The role of mlo in plant immunity and its effect on mycorrhizal colonisation

Mildew Resistance Locus O (mlo) mutants have allowed durable resistance in barley against powdery mildew for over 30 years in the field. However, there is also evidence that it reduces the colonisation of arbuscular mycorrhizal fungi compared to wildtype plants. This may be due to an upregulation of defence-genes such as ROR2 that could lead to a reduction in the beneficial mycorrhizal symbiosis. Although the benefits of mlo in pathogen defence is evident, its role in plant immunity is still poorly understood. This summer, I was given the opportunity to investigate the effect of mlo expression in plant defence and mycorrhization at the John Innes Centre, Norwich.

Firstly, I needed to establish that mlo reduces mycorrhizal colonisation. This involved trying to reproduce results from a research paper by Ruiz-Lozano et al. To do this I (a) grew wild type Ingrid and mlo-5 barley in the glasshouse for 6 weeks (b) blue-ink stained sections of the roots and (c) observed the presence of arbuscules and colonisation using the grid-line intersect method. The results appeared to show that, although there was no significant difference due to sample sizes, there was a 50 % reduction in frequency of arbuscules with no change in colonisation frequency ($p=0.03$). This shows mlo mutants may reduce mycorrhizal colonisation which validates further investigation.

The literature already shows that mlo increases ROR2 expression. However, it could be that MLO has a more integral role in plant immunity (which would further explain the trade-off with beneficial mycorrhiza). To test this, I did an RT-qPCR for the expression of defence genes in unchallenged barley leaves e.g. ROR2, cupredoxin, EFE and RNR3, relative to the constitutively active EF1a. Unfortunately, no pattern could be detected for several of the PAMP-inducible defence genes. However, there did appear to be an increase in ROR2 gene expression in the mlo variants compared to the wildtype.

Although effects on defence in model plants (e.g. Arabidopsis) can be analysed by a variety of other methods in addition to RT-qPCR, they are not easily transferred into barley due to variations in reproducibility. The third part of my project involved optimising a protocol to carry out a ROS (reactive oxygen species) assay in barley leaves and roots to measures oxidative burst in response to stresses. This involved testing different growth locations, PAMP concentrations, presence of phosphate inhibitors and pre-infiltration incubation conditions. I enjoyed having the chance to analyse various research papers and test my own ideas. During this part of the project I came to have a deep appreciation for the scientific process.

During my summer project I was given a lot of freedom to test new protocols and discuss new ideas. I am grateful for the opportunity to learn new skills and be part of such a fantastic research group. Hopefully my results and the protocols I was able to develop will be useful for further study into the effects of mlo on defence and mycorrhization.

I would like to thank the BSPP for giving me the opportunity to be part of such an interesting research project. Also thanks to Dr Chris Ridout, Dr Jeremy Murray, Dr Henk-jan Schooenbeck and Dr Donna Cousins for their help and guidance and making the project so enjoyable.

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