Cultivar-specific long-range chromosome assembly of a *Rhynchosporium* resistant barley landrace to identify the *Rrs1* resistance gene

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Introduction

Barley ranks forth in the worldwide production of cereal crops (faostat.fao.org). Therefore, specific breeding programs for healthy plants are highly demanded. The fungus *Rhynchosporium commune* causes barley scald, a devastating foliar disease that is distributed worldwide and propagates in cool humid climates. The fungus induces scald like lesions causing yield losses reaching up to 40%[1]. Major scald resistance locus *Rrs1* has been mapped repeatedly to the pericentromeric region of chromosome 3H and so far 11 different resistance alleles have been described[2]. Though genetic mapping efforts over decades the underlying resistance gene(s) of *Rrs1* are still unknown.

The barley reference genome has been recently improved[3], however its high amount of repetitive content and potential structural or copy number variations between different varieties make it difficult to pinpoint the underlying gene of important resistance loci, especially if the resistance donor is not the reference cultivar. Spanish barley landrace SBCC145 exhibits outstanding scald resistance[4] but is genetically divergent from reference sequence donor Morex[5] implying nucleotide and/or structural differences. We aim to generate a sequence resource for our resistance donor SBCC145 to identify the $Rrs1_{Rh4}$ resistance gene(s) against barley scald.

Figure 1: *Rhynchosporium commune* spores and typical scald lesions on barley in field (May 2017) and in greenhouse.

Methods

High molecular weight DNA of SBCC145 chromosome 3H was obtained by flow cytometry as described before[6]. A *de novo* assembly was generated using a paired end shotgun library and Meraculous v2.2.4[7] with a k-mer size of 79. To generate proximity ligation data for scaffolding, a Chicago library was prepared as described by Putnam et al. (2016). *De novo* assembly, shotgun and Chicago reads were used as input for HiRise pipeline to scaffold the genome (Fig.2).

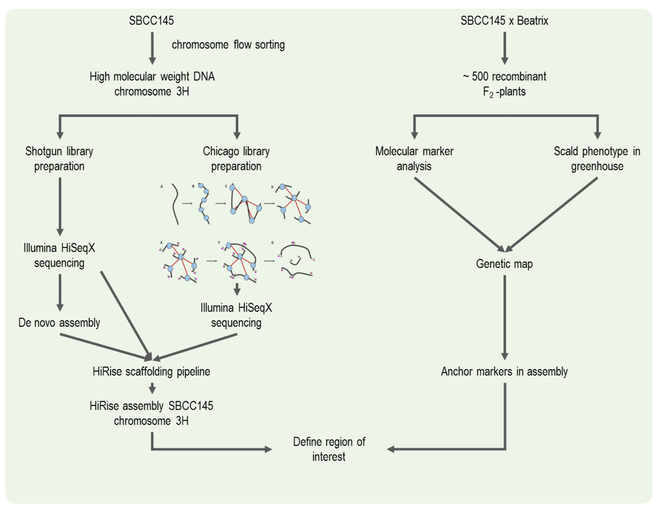

Figure 2: Workflow to identify the *Rrs1* region in SBCC145. Scheme of Chicago library preparation adapted from Putnam et al. 2016.

Scaffolds of the HiRise assembly were aligned to the barley reference sequence of chromosome 3H and visualized as dotplot with minimal cluster size of 5k employing the MUMmer software package version 4.0.0beta2 (https://github.com/mummer4/mummer) (Fig.4). Scaffolds harboring *Rrs1* marker sequences were identified by BLAT v36.x2[8] and sorted for best hits regarding score and % identity. Genetic maps were generated by JoinMap 4[9] based on marker genotype and phenotypic data of 478 F_2-recombinants (Fig.5) from crossing SBCC145 with susceptible spring barley Beatrix.

Alignment to Morex chromosome 3H

High collinearity with no or only small inversions or deletions was found between the SBCC145 3H HiRise assembly and Morex pseudomolecule 3H (Fig.4 A). The *Rrs1* region was verified in the assembly with high contiguity (Fig.4 B).

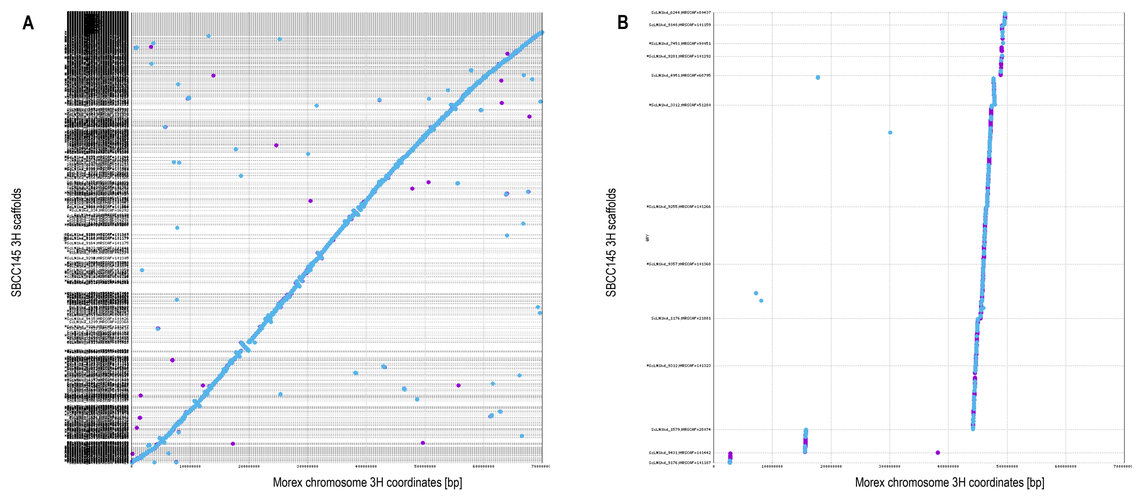

Figure 4: Alignment of SBCC145 3H HiRise assembly to Morex reference sequence.
Dotplot of the total (A) SBCC145 scaffolds or those harboring known *Rrs1* marker sequences (B) aligned to Morex chromosome 3H with a cluster size of 5k. Purple color indicates forward an blue color reverse alignments.

Assembly statistics

	SBCC145 3H *de novo*	SBCC145 3H HiRise	Morex 3H pseudomolecule
Total length [Mb]	438.48	447.38	699
L50	16,073	73	1
N50 [Mb]	0.007	1.603	-
L90	67,480	314	1
N90 [Mb]	0.002	0.288	-

Table 1: Assembly statistics of the SBCC145 3H *de novo* chromosome assembly before and after HiRise scaffolding in comparison to the barley reference sequence (Mascher et al. 2017, doi:10.5447/IPK/2016/34).

Rrs1 region genetic and physical map

Genetic mapping of 18 markers with Kosambi mapping function and LOD of 10 verified the order of published markers for *Rrs1*[4]. MLOC_X3 and AK_X1 are indicated as the closest flanking markers and respective sequences map to two different scaffolds in the SBCC145 3H HiRise assembly.

MLOC_X4 sequence is found in 12 kb distance to MLOC_X3 on scaffold 1 while HV_X_11 is located in 370 kb distance to AK_X1 on scaffold 2. Thus, the physical map rather suggests an inverted order of AK_X1 and MLOC_X4.

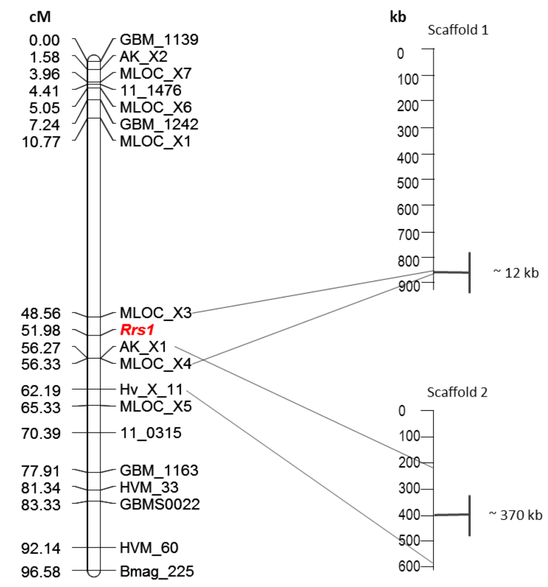

Figure 5: Map of *Rrs1* region on chromosome 3H and marker positions in the SBCC145 HiRise assembly. Genetic distances were derived from 478 F_2-recombinants. *Rrs1* itself was mapped as binary trait.

Gene completeness

To assess gene completeness of the assembly we combined SBCC145 3H assembly with the remaining six Morex pseudomolecules to a hybrid genome and employed Benchmarking Universal Single Copy Orthologs (BUSCO v3) with the embryophyta odb9 dataset[10].

BUSCO scores of the hybrid genome are comparable to that of the barley reference sequence (Fig.3) though 35 % deviation in sequence length (Tab.1).

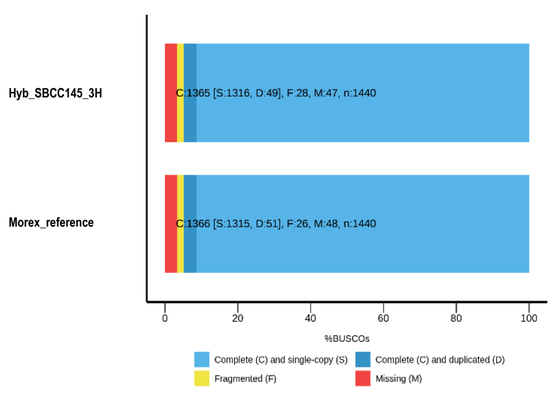

Figure 3: BUSCO assessment of gene completeness. Hyb_SBCC145_3H: SBCC145 chromosome 3H + Morex 1H to 2H + 4H to 7H. Morex_reference Morex chromosome 1H to 7H.

Summary/Outlook

For the highly scald resistant barley landrace SBCC145, we have generated a good quality sequence resource of chromosome 3H in order to identify the *Rrs1* resistance gene(s). Anchoring of marker gene sequences to the assembly narrows down the region of interest to two scaffolds. Thus, novel molecular markers are derived easily and investigation of all kinds of variations between resistant SBCC145 and reference genome of susceptible Morex is possible.

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