



Department of
Agriculture, Fisheries and Food
An Roinn
Talmhaíochta, Iascaigh agus Bia

Research Stimulus Fund

Final Report

“Development of metabolomic based methods to benefit marker assisted breeding in perennial ryegrass”

DAFF Project Ref No: RSF 06-346
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Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDAMENTAL  APPLIED/PRE COMMERCIAL

x	x	
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Key words: (max 4)

Perennial ryegrass, stress, metabolomics, transcriptomics

1. Rationale for Undertaking the Research

There is a substantial grass breeding program at Oak Park developing cultivars for Irish conditions (and export market). 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 of efficiency of introducing desirable combinations of traits due to large resources 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. Research Approach

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 we used transcriptomics and metabolomics approaches to characterize the response of perennial ryegrass to the stress factors described above. Subtractive hybridization libraries (SSH) were constructed for the stress factors 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 SCRI. Plant materials for these approaches used were ecotypes and inbred lines of perennial ryegrass. The inbred lines did not show wide differences in response to stress conditions and we abandoned the diallel crosses of inbred materials after one year of crossing. In a second strand of the project we have studied the segregation within an F2 population to rust resistance 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 we know the genetic location of QTL responsible for variation in individual metabolite levels within the F2 population. These will include many of the metabolites identified as responsive to the environmental stresses we imposed in strand one of the project.

3. Research Achievements

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 has identified perennial ryegrass genes regulated under water stress. This work has been published in an international peer refereed journal.

Selenium is an essential micronutrient for animals and humans, but can be toxic at higher levels. 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 which 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. This work has been published in an international peer refereed journal.

Improving phosphorus (P) nutrient 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. This work has been published in an international peer refereed journal.

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 this project in an existing F₂ mapping population segregating for natural crown rust infection under Irish field conditions. The F₂ 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%). The outcomes of this work have been submitted to an international peer refereed journal.

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 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 outcomes of this work are being prepared for publication.

We have made an attempt to map the primary metabolome of perennial ryegrass to a high resolution genetic map of an F₂ inbred derived mapping population of perennial ryegrass. This work has led 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.

4. Impact of the Research

The outcomes of this research have a high impact on the advancement of plant sciences for the forage grass perennial ryegrass. When the grant application for this project was written little research had been done on the metabolomics of forage species.

We have published the findings of our work in high impact peer reviewed journals, which has increased awareness of the perennial ryegrass research being carried out at Teagasc. This has opened up possibilities to become involved in large consortium applying for European grants and further expand the capabilities of Teagasc in ryegrass research.

The outcomes of the research are forming the basis of further studies identifying functional markers in perennial ryegrass for marker assisted breeding.

This project also increased collaboration amongst Teagasc and SCRI researchers and forged new collaborations (Pete Hedley, SCRI). The project has trained a PhD student and provided further training for a Postdoctoral researcher.

5. Exploitation of the Research

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. Summary of Research Outputs

(a) Intellectual Property applications/licences/patents
Not applicable to this project.

(b) Innovations adopted by industry
Not applicable to this project.

(c) Number of companies in receipt of information
Not applicable to this project.

(d) Outcomes with economic potential
Not applicable to this project.

(e) Outcomes with national/ policy/social/environmental potential
Not applicable to this project.

(f) Peer-reviewed publications, International Journal/Book chapters.

1. Tomaszewski C, Byrne SL, Foito A, , Kildea S, Kopecký D, Dolezel D, Heslop-Harrison JS, Stewart D, Barth S. (2011). Mapping QTL for resistance to natural crown rust infection in an F₂ perennial ryegrass population saturated with DArT markers. *Plant Breeding*, submitted.

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

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

4. 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.

(g) Scientific abstracts or articles including those presented at conferences

1. Investigating Drought Tolerance in a Set of *Lolium perenne* Inbred Lines.

Byrne, S., Foito, A., Stewart, D., Barth, S. Eucarpia fodder crops and amenity grass section meeting. Copenhagen, August 2007. Poster.

2. Identification of QTL for crown rust resistance in perennial ryegrass.

Byrne, S., Foito, A., Stewart, D., Barth, S. Society of Irish Plant Pathologists (SIPP) meeting. Teagasc, Oak Park, Carlow, November 2007. Oral presentation.

3. The Use of SSH To Identify Transcripts under Differential Expression during Peg Induced Drought Stress In *Lolium perenne*. Byrne, S., Foito, A., Stewart, D., Barth, S. Plant and Animal Genome Conference, San Diego, 2008. Poster 815.

4. Mapping Quantitative Trait Loci (QTL) for crown rust resistance in *Lolium perenne*. Byrne, S., Foito, A., Stewart, D., Barth, S. Agricultural Research Forum, Tullamore, March 2008. P12, ISBN 1841705047.

5. Mapping QTL for crown rust resistance in perennial ryegrass. Byrne, S., Foito, A., Stewart, D., Barth, S. Irish Plant Scientists Association Meeting (IPSAM), NUI Maynooth, March 2008. Poster Presentation.

6. Transcriptional and metabolic profiles in *Lolium perenne* L. genotypes with differential physiological responses to a PEG induced drought stress. Byrne, S., Foito, A., Stewart, D., Barth, S. Eucarpia General Congress. Valencia, September 2008. Poster Presentation.

7. Dissecting the genetic nature of crown rust resistance in perennial ryegrass. Byrne, S., Foito, A., Stewart, D., Barth, S. Eucarpia General Congress. Valencia, September 2008. Poster Presentation.

8. Foito, A., Stephen Byrne, S., Barth, S, Shepherd, T. & Stewart D (2009) "Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG-induced water stress." Plant abiotic stress tolerance international conference – Vienna 8-11th February 2009 (poster)

9. Foito, A., Stephen Byrne, S., Barth, S, Shepherd, T. & Stewart D (2009) "Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG-induced water stress." Eucarpia XXVIIIth meeting of the fodder crop and amenity grasses– La Rochelle 11-14th May 2009 (poster)

10. S. Byrne, A. Foito, D. Stewart, S. Barth (Ireland): ‘Omic’ profiling of *Lolium perenne* L. genotypes under different N concentrations. Eucarpia XXVIIIth meeting of the fodder crop and amenity grasses– La Rochelle 11-14th May 2009 (poster)

11. Foito, A., Stephen Byrne, S., Barth, S, Shepherd, T. & Stewart D (2009) “Drought in perennial ryegrass- A metabolomics perspective.” Monogram Network workshop – Bristol 29th April-1st May 2009 (presentation)

(h) National Report
N/A

(i) Popular non-scientific publications

1. Barth S, Byrne S, Foito A, Stewart D (2010). ‘Omics’ for better breeding. *TResearch*, 5(1), 24-25.

2. S. Byrne, A. Foito and S. Barth. Stress Test. *TResearch*, Volume 2, Number 2, Summer 2007, pg21.

(j) Workshops/seminars/ open days at which results were presented (excluding those in (g))

1. Stand at the Oak Park Tillage Open Day on the 27th of June 2007

7. Permanent Researchers

Teagasc	Number of Permanent staff contributing to project	Total Time contribution (months)	Average time contribution per permanent staff member
Susanne Barth	1	6.85	6.85
Derek Stewart	1	6.85	6.85
Total	1	6.85	6.85

8. Researchers Funded by RSF

Type of Researcher	Number	Total Time contribution (months)	Average time
Post Doctorates	1	36	36
Contract Researchers			
PhD postgraduates	1	36	36
Masters postgraduates			

Temporary researcher			
Other	3	7.2	2.4
Total	5	79.2	74.40

9. Postgraduate Research

Total Number of PhD theses: 1

Alexandre Foito 'A metabolomics-based approach to study abiotic stress in *Lolium perenne*'
University of Dundee; PhD thesis submitted June 2010, PhD defended September 2010

10. Project Expenditure

Total expenditure of the project: €450,461.52

Total Award by RSF €438,624.80

Other sources of funding (specify) €
N/A

Breakdown of Total Expenditure

Category	TEAGASC	Name Institution 2	Name Institution 3	Name Institution 4	Total
Contract staff					
Temporary staff	6,142.77				6,142.77
Post doctorates	160,040.36				160,040.36
Post graduates	63,000.00				63,000.00
Consumables	64,911.79				64,911.79
Travel and subsistence	12,382.43				12,382.43
Sub total					
Durable equipment					
Other	50,234.67				50,234.67
Overheads	93,749.50				93,749.50
Total	450,461.52				450,461.52

11. Future Strategies

This project has enabled to showcase that metabolomics are a suitable technique to be applied 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 led to in depth initial insights into the plant metabolism under deficient conditions. This work would need to be followed up and extended to investigate the effect of toxic concentrations of phosphorus to plant metabolism and also for the low nutrient situations how genotypes cope with those. This future work should be coupled to genomics techniques and to 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 to work more directed on cultivars with an improved resistance to this economically important disease.

12. Industry Collaboration

N/A