Scientia Horticulturae 243 (2019) 477–483

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Influence of pollen sources on the expression of FA and TAG biosynthetic pathway genes in seeds of *Paeonia rockii* during the rapid oil accumulation

Lihang Xie, Jiayuan Hu, Qingyu Zhang, Quanfeng Sun, Yanlong Zhang*, Lixin Niu*

National Engineering Research Center for Oil Peony, College of Landscape Architecture and Arts, Northwest A&F University, Yangling 712100, Shaanxi, PR China

ARTICLE INFO

Keywords:
Tree peony
Xenia
Fatty acid composition
Molecular basis
Transcript abundance

ABSTRACT

Tree peony has been widely planted as an excellent oil crop with high proportion of α-linolenic acid (ALA) in its seeds. It was reported that pollen sources have clear effects on fatty acid (FA) composition of its kernels. Nevertheless, the molecular basis of this novel phenomenon is poorly understood. In this study, four *Paeonia suffruticosa* cultivars whose pollen caused significant differences in ALA content of *Paeonia rockii* were selected as pollen donors and grouped under high and low ALA groups. After pollination, total FAs content, composition and expression profiles of 10 genes involved in the FA and triacylglycerol (TAG) biosynthetic pathways were investigated during seed development. During the rapid oil accumulation in seeds, three desaturase genes (SAD, FAD2, FAD3) showed much higher expression in high ALA group, and their differential expression profiles corresponded well with the variations in ALA content among different pollen sources. Overall, pollen sources could affect the expression level of genes associated with fatty acid modification and subsequently affect the composition of FA in the kernels.

1. Introduction

Tree peony (*Paeonia* section *Moutan* DC.) has been planted for ornamental purposes for more than 2000 years in China (Chen and Li, 1998). Chinese tree peony cultivars are geographically classified into four groups including the Northwest, Zhongyuan, Jiangnan, and Southwest groups (Zhang et al., 2007). More recently, *Paeonia rockii* has been promoted for planting in China, since its seeds are identified as a new source of edible oil with abundant unsaturated fatty acids (UFAs), especially ALA which is essential and cannot be synthesized in humans (James, 2001; Zhang et al., 2017).

Paeonia rockii is a cross-pollinated plant without apomixis and has a low rate of self-fertility (Cheng et al., 2005). The sexual reproduction of *Paeonia rockii* mainly relies on exotic pollen. Previous studies have shown that pollen sources have a direct influence on the fruit and seed features of the maternal plants (Darwin, 1868; Focke, 1881). Furthermore, xenia also has clear effects on the chemical compositions of kernels. The total soluble solids of the fruit in date palms (*Phoenix dactylifera* L.) (Rezazadeh et al., 2013) and the amygdalin content in almonds (Sanchez-Perez et al., 2012) could be affected by pollen sources. Kodad reported that xenia could affect fatty acid composition in almond (Kodad et al., 2009). Previously, we also found similar results in tree peony (Xie et al., 2017). However, no report has been found to explain the variation in fatty acid composition of kernel caused by different pollen sources to date.

In higher plants, the mechanism of oil biosynthesis has already been researched widely. Oil biosynthesis in oil crops is composed of two main pathways, the fatty acid (FA) and triacylglycerol (TAG) biosynthetic pathways, which reside in the plastid and endoplasmic reticulum (ER), respectively (Fig. 1) (Bates et al., 2013). The crucial precursor for de novo fatty acid biosynthesis is acetyl-CoA, which is generated from pyruvate by the action of the plastidial pyruvate dehydrogenase complex (PDHC) in a straightforward way (Johnston et al., 1997). The first committed reaction in fatty acid biosynthesis is the formation of malonyl-CoA from acetyl-CoA. Before entering the fatty acid synthesis pathway, malonyl-CoA must be converted to malonyl-ACP, a process catalyzed by malonyl-CoA:ACP transacylase (MCAT). A recurring cycle, including condensation, reduction, and dehydration reactions, directs de novo fatty acid synthesis in the plastid. During the formation of 18-carbon acyl-ACP from malonyl-ACP with acetyl-CoA, the condensation reaction is catalyzed by a set of three specific enzymes called β-ketoacyl-ACP synthases (KAS); in particular, KASII plays a role in the final elongation of the 16-carbon palmitoyl-ACP to the 18-carbon stearoyl-ACP (Pidkowich et al., 2007). In addition to those involved in the condensation reaction, several other enzymes are also required to obtain saturated fatty acids, such as enoyl-ACP reductase (ENR).(Mou

* Corresponding authors.
E-mail addresses: zhangyanlong@nwsuaf.edu.cn (Y. Zhang), niulixin@nwsuaf.edu.cn (L. Niu).

https://doi.org/10.1016/j.scienta.2018.09.002
Received 10 April 2018; Received in revised form 2 September 2018; Accepted 3 September 2018

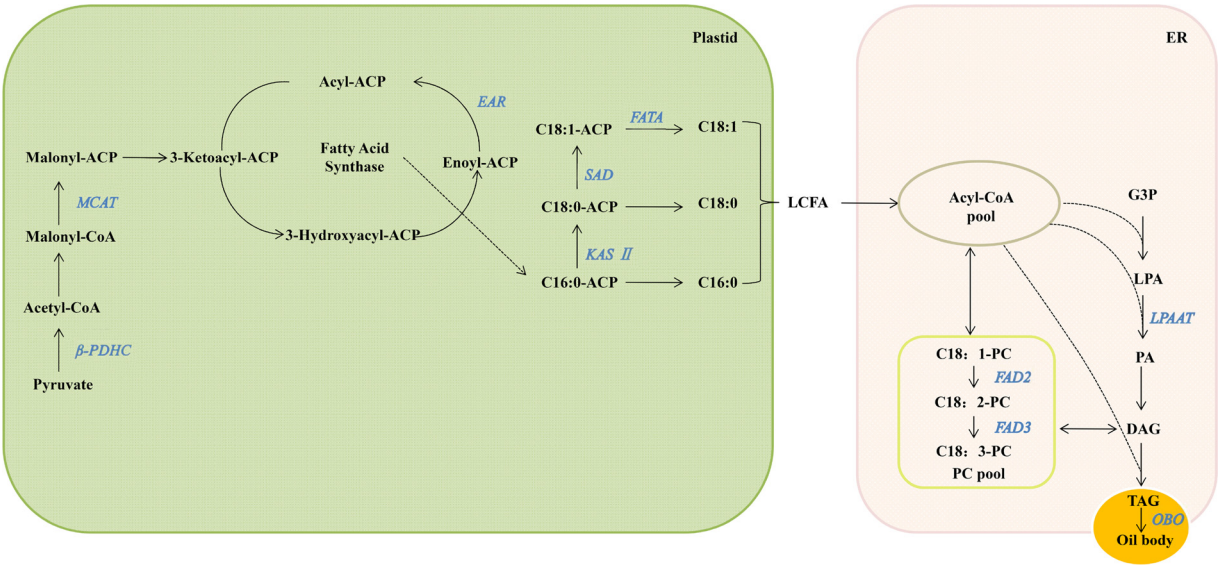

Fig. 1. Overview of major reactions involved in fatty acid and triacylglycerol synthesis.
Abbreviations: substrates are in bold: **ACP**, acyl carrier protein; **DAG**, diacylglycerol; **G3P**, glycerol-3-phosphate; **LCFA**, long chain fatty acid; **LPA**, lyso-phosphatidic acid; PA, phosphatidic acid; **PC**, phosphatidylcholine; **TAG**, triacylglycerol. Key enzymes are in italics; **EAR**, enoyl-ACP reductase; **FAD2**, Δ12 oleic acid desaturase; **FAD3**, Δ15(ω-3) linoleic acid desaturase; **FATA**, acyl-ACP thioesterase A; **KAS II**, ketoacyl-ACP synthase II; **LPAAT**, 1-acylglycerol-3-phosphate acyltransferase; **MCAT**, malonyl-CoAACP transacylase; **OBO**, oil-body oleosin; **SAD**, stearoyl-ACP desaturase; **β-PDHC**, pyruvate dehydrogenase beta subunit.

et al., 2000) The 18:0-ACP produced by elongation can be desaturated to 18:1-ACP by stearoyl-ACP desaturase (SAD) (And and Cahoon, 1998). The process of de novo fatty acid synthesis finishes with the release of fatty acids, which are hydrolyzed by acyl-ACP thioesterase (FAT) in the plastid (Harwood, 1996; Jones et al., 1995). The polyunsaturated fatty acids, including linoleic acid (LA) and α-linolenic acid (ALA), are mainly produced in the endoplasmic reticulum (ER) by reactions catalyzed by oleate desaturase (FAD2) (Okuley et al., 1994) and linoleate desaturase (FAD3) (Browse et al., 1993). The assembly of TAG (also known as the Kennedy pathway) generates phosphatidate (PA) from glycerol-3-phosphate and acyl-CoAs, and this process is catalyzed by the enzymes glycerol-3-phosphate acyltransferase and lysophosphatidic acid acyltransferase (LPAAT) (Bates et al., 2013). After a series of reactions that add the fatty acids to a glycerol backbone, triacylglycerol (TAG) molecules are ultimately formed. These TAGs are then assembled to form oil bodies in the seeds through a process in which oil-body oleosin (OBO) participates (Murphy, 2001). The transcriptional regulation of those genes plays a key role in determining the quality and quantity of plant oils (Zhang et al., 2018). Therefore, we hypothesized that pollen sources might affect the transcript abundances of genes involved in the FA and TAG biosynthetic pathways, and subsequently change the composition of FA in the kernels.

In this study, four *Paeonia suffruticosa* cultivars whose pollen caused clear differences in FA composition of *Paeonia rockii* in two successive years were selected as pollen donors to investigate the possible biochemical and molecular differences caused by different pollen sources. After pollination with different pollen, we performed the total FA content and FA profiling of five seed development stages of four pollination combinations. Further, the transcript profiling of 10 genes from the FA and TAG biosynthetic pathways were performed in crucial stages of rapid lipid accumulation, using real time PCR. Finally, correlation between the expression patterns of studied genes and the FA composition among pollen sources were analyzed comprehensively. The results of this work will also be beneficial to identifying key genes that contributed to the accumulation of polyunsaturated fatty acids.

2. Materials and methods

2.1. Plant material

Fifteen *Paeonia suffruticosa* cultivars with high pollen germination were selected as pollen sources (Table A.1 in Supplementary material). The plants of *Paeonia rockii* with the same genetic origin had been introduced from Feng County, Shaanxi Province, China and grown under the same eco-environmental conditions for ten years in the tree peony germplasm repository at Northwest Agriculture & Forestry University (Yangling, Shaanxi province, China). In the first two years, each pollination treatment was applied to twenty flowers from five uniform trees of *Paeonia rockii*, and pods were hand-collected at the mature stage. In order to minimize the effects of mother plant, flowers from the same tree were pollinated with pollen from selected cultivars in the third year, and nine flowers from three uniform trees were selected for each cultivar, and pods were collected at intervals of twenty days until full maturity, covering a total range of 100 days. The collected samples were immediately frozen in liquid nitrogen and stored at −80 °C for further use.

2.2. Oil extraction and fatty acid analysis

The oil extraction and fatty acid methylation were completed according to the methods described in our previous study (Xie et al., 2017). FAs were analyzed by using a gas chromatograph-mass spectrometer (Thermo Scientific trace 1310 GC-ISQ) equipped with TriPlus RSH robotic sampler (Thermo Scientific). The capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness; Thermo Fisher Scientific, USA) was used in the experiment with helium as the carrier gas. Fatty acid composition was determined using methyl tridecylicacid as an internal standard. All samples were analyzed in quintuplicate at the first two years and in triplicate at the third year.

2.3. Total RNA extraction and cDNA synthesis

Except for oil extraction, seeds from the same pods were randomly selected for the extraction of total RNA in the third year. Total RNA was extracted from the seeds collected at 40, 60 and 80 days after