# PMR 5, a unique plant-specific protein related to cell wall-mediated disease resistance in *Arabidopsis*

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## INTRODUCTION

PMR 5 is a recently described locus identified in a search for plant genes mediating resistance to fungal pathogens.[1,2] Using the small mustard plant *Arabidopsis thaliana* as a genetic model system infected by *Erysiphe cichoracearum* (a causative agent for powdery mildew), we hope to be able to rapidly identify and transfer genes for disease resistance into major crop species, e.g. rice, barley and squash. Over 6000 plant species are infected by powdery mildew[3], and losses worldwide from plant disease outbreaks may exceed $1 billion each year. Cloning[1] of *PMR5* demonstrated that it codes for a membrane-associated 402 aa-protein, unique to plants, with a coding sequence having very few recognizable protein structural motifs; however, the first 22 amino acids of the protein are predicted to serve as an N-terminal target sequence for the endoplasmic reticulum. Compared to wild type plants, the visual phenotype of *pmr5* mutant plants is similar to the previously described *pmr 6* mutation[2], in that the overall size of the plants are reduced, the leaves cup upward, and most importantly have quantitative resistance to powdery mildew fungus. Equally important, neither genotype depended on activation of either the salicylic acid (SA)- or jasmonic acid/ethylene-dependent pathways for defense. To provide some guidance in understanding the underlying molecular lesion, we undertook at Beamline 1.4.3 an IR spectromicroscopic study of leaves from plants homoqygous for mutations in *pmr 5*, a double mutant of *pmr 5 pmr 6*, and plants that had been complemented with DNA for the pmr 6 gene. We supplemented this IR analysis with direct biochemical extractions of the cell walls from similar plants to those used for the IR studies.

## METHODS

*Arabidopsis* seedlings were germinated on agar plates and transplanted after 12 days to soil as described previously.[2] Plants were grown at a density of 2 seedlings / 5" pot, and subjected to continuous light at 22$^{o}$ C temperatures for an additional 2-3 weeks. Four separate genotypes were cultured for IR microscopy simultaneously: (1) *Col-0* wild type plants, (2) *pmr 5* mutants homozygous for a T-DNA insertion, (3) *pmr 5 pmr 6-3* double mutants, and (4) complemented *pmr6::PMR 6* $T_2$'s. The last of the genotypes was added as an additional control; these are plants originally lacking the *PMR6* gene that have been complemented by transforming with a ~6 Kb genomic construct coding for PMR6. Prior to harvest, plants were dark-adapted for 12-18 h to deplete foliar starch pools. Rosette leaves from a dozen or more plants of each genotype were cleared in 1:1 $CHCl_3$/methanol. FT-IR spectra were collected in reflection mode from seedlings at BL

1.4.3 on the Nic-Plan microscope in reflection mode versus a gold standard over the energy range of 4000 to 650 $cm^{-1}$. A variety of cell types were probed, including stomatal cells, vascular bundles and abaxial mesophyll cells. Principal components analysis (PCA) using a covariance matrix approach was applied to contrast: (i) *Col-0* vs *pmr 5*, (ii) *pmr 6:: PMR6* transformants vs *pmr 5*, and (iii) *Col-0* vs *pmr 5 pmr 6-3* double mutants over the "fingerprint region" of 1850 – 750 $cm^{-1}$.

**RESULTS**

Since we previously found that the *pmr 6* mutation altered cell wall composition, we examined the composition of *pmr 5* cell walls using the same technique. FTIR spectra from *pmr 5* cell walls was essentially indistinguishable from *pmr 6* spectra, indicating that both mutations have similar effects on cell walls architecture (**Figure 1**). Like *pmr 6*, *pmr 5* cell walls contain more pectin than wild-type cell walls and had a shift in the energy of cellulose hydrogen-bond networks. These FT-IR results have been confirmed recently by gas-chromatographic/MS analysis of CDTA-extracted pectin fractions isolated from the cell walls of intact plants. Taken together, the data strongly indicate a role for both PMR 5 and PMR 6 in determining cell wall composition. This is particularly significant for PMR 5, the newly described locus, because there was no reason, other than its similarity to PMR 6, to suspect that it was involved in cell wall biochemistry. This highlights the power of FT-IR in metabolomic investigations of plant genomes. As a good control on the background variability of cell wall spectral features, it should be noted the high degree of similarity between true Col-0 wild-type spectra and those of complemented *pmr 6* :: *PMR6* $T_2$ plants (**Figure 1**).

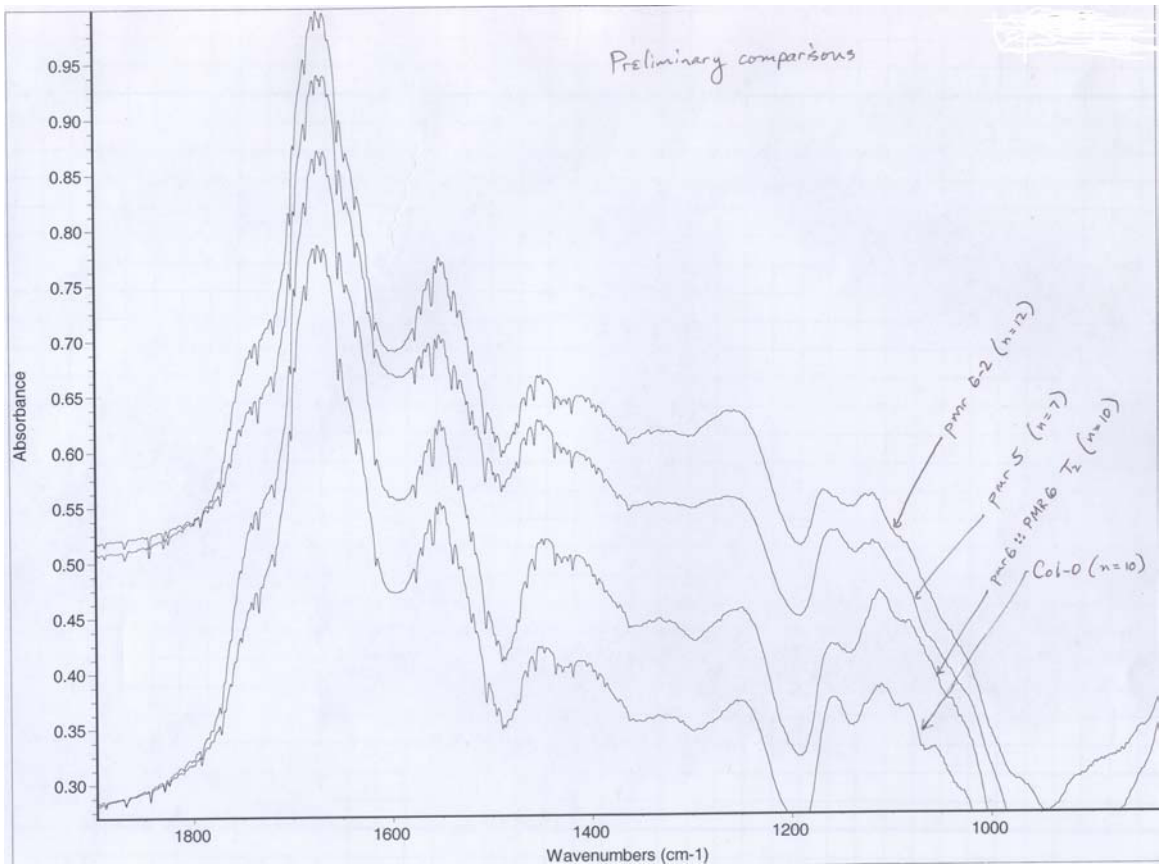


**Figure 1 Fingerprint region FT-IR spectral comparisons of Col-0 wild type plants, homozygous mutants of pmr 5 and pmr 6, and complemented $T_2$s of pmr 6::PMR 6.**

## REFERENCES


1. J. Vogel, T.K. Raab, S.C. Somerville, "Mutations in PMR 5 result in powdery mildew resistance and altered cell wall composition" , Plant Cell (in review, 2003).
2. J. Vogel, T.K. Raab, C.L. Schiff, S.C. Somerville, "*PMR6*, a Pectate Lyase-like gene required for powdery mildew susceptibility in Arabidopsis", Plant Cell **14**, 2095-2106 (2002).
3. P. Schulze-Lefert, J. Vogel, "Closing the ranks to attack by powdery mildew", Trends Plant Sci. **5**, 343-348 (2000).



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