Molecular identification of the causal locus for the petaloid phenotype in *Daucus carota*

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Homeotic alteration phenotype of the flowers in *Daucus carota* are widely used for hybrid breeding, consequently molecular markers tightly-linked to such phenotype are in demand. Here we report the identification of a gene locus responsible for the phenotypic expression of stamen conversion into a petal-like structure, or petaloid. Using a segregating population and sequencing analysis of two bulked populations, we discovered a large contributing peak on the long arm of chromosome 4. *DcMADS2*, a homolog of the B-class floral homeotic gene *PISTILLATA*, found at the center of the peak region, was considered the strongest candidate causal gene. We established PCR primers that could be used to distinguish the two *DcMADS2* alleles linked to each petaloid- and non-petaloid-phenotype.

**Key Words:** carrot, male sterility, petaloid, QTLseq, *DcMADS2*.
PCR Restriction Fragment Length Polymorphism (PCR-RFLP) analysis

A pair of PCR primers (5′-GGGATATTACAGGCTCTCAGCAATG-3′/5′-TTAGGCTGCAAAGGCAAAGG3′) were designed for the PCR amplification of the region surrounding the DraI site on position 22,416,901 of chromosome 4. Tks Gflex DNA Polymerase (TaKaRa) with the following PCR routine: 94°C 1 min; (98°C 10 sec, 55°C 15 sec, 68°C 30 sec) × 35 cycles; 68°C 5 min, was used to amplify the PCR fragment. Five μl of the PCR product was digested by 0.2 μl of DraI (15 U/μl, TaKaRa) with incubation at 37°C for two hours. PCR products were electrophoresed on a 1% agarose gel.

Results and Discussion

We obtained ca. 417 million and ca. 536 million reads from the F and MS bulks via the deep-sequencing runs, respectively. Mean coverage depths of the carrot genome were 132.90 and 169.05 for the F and MS bulks, respectively. Detailed statistics for the sequencing results are presented in Table 1. The total number of SNPs called were 6,836,994 for the F bulk, and 6,629,607 for the MS bulk (Table 1). From the QTLseq analysis, we detected the strongest contributing peak on the long arm of chromosome 4, at approximately the 17.50 to 27.75 mega-base-pair (mbp) position on the pseudomolecule (Fig. 1B, 1C). The window in the center of the peak harbored SNPs between the 22.25 and 22.75 mbp position on the pseudomolecule of chromosome 4 (Fig. 1C).

We hypothesized that a causal gene for the petaloid phenotype is related to the B-class floral homeotic gene. To identify such a candidate, we used the protein sequences of 1,296 gene models within this 17.50 to 27.75 mbp region for BLAST searches against the Arabidopsis thaliana proteins. As a result, we found a gene (LNRQ01000004.1_prot_KZM98268.1_1702 in the Daucus carota genome assembly PRJNA268187) with a homology against A. thaliana AT5G20240, or the PISTILATA gene (Piwarzyk et al. 2007).

The genomic region of this gene spanned from 22,416,479 to 22,417,257 bp region of the pseudochromosome, which is approximately at around the center of this QTL region, meeting the criteria of a suitable candidate. This gene was named as DcMADS2 by a previous study, and was found to be down-regulated in the carpeloid CMS flower (Linke et al. 2003). It is possible that DcMADS2 is the causal gene for the petaloid phenotype investigated in this study.

We searched manually for a SNP by assessing changes in the restriction enzyme digestion patterns within the DcMADS2 genic region. We found a DraI site on position

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total reads</th>
<th>Mapped reads</th>
<th>Mean depth</th>
<th>Number of SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>417,577,082</td>
<td>368,311,807</td>
<td>132.9</td>
<td>6,836,994</td>
</tr>
<tr>
<td>MS</td>
<td>536,062,242</td>
<td>468,086,107</td>
<td>169.05</td>
<td>6,629,607</td>
</tr>
</tbody>
</table>


**QTLseq analysis**

Single nucleotide polymorphisms (SNPs) shared between the two populations were selected using a custom perl script. SNPs with a coverage depth between 75 to 225 were used for further analysis. We used the G’ score based statistical framework proposed in a previous study (Magwene et al. 2011) to detect QTLs. G’ is a modified version of G statistic scores that calculates the SNP segregation distortion between the two segregant bulks. The window size for the G’ calculation was set at 500 kilo-base-pair (kbp), and the distance between the adjacent window was set at 250 kbp. The G’ score was calculated using a custom perl script. Series of scripts used for this workflow are deposited in Open Science Framework (https://osf.io). Manhattan plots were created using the R software (R Core Team 2017).
QTLseq analysis of the petaloid phenotype in carrots

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Literature Cited


22,416,901 bp of chromosome 4 in the MS bulk, whereas a series of SNPs were found on this site in the F bulk. To enable detection of PCR-RFLP, we designed PCR primers that could amplify the DNA fragment surrounding this site. As expected, Dral-treatment yielded complete digestion of the PCR fragments from an MS individual, whereas a mixture of digested and undigested bands from an F individual was detected (Fig. 2).

In this study we found the QTL responsible for the carrot petaloid phenotype analyzed in this study. It is possible that this QTL is related to the petaloid previously reported as the phenotype of CMS. However, the locus we identified is distinct from the fertility restorer locus previously reported on chromosome 9 (Alessandro et al. 2013). Breeding programs should benefit from the DNA marker established in this study. Future studies may aim to clarify the role of DcMADS2 in the expression of the petaloid phenotype.

Fig. 2. The PCR-RFLP analysis of DcMADS2. M indicates a 100-bp-sized DNA ladder marker. F: fertile individual. MS: male-sterile individual. Restriction enzyme Dral was used to digest the PCR product.