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Search for fertility restoring genes in carrots

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Introduction

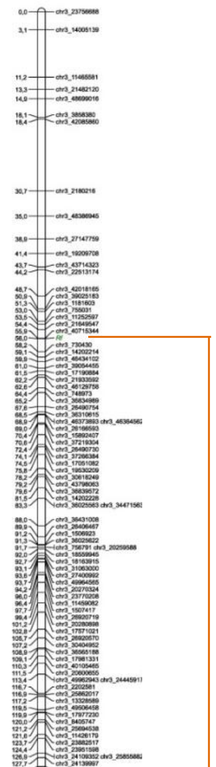
In cultivated carrots cytoplasmic male sterility (CMS) is induced by either the Sa- or Sp-cytoplasm. The former is responsible for the brown anther sterility, the latter causes CMS of the petaloid type. I was reported that mitochondria of the Sp-cytoplasm contained an abnormal *atp9* gene and that expression of this gene was associated with the male-sterile phenotype (Szklarczyk et al. 2014). The present work is aimed at identification of candidate fertility restorer (*Rf*) genes for both sterilizing cytoplasms in carrots.

Plant material

For the purpose of this study four mapping populations were developed – they all segregated into male-sterile and male-fertile (restored) plants. Two populations (536 and 538) carried the Sa-cytoplasm and two populations (170 and 510) – the Sp-cytoplasm. Each population resulted from a cross between a male-sterile and a male-fertile plant – both originating from the same CMS line characterized by occasional segregation of male-fertile plants.

Methods

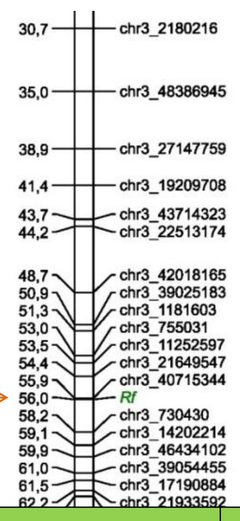
Individual plants from these populations were subjected to targeted genotyping by sequencing (tGBS) based on the PE150 Illumina sequencing. The obtained sequence reads were mapped to either chromosome 3 (populations 536 and 538) or chromosome 9 (populations 170 and 510) using BWA (Durbin and Li 2009). 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 (Chi^2 test, p>0.7, MS Excel), elimination of polymorphisms spaced less than 100 kb (custom Awk script, W. Wesołowski, optional). The filtrated polymorphisms were used for linkage regression mapping with JoinMap (Van Ooijen 2006).



Tab. 1. Number of polymorphisms from chromosome 3 on different filtration steps for population 538.

Filtration stage	No. of polymorphisms
All polymorphisms generated by Platypus	2 851 996
High quality bi-allelic polymorphisms	755 170
Polymorphisms with at max. 5 unidentified genotypes	202 495
Polymorphisms with 1 : 1 segregation	249

Fig. 1. Genetic map of sequence polymorphisms from chromosome 3 for population 538 (S_a-cytoplasm). Arrow – position of the *Rf* gene.



Polymorphism	Polymorphism location [cM]	Nearest PPR gene	Distance from the nearest PPR gene [bp]
chr3_43714323	43.746	LOC108215037	212 750
chr3_22513174	44.194	LOC108211424	46 304
chr3_42018165	48.709	LOC108214534	332 978
chr3_39025183	50.931	LOC108211887	830 449
chr3_1181603	51.322	LOC108214244	14 676
chr3_755031	52.987	LOC108214244	441 248
chr3_11252597	53.458	LOC108213741	1 342 403
chr3_21649547	54.383	LOC108211424	909 931
chr3_40715344	55.896	LOC108211887	856 535
Phenotype (restorer)	55.953	–	–
chr3_730430	58.230	LOC108214244	465 849
chr3_14202214	59.122	LOC108214336	1 164 796
chr3_46434102	59.940	LOC108213200	131 378
chr3_39054455	60.963	LOC108211887	801 177
chr3_17190884	61.543	LOC108215332	273 133
chr3_21933592	62.207	LOC108211424	625 886
chr3_46129758	62.605	LOC108214467	161 453
chr3_748973	64.446	LOC108214244	447 306
chr3_36834989	65.237	LOC108210383	605 680

Tab. 2. PPR genes neighboring sequence polymorphisms linked to the restorer from population 538.

Results

Typically for a single plant approx. 40 M sequence reads were generated which allowed for massive identification of sequence polymorphisms from chromosomes 3 (populations with the Sa-cytoplasm) and 9 (populations with the Sp-cytoplasm). These chromosomes were chosen based on preliminary linkage data produced earlier with the use of conventional GBS. Prior to linkage mapping the identified polymorphism were subjected to thorough filtration (Tab. 1). The created linkage maps of chromosome 3 had 169/185 loci – including the restorer locus – with the density of 0.7 loci/cM (Fig. 1). The *Rf* locus was also present in the maps obtained for chromosome 9, they contained 97/159 loci with the density of 1.5/1.4 loci/cM. Since physical location of the mapped polymorphisms was known, for each of them it was possible to pick the nearest PPR gene. Therefore, for each analyzed population it was possible to select PPR genes corresponding to DNA polymorphisms most tightly linked to the *Rf* locus (Tab. 2). In the further analyses these PPR genes will serve as candidate restorer genes.

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