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The effect of copy number variation of fatty acid synthesis genes on Brassica oil profiles

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Background:

One method to expand the gene pool of rapeseed is to resynthesise *B. napus* (2n = AACC) by interspecific crosses between the diploid progenitor species B. rapa (2n = AA) and B. oleracea (2n = CC). Meiotic crossovers between A- and C-genome chromosomes produce gametes heterozygous for non-reciprocal translocations (chromosome segments with A -> C or C -> A replacements), so-called "duplication-deletion" events. These duplications and deletions are subsequently established in populations via self-pollination, resulting in fixed copy number variation (CNV). Some important adaptive and agronomic characteristics, such as grain yield, frost tolerance, and flowering time, associated with CNVs. However, there is to date no study of the effect of CNV on fatty acid profiles in Brassica.

Objective:

This research aims to explore the impact of genomic structural variation and varying copy numbers of fatty acid genes or gene regulators, as well as complete or partial deletions of these genes or gene regulators, on oil profiles in resynthesised rapeseed.

Methods:

We identified key genes involved in lipid biosynthesis in Arabidopsis and their homologs in Brassica species. The copy number of these genes in *B. napus* was determined and identified to range from 1 to 13 copies. The exact location of different genes in the reference genome and also in relation to existing genotype (Illumina Infinium 60K SNP array) data of available plant material (resynthesized rapeseed lines) was identified to check if these genes were located in CNV regions. Two groups of 36 plants derived from individual (allelically homozygous) parent plants putatively heterozygous for CNVs involving fatty acid biosynthesis genes were grown out in order to assess any differences in oil profiles and gene expression between individuals in progeny sets.

Results:

Seven genes were present in CNV regions. Of these, GPAT7, with copies on chromosomes A10 and C09, was selected as the best potential candidate for future functional and oil profile analysis. Among 129 resynthesized lines, two genotypes (A9C47 and A4C47) had a single copy of a chromosome region on A10 where the GPAT7 gene copy was located, and no detectable corresponding extra copy of the C9 region containing the GPAT7 gene. Therefore, these genotypes were expected to produce self-pollinated progeny segregating for 0, 1 or 2 copies of this region, such that the effect on the oil profile of having a different number of copies of GPAT7 can be studied. Primer pairs for amplifying Bna.GPAT7.A10 and Bna.GPAT7.C09 were designed to assess presence of each gene copy. After 30 days of flowering, leaf tissue and developing seeds were collected to extract RNA to detect GPAT7 gene expression. Gene expression and mature seed oil profile analyses are ongoing.

Conclusions:

The effect of copy number variation of fatty acid biosynthesis genes has never been investigated in Brassica. Resynthesised rapeseed lines show frequent, segregating CNVs and hence may comprise an interesting system to investigate these effects. Work is in progress to determine the effect of different copy numbers of GPAT7, a fatty acid biosynthesis gene, on oil profiles in resynthesized rapeseed.