

New aspects of fertility restoration in beets

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Introduction

In cultivated beets (*Beta vulgaris* L. subsp. *vulgaris*) cytoplasmic male sterility (CMS) is conditioned by the presence of the sterilizing Owen's cytoplasm (S-cytoplasm). Two nuclear genes are known to suppress its sterilizing effect - X (*Rf1*) from chromosome 3 and Z (*Rf2*) from chromosome 4..

Plant material

We analyzed two populations carrying the S-cytoplasm and segregating into male-sterile and male-fertile plants. According to our preliminary data in these populations - table beet 506 and sugar beet S04 786 - fertility restoration was caused by a gene from chromosome 3 and 4, respectively. The plant material was developed in KHBC Straszów, Poland (population S04 786) and Plantico Zielonki, Poland (population 506).

Methods

Individual plants from population S04 786 were subjected to NGS genotyping based on the PE150 Illumina sequencing. The obtained sequence reads were mapped to chromosome 4 using BWA (Durbin and Li 2009). The resulting SAM files were converted into the binary form (BAM files) using SAMtools (Li et al. 2009). This program was also used for sorting the BAM files and for preparing their indexes. The processed BAM files were analyzed with Platypus (Rimmer et al. 2014). The resulting VCF files contained information about all identified sequence polymorphisms which subsequently were subjected to the following filtration steps: elimination of low quality polymorphisms (VCFtools, Danecek et al. 2011), selection of bi-allelic polymorphisms (VCFtools), elimination of polymorphisms with more than five unidentified genotypes (custom Awk script, W. Wesołowski), selection of polymorphisms with 1 : 1 segregation (Chi2 test, p>0.7, MS Excel), elimination of polymorphisms spaced less than 10 kb (custom Awk script, W. Wesołowski). The filtered polymorphisms were used for linkage regression mapping with JoinMap (Van Ooijen 2006).

Individual plants from population 506 were genotyped with two DNA markers:

- Rf1-Morf - anchored in the *Rf1* locus,
- 3-363/TaqI - anchored in other location of chromosome 3.

Results

Population 506 was genotyped with two molecular markers - the first (Rf1-Morf) was anchored within the X (*Rf1*) locus and did not differentiate male-sterile and male-fertile plants. The second marker (3-363/TaqI) was from other location on chromosome 3 and it showed co-segregation (71,2 %) with the phenotype. These results indicate that on chromosome 3 in addition to X (*Rf1*) there is yet another fertility restoring gene.(Figs. 1 and 2).

Population S04 786 was subjected to NGS-based genotyping - thorough selection allowed identification of 495 sequence polymorphisms from chromosome 4. A high density linkage map of chromosome 4 was constructed with the use of these polymorphisms and phenotypic segregation. Based on the physical location of polymorphisms closely linked to the phenotypic locus several PPR protein-coding genes were selected as candidates for the Z (*Rf2*) restorer. (Fig. 3, Tabs. 2 and 2).

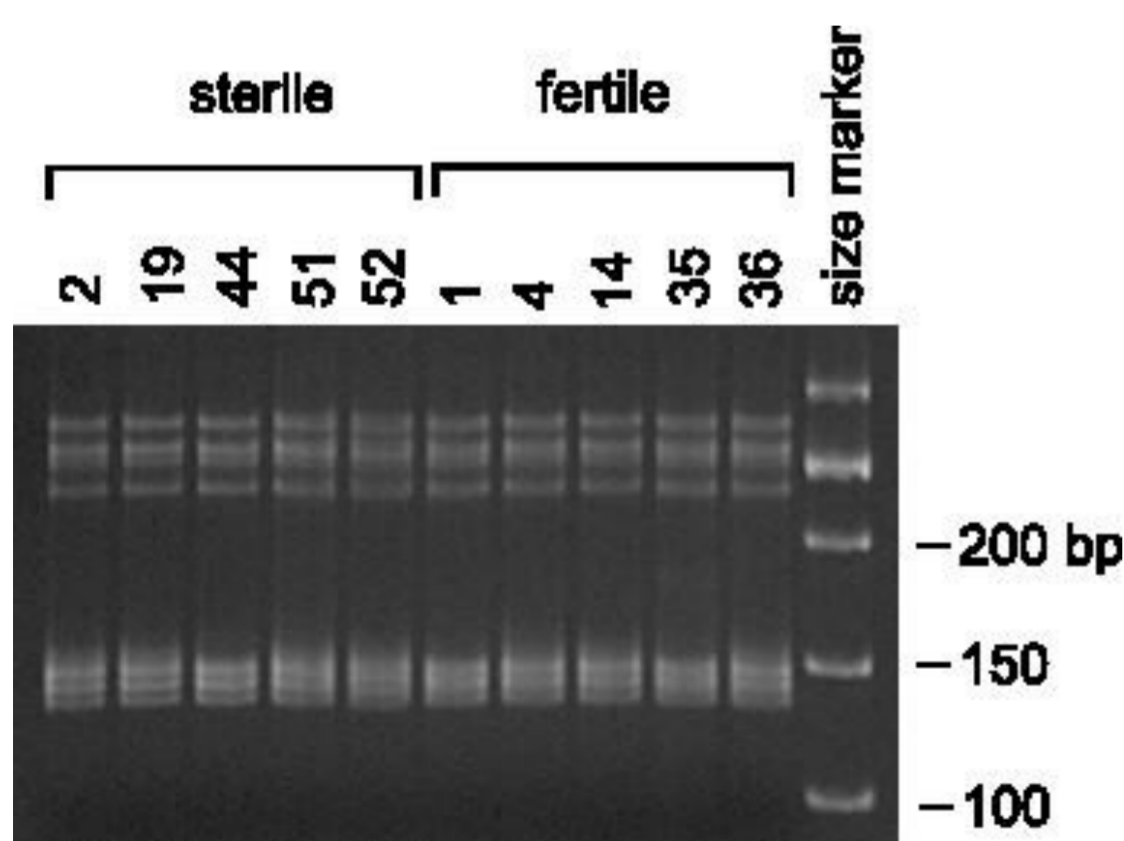


Fig. 1. Genotyping with marker Rf1-Morf for selected male-sterile and male-fertile plants from population 506.



Fig. 2. Genotyping with marker 3-363/TaqI for selected plants from population 506.

Tab. 1. Number of polymorphisms from chromosome 4 on different filtration steps for population S04 786.

| Filtration stage | No. of polymorphisms |
|---|----------------------|
| All polymorphisms generated by Platypus | 2 676 049 |
| High quality bi-allelic polymorphisms | 546 237 |
| Polymorphisms with at max. 5 unidentified genotypes | 150 406 |
| Polymorphisms with 1 : 1 segregation | 732 |
| Polymorphisms spaced with 10 kb | 495 |

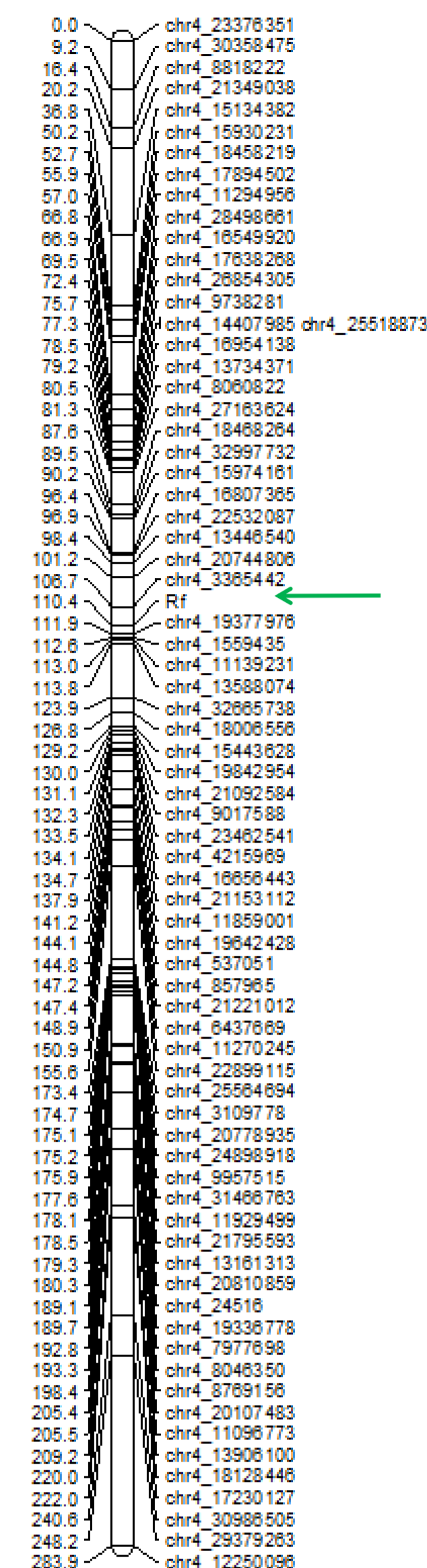


Fig. 3. Genetic map of sequence polymorphisms from chromosome 4 for population S04 786. Arrow - position of the *Rf* gene.

Tab. 2. PPR genes neighboring sequence polymorphisms linked to the restorer from population S04 786.

| Polymorphism | Polymorphism location [cM] | Nearest PPR gene | Distance from the nearest PPR gene [bp] |
|-------------------------|----------------------------|------------------|---|
| chr4_27163624 | 81,335 | LOC104891448 | 1950348 |
| chr4_18468264 | 87,607 | LOC104891215 | 610452 |
| chr4_32997732 | 89,53 | LOC104891881 | 578509 |
| chr4_15974161 | 90,169 | LOC104890958 | 2415859 |
| chr4_16807365 | 96,422 | LOC104891215 | 2271351 |
| chr4_22532087 | 96,901 | LOC104891273 | 1708765 |
| chr4_13446540 | 98,445 | LOC104890958 | 103982 |
| chr4_20744806 | 101,178 | LOC104891273 | 76317 |
| chr4_3365442 | 106,663 | LOC104890121 | 183884 |
| Phenotype (<i>Rf</i>) | 110,367 | - | - |
| chr4_19377976 | 111,902 | LOC104891235 | 184480 |
| chr4_1559435 | 112,607 | LOC104889967 | 81538 |
| chr4_11139231 | 112,986 | LOC104890819 | 118337 |
| chr4_13588074 | 113,819 | LOC104890958 | 29772 |
| chr4_32665738 | 123,907 | LOC104891881 | 246515 |
| chr4_18006556 | 126,75 | LOC104891215 | 1072160 |
| chr4_15443628 | 129,181 | LOC104890958 | 1885326 |
| chr4_19842954 | 129,957 | LOC104891235 | 278587 |
| chr4_21092584 | 131,079 | LOC104891273 | 269262 |

Literature

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