Identification of PAMPs originating from Downy Mildew Pathogen *Hyaloperonospora parasitica*

Sophie Laflin, Nick Holton, Mahmut Tör

Warwick HRI, University of Warwick, Wellesborne, Warwick, Warwickshire, CV35 9EF

Introduction

Pathogen Associated Molecular Patterns (PAMPs) are highly conserved molecules, unique to microbes and conserved within a given class of microbes (pathogens and non pathogens)[1]. These are recognised as foreign by plant pattern recognition receptors, inducing an immune response termed PAMP-triggered immunity. Known bacterial PAMPs include flagellin and Ef-Tu, no PAMPs have been identified for the oomycete plant pathogen *Hyaloperonospora parasitica*.

The aim of this project was to establish a system for identifying PAMPs.

***Figure 1 - Hyaloperonospora parasitica* is an obligate pathogen of the plant Arabidopsis thaliana. Notice the white spores on the leaf.**

Methods

In order to test for PAMPs, it was necessary to detect when an immune response was generated. Transgenic PR-1-GUS plants were used for this purpose, containing the promoter of Pathogenesis Related gene 1 (PR-1), one of the many genes induced during the plant immune response, fused with the GUS gene for the enzyme beta-glucuronidase. Assaying for GUS activity using the enzyme substrate X-Gluc, to produce blue staining in the plant, revealed when an immune response had been generated.

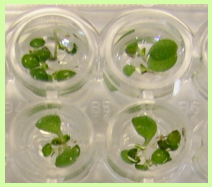


Figure 2 – Six day old seedlings. Treatment added to liquid medium.

Figure 3 – GUS assay 48 hours after treatment. No staining in top row, blue staining shown in bottom row.

Pathogen Spores

Spores from three isolates of *H. parasitica*, Noks1, Cala2 & Emoy2, either untreated or heat killed, used as treatments. Intense staining seen in untreated spores wells (Nu, Cu, Eu); very faint staining caused by heat killed spores in some seedlings.

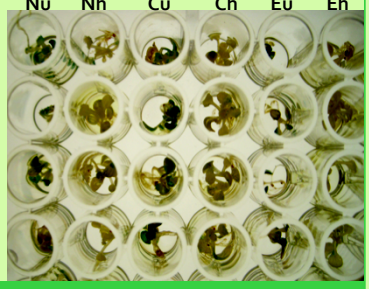
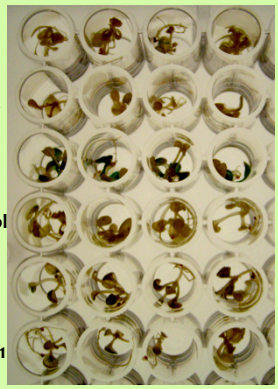


Figure 4 – GUS stained PR-1-GUS seedlings treated with untreated and heat killed spores. Nu: Untreated Noks1 spores; Nh: Heat killed Noks1 spores; Cu: Untreated Cala2 spores; Ch: Heat killed Cala2 spores; Eu: Untreated Emoy2 spores; Eh: Heat killed Emoy2 spores.

Bacterial PAMPs and Plant Hormones



Strong staining observed when treated with SA; weaker staining in leaf veins treated with flagellin & Atpep1.

Figure 5 – GUS stained PR-1-GUS seedlings treated with plant hormones and bacterial PAMPs. IAA: indoleacetic acid; MEJA: methyl jasmonate; SA: salicylic acid; Flg22: flagellin peptide; Atpep1: plant peptide.

Plant Material

Plant protein samples extracted from Arabidopsis leaves used to treat seedlings. GUS staining observed in all treatment conditions, most intense in UI, II and IS.

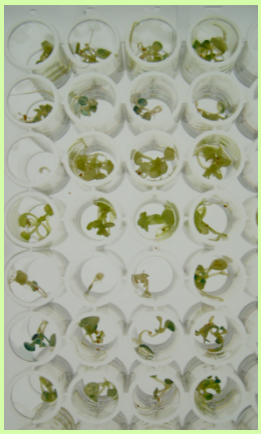
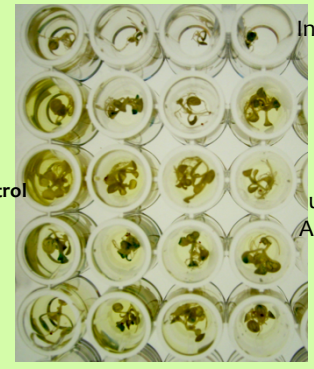


Figure 6 – GUS stained PR-1 seedlings treated with plant material extracts. UT: uninfected plant total protein; UI: uninfected plant insoluble protein; US: uninfected plant soluble protein; IT: Infected plant total protein; II: Infected plant insoluble protein; IS: Infected plant soluble protein.

Intercellular Wash Fluid



Intercellular wash fluid obtained from infected and uninfected Arabidopsis leaves. Each sample caused some GUS staining in PR-1 seedlings.

Figure 7 – GUS stained PR-1 seedlings treated with intercellular wash fluid. U1: Uninfected, first centrifuge; U2: Uninfected, second centrifuge; I1: Infected, first centrifuge; I2: Infected second centrifuge.

Conclusions

H. parasitica spores all induced GUS expression in PR-1 seedlings.
Heat treated spore induced GUS expression in some PR-1 seedlings but at a much lower level.
Flg22 and Atpep1 induced GUS expression in PR-1 seedlings.
SA greatly induced GUS expression in PR-1.
Uninfected and infected plant extracts induce GUS expression.
Intercellular wash fluid from infected and uninfected plants induced GUS expression in PR-1 seedlings.

Further Work

- Try heat killing spores for longer to see if GUS expression is prevented.
- Try separating plant proteins in size order to identify which proteins are inducing GUS expression.
- Use a quantitative assay to determine differences in the level of GUS expression caused by the different treatments.

URSS Experience

The scheme has given me an insight into the world of academic research; I have learnt about how research problems are approached, attended and contributed to group meetings, developed and practiced my laboratory skills and gained invaluable experience which will help me when applying for jobs. This placement has also been a chance to explore one of the career paths available to me after graduation, of which I previously had very little knowledge.

THE UNIVERSITY OF WARWICK

URSS

Warwick HRI

References:

[1]Knoth, C. and Eulgem, T. (2008) **The oomycete response gene LURP1 is required for defence against Hyaloperonospora parasitica in Arabidopsis thaliana.** The Plant Journal 55, 53-64.

[2]Tör, M. (2008) **Tapping into molecular conversation between oomycete plant pathogens and their hosts.** European Journal of Plant Pathology 122, 57-69.