Whealbi

Wheat and barley Legacy for Breeding Improvement

Grant agreement number: FP7-613556

Collaborative Project
SEVENTH FRAMEWORK PROGRAMME

Deliverable D1.1
1st report on DNA extractions of wheat and barley accessions (512 of each) provided for the project

Due date: M6

Actual submission date: M11

Project start date: January 1st, 2014 **Duration:** 60 months

Workpackage concerned: 1

Concerned workpackage leader: IPK

Dissemination level: PU

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Summary

Objectives
Work package 1 is providing the Plant Material for DNA extraction (D1.1) and Genotyping as well as for sowing the common garden experiments for phenotyping at different environments (D1.2). This work package will also deliver seeds for subsequent analyses in different WP as an output of the garden experiments (D1.3).

Rationale:
Seeds of all accessions were to be propagated at the site of the respective contributors (WHEALBI partner) from stocks that were produced by 2 generations of single seed descent (SSD) to ensure for sufficient homogeneity of the accessions.
The practice of propagation was dependent on the established routines at the respective partner sites. IPK served as the central hub for collecting aliquots of multiplied seeds for sending to JHI for DNA extraction and the different garden sites for planting. JHI is preparing DNA from all accessions to be distributed to PTP and TGAC for performing ExCap experiments for all barley or wheat accessions, respectively

Teams involved:
IPK, JHI, PTP,TGAC

DNAs extraction of >500 wheat and >500 barley accessions

1.1. Seeds and germination

A small number of seeds (about 5) were received from IPK in January 2014. Single seeds were planted in trays under controlled conditions in a heated glasshouse at the James Hutton Institute.

Two to three cm of leaf material was harvested from a single germinating seed for DNA extraction using Qiagen DNeasy plant mini kit.

1.2. DNA extraction

DNA was extracted following the manufacturer's instructions with several modifications. Firstly leaf material was ground using Eppendorf pestles in the initial buffer (AP1) and secondly DNA was eluted in 70 μl and this was used to re-elute a second time providing a total volume of approximately 70 μl. DNA was gel quantified against standard lambda DNA and most DNAs were between 50ng to 100 ng/ μl. This procedure was carried out for the selected 512 wheat and barley accessions (see attachment).

1.3. Aliquots sent for exome capture – initial testing

Barley

DNA (40 μl) μl aliquots of 8 barley accessions were sent to PTP in March 2014 for initial standardising of protocols for exome capture. DNA was quantified using TapeStation in order to verify the gDNA integrity and yield (Table 1). Libraries were prepared, pooled, hybridised and sequenced according to the published protocols (Mascher et al. 2013 Plant J 76: 494-505).

Table 1

		Picogreen	Nanodrop	
	Accession	**ng/ul**	**260/280**	**260/230**
1	20	46,87	1,81	1,52
2	21	112,89	1,83	1,91
3	22	68,37	1,88	1,67
4	23	103,15	1,84	1,88
5	24	98,82	1,9	1,75
6	25	89,31	1,92	1,67
7	26	71,42	1,85	1,58
8	27	81,03	1,73	1,6

From the initial results a further set of 64 DNAs were sent to PTP and to date these have been successfully exome captured and sequenced. DNA from the remaining accessions has been sent to PTP, although there are a few issues with DNA concentration and 16 DNAs are being re-extracted.

Wheat

The wheat exome pilot study has been delayed for several reasons. During the Kick Off meeting we discussed which of the two available capture arrays to use for wheat (to date there is only one array for barley) and following considerable consultation we agreed to use the original capture array, which subsequently had to be purchased and tested at TGAC. Furthermore TGAC have been installing a new sequencer and this has delayed processing the wheat accession. However, we now have barcoded plates and the first plate of DNA for testing (comprising different ploidy wheats) will be delivered middle of December 2014.

Conclusion

DNAs have been provided for 500 wheat and 500 barley accessions and we are in the process of capturing and sequencing. D1.1 has been delayed but is accomplished now.

Attachments:
1) Final list of wheat and barley accessions