Title

"Evaluating modes of resistance to *Plasmodiophora brassicae* in *Arabidopsis thaliana"*

Abstract

Clubroot disease caused by the obligate biotroph *Plasmodiophora brassicae* (*P. brassicae*) is one of the most economically important diseases of brassica crops. The characteristic symptom of the disease is the development of galls on roots and hypocotyls of infected plants, this leads to serious water and nutrient imbalance and results in massive yield loss. A limited set of resistant cultivars have been developed to manage the disease. As pathotypes capable of breaking resistance against the resistant cultivars spread to wider geography, there is an urgent need to find out new sources of resistance against the disease.

To tackle the problem, 142 natural accessions of *Arabidopsis thaliana* were screened with a P1+ *P. brassicae* pathotype collected from the West Pomerania region of Poland. Arabidopsis, being a member of the Brassicaceae group of plants, is prone to clubroot infection. Due to the availability of a wide variety of genomic data for Arabidopsis, it was possible to carry out a Genome Wide Association Study (GWAS) which resulted in identification of a significant SNP on chromosome 1, linked with resistance to clubroot disease. This SNP coincided with a previously identified locus named *RPB1* (*Resistance to Plasmodiophora brassicae 1*) absent in the Col-0 reference genome. *RPB1* codes for a small protein of 16 kDa (148 aa) with no known domains present. The role of *RPB1* was characterized by transferring the gene into susceptible Col-0 under various expression conditions. No transgenic Arabidopsis plant could be recovered following repeated transformation with *RPB1* under control of a 35S promoter. *Agrobacterium* mediated transient expression in tobacco leaves with this *35S::RPB1* construct resulted in localized cell death (but not for a GFP-tagged *RPB1* version) prompting the hypothesis that overexpression of *RPB1* in Arabidopsis results in embryo lethality. Three transgenic lines overexpressing a GFP-tagged *RPB1* under control of the *35S* promoter gave rise to T3 transgenic plants which displayed a wide range of phenotypical variety in terms of plant size and symptoms of autoimmune lesions. Measurement of *GFP-RPB1* expression in transgenic plants inversely correlated with rosette size, *P. brassicae* pathogen load and

clubroot gall diameter, while positively correlating with expression of the salicylic acid signalling marker *PR1* in aerial tissue. Thus, it can be assumed that attaching a GFP tag on RPB1 results in interference with its immune signalling capability, but the overexpressing plants still exhibit stunting due to some degree of defense signalling activation. Finally, the *RPB1* gene from resistance accession Est-1 along with its 1 kb upstream promoter region was transferred to the susceptible Col-0 accession. Clubroot infection resulted in activation of the promoter which indicates that *RPB1* is not involved in direct recognition of pathogen virulence factors. Col-0 transgenic plants harboring *RPB1* under its native promoter did not exhibit resistance comparable to Est-1, though pathogen titer was significantly reduced in three of five lines examined. While *RPB1* is involved in clubroot disease resistance, there seems to be additional components missing from susceptible Col-0 to confer full resistance.

Early changes in the transcriptome of susceptible Col-0 and resistant Est-1 accessions were profiled by mRNA-Seq analysis. The jasmonic acid mediated signalling pathway dominated the Col-0 transcriptome, while salicylic acid mediated signalling was the prominent signature of the Est-1 transcriptome. Genes upregulated in Est-1 in response to *P. brassicae* included a wide variety of immune responsive genes showing an ETI response upon pathogen infection. The Col-0 transcriptome carries changes in a few development related pathways that have been previously identified in other gall forming pathogens, showing how diverse pathogens have evolved to hijack similar pathways.

Apart from characterizing resistance responses from the host perspective, the pathogen's ability to mask its surface chitin moieties was also investigated. By comparing transcriptome datasets at various timepoints of the infection, it was noted that chitin responses in the host are substantially repressed at later stages of infection. A putative chitin binding virulence factor, *PBRA_005081*, was cloned from the pathogen and ectopically expressed in Arabidopsis. Transgenic lines exhibited increased pathogen load and reduced water content upon infection, indicating enhanced disease progression.

In summary, exploitation of Arabidopsis natural accessions resulted in identification of the *RPB1* gene involved in clubroot resistance and its immunogenic properties when transferred to susceptible accession Col-0. A putative virulence factor was also identified which might be used by the pathogen for protection and production of its spores.