Status report of AU activities in the Robusta project in 2017

Potato drought experiment

The aim of this study was to conduct a potato time-course drought experiment to study the epigenetic changes during drought.

Plant material and experimental design

The two contrasting drought tolerance potato varieties, Desiree and Folva, were used for the study. The experiment was performed in a greenhouse under controlled conditions. Flower Power sensors were used to monitor and collect data on soil moisture, sunlight, temperature, and fertilizer. Potato tubers were layed in soil in 10 liter pots the 10th of April, and the drought stress treatment was started June 6th, 2017.

Leaves for DNA isolation for epigenetic analysis were collected at the start of the drought experiment and every second day for a three week period. Figure 1 shows the effect of the drought stress after 21 days of drought.

Figure 1. Folva drought stress experiment. Plants 1, 3, and 5 are control plants and the plants 2, 4, and 6 are drought stressed plants.

A total of 96 samples were selected for further analysis. DNA was isolated using the CTAB method and the DNA concentration was normalized for all samples. Two samples were initially selected for a pilot study aimed at identifying the optimal enzyme for epigenetic analysis using three different enzyme combinations. Sequencing libraries were constructed and samples were sequenced on the Illumina platform as 150bp paired-end sequences. The stats of this work is that we have received the sequencing data from the sequencing

provider and the data analysis is ongoing at the moment. Following the identification of the optimal enzyme combination all 96 samples from the potato drought experiment will be subjected to epigenetic analysis.

Status of barley experiments

A total of 64 barley lines in eight replicates are included in the barley experiment at the RadiMax facility during the 2017 growth season. A total of 600 barley samples were collected from four replicates (control and drought stressed flag leaves) in the beginning of July, 2017. This was a very laborious process that took three days for four persons.

Sample processing

Samples were snap frozen in liquid nitrogen and homogenized. The homogenized samples were divided in two and used for DNA and RNA isolation, respectively. DNA was isolated from all 600 samples while RNA was isolated form 150 samples (one replicate; drought and control). The 150 RNA samples have been sent for RNA sequencing and we are waiting for the sequencing data to arrive.

Two barley samples were initially selected for a pilot study aimed at identifying the optimal enzyme for epigenetic analysis using three different enzyme combinations. Sequencing libraries were constructed and samples were sequenced on the Illumina platform as 150bp paired-end sequences and the optimal enzyme combination for epigenetic analysis were identified. Subsequently, a total of 150 DNA samples (one replicate; drought and control) were prepared for epigenetic analysis. The samples were sequenced as 150bp paired-end sequencing and the analysis of the 150 epigenetic samples is ongoing at the moment.

Status of ryegrass experiments

A total of 300 ryegrass populations are included in the ryegras experiment at the RadiMax facility during the 2017 growth season. A total of 1200 ryegras samples were collected from four replicates (control and drought stressed flag leaves) before the third cut in August, 2017. This was a very laborious process that took four days for four persons.

Sample processing

Samples were snap frozen in liquid nitrogen and homogenized. The homogenized samples were divided in two and used for DNA and RNA isolation, respectively. DNA was isolated from all 1200 samples. Due to funding limitations it is only possible to perform RNA sequencing of 150 samples (one replicate; drought and control). We will use contrasting populations for root depth, however, the identification of the samples has been delayed because the phenotyping results that has to be provided by Copenhagen University is delayed.

Two ryegrass samples were initially selected for a pilot study aimed at identifying the optimal enzyme for epigenetic analysis using three different enzyme combinations. Sequencing libraries were constructed and samples were sequenced on the Illumina platform as 150bp paired-end sequences and the optimal enzyme combination for epigenetic analysis were identified (Figure 2). The graph aims to explain how the sequencing

power is distributed over the contigs. As can be seen from the figure the expected minimum coverage per contig for one on the combinations does not drop as steeply as it does for the other 2 enzyme combinations.

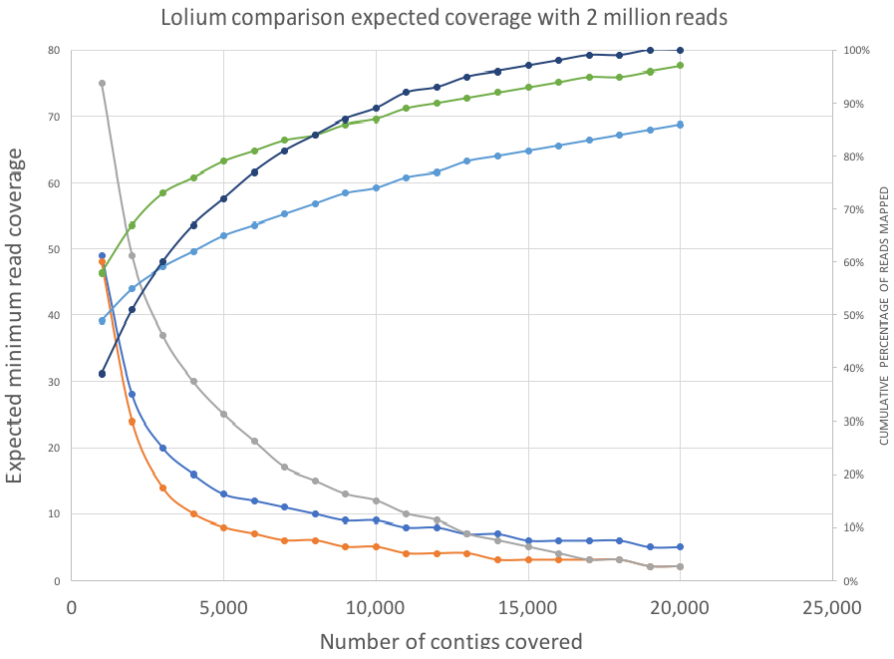

Figure 2. Identification of the optimal enzyme combination for epigenetic analysis in ryegrass.

Subsequently, a total of 600 DNA samples (one replicate; drought and control) were prepared for epigenetic analysis. The library construction is currently ongoing and will be completed by the end of the year.

Milestones achieved in 2017

- Sample collection of 600 barley samples from the RadiMax facility
- Sample collection of 1200 ryegrass samples from the RadiMax facility
- Sample processing (grinding etc.) of 1800 ryegrass and barley samples
- DNA isolation from 1200 barley and ryegrass samples
- RNA isolation and sequencing of 150 barley samples
- Epigenetic library construction and sequencing of 150 barley samples
- Epigenetic pilot studies aimed at identifying the optimal enzyme combination for epigenetic analysis in ryegrass, potato and barley
- Potato time-course drought experiment completed
- Identification of the optimal enzyme combination for epigenetic analysis for barley
- Identification of the optimal enzyme combination for epigenetic analysis for potato
- Identification of the optimal enzyme combination for epigenetic analysis for ryegrass