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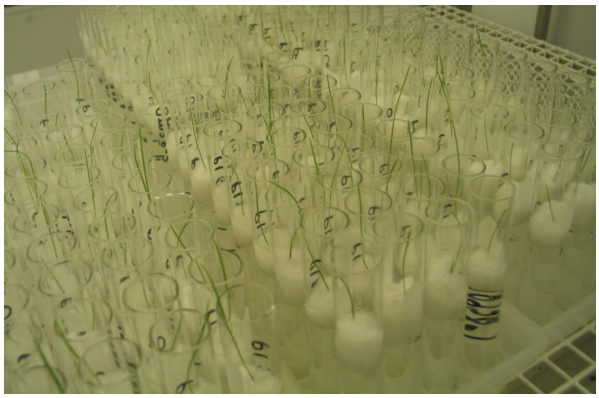
Development of metabolomics based methods to benefit marker assisted breeding in perennial ryegrass



Key external stakeholders:

Plant geneticists, grass breeders, general public

Practical implications for stakeholders:

This project has demonstrated that metabolomics is a suitable technique for application to outbreeding forage species.

- In combination with targeted physiological experiments we have identified key metabolites involved in several abiotic stress conditions in perennial ryegrass. This metabolomics work on nitrogen and phosphorus acquisition and usage efficiency has lead to in depth initial insights into plant metabolism under deficient conditions.
- Follow up work would need to investigate the effect of toxic concentrations of phosphorus on plant metabolism and also how genotypes cope with low nutrient situations. This future work should be coupled with genomics techniques and modelling approaches to maximise the gain in information acquired which could lead in the longer term to cultivars with improved nutrient efficiency.
- Also an investment into the genetics of crown rust resistance and to sources and strains of crown rust would help with the development of cultivars with an improved resistance to this economically important disease.

Main results:

- Key metabolites and transcripts in the response of perennial ryegrass to low phosphorus supply and drought stress were identified.
- Key metabolites in the response of perennial ryegrass to different levels of nitrogen supply were identified.
- Key transcripts in the response of perennial ryegrass to toxic levels of selenium were identified.
- A metabolite quantitative trait loci (mQTL) study identified and genetically mapped QTL associated with quality traits.

Opportunity / Benefit:

- The research completed in this project is of a ‘public good’ and strategic nature. The outcomes of this project provide an excellent base to study in greater depth insights into plant metabolism under deficient conditions for nitrogen and phosphorus acquisition and usage efficiency.

Collaborating Institutions:

The Hutton Institute, Dundee, Scotland
The University of Dundee, Scotland
Biomathematics and Statistic Scotland

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1. Project background:

Perennial ryegrass is of economic importance to both Irish agriculture (90% of the agricultural land) and the leisure industries. The breeder's objective is to produce cultivars with a diverse range of characteristics for a range of specific uses. Major breeding goals include; improving the nutritive quality as an animal feed, nitrogen and phosphate use efficiency for a more safe and sustainable use of resources, tolerance to selenium a localized problem in many areas of Ireland, and adaptability to changing environmental factors including susceptibility to drought and disease resistance. Conventional selection during breeding puts a limit on the efficiency of introducing desirable combinations of traits due to the large resource required to phenotype the breeding germplasm. This constraint severely limits the ability of plant breeders to target specific environmentally valuable traits in new cultivars. The development of functional molecular markers to select for these specific traits may reduce the need for costly phenotypic selection at certain times during the breeding cycle. The development of functional marker technologies can be accelerated by the application of metabolomics technology to understand a plants response to these environmental changes.

2. Questions addressed by the project:

The project aimed to investigate if metabolomics methods can be used as a suitable technique to be applied to outbreeding forage species to aid the identification of key metabolites affected under environmental stress conditions. This was achieved by asking 2 key questions:

- Is it possible to identify key metabolites and transcripts for environmental stress conditions such as drought stress, nitrogen and phosphorus deficiency in the outbreeding plant species perennial ryegrass?
- Is it possible to place key metabolites on a genetic map to make those accessible for further molecular marker assisted breeding strategies?

3. The experimental studies:

The primary objectives of this project was to apply metabolomics technology to understand the response of perennial ryegrass to environmental change, and combine this data with both transcriptomic and genomic data with the purpose of developing functional markers for perennial ryegrass breeding.

A stepwise approach was applied to investigate the genetic basis of phenotypic and metabolic plasticity to abiotic stress factors for perennial ryegrass inbred lines, a segregating population and wildtype accessions. The stress testing (water stress, nitrogen, phosphorus and selenium) was carried out in hydroponics to enable the targeted application of one stress factor at a time. Hydroponics allowed the genetic characterization and metabolite profiling of above and below ground biomass (roots and shoots). The single biotic stress studied (crown rust infection) was monitored under field conditions and natural infection.

In the first strand of the project transcriptomics and metabolomics approaches were used to characterize the response of perennial ryegrass to the stress factors described above. Subtractive hybridization libraries (SSH) were constructed for the stress factors of drought and nitrogen. These libraries were enriched for genes regulated by the stress factor. Candidate genes were subsequently selected to carry out expression profiling via real-time PCR. To study the response of perennial ryegrass to selenium toxicity we modified the SSH screening technique by using next generation sequencing technology (NGS) to sequence the entire libraries of selenium stress regulated genes. Using this approach we were able to identify genes and gene families regulated upon selenium stress. A different approach was taken for the stress factor phosphorus, where we tested the possibility of using a 44K barley micro array to study gene expression in perennial ryegrass. This proved possible and we subsequently used the array to characterize changes in the transcriptome under low phosphorus. For metabolomics analysis, plant material grown under various stress conditions were harvested, flash frozen in liquid nitrogen and polar and non-polar extracts were analysed using a range of metabolomics techniques at the Hutton Institute. Plant materials for these approaches used

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were ecotypes and inbred lines of perennial ryegrass. The inbred lines did not show wide differences in response to stress conditions and work was stopped on the diallel crosses of inbred materials after one year of crossing.
In a second strand of the project the segregation within an F2 population to rust resistance was studied in order to identify QTL associated with the trait. The F2 population was previously used to map biomass QTL and we had an existing genetic linkage map. In order to enhance this existing genetic map we employed DArT marker technology to improve marker density. We looked at the metabolomics profiles of each plant within the entire F2 population under field conditions, with the goal of mapping individual metabolites as quantitative traits (mQTL). In this way the genetic location of QTL responsible for variation in individual metabolite levels within the F2 population could be identified. These include many of the metabolites identified as responsive to the environmental stresses which were imposed in strand one of the project.

4. Main results:

Metabolic profiling was carried out in perennial ryegrass to uncover mechanisms involved in the plants response to water stress. When leaf and root materials from two genotypes, with a contrasting water stress response, were analysed by GC-MS, a clear difference in the metabolic profiles of the leaf tissue under water stress was observed. Differences were principally due to a reduction in fatty acid levels in the more susceptible Cashel genotype and an increase in sugars and compatible solutes in the more tolerant PI 462336 genotype. Sugars with a significant increase included: raffinose, trehalose, glucose, fructose and maltose. Increasing the ability of perennial ryegrass to accumulate these sugars in response to a water deficit may lead to more tolerant varieties. The metabolomics approach was combined with a transcriptomics approach in the water stress tolerant genotype PI 462336, which identified perennial ryegrass genes regulated under water stress.

Selenium is an essential micronutrient for animals and humans, but can be toxic at high concentrations. Manipulation of Se metabolism in plants may enable plants to be tailored to enhance Se content for human and animal consumption and to decontaminate Se polluted soils. In this project, we generated subtracted cDNA libraries from perennial ryegrass roots and leaves, enriched for genes whose expression is enhanced under toxic levels of selenium. The libraries were sequenced using next generation sequencing technologies to characterize the pool of enriched genes. Within these subtracted libraries, there were a large number of genes involved in the calcium-calmodulin signaling network. Furthermore, in the leaf subtracted cDNA library, we identified 28 ABC transporters. Subsequent expression analysis by quantitative RT-PCR demonstrated the significant accumulation of these transcripts in the leaf tissue of perennial ryegrass under toxic levels of Se. These results suggest a role for ABC transporters in selenium movement and accumulation in perennial ryegrass.

Improving phosphorus (P) nutrient use efficiency in *Lolium perenne* (perennial ryegrass) is likely to result in considerable economic and ecological benefits. To date, research into the molecular and biochemical response of perennial ryegrass to P deficiency has been limited, particularly in relation to the early response mechanisms. This study performed as part of this project aimed to identify molecular mechanisms activated in response to the initial stages of P deficiency. A barley microarray was successfully used to study gene expression in perennial ryegrass and this was complemented with gas chromatography-mass spectrometry metabolic profiling to obtain an overview of the plant response to early stages of P deficiency. After 24 h of P deficiency, internal phosphate concentrations were reduced and significant alterations were detected in the metabolome and transcriptome of two perennial ryegrass genotypes. Results indicated a replacement of phospholipids with sulfolipids and the utilization of glycolytic bypasses in response to P deficiency in perennial ryegrass.

Crown rust caused by the fungal biotroph, *Puccinia coronata*, is an economically destructive disease of perennial ryegrass. To identify genetic loci associated with resistance to this disease, Quantitative trait loci (QTL) mapping was performed in an existing F2 mapping population segregating for natural crown rust infection under Irish field conditions. The F_2 population, consisting of 325 genotypes was saturated with DArT markers to improve map coverage and density. This high density map was used to locate QTL associated with the differences in crown rust susceptibility identified within the population. QTL on linkage groups 2, 3, 4, and 7 were successfully identified, with the QTL on linkage group 2 explaining the largest percentage of the phenotypic variance (13.9%).

Nitrogen use efficiency (NUE) is a key objective in perennial ryegrass breeding in order to produce economically and environmentally sustainable varieties. We performed an in depth study looking at the

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changes in the phenotype and metabolism of seven perennial ryegrass genotypes to altering concentrations of nitrogen. This allowed us to identify biochemical processes being altered as external nitrogen concentrations were altered.

The primary metabolome of perennial ryegrass was mapped into a high resolution genetic map of an F2 inbred derived mapping population. This work has lead to the identification of quantitative trait loci controlling the accumulation of individual metabolites. This allows us to look at the genetic control of these metabolites, including the metabolites we have identified above as being responsive to environmental change. This work is forming the basis of further studies at Oak Park. The publication of the outcomes of this work is in preparation.

5. Opportunity/Benefit:

The research completed in this project is of a 'public good' nature. As such it will deliver a direct economic benefit to the forage sector. While the research has not delivered patents or intellectual property it has demonstrated that significant research outputs can be achieved in a small frame.

6. Dissemination:

The project resulted in a number of scientific publications and presentations at meetings including the plant breeding sector. The project was also presented to visitor groups and at Open Days in Oak Park.

Main publications:

Foito A., Byrne S.L., Hackett C., Hancock R.D., Stewart D. and Barth S. (2012) 'Short-term response in leaf metabolism of perennial ryegrass (*Lolium perenne*) to alterations in nitrogen supply' Metabolomics (DOI: 10.1007/s11306-012-0435-3)

Tomaszewski C., Byrne S.L., Foito A., Kildea S., Kopecký D., Doležel J., Heslop-Harrison P.(J.S.), Stewart D. and Barth S. (2012) 'Genetic linkage mapping in an F2 perennial ryegrass population using DArT markers' *Plant Breeding*, 131: 345—349 (DOI: 10.1111/j.1439-0523.2011.01944.x)

Byrne S, Foito A, Hedley P, Morris J, Stewart D and Barth S. (2011) 'Early response mechanisms of perennial ryegrass to phosphorus deficiency' *Annals of Botany*, 107: 243-254.

Byrne S, Durandeau K, Nagy I and Barth S (2010) 'Identification of ABC transporters from *Lolium perenne* that are regulated by toxic levels of Selenium' *Planta* 231: 4, 901 - 911.

Foito A, Byrne S, Shepard T, Stewart D, Barth S (2009) 'Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG induced water stress' *Plant Biotechnology Journal* 7:8, 719-732.

Popular publications:

Byrne S., Foito A., Stewart D. and Barth S. (2010) 'Omics' for better breeding. *TResearch* 5: 24-25.

Barth S., Byrne S., Anhalt U. and Yang B. (2007) Applying 'omics' technologies to grassland improvement. *TResearch* 2 (2), p 22-23.

Byrne S., Barth S. and Foito A. (2007) Stress Test. *TResearch* 2 (2), p 21.

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