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Monitoring of changes in pastoral swards under selection over time

Key external stakeholders:
Plant geneticists, agronomists, grass breeders, general public

Practical implications for stakeholders:
The outcome of this project is of strategic importance. The primary stakeholders include the Teagasc perennial ryegrass breeder, agronomists working in grasslands science and ecologists.

- Pastoral production systems are profoundly important to the Irish economy. In pastoral production systems, swards are grazed intensively, and sward productivity declines over time. The speed with which this decline occurs is described as the persistency of varieties.
- Breeding grasses with good persistency, a slow decline in productivity over time, is a key goal for perennial ryegrass breeders.
- The central question to be addressed during this fellowship was whether changes in the population structure of perennial ryegrass varieties subjected to grazing can be tracked using novel, cutting-edge genome-wide marker technologies to monitor shifts in allele frequency in these populations. If so this would provide a method to measure the impact of the environment on populations which have very little observable differences in phenotypes amongst individuals of these populations.

Main results:
The experiments were based on one artificial seed mixture experiment in the glasshouse and on two sets of established field experiments.

- Seeded mixtures experiment (glasshouse): Overall, each seeded mixture could clearly be identified with K-means clustering, even mixtures of 96.5% of one variety mixed with 3.5% of a second variety could be deciphered from each other.
- In both field experiments very high SNP coverage could be obtained (in excess of 20 000 SNP). However for both field experiments more variation was observed within technical replicates than between blocks, even amongst the original seed lot samples.
- For the two field experiments discriminant analyses using multidimensional scaling failed to show any clear patterns of variation. This means that in artificial mixtures under glasshouse conditions the molecular fingerprinting worked at high precision, but not under field experimental conditions.

Opportunity / Benefit:
The project is of strategic nature. The project devises on the precision of fingerprinting techniques in seeded mixtures and provides baseline data for further development of biometric approaches in variety assessment with a focus on molecular fingerprinting techniques. The project also devises a sampling method to collect representative samples from field experiments for genetic and ecological studies.

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1. Project background:

Unlike varieties in many crops in which all plants of a variety are nearly genetically identical individual perennial ryegrass cultivars are effectively genetically divergent populations of individuals that have been selected to have broadly similar characteristics. In addition as opposed to annual crops perennial ryegrass cultivars are expected to remain in the field over a near decadal period before re-seeding. Once consequence of these factors is that once planted the composition of perennial ryegrass swards can change over time in response to environmental variation and management practice.

2. Questions addressed by the project:

The overall project objectives were to investigate if with ultra-high resolution genetic fingerprinting techniques and available bioinformatics pipelines changes in the genetic composition of grass populations under selection as in differently treated swards could be found. Specific questions were:
-What are the optimal sample collection strategies to obtain a representative sample of a sward?
-Can we distinguish changes in allele frequencies amongst perennial ryegrass varieties in artificial mixtures?
-Can we transfer these methodologies to field studies?

3. The experimental studies:

Seeded mixtures: A total of six diploid perennial ryegrass breeding lines were used to produce seven different mixtures. Four technical replicates were made per mix for a total of 52 samples. Before the second leaf was out (after around 14 days), the resulting lawn was cut back to 3 cm using a cutting guide, and the cuttings discarded, 1 cm of leaves was collected from the whole pot. 2) Seeding rate experiment: Four commercial varieties were sown in spring 2013 in plots of 5 x 1.5 m in Teagasc Moorepark Research Centre, Fermoy, Co. Cork. The study consisted of a randomised block design, with three different seeding rates (High: 37 kg/ha, Medium: 22 kg/ha and Low: 7.5 kg/ha) and four replicates per treatment. Plots were mechanically cut nine times a year during the growing season using a Haldrup grass harvester. The sampling strategy consisted of throwing a transect of 1m at random on the plot and to collect a handful of grass every 10 cm along it. This procedure was repeated 3 times per plot, corresponding to 4 technical replicates per plot. In total, 16 samples/variety/seeding rate (4 technical replicates x 4 blocks) were collected. In parallel, original seed batches that were used in the experiment were sown in pots. One gram of seeds was sown in 11 x 11 cm pots, and four replicates per variety were sown. Two weeks later, leaf blades were cut at the same height and approximately 1 cm was collected. 3) Animal grazing experiment: A mix of 59 varieties were sown on plots of 8 x 3 m following a randomised block design with 3 replicates in Teagasc Moorepark Research Centre, Fermoy, Co. Cork. The varieties are a mix of both recommended and candidates for recommendation by DAFM and they were sown at a rate of 37 kg/ha. The experiment was grazed nine times a year between March and November. From this experiment, eight varieties were collected. The sampling strategy was the same as per above experiment, to obtain 12 samples/variety (4 technical replicates per plot x 3 blocks). Original seed batches were sown and collected following the same method as per above experiment to obtain four technical replicates. We have used next generation sequencing technologies to compare allele frequencies in these seed populations.

4. Main results:

Experiment 1 (Seeded mixtures): More than 372 million reads were generated and more than 90% of them passed filters. Between 1.5 and 7.2 million reads were found per individual. After read alignment and filtering, a total dataset with 30,747 putative SNPs was identified. Multidimensional scaling and K-means clustering gave the same clustering of individuals with both Euclidean and Nei distance matrices. Overall, each mixture could clearly be identified with K-means clustering, even mixture 1 (96.5% of variety A and 3.5% of variety B) could be deciphered from family A. The more the percentage of B in mixtures 1 to 4, the

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closest the replicates are from family B with multidimensional scaling.

Experiment 2 (Seeding rate): After read alignment and filtering, a wealth of genetic diversity was found amongst varieties ranging between 32,777 and 40,805 SNP.

Experiment 3 (Animal grazing): After read alignment and filtering, 20,960 putative SNPs were discovered for the diploid samples, and 24,425 putative SNPs for the tetraploid samples. More variation was observed within technical replicates than between blocks, even among the original seed lot samples.

For both field experiments after evaluation of the genetic composition with biometric methods using discriminant analyses with multidimensional scaling we did not find any clear patterns of variation between treatments. In both field experiments more variation was observed within technical replicates than between blocks and also between treatments. Also the application of further biometric tests (MDS for different read depths between 5 and 30) did not improve the distinctness of the different treatments based on allele frequencies. Also Pearson correlations between the median of the replicates of the original seed lot and the different treatments showed no significant differences for each variety.

5. Opportunity/Benefit:

This project constitutes the first demonstration of the use of genome-wide marker technology to track allelic frequency shifts in perennial ryegrass swards over time. This is significant because if this method would work under field conditions the impact of the environment on plan populations could be accurately measured.

It was also the starting point of a new approach for negative selection against productivity-limiting alleles that could be embedded into future Genomic Selection (GS) based approaches for perennial ryegrass improvement. After the initial experiment 1, we were quite hopeful with the discriminating power of the technique, as every mixture could be identified with Kmeans clustering. However, both field experiments carried out failed to show any pattern in the changes. More changes were detected within replicates than between treatments or blocks, and this was consistent for each variety for both experiments, irrespective of the different treatments. It is possible that the raw data generated in these experiments can provide discrimination of changes in allele frequencies over time also in field samples if biometrics approaches for population data improve over time. Questions to be addressed in the future are: (1) Was there no selection over time period tested, (2) was there selection for random genotypes under field conditions, (3) were the molecular technique or the biometrics approaches insufficient to allow for a detailed resolution of alleles amongst swards?

6. Dissemination: n/a

7. Main publications:

Aude Perdereau presented a poster at the Monogram Meeting in Cambridge in March 2016 and a poster the Eucarpia General Congress in Zurich in September 2017.

Popular publications: n/a

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