ENHANCED RESISTANCE TO *Xylella fastidiosa* IN TRANSGENIC SWEET ORANGE PLANTS EXPRESSING THE *STX IA* GENE

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INTRODUCTION

Citrus is susceptible to a large number of diseases caused by plant pathogens. Among the diseases of economic importance currently present in Brazil are include bacterial disease with citrus canker, citrus variegated chlorosis (CVC) and recently huanglongbing (HLB). These bacterial diseases have the potential to significantly increase production costs and thus decrease profitability for Brazilian growers.

Citrus variegated chlorosis (CVC) is an economically important and destructive disease caused by the bacterium *Xylella fastidiosa* Wells (Chagas et al., 1993). The bacterium is transmitted by xylem-feeding sharpshooter leafhoppers (*Homoptera: Cicadellidae, Cicadellinae)* (Roberto et al., 1996), and by natural root grafts and top grafting with infected budsticks (He et al., 2000). The presence of the bacterium in the xylem vessels makes difficult the development of chemical or biological control measures. The use of resistant varieties is likely to be the most appropriated control method for this disease and genetic transformation can be a viable tool in citrus breeding programs (Colleta-Filho and Machado, 2002).

Plant transformation with antibacterial peptides has been used to enhance pathogen resistance in different plants. Sarcotoxin IA (STX IA) is an antibacterial peptide that is secreted by a meat-fly *Sarcophaga peregrina* and belongs to the peptide group that interacts with bacterial cellular membrane causing an electrochemical potential loss (Okada and Natori, 1983). The objective of this study was to evaluate the response of sweet orange plants transformed with the antimicrobial peptide gene *STX IA* to CVC.

Materials and Methods

Experiments were conducted in greenhouse of the Instituto Agronômico do Paraná - IAPAR. For this study, five independent events of sweet orange cv. Pera (STX-3, STX-5, STX-11, STX12, STX-13), transformed with the vector pST10 containing the *STX IA* gene under control of CaMV 35S promoter and the signal peptide from tobacco PR1a, were included (Bespalhok et al., 2001). For each transformation event, five plants with similar size were inoculated with the CVC bacterium. For inoculation, segments of 2 to 4 cm from citrus plant infected with *X. fastidiosa* were used for grafting. The segments were inserted in the tested plants and wrapped with 2 cm wide transparent plastic strips. The plastic strips were removed after 40 days. Non-transformed plants of the same cultivar were used as control.

The plants were examined regularly for CVC symptom development and evaluated by PCR at six and thirteen months after inoculation. For PCR analysis, CVC-specific primers RST31-RST33 and RST31-RST33 internal were used in attempts to amplify a fragment of the rpoD gene that encodes the sigma subunit of the RNA polymerase (Minsavage *et al.* 1994).

Results and Discussion

The sarcotoxin IA-expressing plants showed normal growth and development, which suggests that the toxin is not deleterious to the host plant. Six months after inoculation, the presence of the CVC bacterium was detected in the control plants by PCR. The plants showed symptoms of the disease approximately thirteen months after inoculation. Just one plant of the STX-3, STX-11 and STX-12 transgenic events developed CVC symptoms (Fig. 1 and 2). In contrast, three out of five plants of the STX-5 and STX-13 transgenic events showed CVC symptoms (Fig. 2).

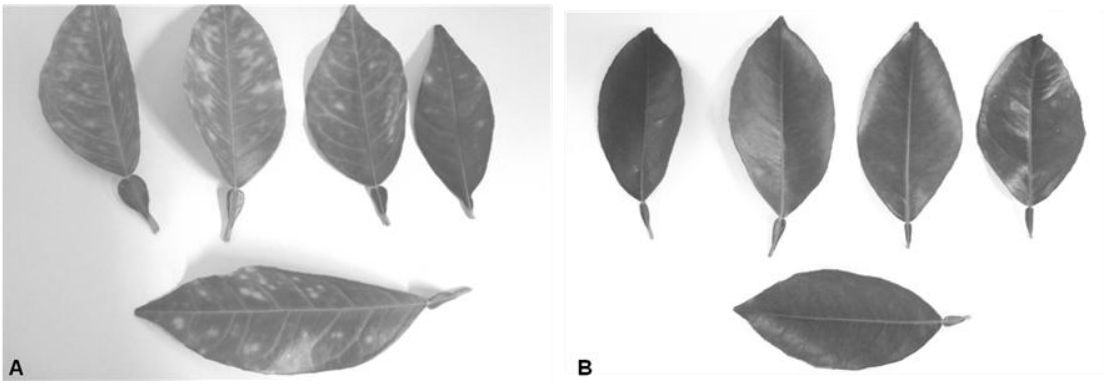

Figure 1. Symptoms of CVC in leaves of citrus plants inoculated with *Xylella fastidiosa*. A) Non-transformed control plants and B) transgenic plants (STX-12).

Sarcotoxin IA is an antimicrobial peptide with high level of positively charged residues, which associates preferentially with negatively bacterial membranes. We hypothesize that the incomplete resistance of the transgenic events could be related to the inoculums of the CVC bacterium used for challenging the plants or to the non sufficient expression of sarcotoxin IA to inhibit the bacteria in different plants of each event.

After thirteen months, symptoms on leaves were variable, depending on the transgenic event. Some transformed and also non-transformed control plants showed PCR amplification of the rpoD gene but did not show symptoms of CVC (Fig. 2). The symptom variation in the plants is probably due to the uneven distribution of the bacterium in the plant. On the other hand, PCR is highly sensitive technique that allows detection of the bacterium even under low population of the pathogen in the host as in the case of the transgenic plants expressing the *Sarcotoxin IA* gene.

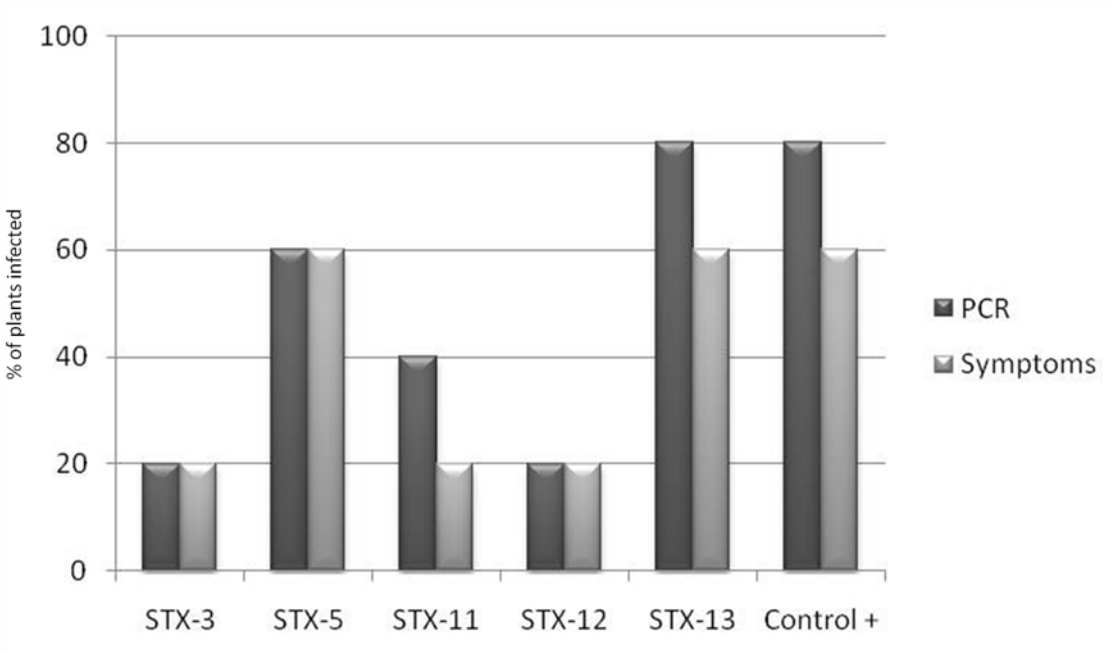

Figure 2. Frequency of CVC disease of plants of transgenic events and non-transformed control plants thirteen months after inoculation with *Xylella fastidiosa.*

Conclusion

The results show an improvement in resistance to CVC of two transgenic events, STX-3 and STX-12, still that the concentration inoculum of the CVC bacterium was unknown. Although further field experiments are necessary to evaluate the performance of transgenic events, this work indicates that it is possible to enhance resistance to CVC by using antimicrobial peptide genes.

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