Technology Updates **Crops, Environment and Land Use**

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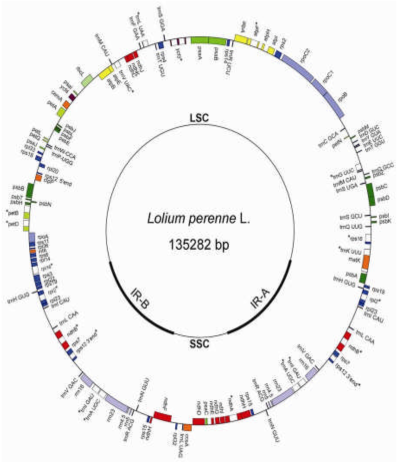
Perennial ryegrass organelle genomics



Key external stakeholders:

Plant geneticists, grass breeders, general public

Practical implications for stakeholders:

The outcome of this project is of strategic nature. Main stakeholders at this time point are plant geneticists trying to unravel phenomena related to the organelle genomes of plants. Outcomes of this project form the basis for these studies.
Information gained on both perennial ryegrass organelle genomes is now available to carry out more applied research.

Main results:

- A high quality assembly of the perennial ryegrass chloroplast genome has been produced and made publically available.
- A high quality draft assembly of the perennial ryegrass mitochondrial genome has been produced.
- Molecular chloroplast markers have been developed which are useful to distinguish grass species and to assess genetic diversity in grass species. These markers are available to the breeding community.
- A robust regeneration protocol for perennial ryegrass amenable for genetic engineering studies was developed.
- Evidence for horizontal gene transfer between perennial ryegrass and a rhizosphere fungus was found.

Opportunity / Benefit:

The project is of strategic nature. To date only very few mitochondrial genomes of monocot plant species have been sequenced. Phylogenetic studies at high taxonomic ranks can be conducted and structural rearrangements and sequence evolution to understand patterns and processes of molecular evolution of the mitochondrial genome in grasses can be studied. The project results will allow investigation of the interaction with, and susceptibility to, endophytic fungi of perennial ryegrass in connection to plant nuclear and mitochondrial gene transfer or horizontal gene transfer between the fungus and the plant. The most studied plant mitochondrial mutation causes cytoplasmic male sterility (CMS), a tool widely used in plant breeding which prevents the formation of viable pollen.

Collaborating Institutions:

National University of Ireland Maynooth
Trinity College Dublin

Contact Susanne Barth Email: susanne.barth@teagasc.ie.

Teagasc project team:
Dr. Susanne Barth (PI)
Dr. Kerstin Diekmann
Dr. Rob van den Bekerom

External collaborators:
Prof. Phil Dix, NUIM Maynooth
Prof. Trevor Hodkinson, TCD
Prof. Ken Wolfe, TCD

1. Project background:

Perennial ryegrass (*Lolium perenne*) is an important pasture grass species for the temperate regions of the world. It is especially important for Ireland since a vast majority of Irish agriculture depends on the productivity of this species. It is grown mainly in the United States, Europe, Australia and New Zealand as a forage crop and it is also an important turf grass. Perennial ryegrass belongs to the family Poaceae and is related to the other economically important and extensively studied crop species like wheat, rice and maize. Perennial ryegrass has a nuclear genome size of about 2300 Mbp which is distributed over seven haploid chromosomes. The organelle genomes of perennial ryegrass, chloroplast and mitochondria, have not been sequenced yet. These genomes are small in comparison to the nuclear genome (estimated size of chloroplast genome 150kbp; mitochondrial genome 400kbp), however they are both important in their biological functions. Modifications in both genomes are amenable to enhance productivity of grasslands by genetic engineering of the chloroplast genome and via the more directed exploitation of heterosis via cytoplasmic male sterile (cms) lines which are based on incompatibilities between the nuclear and the mitochondrial genome. The research is of a public good nature. In the long term the outcomes of this research will support sustainable agricultural production practices.

2. Questions addressed by the project:

The overall project objectives were to sequence the whole genome of the perennial ryegrass chloroplast and mitochondrial genomes. Questions addressed were
-How completely can the chloroplast and mitochondrial *Lolium perenne* genomes be assembled?
-How can the sequence information of both genomes be described and exploited?
-Can we better understand the molecular evolution of these genomes and is there evidence of horizontal gene transfer?
-How useful are the genomes for examining the molecular evolution of chloroplast and mitochondrial genes in comparison to other grasses?
-Can we develop a robust regeneration protocol for perennial ryegrass to facilitate the transformation of *Lolium perenne* chloroplasts?

3. The experimental studies:

The perennial ryegrass chloroplast genome was shotgun sequenced and assembled using the cultivar ‘Cashel’. It was necessary to develop a chloroplast DNA extraction protocol for grasses. Molecular markers from the chloroplast genome were developed and tested on a number of related grass species. The perennial ryegrass mitochondrial genome from cultivar ‘Shandon’ was shotgun sequenced and a high quality draft assembly was made. Evidence for horizontal gene transfer between *Glomus intraradices* and *Lolium perenne* was investigated. A robust regeneration protocol for perennial ryegrass was developed. RNA editing in the chloroplast genome of stressed and non-stressed plants from different genotypes was studied.

4. Main results:

A robust protocol to extract highly purified chloroplast DNA from grasses was developed. A complete chloroplast genome draft was obtained and molecular markers from this genome were developed and tested on *Lolium perenne* and related grass species. Several of the markers developed proved to be useful to distinguish *Lolium perenne* from *Lolium multiflorum* and other grass species and to assess genetic diversity in *Lolium*. The influence of stress on RNA editing in the chloroplast genome was conducted in several gentypes, genotype was found to be more influential on RNA editing than environmental stress (drought). A high quality draft assembly of the perennial ryegrass mitochondrial genome was made and evidence for horizontal gene transfer was found. The sequence information can be used now for a multitude of studies on the strategic and applied side of science.
A robust regeneration protocol for *Lolium perenne* was established and can be used in the future for genetic engineering of the chloroplast genome of *Lolium perenne*.

Contact Susanne Barth Email: susanne.barth@teagasc.ie.

5. Opportunity/Benefit:

The project is of strategic nature and will benefit mainly strategic research with a view to using the knowledge gained in plant breeding. The sequence information of the *Lolium perenne* chloroplast genome provides the opportunity to study the specific modes of gene expression, including cis- and trans splicing and RNA editing. The interaction with and susceptibility to endophytic fungi of perennial ryegrass particularly in connection to plant nuclear and mitochondrial gene transfer or horizontal gene transfer between the fungus and the plant can now be investigated. Phylogenetic studies at high taxonomic ranks can be conducted and structural rearrangements and sequence evolution to understand patterns and processes of molecular evolution of the mitochondrial genome in grasses can be studied. The project also provides the bioinformatics basis for the construction of chloroplast transformation vectors to use plants as cell factories for the production of recombinant proteins. The expression of severe and effective changes in phenotypes can be a result of the high recombination rate in the mitochondrial genome. The most studied plant mitochondrial mutation causes cytoplasmic male sterility (CMS), which prevents the formation of viable pollen. CMS is a valuable and economic tool to create directed heterosis (hybrid vigor) and to reproduce identicle genetic line constitutions.

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, at Open Days in Oak Park and at Food Harvest in Athenry in 2008.

Main publications:

Diekmann K, Hodkinson T.R. and Barth S. (2012) New chloroplast microsatellite markers prove to be suitable for assessing genetic diversity of *Lolium perenne* and other related grass species. *Annals of Botany* 110: 1327 – 1339 (DOI:10.1093/aob/mcs044).

Diekmann K., Hodkinson T.R., Wolfe K.H. and Barth S. (2012) First insights into the mitochondrial genome of perennial ryegrass (*Lolium perenne*) in '*Breeding Strategies for sustainable forage and turf grass improvement*' (Barth S and Milbourne D eds) Springer, Dordrecht, 2012 p 141 - 146 (ISBN 978-94-007-4554-4) (DOI 10.1007/978-94-007-4555-1)

Diekmann K., Hodkinson T.R., Wolfe K.H., van den Bekerom R., Dix P.J., and Barth S. (2010) The complete chloroplast genome sequence of perennial ryegrass (*Lolium perenne* L.) reveals useful polymorphisms among European ecotypes. In: *Sustainable use of genetic diversity in forage and turf breeding* (Huyghe C ed), 409-413, Springer, Berlin (DOI: 10.1007/978-90-481-8706-5_59).

Diekmann K., Hodkinson T.R., Wolfe K.H., Bekerom R.v.d., Dix P.J. and Barth, S. (2009) Complete chloroplast genome sequence of a major allogamous forage grass species, perennial ryegrass (*Lolium perenne* L.), *DNA Research* 16(3): 165-176.

Diekmann K., Hodkinson T.R., Fricke E., and Barth S. (2008) An optimized Chloroplast DNA Extraction Protocol for Grasses (Poaceae) Proves suitable for Whole Plastid Genome Sequencing and SNP Detection. *PloS One* 3(7): e2813. DOI:10.1371/journal.pone.0002813.

7. Compiled by: Dr. Susanne Barth

Contact Susanne Barth Email: susanne.barth@teagasc.ie.