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Effects of specific organs on seed oil accumulation in *Brassica napus* L.

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ABSTRACT

Seed oil content is an important agricultural characteristic in rapeseed breeding. Genetic analysis shows that the mother plant and the embryo play critical roles in regulating seed oil accumulation. However, the overwhelming majority of previous studies have focused on oil synthesis in the developing seed of rapeseed. In this study, to elucidate the roles of reproductive organs on oil accumulation, silique, ovule, and embryo from three rapeseed lines with high oil content (zy036, 6F313, and 61616) were cultured *in vitro*. The results suggest that zy036 silique wall, 6F313 seed coat, and 61616 embryo have positive impacts on the seed oil accumulation. In zy036, our previous studies show that high photosynthetic activity of the silique wall contributes to seed oil accumulation (Hua et al., 2012). Herein, by transcriptome sequencing and sucrose detection, we found that sugar transport in 6F313 seed coat might regulate the efficiency of oil synthesis by controlling sugar concentration in ovules. In 61616 embryos, high oil accumulation efficiency was partly induced by the elevated expression of fatty-acid biosynthesis-related genes. Our investigations show three organ-specific mechanisms regulating oil synthesis in rapeseed. This study provides new insights into the factors affecting seed oil accumulation in rapeseed and other oil crops.

1. Introduction

Vegetable seed oils, which exist mainly as triacylglycerol, are important commodities worldwide in the food, animal agriculture, and chemical industries, and represent a renewable energy source [1–3]. Global consumption of vegetable oils has increased by >50% in the past decade, while the prices of these products have doubled [4]. Therefore, improving oil production is now widely acknowledged as an important goal of oil-crop breeding [5]. Because rapeseed (*Brassica napus* L.) is the third largest source of vegetable oil (global production of rapeseed oil was 23.91 million tons during 2012–2013), there is great scientific interest in the mechanisms regulating its oil synthesis and seed oil content [5].

Seed oil content is a complicated quantitative trait under the control of three genetic effects (embryonic, cytoplasmic, and maternal) plus interactions between the genotype and environment [6,7]. During the past decade, the growth in knowledge regarding lipid biosynthetic pathways in embryos has accelerated our understanding of seed oil biosynthesis and bioaccumulation, especially in the model plant *Arabidopsis thaliana* [8–10]. In addition to the generally accepted fatty acid and triacylglycerol biosynthetic pathways [11,12], the transcriptional regulatory network that controls *Arabidopsis* seed development plays an important role in the regulation of oil synthesis [13]. In this network, the transcriptional activator LEAFY COTYLEDON 1 (LEC1) and the downstream partner WRINKLED1 have been implicated in the control of genes that encode enzymes involved in plastidial glycolysis and fatty acid biosynthesis [14–16]. The vast majority of these studies have centered on oil synthesis in developing embryos.

Genetic-based studies have shown that maternal effects play a critical role in controlling seed oil content in flax (*Linum* spp.) [17], soybean (*Glycine max*) [18], and *Arabidopsis* [19]. However, additional studies concerning the underlying mechanisms of these effects are essential to fully understand oil synthesis regulation. In rapeseed, oil content is controlled mainly by heritable maternal and cytoplasmic genetic effects [7]. The seed oil content in F1 hybrid rapeseed is primarily associated with the maternal parent [20]. Photosynthesis in the silique wall has also been found to influence the final seed oil content in the context of the maternal genotype [21]. In developing rapeseed seeds, plastidial, mitochondrial, and

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cytosolic pathways contribute to fatty acid biosynthesis [22–24]. Despite the progress that has been made in understanding some aspects of oil synthesis, we still know relatively little about how lipid synthesis is regulated and how oil accumulates in and outside of seeds, and this lack of understanding hinders the development of high-oil (HO) lines [5,25].

In vitro plant tissue culture, which allows for tissue uniformity and environmental control, is an excellent system for investigating tissue development. Ovary and ovule culture for embryo rescue has enabled interspecific *Brassica* hybrids and intergeneric hybrids of *B. napus* and *Sinapis alba* to overcome incongruity barriers [26–30]. *In vitro* culture was also used to compare metabolic fluxes and enzyme activities in embryos under different genetic conditions [31]. For this study, we assessed the genetic diversity and population structure of 93 rapeseed lines with varying oil content and cultured *in vitro* the silique, ovule, and embryo of three HO lines zy036, 6F313, and 61616. By comparing oil levels as well as the transcriptional profiles in the cultured seed and embryo, we identified the primary pathways affecting oil synthesis in different organs.

2. Materials and methods

2.1. Plant materials and growth conditions

In September of 2010, a total of 93 rapeseed lines were planted in field in the city of Wuhan. Eighteen of the 93 rapeseed lines, including three HO lines (zy036, 61616, and 6F313) and two low oil (LO) lines (51070 and 93275), were planted repeatedly in the same field in 2011 and 2012. Six plants of each line were used for the detection of oil content.

2.2. Leaf cutting and carpopodium girdling experiments

At the beginning of blooming stage, leaves (including the sessile leaves) were cut alternately from the bottom up along the entire height of plant. In the girdling experiment, the carpopodium girdling process was conducted based on the method introduced by Hua et al. [21]. Six plants were included in this experiment for every rapeseed line.

2.3. Population structure analysis

The 96-plex OPA (oligonucleotide pool assay) was used in this study which consisted of 42 A-genome SNPs and 54 C-genome SNPs that were distributed evenly genome-wide [32]. POPGENE 1.32 was used to analyze major allele frequency, genetic diversity, and polymorphism information content of the 93 rapeseed lines [33]. The similarity matrix was subjected to cluster analysis using the unweighted pair-group method with the arithmetic-average algorithm in NTSYSSpc version 2.10e [34].

2.4. Rapeseed in vitro tissue culture conditions

To determine the suitable sucrose concentration for rapeseed tissue culture, siliques from seven developmental stages (10, 14, 18, 22, 26, 30, and 34 d after flowering [daf]) were sampled from 6F313 line grown in the field. Sucrose contents in silique walls and ovules were measured according to the method reported by Roe [35].

For *in vitro* culture, young siliques of 10 d (silique culture), 15 d (ovule culture), and 20 d (embryo culture) after pollination were sampled. The fresh pods were disinfected as follows: a 30-s wash in a 75% (v/v) alcohol solution, a 3-min rinse in sterile water, a 12-min wash with shaking in a 0.1% (w/v) $HgCl_2$ solution, three rinses in sterile water for 5 min, and then the pods were blotted dry with sterile filter paper.

The pod culture followed the procedure described by Musgrave et al. [30]. To summarize briefly, a small piece of the bottom carpopodium was removed and four siliques were placed in flat-bottom test tubes containing 10 ml of liquid 1/2 Murashige and Skoog medium (MS) (PhytoTechnology Laboratory, Shawnee Mission, KS, USA) with different sucrose concentrations (w/v). The test tubes were incubated under a 12-h artificial light (24 °C; photon flux density, 100–120 μmol/m^2 s)/12-h dark (22 °C) cycle. To ensure nutritional adequacy, every 5 d the medium was replaced with fresh MS and a small piece of the bottom carpopodium was removed. Pods were cultured for 14 d before harvest.

For ovule culture, ovules with funicles were carefully dissected from sterile pods and placed in culture plates with 2 ml of liquid Gamborg B5 medium (PhytoTechnology Laboratory) containing different sucrose concentrations (w/v). The plates were incubated under a 12-h artificial light (24 °C; photon flux density, 50–60 μmol/m^2 s)/12-h dark (22 °C) cycle. To ensure nutritional adequacy, every 5 d medium was replaced. Ovules were cultured for 14 d before harvest.

For embryo culture, embryos (~3.0 mm in diameter) at early stage of oil accumulation were dissected from ovules of 20 daf rapeseed pods and transferred into solid 1/2 MS (0.8% agar, w/v) containing different sucrose concentrations. The plates were sealed with Nescofilm and incubated under a 12-h artificial light (24 °C; photon flux density, 20–30 μmol/m^2 s)/12-h dark (22 °C) cycle. After a 14-d incubation, the embryos were harvested. All full-cycle experiments for *in vitro* culture were repeated at least four times.

2.5. Detection of oil content in seed and embryo

Siliques, ovules, and embryos harvested from plants grown in the field and from *in vitro* cultures were dried for 3 d at 40 °C. The seed and embryo oil contents were determined by nuclear magnetic resonance spectroscopy using an NMR PQ001 system (Niumag, ShangHai, China). The seed mass range used for this study was 0.1–1.2 g

2.6. Total RNA extraction and reverse transcription reaction

Total RNAs from seed coats of 6F313 and 51070 at 20 daf and the 5 d cultured embryos of zy036, 6F313, and 61616 (with 6% sucrose) were extracted using the Plant Mini RNeasy kit (Qiagen, Düsseldorf, Germany). The reverse transcription reaction was performed using the First Strand cDNA Synthesis Kit for RT-PCR (Takara, Dalian, China).

2.7. Transcriptome sequencing of seed coat mRNA from 6F313 and 51070

The trancriptomes of total seed coat were sequenced using the Illumina HiSeqTM 2000 platform (the Beijing Genomics Institute, Shenzhen, China). Gene expression levels were calculated using the reads per kilobase per million (RPKM) method [36]. Rapeseed genes were annotated as orthologs with those from *B. oleracea*, *B. rapa*, and *Arabidopsis*. To screen the differentially expressed genes (DEGs), we tested *P*-values corresponding to differential gene expression based on the method introduced by Audic and Claverie [37]. Corrections for false positives (Type I errors) and false negatives (Type II errors) were performed using the FDR method [38]. Finally, we chose FDR ≤ 0.001 and the absolute value of Log2Ratio ≥ 1 as the threshold to judge the significance of gene expression difference in the seed coats of 6F313 and 51070. The DEGs were submitted to the website of