Genotyping-by-Sequencing of a set of diverse spring barley (*Hordeum vulgare*) accessions

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Research Objective

The aim of the work presented was to saturate a set of 192 spring barley accessions with a high density of SNP markers using Genotyping-by-Sequencing (GBS). The set of barley accessions from different origins represents a broad spectrum of the genetic diversity and exhibits low linkage disequilibrium making it useful for high-resolution association mapping studies. Indeed, the set of barley accessions has previously been genotyped with 45 SSR markers, 1536 Illumina GoldenGate markers, 1935 DArT markers and the 9k iSelect chip (Haseneyer et al. 2010, Plant Breeding 129:271-279; Pasam et al. 2012, BMC Plant Biology 12:16; Comadran et al. 2012, Nat Genet 44:1388-1392). However, a higher number of SNP markers is needed at a density that reflects genome-wide linkage disequilibrium structure and haplotype diversity.

Genotyping-by-Sequencing Experiment and Bioinformatics

The GBS experiment (Wendler et al. 2014, Plant Biotechnol J, in press) comprised the construction of a reduced-representation 192-plex library and its sequencing on an Illumina HiSeq 2000 (1 lane,1 x 100 cycles). Data obtained were analyzed using a reference-based approach (*Fig. 1*).

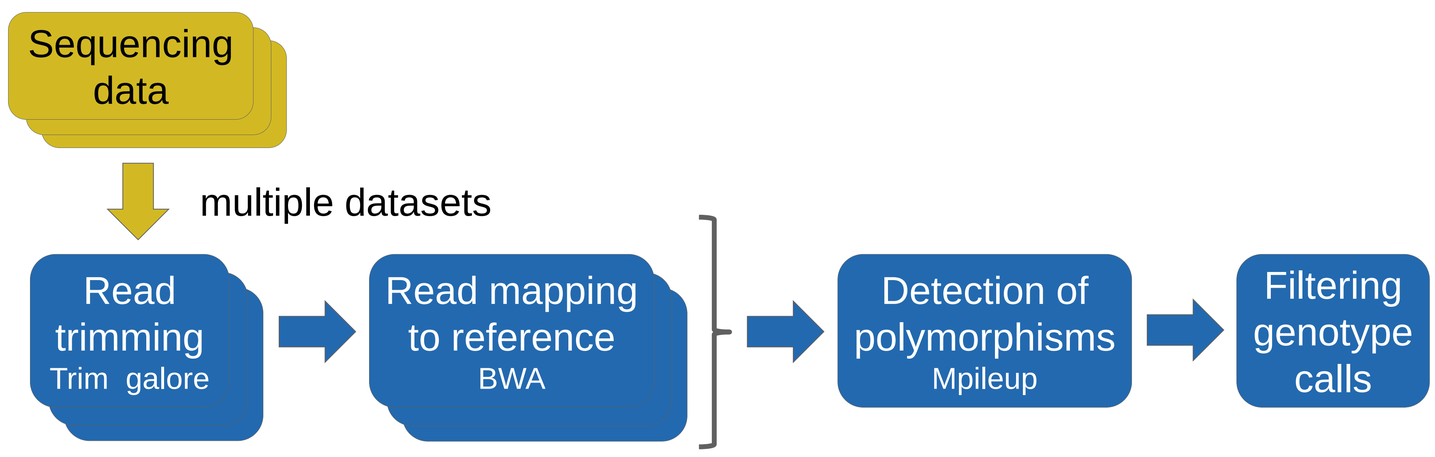

Figure 1
Reference-based computational pipeline for the analysis of 20GB of sequencing data and anchoring of SNPs according to:
1) Mascher et al. 2013, PLoS ONE 8(10): e76925. doi:10.1371/journal.pone.0076925.
2) Mascher et al. 2013, The Plant J 76, 718-727.

Results

The library sequenced was well balanced and sequencing data were of very good quality (*Fig. 2*). Using GBS, 7,439 bi-allelic, high-quality SNP markers could be generated. A high proportion (about 82%; *Fig. 3*) of the SNPs was anchored to the integrated physical and genetic map of barley (IBSC 2012, Nature 491:711-716). Based on the SNPs detected major structuring in the set of barley accessions was revealed reflecting row type (*Fig. 4*).

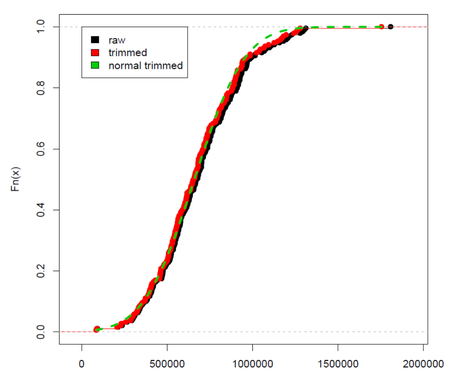

Figure 2
Cumulative distribution of the number of reads (x) per accession for raw (*black*) and trimmed (*red*) reads. Data is normally distributed (*green*).

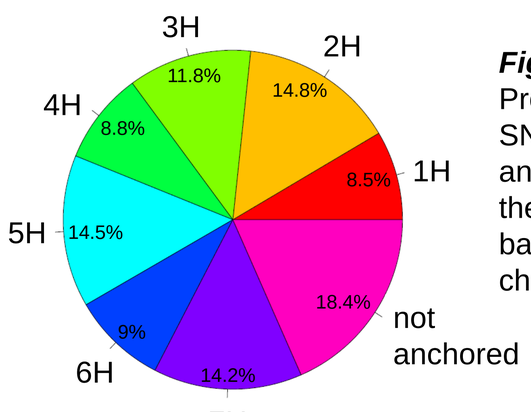

Figure 3
Proportions of SNPs anchored to the seven barley chromosomes.

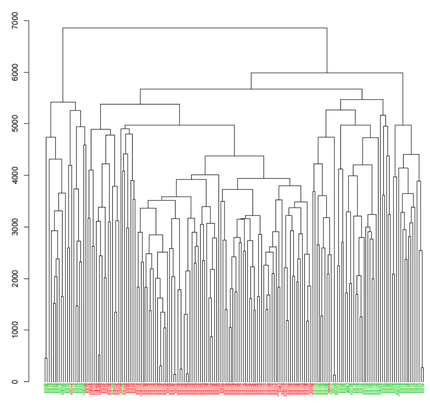

Figure 4
Dendrogram of a hierarchical clustering of the barley accessions with single-linkage clustering based on the 7,439 SNPs. Accessions are colored according to row type (*red*: two-rowed, *green*: six-rowed).

Outlook

It is expected that the saturation of the set of diverse barley accessions with a high density of GBS markers will improve whole-genome association mapping in terms of locating candidate genes. Furthermore, association mapping will provide markers linked to genes of interest that are targeted in barley breeding.

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