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Coordinate changes in gene expression and triacylglycerol composition in the developing seeds of oilseed rape (*Brassica napus*) and turnip rape (*Brassica rapa*)

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ABSTRACT

Crop production for vegetable oil in the northern latitudes utilises oilseed rape (*Brassica napus* subsp. *oleifera*) and turnip rape (*B. rapa* subsp. *oleifera*), having similar oil compositions. The oil consists mostly of triacylglycerols, which are synthesised during seed development. In this study, we characterised the oil composition and the expression levels of genes involved in triacylglycerol biosynthesis in the developing seeds in optimal, low temperature (15 °C) and short day (12-h day length) conditions. Gene expression levels of several genes were altered during seed development. Low temperature and short day treatments increased the level of 9,12,15-octadecatrienoic acid (18:3*n*−3) in turnip rape and short day treatment decreased the total oil content in both species. This study gives a novel view on seed oil biosynthesis under different growth conditions, bringing together gene expression levels of the triacylglycerol biosynthesis pathway and oil composition over a time series in two related oilseed species.

1. Introduction

Two oilseed species of *Brassica* are cultivated in the northern climate regions of the world: oilseed rape (OR, *Brassica napus* L. subsp. *oleifera*) for its higher yield and turnip rape (TR, *Brassica rapa* L. subsp. *oleifera*) for its shorter growing period necessary in cold climate where the summer is short. *B. napus* (AACC, $2n = 38$) is an allopolyploid crossing between *B. rapa* (AA, $2n = 20$) and *Brassica oleracea* (CC, $2n = 18$). The oil composition of the two oilseed species is highly similar and their oils are typically mixed and sold as one product (rapeseed oil). Rapeseed oil contains a nutritionally optimal omega-6/omega-3 fatty acid ratio and very little saturated fatty acids. Typically, the most abundant fatty acid is 9-octadecenoic acid (18:1*n*–9, 60%), followed by 9,12-octadecadienoic acid (18:2*n*–6, 20%) and 9,12,15-octadecatrienoic acid (18:3*n*–3, 10%) (Gunstone, Harwood, & Dijkstra, 2007).

The most abundant component in rapeseed oil is the mixture of triacylglycerols (TAGs). Their synthesis is divided between plastid, cytosol and endoplasmic reticulum (ER) following the eukaryotic lipid synthesis pathway (Bates, Stymne, & Ohlrogge, 2013). TAGs are synthesised from acyl-CoA and *sn*-glycerol-3-phosphate *via* the Kennedy pathway (Kennedy, 1961) or *via* phosphatidylcholine in ER. The key enzymes of the Kennedy pathway include *sn*-glycerol-3-phosphate acyltransferase (GPAT), which adds the first fatty acid chain in the *sn*-1 position of the glycerol backbone, lysophosphatidic acid acyltransferase (LPAAT), which adds the second chain in *sn*-2, phosphatidate phosphatase (PAP), which removes the orthophosphate yielding diacylglycerol (DAG), and finally diacylglycerol acyltransferase (DGAT), which adds the third chain in *sn*-3 (Kennedy, 1961). In an alternative route, cholinephosphotransferase (CPT) converts DAG into phosphatidylcholine and phospholipid:diacylglycerol acyltransferase (PDAT) converts it further into TAG. According to recent results, an additional enzyme (phosphatidylcholine:diacylglycerol cholinephosphotransferase, PDCT) has a function in connecting phosphatidylcholine with diacylglycerol in *Arabidopsis* (Lu, Xin, Ren, Miquel, & Browse, 2009). Fatty acid desaturation occurs through three enzymes starting at plastid with Δ^9-desaturase using acyl-acyl carrier protein as a substrate, and further at ER using phosphatidylcholine as substrate with fatty acid desaturases 2 (FAD2) and 3 (FAD3), which

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desaturate fatty acids in phosphatidylcholine. Other plant desaturases exist, but do not function in TAG biosynthesis. Two enzymes transfer fatty acids between the acyl-CoA pool and phosphatidylcholine during TAG biosynthesis, namely lysophosphatidylcholine acyltransferase (LPCAT) transferring fatty acids into phosphatidylcholine and phospholipase A_2 (PLA_2) removing fatty acids from phosphatidylcholine into the acyl-CoA pool.

Several of the TAG biosynthesis pathway genes have been characterised, but in some cases the function of the different homologues is unknown (Bates et al., 2013). There are nine genes encoding a protein with GPAT function in plants, but the GPAT involved in the Kennedy pathway remains uncertain, although it may be *GPAT9* (Bates et al., 2013). There are four genes for LPAAT in *Brassica* sp. and three genes for DGAT in plants, but the exact functions of the isoenzymes in TAG biosynthesis are unknown, while PAP has several candidate genes, but none of them have been proven to function in the Kennedy pathway (Bates et al., 2013). The genes for Δ^9-desaturase, FAD2, FAD3, PDAT1, and two isoenzymes of LPCAT are known.

Historically, rapeseed oil contained a high amount of 13-docosenoic acid (erucic acid, 22:1*n*–9), making it unsuitable for human consumption, although having important non-food uses. Low erucic acid rape was achieved through plant breeding and the contemporary rapeseed oil contains only traces of erucic acid, while the bulk is 9-octadecenoic acid (18:1*n*–9). The 3-ketoacyl-CoA synthase (KCS) elongates fatty acids to 20 and 22 carbons, producing erucic acid in the high erucic acid cultivars. Mutations in the fatty acid elongase 1 (*FAE1*)/*KCS18* gene are responsible for the low erucic acid trait (Puyaubert et al., 2005).

Environmental stresses of different kinds commonly alter biosynthetic pathways of plants. Abiotic stresses may include for example low or high temperature, availability of nutrients and water, or availability of light. Many of the aforementioned kinds of stresses can influence the fatty acid composition of plants (Upchurch, 2008). Sometimes the altered fatty acid composition may be beneficial to human nutrition, for example through an increased amount of polyunsaturated fatty acids.

Until now, there has been a lack of studies that would combine targeted examination of gene expression, oil composition and different growth conditions together during seed development in oilseed *Brassicas*. We hypothesised that gene expression levels of the studied genes will change during seed development and that TAG composition will be affected by abiotic stresses. The plants were grown in controlled growth chambers, where they were exposed to low temperature (15 °C, 16-h day length), and to short day conditions (20 °C, 12-h day length). The seeds of these plants were compared to plants grown in two different optimal temperature (22 or 20 °C) and day length (16 h or 16–19 h) conditions. Samples were collected from developing seeds starting 2 weeks after flowering (WAF). Gene expression levels, fatty acid composition and TAG composition was followed in a time series experiment in all the different conditions. Gene expression levels were found to be affected by seed development and some coexpressed genes were detected. Low temperature and short day length stress increased the amount of polyunsaturated fatty acids.

2. Materials and methods

2.1. Plant materials

Certified seed of OR (*B. napus* subsp. *oleifera* L.) 'Marie' (a self-pollinating doubled haploid summer cultivar) was obtained from Boreal Plant Breeding Ltd., Jokioinen, Finland and certified seed of TR (*B. rapa* subsp. *oleifera* L.) 'SW Petita' (a cross-pollinating population cultivar of summer type) from K-maatalous Experimental Farm, Hämeenlinna, Finland. The seeds were sown on a soil mixture (Taimimulta, Kekkilä, Vantaa, Finland) in pots of 17 cm in diameter (eight plants/pot). Two pots of each cultivar were sown for each growth condition and all plants were kept in the same growth room until flowering. The conditions of the growth room are detailed in Table 1. The plants were watered twice a week and fertilised once a week with a 0.1% solution of a 13-7-20 N-P-K fertilizer (Puutarhan Kesä, Yara, Espoo, Finland) until 2 WAF, and after that only watered.

We avoided the photoperiod effect on flowering by keeping the plants in the same growth room until flowering started. When flowers started to appear (*ca.* 30 days after germination for TR and 50 days after germination for OR), a third of the plants were transferred to a low temperature treatment growth chamber (15 °C, Table 1) and another third to a short day treatment in a growth room (Table 1) where they received light from fluorescent lamps, while the remaining third remained in the original growth room. Individual flowers were tagged on the day of opening and pollination was achieved by touching them with other flowers of the same cultivar. The plants were separated with a fabric cover to hinder cross-pollination.

A regular greenhouse environment was used as an additional control condition. There the plants received unsupplemented natural daylight from March to early July ranging from 13 h of light at germination time to 16 h at flowering and to 19 h at ripening. The room was located on the northern side of the greenhouse, keeping the sunlight indirect and reducing overheating of the room (detailed conditions in Table 1). Fertilisation and watering were as above. Flowering started in the greenhouse approximately 45 days after germination for TR and 50 days after germination for OR.

The plant experiment was conducted three times with seeds sown at different days. The seed ripening started in control conditions 5–7 WAF, while in the low temperature and short day conditions the beginning of ripening was delayed until 7–8 WAF. Seeds were harvested once a week 2–7 WAF until the beginning of ripening and the samples (Table 1) containing all the seeds of one silique each minus any germinated seeds were flash frozen in liquid nitrogen. Flexibility of ±2 days from the recorded flowering date was allowed for the weekly time point (for example, 2 WAF equals 12–16 days after flowering). In the first collection time point (2 WAF for control conditions and 3 WAF for stress conditions, Table 1)

Table 1
Samples collected for the study. OR oilseed rape, TR turnip rape, *x* sample collected, – sample not collected, because seeds were either too small or already ripe.

Treatment	Species	Cultivar	Temperature day/night	Light cycle day/night	Light intensity μmol m^{-2} s^{-1}	2 WAF	3 WAF	4 WAF	5 WAF	6 WAF	7 WAF	Ripe
Short	OR	Marie	20 °C/20 °C	12 h/12 h	200	–	x	x	x	x	–	x
Day	TR	SW Petita	20 °C/20 °C	12 h/12 h	200	–	x	x	x	x	–	x
Low	OR	Marie	15 °C/15 °C	16 h/8 h	200	–	x	x	x	x	–	x
Temp.	TR	SW Petita	15 °C/15 °C	16 h/8 h	200	–	x	x	x	x	x	x
Growth	OR	Marie	22 °C/22 °C	16 h/8 h	150–300	x	x	x	x	–	–	x
room	TR	SW Petita	22 °C/22 °C	16 h/8 h	150–300	x	x	x	–	–	–	x
Green-	OR	Marie	20 °C/15 °C	16 h/8 h	200–500	x	x	x	x	x	–	x
House	TR	SW Petita	20 °C/15 °C	16 h/8 h	200–500	x	x	x	x	–	–	x