Characterization of pea defense mechanisms against *Fusarium oxysporum*

Nicolas Rispail[1], Mustafa Bani[2], Alessio Cimmino[3], Alejandro Pérez-De-Luque[4], Antonio Evidente[3] and **Diego Rubiales[1]**

[1]Institute for Sustainable Agriculture, Córdoba, Spain; [2]Ecole Nationale Supérieure de Biotechnologie, Constantine, Algeria;
[3]Università di Napoli Federico II, Naples, Italy; [4]IFAPA Centro Alameda del Obispo, Córdoba, Spain.

Introduction

Fusarium oxysporum f. sp. *pisi* (*Fop*) is a ubiquitous soil pathogen reducing field pea (*Pisum sativum*) yield worldwide (1-2). Monogenic resistance have been already described and successfully used in breeding. However, monogenic resistance is at risk of breakdown by the constant evolution of the pathogen that already led to the emergence of four distinct *Fop* races. To improve the durability of resistance, its genetic base should be broadened and a better knowledge on the defense mechanisms efficient against *Fop* should be gathered. In a recent screening of a collection of wild *Pisum* relatives, we identified several pea accessions with complete or partial resistance to *Fop* race 2 isolates (2). Here, we describe several experiments to characterize the resistance mechanisms underlying resistance in these sources mainly focussing on the mechanisms acting before root penetration by studying their root exudates (3) and those acting after penetration through an histological approach (4).

A. Characterisation of the mechanisms acting before penetration

Pre-penetration mechanisms are those that interfere with the pathogen recognition of its host. These mechanisms can act by impeding conidia germination or appressoria formation. They might arose from the lack of germination stimuli or the secretion of germination inhibitors (5). Root exudates are important determinants of plant-rhizosphere interaction that modulate germination and growth of soil microorganisms (6). Composition of root exudates is thus likely to contribute to pre-penetration resistance in some host accessions.

To determine whether some pea accessions secreted specific compounds impacting on Fop development, we collected the root exudate from a selection of 12 wild pea accessions with differential responses to *Fop*. The root exudates, collected by transferring 15 days-old plants in sterile water for two days, were then tested on *Fop* germination by incubating 5 x 10^5 *Fop* spores in 1 ml root exudates for 12h (3).

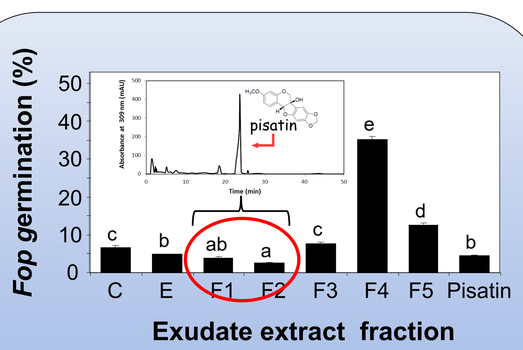

Figure 2. Effect of root extract fraction on *Fop* germination. Insert chromatographic separation of fraction 1 and 2.

Significant differences in the extent of *Fop* germination ($p < 0.001$) were detected between root exudates (Fig. 1). Most root exudates stimulated *Fop* germination inducing from 30% to 67% of germination. By contrast the **root exudates of three pea accessions**, JI 1412, JI 2480 and P42 **had no effect or inhibited *Fop* germination** (Fig. 1). Interestingly, only 6.4% of *Fop* spore germinated in the root exudate of the partially resistant accession JI 2480 which was significantly lower than the control in water (Fig. 1). This indicate that **some pea accessions possess a pre-penetration resistance mechanism that might contribute to reduce and delay infection** (3).

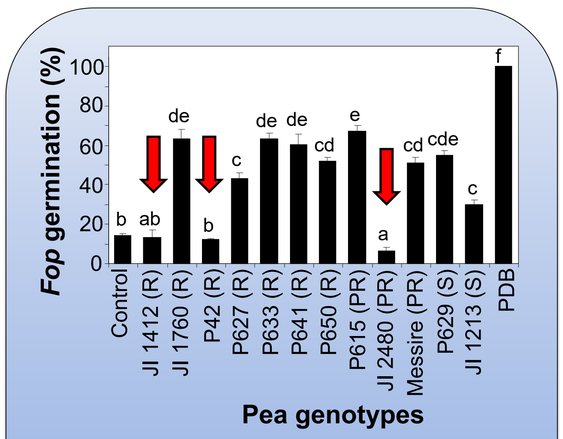

Figure 1. Effect of pea root exudates on *Fop* germination

To identify the inhibitory molecules, crude root exudates from Messire was extracted with ethyl acetate and fractionated by chromatography in 5 homogeneous fractions. **Two of these fractions (F1 and F2) inhibited *Fop* germination** to the same extent as the whole extract. Further analysis revealed that these fractions were **mainly composed of pisatin**, the main pea phytoalexin (Fig. 2). Interestingly, **pure pisatin** at 50 ppm **inhibited *Fop* germination** to a similar extent as the whole extract (Fig. 2). In addition, we observed a significant negative correlation between pisatin content in root exudates and their effect on *Fop* germination (Fig. 3). This strongly support **a role of pisatin in the pre-penetration mechanism** that reduce pathogen germination and so contribute to delay pathogen population build up in the soil (3).

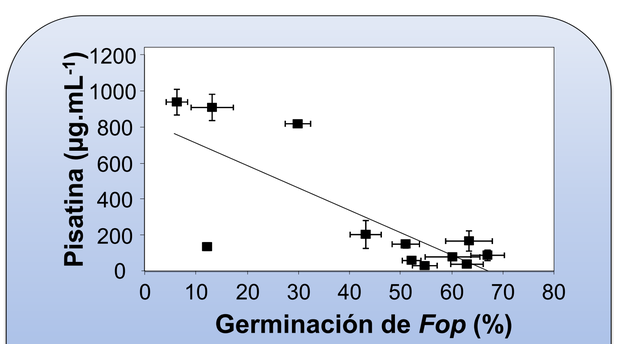

Figure 3. Relationship between the extent of *Fop* germination induced by the root exudates and their pisatin content.

B. Characterisation of the mechanisms acting at and after penetration

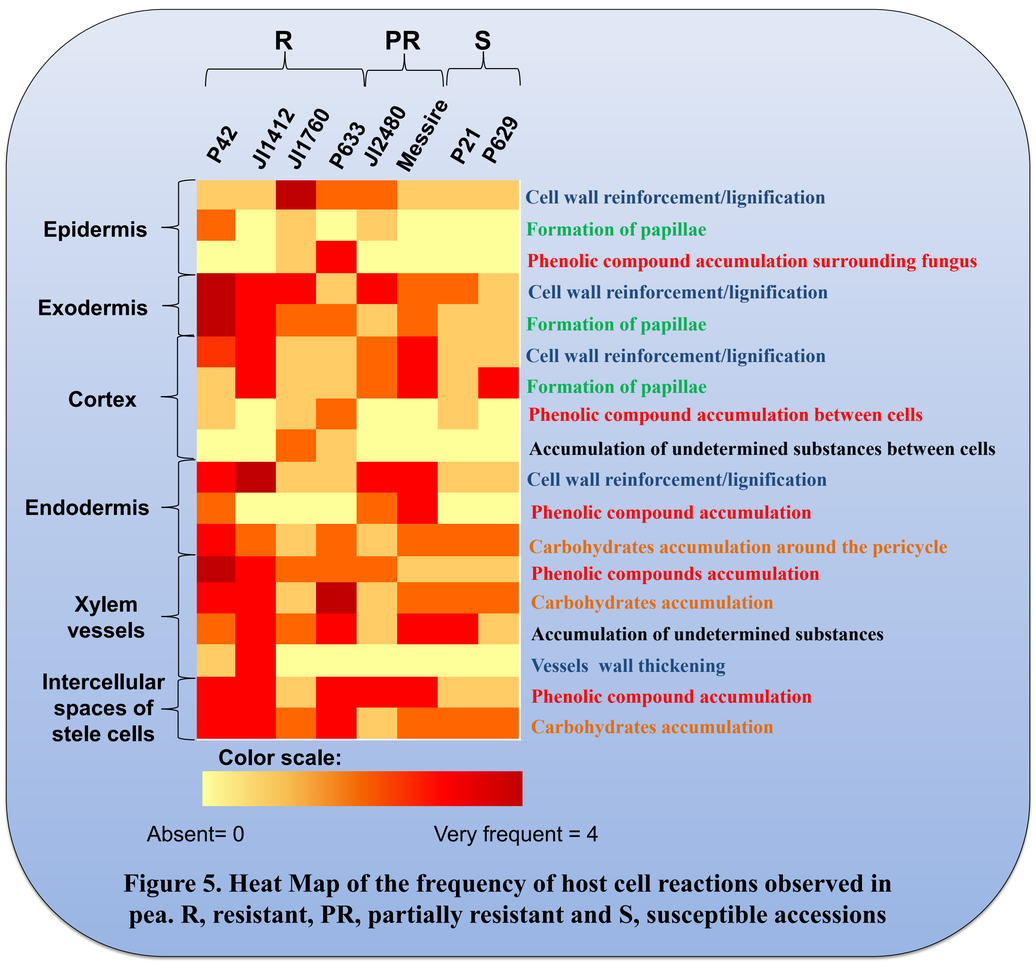

Figure 5. Heat Map of the frequency of host cell reactions observed in pea. R, resistant, PR, partially resistant and S, susceptible accessions

Fusarium wilt infection is initiated after germination by the attachment to and subsequent penetration of host root by infective hypha. Hypha were shown to enter host root both inter and intracellularly without the formation of any specialized structure. Upon penetration, the infective hypha grow through the cortex to the vascular bundle to extensively develop within xylem vessels using them to colonize the upper part of the plant (7). Several defense mechanisms blocking *F. oxysporum* infection have been described in plants. The most efficient mechanisms are the formation of chemical and physical barriers at site of penetration and within vascular tissue throught phytoalexin accumulation, lignification and accumulation of gums, gels or tyloses within xylem cells (7-8).

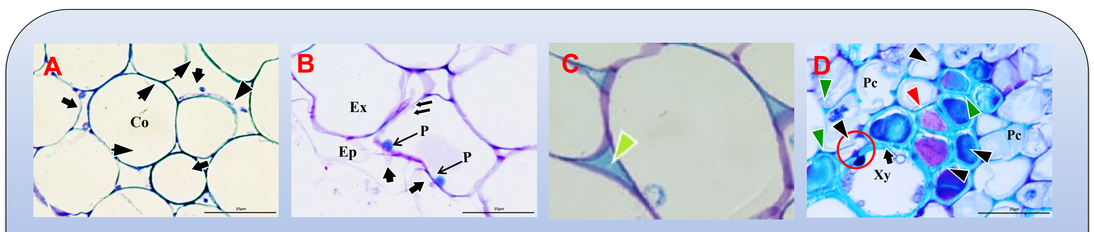

Figure 4. Transverse thin section of resin embedded pea root tissues showing the main resistance mechanisms detected in pea against *Fop.* A, cell wall strengthening, B, papilla formation at attacked site, C and D, accumulation of toxic substances (mainly phenolics and carbohydrates) between and within cells

To determine the defense mechanisms underlying the resistance of the pea accessions, **a detailed histological study was performed on 8 accessions** with differential response to the disease (4). For this 15 days old plants were inoculated with *Fop* for 7 days. Infected roots were cut to small segments, fixed in Karnowsky fixation solution, embedded in resin which were used to prepare the toluidine blue (TBO) stained transverse semi-thin sections (2µm).

Close examination of the root sections indicated that ***Fop* progression was efficiently blocked by the establishment of three defense mechanisms**, **cell wall strengthening** by lignification and/or suberisation (Fig. 4A), **papilla formation** at attacked sites (Fig. 4B) and **accumulation of phenolics and carbohydrates** within and between cells (Fig. 4C and 4D). Each of these mechanisms were detected at different region within the root tissues. **These mechanisms were detected in all accessions** albeit at different frequency revealing that **each resistant accessions** used these three mechanisms differently to **develop its own defense strategy** (Fig. 5). This open great opportunity for resistance breeding by combining these distinct defense strategy in a single elite pea cultivars.

Concluding Remarks

- ***Fop* resistance is mediated by pre- and post-penetration mechanisms**
- **Pisatin secretion in root exudate contribute to a pre-penetration mechanisms reducing pathogen germination**
- **Post-penetration mechanisms are mediated by three main mechanisms: papilla-formation, cell wall strengthening and accumulation of toxic substances within and between cells.**
- **Each resistant accessions set up its own defense strategy**

Research was supported by Spanish project AGL2014-52871-R and Marie Curie program, and by EU-FP7-LEGATO

References

(1) Rubiales et al., 2015. Crit Rev Plant Sci 34,195–236
(2) Bani et al., 2012. Plant Pathol 61, 532-542
(3) Bani et al., 2017. Plant Pathol. (Accepted pending revision)
(4) Bani et al., 2017. Front. Plant Sci. (Accepted pending revision)
(5) Rubiales and Niks, 1996. Physiol. Mol. Plant Pathol. 49, 89-101
(6) Bais et al., 2006. Annu. Rev. Plant Biol 57, 233-266
(7) Bishop and Cooper, 1983. Physiol. Plant Pathol. 22, 15-27
(8) Bishop and Cooper, 1983. Physiol. Plant Pathol. 23, 323-343