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The use of GBS-transcriptomics for mapping fertility restorers in onions

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Introduction

Hybrid varieties of onion (*Allium cepa* L.) are produced using cytoplasmic male-sterile (CMS) maternal lines. Reproduction of a CMS line requires a specific pollen donor – a maintainer line. With respect to the nuclear genome these lines (CMS and the respective maintainer) should be isogenic, but they carry different cytoplasms – sterilizing and normal, respectively. In breeding programs selection of maintaining genotypes may pose a problem due to the widespread occurrence of fertility restoring genes (*Rf* genes) that suppress the action of the sterilizing cytoplasm.

Plant material

In order to characterize such fertility restorers in onion we developed three populations – 107a, 107b and 441 – carrying the sterilizing cytoplasm and segregating into male-sterile and male-fertile plants. Each population resulted from crossing a male-sterile and a male-fertile plant – both belonging to the same segregating family.

Methods

For individual plants of these populations total cellular RNA was isolated from leaves (using commercial on-column RNA purification kits). The resulting RNA preparations were used for production of cDNA sequencing libraries that were subjected to PE150 Illumina sequencing [outsourced to Novogene (UK) Company Limited].

Sequencing statistics was calculated using proprietary scripts written by W. Wesołowski. Based on the pollen viability testing 10 most male-sterile and 10 most male-fertile plants were selected. The FASTQ files of the plants belonging to one group were merged using appropriate Bash commands. QTL identification was performed with the use of QTL-seq (Takagi et al. 2013) according to the procedure described on the GitHub page of this software*. As a reference sequence the assembly submitted by the Northwestern Polytechnical University was used (GCA_030765085.1). Due to the large size in this assembly each chromosome is divided into two or three parts. In these analyses as a parent substitute a single male-sterile plant was used. So far the QTL-seq analysis was performed only for population 441.

* https://github.com/YuSugihara/QTL-seq?tab=readme-ov-file#example-1--run-qtl-seq-from-fastq-without-trimming

Results

For each analyzed population ca. 700 M (640-770) sequencing reads were obtained in one direction (either forward or reverse) – this corresponded to ca. 100 B (95-115) of sequenced nucleotides. On average a single plant yielded approx. 4 M reads, which corresponded to 600 M nucleotides sequenced in one direction. For all analyzed populations the average Phred score was very high and exceeded 68. These populations were also similar with respect to their GC content, which ranged from 41,7 to 42,5 % (tab. 1).

Tab. 1. Statistical parameters of sequencing reads – population 441.

Parameter		Value
Summarized read length**		578 924 479,50
Read number**		3 859 496,5
Read length	min.	150
	max.	150
	average	150
% GC		42,5
N50		150
N95		150
% Q20		96,7
% Q30		91,4
Phred	min.	35,00
	max.	70,00
	average	68,5

** for reads in one direction – forward or reverse

In QTL-seq analysis altogether 101 sequence polymorphisms showed correlation with pollen viability (the measure for fertility restoration, statistical significance under the null hypothesis of no QTL: P < 0.01) - 5 indels and 96 SNPs. The were found in all chromosomes except chromosome 7. However, the majority of them were form chromosome 1 (66 polymorphisms) cumulating mostly in its first part (61) where they comprised a region of 671 Mb. For other chromosomes the number of sequence polymorphisms ranged from 1 (chromosome 6) to 10 (chromosome 5). These results indicate that the major gene controlling fertility restoration in population 441 is located in chromosome 1 (fig. 1).

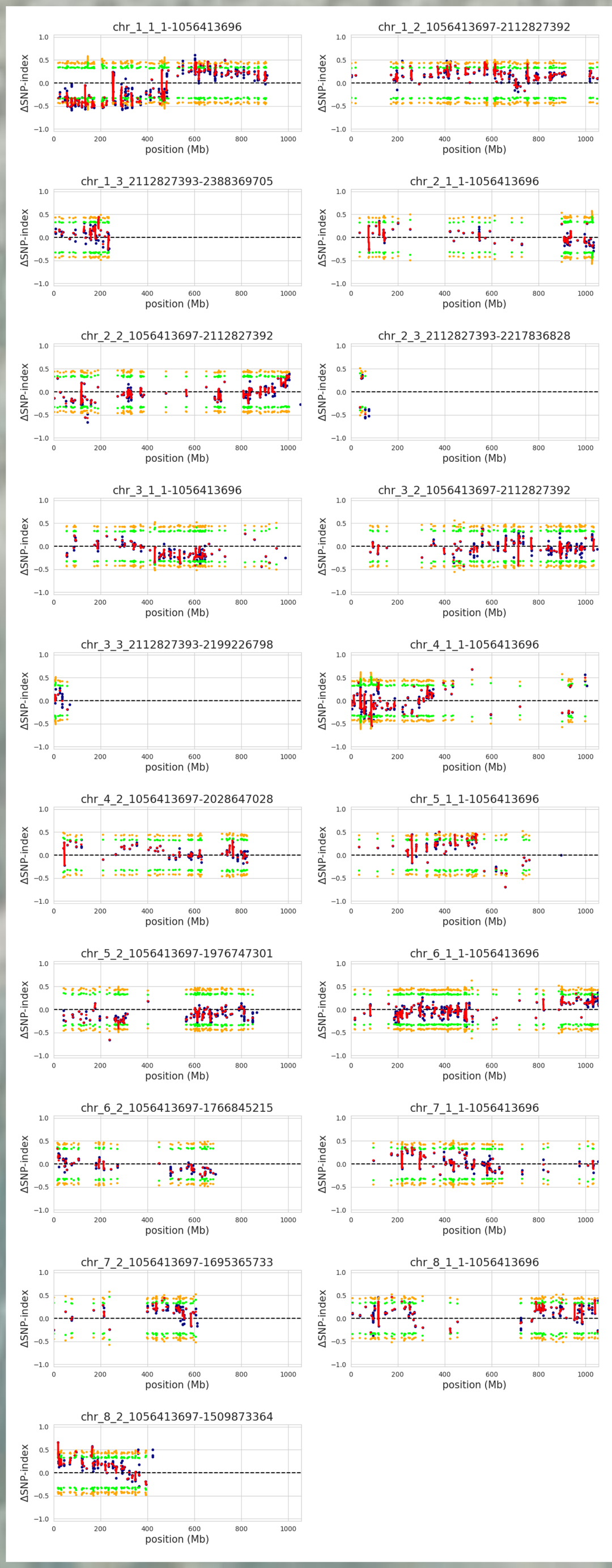

Fig. 1. Identification of genomic regions controlling fertility restoration (pollen viability) - ΔSNP index plot of divided onion chromosomes with statistical confidence interval under the null hypothesis of no QTLs (orange, P < 0.01; and green, P < 0.05).

Literature

Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C, Uemura A, Utsushi H, Tamiru M, Takuno S, Innan H, Cano LM, Kamoun S, Terauchi R (2013) QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J 74:174-183

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