Vicente C et al., Exploring the relation between virulence and oxidative stress response of *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus.* In: Schröder, T. (ed.), Pine Wilt Disease Conference 2013, pp. 93-94, Braunschweig, ISSN: 1866-590X

Exploring the relation between virulence and oxidative stress response of *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*

Vicente C, Ikuyo Y, Mota M, Hasegawa K

ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Departamento de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal
Email: cvicente@uevora.pt

Tolerance to host-mediated oxidative stress (OS) conditions is an essential characteristic of plant-parasitic organisms. Susceptible *Pinus thunbergii* reacts to *Bursaphelenchus xylophilus* invasion with a strong oxidative burst (Hirao *et al*., 2012), which may indicate that virulent *B. xylophilus* must possess an efficient antioxidant system to cope with these conditions. Previous studies have suggested that PRX (2-cysteine peroxiredoxin), GST (glutathione S-transferase) and GAPDH, all localized in the surface coat, are potential scanvengers of *B. xyophilus* to plant reactive oxygen species (ROS) (Shinya *et al*., 2010; Li *et al*., 2011). More recently, 12 anti-oxidant proteins were identified in the *B. xylophilus* secreotome after plant extract stimuli, emphazing their importance in the control of global oxidative stress of *B. xylophilus* (Shinya et al., 2013). In this work, our main concern was to study of OS tolerance of *B. xylophilus* isolates and *B. mucronatus* and the relation with their pathogenicity (virulence level) to susceptible pine species. Previous results (Vicente *et al., submitted*) have already suggested a relation virulence-OS tolerance among *B. xylophilus* isolates virulent Ka4 and avirulent C14-5. So, firstly, three *B. xylophilus* isolates, Ka4 and T4 (virulent) and C14-5 (avirulent), and one *B. mucronatus* (avirulent) were tested for OS tolerance using hydrogen peroxide as oxidative agent, in concentrations ranging from 0-40 mM H_2O_2. After 24h-exposure to this oxidant agent, nematode survival was checked. A clear difference between virulent and avirulent isolates was recorded in OS conditions, even in the lowest H_2O_2 concentration. The virulent isolates (Ka4 and T4) presented lower mortality percentage in all concentrations than avirulent ones (C14-5 and *B. mucronatus*). Statistical differences between Ka4 and T4 were also found until 30mM H_2O_2 treatment, being Ka4 the most resistant isolate. Concerning avirulent isolates, mortality percentage was higher than 90% in all concentrations, with no statistical differences found between C14-5 and *B. mucronatus*. Next, we assessed transcription levels of 5 main antioxidant and detoxifying enzymes during the OS conditions (15mM H_2O_2, 24h-exposure), and compared with normal conditions (no stress applied) by qRT-PCR. The following enzyme genes were analysed:

CTL (catalases, *Bxy-ctl-1* and *Bxy-ctl-2*), SOD (superoxide dismutase, *Bxy-sod-1*, *Bxy-sod-2* and *Bxy-sod-3*), GXP (glutathione peroxidase, *Bxy-gxp-1*, *Bxy-gxp-2* and *Bxy-gxp-3*), GST (glutathione S-transferase, *Bxy-gst-1* and *Bxy-gst-3*) and PRDX (peroxiredoxin, *Bxy-prdx-2*). In the case of *B. mucronatus*, this analysis was not possible to conduct since no information is available about its genome. From the selected enzymes, only *Bxy-ctl-1* and *-ctl-2* were significantly upregulated ($P<0.05$) in virulent isolates Ka4 and T4. In the case of C14-5, only *Bxy-ctl-2* was significantly downregulated ($P<0.05$) in comparison with normal conditions. For SODs and GPXs, there were no statistical differences between isolates, although we could assess that *Bxy-sod-1* and *-2* were nearly 1-fold upregulated for T4; *Bxy-sod-3*, *Bxy-gxp-2* and *Bxy-gxp-3* for Ka4 and T4 were expressed at the same level than normal conditions; and that *Bxy-sod-2* and *-3*, and *Bxy-gxp-2* and *-3* were downregulated for avirulent C14-5. Concerning the detoxifying enzymes GST and PRDX: *Bxy-gst-1* of isolates Ka4 and T4 were, respectively, downregulated and unchanged under OS conditions, and that expression of *Bxy-prdx-2* for both virulent isolates was suppressed in stress conditions. In contrast, *Bxy-gst-1* of avirulent C14-5 was upregulated in OS conditions and *Bxy-prdx-2* remained unaltered. GST-3 was not detected in all isolates. Following, we will analyse gene expression of these enzymes in *in vivo* conditions for all *B. xylophilus* isolates to ascertain the global oxidative status of the nematode as result of natural oxidative stress conditions. We were able check 100% sequence similarity of coding sequences of CTLs, SODs and GXPs for Ka4, T4 and C14-5, suggesting that if different enzymatic activities are presented may be due to posttranslational modifications.

Based in these results, we hypothese a possible positive correlation between the level of OS tolerance and the level of virulence of *B. xylophilus*, which can be further investigated as a virulence marker.

REFERENCES

Hirao T, Fukatsu E, Watanabe A. (2012) Characterization of resistance to pine wood nematode infection in *Pinus thunbergii* using suppression subtractive hybridization. *BMC plant biology* 12:13.

Li Z, Liu X, Chu Y, Wang Y, Zhang Q, Zhou X (2011) Cloning and characterization of a 2-Cys peroxiredoxin in the pine wood nematode, *Bursaphelenchus xylophilus*, a putative genetic factor facilitating the infestation. *International Journal of Biological Sciences* 7:823-836.

Shinya R, Morisaka H, Takeuchi Y, Ueda M, Futai K (2010) Comparison of the surface coat proteins of the pine wood nematode appeared during host pine infection and in vitro culture by a proteomic approach. *Phytopathology* 100, 1289–97.

Shinya R, Morisaka H, Kikuchi T, Takeuchi Y, Ueda M, Futai K (2013) Secretome analysis of pine wood nematode *Bursaphelenchus xylophilus* reveals the tangled roots of parasitism and its potential for molecular mimicry. *PloS One* 8:e67377.

Vicente C S L, Ikeyo Y, Mota M, Hasegawa K. Pinewood nematode-associated bacteria contribute to oxidative stress resistance of *Bursaphelenchus xylophilus*. *BMC Microbiology submitted*.