Studies on differential susceptibility of 'Rocha' pear clones and Portuguese varieties of pear and apple to fire blight - tools for the sustainability of fruit chain production

Suscetibilidade diferencial de clones de pereira 'Rocha' e de variedades autóctones de pereira e macieira à doença do Fogo Bacteriano em Portugal demonstram potencial como alternativas para a sustentabilidade da fileira

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Abstract

Fire blight was officially identified for the first time in Portugal, in 2011, affecting pear cultivar 'Rocha', the most important Portuguese variety of pear, with high economic impact in the production chain and trade. Since then the bacterium has destroyed new orchards in several areas of pear and apple production in the country. Nurseries and phytogenetic resources of several important *in vivo* collections have also been affected and are considered endangered. Severe disease symptoms on branches, flowers and fruits are also present in several other autochthones pear and apple varieties, leading to the death of trees under the climatic and cultural conditions prevalent in Portugal. Coordinated efforts from all fruit production chain actors, including farmers, phytosanitary authorities and research institutions, produced tools to overcome disease impact and build economic and social sustainability of this activity. Within the frame of the national research project Proder InovPomo (2014-2017), the phytogenetic resources of two official collections of pear and apple trees were assessed towards susceptibility to fire blight disease, to select and preserve the individuals combining the best agronomic features as well as higher tolerance to fire blight. The results achieved are essential for selection and preservation of Portuguese phytogenetic resources and biodiversity, as well as for delivering high quality planting materials to farmers for the establishment of new orchards, especially in fruit production areas where fire blight disease is present.

Keywords: Disease management and control, *Erwinia amylovora*, pathogenicity.

Resumo

A doença do Fogo Bacteriano das Rosáceas foi oficialmente identificada pela primeira vez em Portugal em 2011, afetando com grande impacto económico devido a quebras

provocadas na produção e no volume de exportações, os pomares de pereira da cultivar 'Rocha', a mais importante variedade portuguesa. Desde então a bactéria tem vindo a destruir novos pomares em várias áreas de produção de peras e maçãs em Portugal. Os viveiros e importantes coleções *in vivo* de recursos fitogenéticos autóctones também foram afetados, encontrando-se em risco. Na generalidade observam-se sintomas muito característicos da doença nos ramos, flores e frutos de distintas variedades autóctones, que conduzem à morte das árvores afetadas, nas condições climáticas prevalentes nas regiões mais importantes de produção de pomóideas em Portugal. Os vários intervenientes da cadeia de produção, incluindo fruticultores, autoridade fitossanitária e instituições de investigação implementaram ferramentas para mitigar o impacto económico e social causado pela doença e conduzir à sustentabilidade desta atividade económica. O trabalho desenvolvido no âmbito do projeto de investigação nacional Proder InovPomo (2014-2017), permitiu identificar e avaliar a suscetibilidade de duas coleções vivas de recursos fitogenéticos autóctones de macieira e pereira, em risco, avaliando a sua suscetibilidade a *Erwinia amylovora*, com o objetivo de selecionar e preservar os indivíduos com as melhores características agronómicas, evidenciando maior tolerância à doença do Fogo Bacteriano das Rosáceas.

Palavras-chave: Controlo fitossanitário, Fogo bacteriano, *Erwinia amylovora*, patogenicidade

INTRODUCTION

Fire blight disease has a severe impact in the production and trade of Portuguese apple and pear, namely in what concerns pear from cultivar 'Rocha'.

Since 2011, *Erwinia amylovora* has destructed nurseries and orchards from distinct production areas of the country (Cruz, 2011). Nurseries and phytogenetic resources are endangered as most of them are settled in these affected areas (Cruz, 2012).

Similar problems aroused in the main European pomme production areas where apple and pear genetic resources were also assessed (Thibault *et al*., 1987; Szobiczewski *et al*., 2011; Gassmann *et al*., 2014) with very promising results, identifying accessions to be included in new breeding programs as tolerant parents, or displaying good qualities for industry purposes, contributing moreover to maintain biodiversity.

Within the framework of Proder InovPomo Project (PA49448) (2014-2017), the phytogenetic resources from two official *in vivo* collections of autochthones varieties of pear and apple were assessed towards the presence of *E. amylovora* and further studied to identify the existence of differential susceptibility to fire blight disease, with the objective of selecting and preserving individuals displaying higher levels of tolerance to the disease.

The results gathered during the time lapse of this project allowed identifying distinct levels of susceptibility to *E. amylovora* within the 80 pear 'Rocha' clones tested under confinement conditions as well as several national varieties of pear and apple able to serve as alternatives for sustainability of the fruit chain in the main production areas, adapted to Portuguese climatic conditions. This work also allowed the preservation of the

important endangered phytogenetic resources and perspective the study molecular mechanisms associated to specific plant immunity.

MATERIALS AND METHODS

Bacterial strains

Erwinia amylovora strain CPBF 1307, isolated in Portugal and collected from *Pyrus communis* cv. 'Rocha', in 2010, in the Alcobaça area, previously characterized and conserved at -80ºC, was used as positive control for diagnostic analysis as well as for susceptibility trials implemented under environmental controlled and appropriate confinement conditions.

The detection and identification of the bacterium was performed according to EPPO standard PM 7/21(2) (EPPO, 2013). Susceptibility tests were performed according to Parprstein *et al.* (2014) and EPPO standard PM 7/21(1) (EPPO, 2004).

The bacterial strain selected was grown in KMB agar (King *et al*., 1954), for 48hours at 24ºC, streaked to check for purity and further tested using conventional PCR, following Obradovic *et al.* (2007) modified by Gottsberg (PM 7/21(2)) (EPPO, 2013). The bacterial inoculum was prepared in phosphate buffer saline 10mM at a concentration of approximately 10^8 cells/ml (OD=0.1 at 600nm).

Biological tests

Biological tests were performed along the three years of the project and repeated for confirmation of preliminary results achieved.

Young branches of the year from 72 clones of pear cv. 'Rocha' collected at Quinta de S. Gonçalo (Caldas da Rainha) and eight clones collected at Quinta do Olival Fechado (INIAV, Alcobaça) were firstly tested to determine the presence of disease, and used further to evaluate susceptibility to *Erwinia amylovora*.

Comparative analysis of susceptibility towards fire blight disease was also tested among 15 Portuguese pear varieties, other than 'Rocha' and 16 autochthones apple varieties.

Ten branches from each of the selected trees genomically characterized for identity confirmation, with ca. 30cm long were collected at the end of spring, wrapped in paper and transported to the laboratory, refrigerated, in closed polyethylene bags. Four replicate branches from each clone/variety were surface disinfected with 70% ethanol for 30seconds and further washed for three times in sterile distilled water, and dried. All branches were cut aseptically at the bottom and at the tip with a sterile scalpel, immediately introduced in micropropagation tubes containing 0,1% agar for inoculation (EPPO standard PM 7/21(1), 2004).

All branches replicates with approximately 20cm long were inoculated at the tip in a flow chamber with 10µL of the bacterial suspension, immediately closed and transferred to a confinement chamber with 24ºC/18ºC (day/night) and 16-hour photoperiod for incubation, during three weeks. Plant susceptibility to fire blight was assessed 21 days after inoculation (DAI), based on the lesion length on shoots. 10% of replicates randomly chosen were used to determine the reproducibility of the inoculation method. One-way ANOVA data analysis was performed using software R.

RESULTS AND DISCUSSION

The selected plants were confirmed as free of *E. amylovora* according to the diagnostic procedures used, and by this reason used for branch inoculation.

Results from branch inoculations revealed significantly distinct susceptibility levels of the 80 pear clones tested to the bacterium ($p<0,05$) (Figure 1).

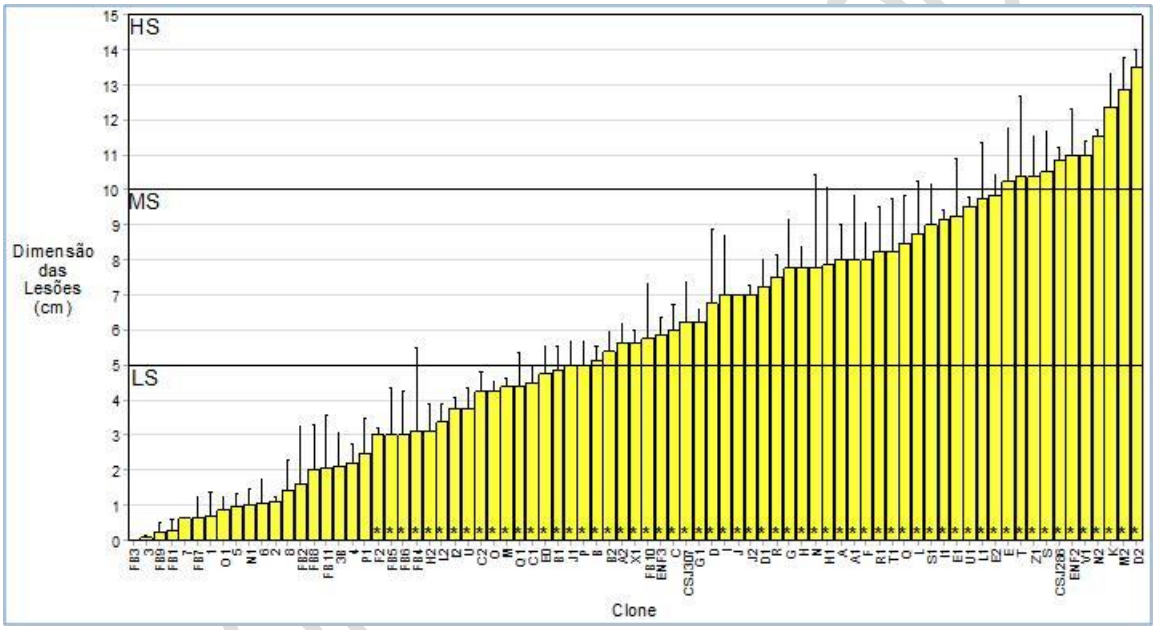

Figure 1 – Susceptibility study to *Erwinia amylovora* of 80 *Pyrus* sp. 'Rocha' clones estimated by ANOVA using R software comparing the length (cm) of the lesions measured 21 DAI (days after inoculation) ($p<0,05$; $\rho=0,84$). Low susceptibility (LS); Medium susceptibility (MS); High susceptibility (HS).

Comparative analysis of susceptibility among 15 Portuguese varieties of pear, other than 'Rocha', highlighted a differential response to *E. amylovora*, revealing that four of them, 'Pera Bonita', 'São Bartolomeu', 'Carvalhal' and 'Aguinha', presented medium to high susceptibility to the bacterium. Inversely, the remaining 11 varieties, displayed lower susceptibility levels, showing to be potential alternatives to pear 'Rocha' (Figure 2). This

variety displayed levels of disease susceptibility higher than the average of the 15 pear varieties tested, after assessing the average of all clones tested (Figure 4).

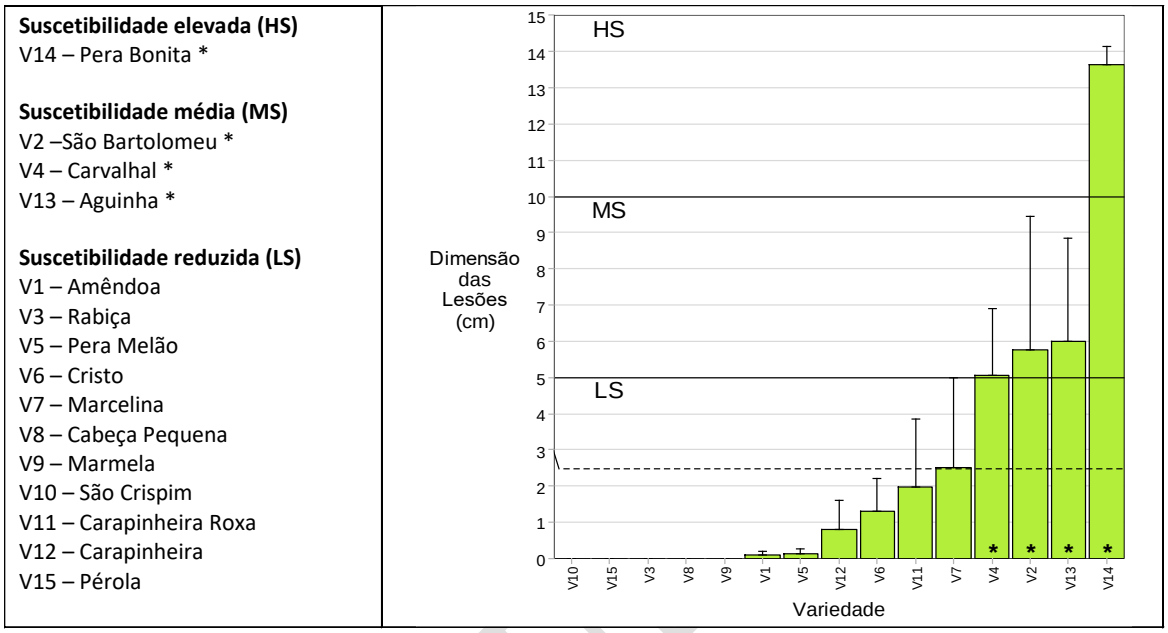

Figure 2 – Susceptibility study to *Erwinia amylovora* of 15 *Pyrus* sp. traditional varieties estimated by ANOVA using R software comparing the length (cm) of the lesions measured 21 DAI (days after inoculation) ($p<0,05$; $\rho=0,84$). Low susceptibility (LS); Medium susceptibility (MS); High susceptibility (HS).

Similarly, 16 apple varieties, also evaluated towards fire blight susceptibility, displayed, in general, lower levels of disease symptoms three weeks after inoculation, when compared to pear varieties, showing low to medium susceptibility under our trial conditions ($p<0,05$) (Figure 3). Eight of these apple varieties showed no visible symptoms at 21 DAI. In addition, viable cells of the bacterium were not recovered after re-isolation from the inoculated branches. Among the authoctones apple varieties, the most susceptible was ’Bravo Esmolfe‘, one of the most popular in the country.

Suscetibilidade média (MS)
M14 – Pero Camões *
M11 – Bravo Esmolfe *

Suscetibilidade reduzida (LS)
M15 – Camoesa Rosa do Menezes *
M16 – riscadinha de Palmela *
M1 – Espelho
M2 – Pardo Lindo
M3 – Pero de Borbela
M4 – Pero Limão
M5 – Pero burro
M6 – Porta da Loja
M7 – Pero Sousa
M8 – Pero da Malveira
M9 – Malápio
M10 – Camoesa de Sta. Maria
M12 – Casa Nova de Alcobaça
M13 – Pero Coimbra

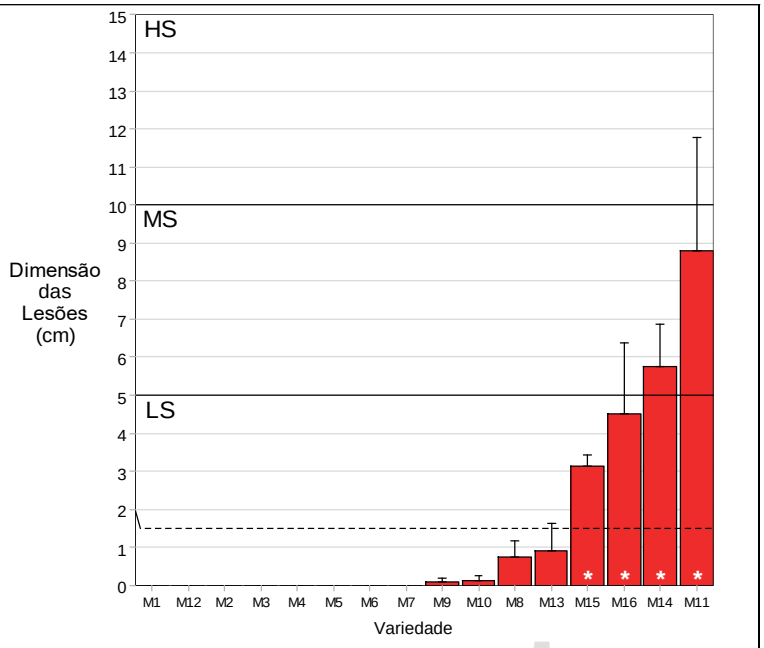

Figure 3 – Susceptibility study to *Erwinia amylovora* of 16 *Malus* sp. traditional varieties estimated by ANOVA using R software comparing the length (cm) of the lesions measured 21 DAI (days after inoculation) ($p<0,05$; $\rho=0,84$). Low susceptibility (LS); Medium susceptibility (MS); High susceptibility (HS).

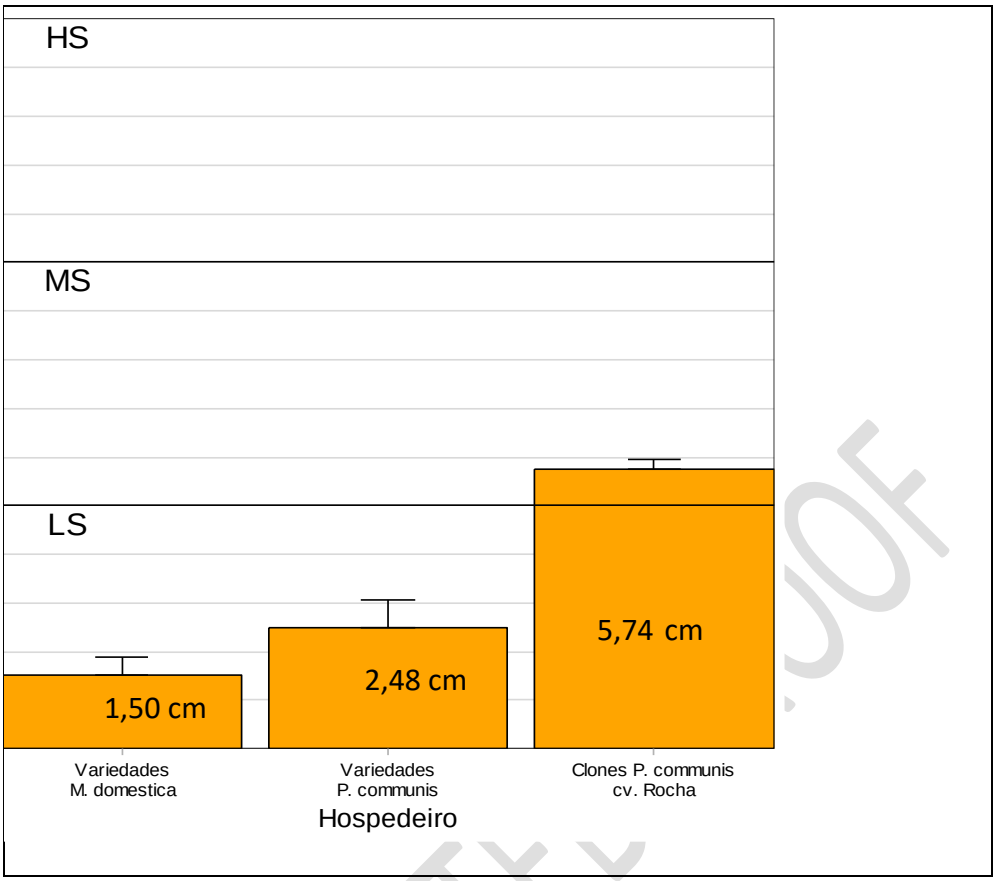

Figure 4 – Global susceptibility analysis to *Erwinia amylovora* of Portuguese *Pyrus* sp. clones and varieties and *Malus* sp. varieties estimated by ANOVA using R software comparing the length of the lesions (cm) measured 21 DAI (days after inoculation) ($p<0,05$; $\rho=0,84$). Low susceptibility (LS); Medium susceptibility (MS); High susceptibility (HS).

Global comparative analysis allowed to underline distinct levels of susceptibility to fire blight agent – *Erwinia amylovora* – and to demonstrate the potential of *Pyrus* sp. and *Malus* sp. phytogenetic resources as alternatives for the establishment of new orchards, controlling high levels of basal inoculum in areas contiguous to contaminated delimited areas where the disease is known to occur (Figure 4). Parallel studies in other European countries lead to the identification of similar results, with distinct levels of susceptibility for different sets of accessions and autochthones varieties of pear and apple (Paprstein *et al*., 2014; Sedlak *et al*., 2014).

CONCLUSIONS

Recent studies confirm the benefits of this approach as a tool to identify resistance sources, and to identify alternatives to commercial varieties preventing dispersal of the bacterium and sustainability of the fruit production chain in a short time lapse (Szobiczewski *et al*., 2011; Gassmann *et al*., 2014).

Evaluation of the presence of *Erwinia amylovora* in these *in vivo* collections allowed the sanitation and preservation of relevant ecological biodiversity and long-term conservation at the national germplasm bank (Banco Português de Germoplasma Vegetal – BPGV), by *in vitro* propagation.

Differential susceptibility of this resources towards the causal agent of fire blight disease, also allowed identifying the existence of climate adapted autochthones varieties of pear and apple, as well as pear 'Rocha' clones as solid alternatives for the sustainability of Portuguese fruit chain production.

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