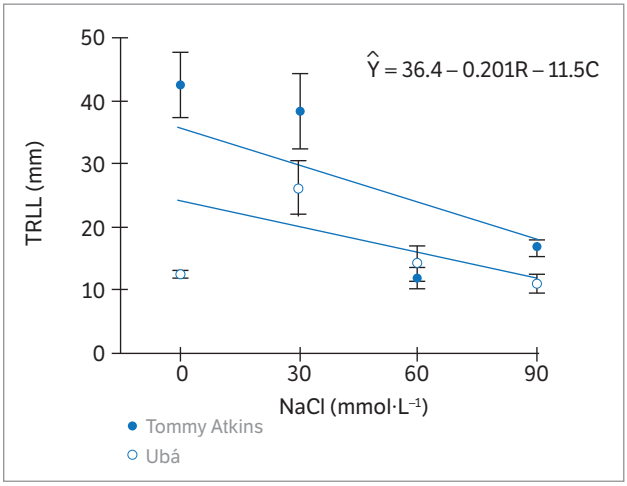

Figure 1. Total relative lesion length (mm) evaluated on stem tissues of plants from cultivars Tommy Atkins and Ubá exposed to different sodium chloride (NaCl) concentrations and inoculated with *Ceratocystis fimbriata*.

There was a differential response of the 2 cultivars to Na concentration on the stem in function of the NaCl doses while, for the Cl concentration on the stem, they showed a similar pattern ($p < 0.05$) (Figure 2). Data from Na concentration was best fitted to the quadratic model (Figure 2). Plants from cultivars Ubá and Tommy Atkins showed a great Na concentration at the doses of 30 and 60 mmol NaCl·L^{-1}, respectively (Figure 2). Data from Cl concentration was best fitted to the linear model (Figure 2). The Cl concentration on stem of plants from cultivars Tommy Atkins and Ubá increased from 0.25 to 0.97 dag·kg^{-1} →

when the NaCl doses increased from 0 to 90 mmol·L^{-1} NaCl (Figure 2).

The results from the present study confirm the findings from Araujo et al. (2014) that plants from cultivar Ubá are more resistant to mango wilt than plants from cultivar Tommy Atkins. However, when plants from cultivars Tommy Atkins and Ubá were exposed to the highest doses of NaCl, the TRLL was dramatically reduced, especially for the former cultivar. According to Dixon and Paiva (1995), plants respond to abiotic and biotic types of stresses by increasing the concentration of pre-existing antimicrobial compounds. Therefore, it is plausible to postulate that mango plants exposed to salt stress have the phenylpropanoid pathway stimulated and, consequently, respond against *C. fimbriata* infection in a more efficient way. Araujo et al. (2015) observed that preventive spraying of susceptible mango plants from cultivar Palmer with acibenzolar-S-methyl and potassium phosphite reduced mango wilt development and increased the concentrations of alkaloids: theobromine and 7-methylxanthine, and of the phenolics: catechin, epicatechin, epigallocatechin, gallic acid, myricetin, *p*-coumaric acid, *p*-hydroxybenzoic acid, phloridzin, sinapinic acid, and salicylhydroxamic acid. According to Dileo et al. (2010), plants submitted to salt stress show a rapid and a transient systemic increase in levels of abscisic acid (ABA), which can influence disease response pathways. It is know that ABA is a primary dehydration-responsive messenger and can increase either the susceptibility or the resistance of plants to pathogens of different life styles (Dileo et al. 2010).

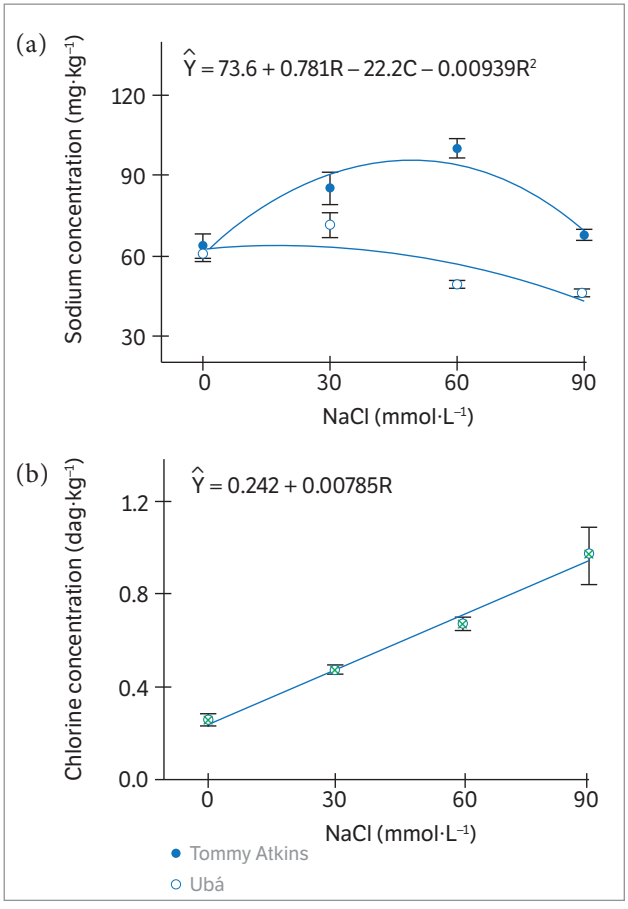

Figure 2. Concentrations of sodium (a) and chlorine (b) on stem tissues of plants from cultivars Tommy Atkins and Ubá exposed to different sodium chloride (NaCl) concentrations and inoculated with *Ceratocystis fimbriata*. Data from chlorine concentration is the average of the 2 cultivars.

In the present study, even though plants from cultivar Tommy Atkins were more susceptible than plants from cultivar Ubá, based on the TRLL values, plants from the former cultivar exposed to the highest NaCl doses were able to counteract the infection process of *C. fimbriata* more efficiently. Some host induced defense mechanisms, such as the synthesis of pathogenesis-related proteins and the production of phenolic-like compounds, are intrinsically related to the plant genotype (Steiner and Schönbeck 1995). According to Araujo et al. (2014), the rapid and high accumulation of phenolic-like compounds contributed to reduce the colonization of *C. fimbriata* on the stem tissue of mango plants from cultivar Ubá compared to plants from cultivar Tommy Atkins. Bispo et al. (2015) reported that plants from cultivar Tommy Atkins exhibited an increase in the activities of enzymes and in the concentration of metabolites related to the oxidative stress responses in comparison with plants from cultivar Ubá. The greatest Na concentration in the stem tissue of plants from cultivar Tommy Atkins that received solutions containing 30, 60, and 90 mmol·L^{-1} NaCl may explain, at least in part, their better response against *C. fimbriata* infection in comparison with plants from cultivar Ubá.

Plants submitted to intense salinity condition are often more resistant to diseases (Dileo et al. 2010). For some host-pathogen interactions, the accumulation of soluble salts in the roots zone makes the plants respond in a similar way when subjected to water stress, resulting, therefore, in an inhibition of cells expansion and division, stomatal closure, decrease in transpiration and xylem flow as well as an increase in the mesophyll resistance and cuticle thickness (Shannon →

1997; Carillo et al. 2011). At the microscopic level, Araujo et al. (2014, 2015) observed that the formation of a barrier of strength in parenchyma cells and rapid deposition of tyloses impregnated with phenolics on the stem of plants from resistant mango cultivars contributed to reduce the colonization of the xylem vessels by *C. fimbriata*. In the present study, is it plausible to postulate that plants exposed to the highest NaCl doses showed symptoms of water stress such as an increase in cell resistance and inhibition of the symplastic xylem, which probably hampered the colonization of the stem tissue by *C. fimbriata*.

In conclusion, even though plants from the 2 cultivars showed difference in Na concentration on their stem tissue, but similar Cl concentration, it can be postulated that they became more resistant to mango wilt due to a potentiation of the phenylpropanoid pathway. It is even possible to consider the role played by water stress to hamper the colonization of the stem tissue by *C. fimbriata* in a scenario where the salt stress was kept at its maximum. Moreover, it is important to emphasize that further studies at microscopic, biochemical and physiological levels are needed to bring new insights into the mechanisms involved in mango resistance against *C. fimbriata* infection in a salt stress environment.

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