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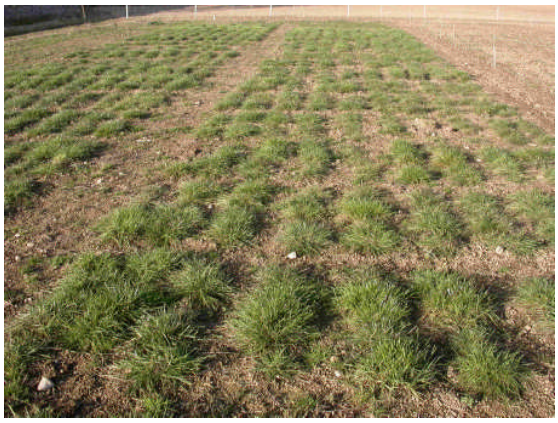
Exploring the extent of genetic diversity in Irish and European *Lolium* accessions



Key external stakeholders:

Grass breeders, seed producers, policy makers in genetic resources management, general public

Practical implications for stakeholders:

The project has found

- Irish *Lolium* accessions with high water soluble carbohydrate content which could be incorporated in breeding programmes.
- Novel molecular markers which grouped genepools of Irish and European *Lolium* accessions, information which is useful for breeding.
- Rachis length has been proven to be a good predictor of seed yield.

Main results:

A subset of the Teagasc Irish *Lolium perenne* genetic resources collection was characterised with a range of techniques. Morphological measurements of a number of agronomically important traits were carried out (heading date, seed yield components, biomass yield components) and water soluble carbohydrates (WSC), protein contents and dry matter at different time points during the growth season were measured. Several novel chloroplast markers were developed. These markers and other markers have been used to group the material into gene pools. The project resulted in a well characterized subset of the Irish *Lolium perenne* genetic resources collection.

Opportunity / Benefit:

The characterization of plant genetic resources is necessary to make these resources available for use in pre-breeding and breeding and also to create an inventory of phenotypes and genotypes for future uses.

Collaborating Institutions:

Trinity College Dublin

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Teagasc project team: Dr. Susanne Barth (PI)
Dr. Andreas Froehlich (WSC, proteins)
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1. Project background:

Lolium perenne L. is a strict outbreeder with a strong two locus controlled self-incompatibility system. Therefore, each individual plant of an ecotype/cultivar has a heterogenous genotype. Genetic diversity can be created either by generation of novel alleles by mutation or by recombination of existing alleles. Plant breeding uses recombination of existing alleles for the creation of more promising cultivars. In nature a broad range of alleles exists which has been greatly reduced in elite breeding material during the selection process. Rare wild alleles can contribute to the improvement of a variety for quality and disease resistance traits. Understanding the number of alleles existing in natural wild populations and populations of cultivars is thus very desirable.

The markers used in breeding programmes according to the standards of the International Union for the protection of new varieties of plants (UPOV) for the distinction of cultivars are still morphological markers. However, with the selection for homogenous populations it is getting more difficult to distinguish cultivars. While isozyme markers were added to the above standards, their application is restricted by their small number. Further valuable morphological markers can be the quality components of cultivars, e.g. their water soluble carbohydrate content, which is a very important trait in improving cultivars for improved feeding value. Additionally molecular markers offer an opportunity to investigate genetic diversity in more detail. This project used existing codominant nuclear microsatellite (SSR) markers and developed novel chloroplast microsatellite markers (cpSSR) to study genetic diversity in Irish and European *Lolium* accessions.

No publications on the genetic diversity of ecotypes from Ireland and the British Isles have been reported in the literature. However, a prerequisite to successfully exploiting the potential of ecotypes for a breeding programme knowledge of the diversity is required.

2. Questions addressed by the project:

(1) Estimate the level of genetic diversity between and among ecotypes of *Lolium perenne* originating in Ireland using molecular markers and group them in a European context.

(2) Collect and use morphological/phenological and geographic data on Irish and European accessions for further characterisation and correlation with molecular data.

(3) Carry out quality measurements in *Lolium perenne* material from distinct gene pools.

3. The experimental studies:

The Irish ecotypes were selected from the Teagasc Oak Park collection holding 419 *L. perenne* accessions collected from old Irish pasture ecosystems Accessions were grown from seed and planted as spaced plants in the field in Oak Park (Lime field) in 2003 and 2004. For water soluble carbohydrate (WSC) analysis samples were harvested over five time points during the growth season in 2004 from May to October. WSC were analysed using HPLC and protein contents were assessed using LECO. Novel cpSSR markers were developed (McGrath *et al*. 2006) and were used to estimate the extent of genetic diversity in the cytoplasm in Irish and non Irish *Lolium* accessions. Nuclear SSR markers were employed to study the extent of genetic diversity in the nuclear genome of *Lolium perenne* accessions. Morphological characters were recorded in the field and samples taken into the lab for detailed analysis of seed yield characters.

4. Main results:

All the analysis found high levels of diversity in the Irish *Lolium perenne* collection and most variation was found within populations, a reflection of the allogamous breeding system of *L. perenne.* A new set of

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chloroplast SSR markers was developed and the data collected with these markers showed extremely high variation and were able to successfully determine cytoplasmic genepools of *Lolium* for breeding purposes. They were also able to distinguish Irish ecotypes from European ecotypes via principle components analysis (PCA), UPGMA dendrograms and in analyses of molecular variance (AMOVA). The results support a hypothetical migration route of *L. perenne* from the Near East, across the Mediterranean and north to Britain and Ireland. In vegetative and reproductive morphological characters, most variation (e.g. 31% for glume length in ecotypes) was partitioned within populations rather than among populations indicating high levels of inter-populational gene flow. Morphological characters were also able to separate ecotypes from cultivars via PCA and UPGMA dendrograms. Strong relationships were seen between reproductive characters such as between rachis length and spikelets per spike, florets per spikelet and glume length; Based on regression modeling the use of rachis length as a predictor of reproductive performance was suggested. Individual ecotypes showed exceptional values in biochemical characters such as water soluble carbohydrate (WSC) and the values oscillated widely and were closely correlated to cutting time. Most variation was attributed to environmental factors and less was attributed to genotypic differences. Some Irish genotypes showed potential to be used in future breeding programmes for elevated WSC content. Nuclear SSR markers were also applied to the collection of ecotypes and varieties. The results showed the majority of populations deviated from Hardy Weinberg equilibrium, and had excesses of homozygotes. Levels of linkage disequilibrium between pairs of nuclear SSR markers were low, indicating that the markers can be used in association studies to determine if any of them have associations with phenotypic traits analyzed in this study.

5. Opportunity/Benefit:

The characterization of plant genetic resources is necessary to make these resources available for use in pre-breeding and breeding and also to create an inventory of phenotypes and genotypes for future use. Only ~ 1/10th of the Oak Park *Lolium perenne* collection was characterized, but it gives an indication of its diversity and potential value.

6. Dissemination:

The project resulted in a number of scientific publications and a popular scientific publication. The project was also presented to visitor groups, at Open Days in Oak Park and as part of a genetic resources event organised by DAFM in 2008.

Main publications:

McGrath S., Hodkinson T.R., Charles M.T., Zen D. and Barth S. (2010) 'Variation in inflorescence characters and inflorescence development in ecotypes and cultivars of *Lolium perenne* L.' *Grass and Forage Science* 65: 398 - 409

McGrath S., Hodkinson T.R. and Barth, S. (2007) 'Extremely high cytoplasmic diversity in natural and breeding populations of *Lolium* (Poaceae).' *Heredity* 99:531 - 544

McGrath S., Hodkinson T.R., Salamin N. and Barth S. (2006) 'Development and testing of novel chloroplast microsatellite markers for *Lolium perenne* and other grasses (Poaceae) from *de novo* sequencing and *in silico* sequences.' *Molecular Ecology Notes* 6: 449 - 452

Bolaric S., Barth S., Melchinger A.E. and Posselt U.K. (2005c): 'Molecular characterisation of genetic diversity in European germplasm of perennial ryegrass.' *Euphytica* 146: 39 - 44

Bolaric S., Barth S., Melchinger A.E. and Posselt U.K. (2005b) 'Molecular genetic diversity within and among German ecotypes in comparison to European perennial ryegrass cultivars.' *Plant Breeding* 124: 257 - 262

Bolaric, S., Barth, S., Melchinger, A.E. & Posselt, U.K. (2005a) 'Genetic diversity in European perennial ryegrass cultivars investigated with RAPD markers.' *Plant Breeding* 124: 161-166

Popular publications:

Barth S., McGrath S., Fröhlich A. and Hodkinson T.R. (2007) 'The importance of being diverse. Why is diversity in a plant species important?' *TResearch* 2 (4), 22 - 23

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