Early-response mechanisms of perennial ryegrass (*Lolium perenne* L.) to phosphorus deficiency

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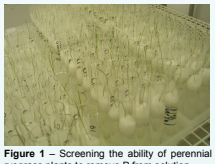

Figure 1 – Screening the ability of perennial ryegrass plants to remove P from solution.

Screening

Omic profiling

Conclusions

Future Work

Background:

Phosphorus (P) is an essential macronutrient required by plants for growth and development. However, P sources are limited and are expected to be depleted by the end of the century. Perennial ryegrass is the major grassland species present in the British isles and accounts for a significant proportion of P fertilizer consumption. Therefore, it is likely that an improvement in phosphorus usage efficiency will result in significant benefits. In order to analyse the response of perennial ryegrass plants to limiting-P conditions a metabolite profiling approach was used in combination with transcriptomics.

Screening:

36 different ecotypes were screened for their ability to remove P levels from solution during a period of 3 days (Figure 1). The values of P concentration removed from the initial solution were determined using a molybdenum blue assay and are represented in Figure 2.

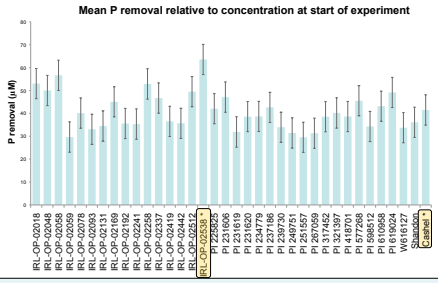

Figure 2 - mean values for P_i removal from solution for 36 genotypes. The two genotypes selected from this screen (highlighted in yellow) were propagated for the experiments described below. The results are for P_i removal relative to amount of P in starting solution (0.31mM of KH_2PO_4), which was used for serial dilutions in constructing the standard curve for the molybdenum blue assay. The standard error of difference is 6.606.

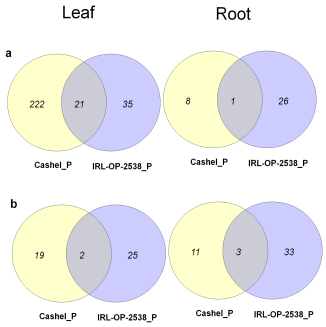

Figure 3- a) Number of genes from barley array hybridisations with ≥ 2-fold change in expression ($p<0.05$) under limited phosphorus for each genotype. b) Number of metabolites with significant fold change ($p<0.05$) under limited phosphorus for each genotype. Leaf tissue on left and root tissue on right.

Transcript and Metabolite profiling:

Genotypes 2538 and Cashel were grown in a hydroponics system and allowed to establish for a period of 2 weeks. Plants were then exposed to either a nutrient solution with control levels of P (0.31 mM of KH_2PO_4) or a solution with reduced levels of P (0.016 mM of KH_2PO_4) for 24h before material from leaves and roots was harvested. Metabolite profiling of both tissues was performed following extraction and derivatization of both polar and non-polar fractions (Foito *et al.*, 2009) in a gas-chromatography coupled Thermo Finnigan DSQ-MS system. Transcript profiling was performed in a barley microarray chip with 6000-7000 of hybridizations with cDNA to produce an acceptable signal.

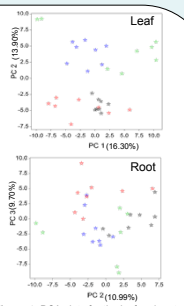

Figure 4- PCA plots for the leaf and root metabolite profiling analysis. Cashel_P grown under P limiting conditions - ★; Cashel_P grown under P sufficient conditions -★; IRL-OP-2538_P grown under P limiting conditions - ★; IRL-OP-2538_P grown under P sufficient conditions -★.

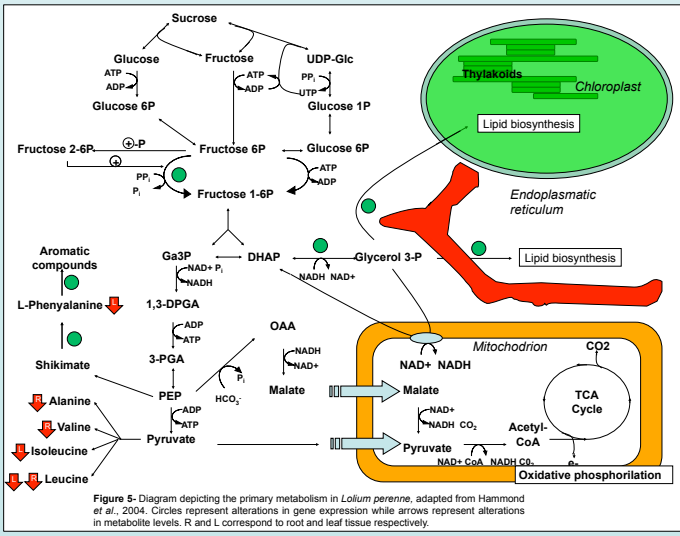

Figure 5- Diagram depicting the primary metabolism in *Lolium perenne*, adapted from Hammond *et al.*, 2004. Circles represent alterations in gene expression while arrows represent alterations in metabolite levels. R and L correspond to root and leaf tissue respectively.

Conclusions:

The induction of P-limitation in perennial ryegrass appears to elicit signalling mechanism as uncovered by transcript profiling (Phospholipase C, calcium mediated signalling and ids-4-like gene). Furthermore, the lipid metabolism seems to be affected both at the transcriptional and metabolic level, suggesting a replacement of membrane phospholipid by non-phosphorus membrane lipids. The transcript and metabolite profiles suggested the involvement of two glycolitic bypasses. In addition to this the expression of genes involved in the biosynthesis of secondary aromatic precursors seems to be affected which appears to be correlated by metabolite data. Analysis of both tissues revealed alteration of source/sink relationships within the plant.

Acknowledgements

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