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De novo transcriptome sequencing of *Torreya grandis* reveals gene regulation in sciadonic acid biosynthesis pathway

Jiasheng Wu[a],*, Jiandiao Huang[a], Yiwei Hong[a], Haizhen Zhang[b], Mingzhu Ding[a], Heqiang Lou[a], Yuanyuan Hu[a], Weiwu Yu[a], Lili Song[a],*

[a] State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou, Zhejiang 311300, China
[b] Hubin Administration of Hangzhou West Lake Scenic Area, Hangzhou Municipal Bureau of Landscape and Cultural Relic, Hangzhou 310001, China

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ABSTRACT

Torreya grandis (*T. grandis*), a conifer of the Cephalotaxaceae family, yields rare and unique dried fruits of great nutritional value that contains a high level of novel UFA, sciadonic acid. The composition of sciadonic acid in different tissues and cultivars, and the molecular mechanisms underlying the biosynthesis of sciadonic acid in *T. grandis*, both of which remain unknown however, were investigated. Two cultivars, *T. grandis cv.* Yuanfei and Xifei were used to reveal in comparison the fatty acid composition of roots, stems, leaves, arils and kernels. It was found that the Yuanfei kernels had the highest levels of sciadonic acid with 17% in oil. In transcriptome analysis, a total of 80,235 and 82,454 unigenes were generated from five libraries of Yuanfei and Xifei cultivars, respectively. KEGG orthology enrichment of differentially expressed genes showed that lipid metabolism pathways were the most highly represented. In particular, six candidate unigenes (Δ^6-elongase and Δ^5-desaturase) encoding sciadonic acid elongase and desaturases with high expression levels in Yuanfei and Xifei kernels were identified. This study provides the first comprehensive genomic analysis of sciadonic acid biosynthesis and contributes to the understanding of the molecular mechanisms responsible for *de novo* fatty acid biosynthesis and high sciadonic acid accumulation in gymnosperm species.

1. Introduction

Unsaturated fatty acids (UFA) are a major focus in the fields of nutrition and medicine due to their preventative effects on angiosclerosis, obesity, certain types of cancers, and neurological disorders. The formation of UFA has evolved during *de novo* fatty acid synthesis and fatty acid elongation and desaturation pathways (Beisson et al., 2003; Harwood, 1996; Ohlrogge and Browse, 1995), subsequently linking three carbons of the glycerol backbone, accumulating as triacylglycerols (TAGs) in plant seeds (Brown et al., 2012). Currently, investigation into fatty acid synthesis in plants has focused almost entirely on oil seeds in angiosperm species, such as Arabidopsis, Pecan and Tree peony (Huang et al., 2017; Li et al., 2015; Li-Beisson et al., 2010). UFA biosynthesis in gymnosperm species, which are recommended as an important oil seed resource, is difficult to study because they have very large effective population sizes and their genomes are highly heterozygous (Nystedt et al., 2013). Sciadonic acid, also known as Cis-5,11,14 eicosatrienoic acid, is a newly identified type of non-methylene-interrupted polyunsaturated fatty acid (NMI-PUFA) with a Δ^{5cis}-ethylenic bond, and is considered to be characteristic of gymnosperms (Wolff, 1999). Sciadonic acid was initially discovered by Itabashi and Takagi (1982) in the seeds, arils, and leaf lipids of *Taxus cuspidate* (Japanese yew) (Itabashi and Takagi, 1982). Sciadonic acid mainly occurs in seed oils of various gymnosperm (conifer) species, such as *Podocarpus andinus*, *Sciadopitys verticillata*, and *Podocarpus nagi* (Berger et al., 2002; Wolff et al., 1999a,b), although the content and profile vary depending on the family, genus, or even the species. Sciadonic acid as a taxonomic marker has likely been around for over 250 million years and accompanied the emergence, radiation, and expansion of gymnosperms, whereas almost all angiosperm species have lost the capability to introduce supplementary Δ^5-desaturation in unsaturated fatty acids in C20 acids dating back to the Devonian period (Wolff, 1999; Aitzetmuller, 1995). Recently, considerable research on increasing sciadonic acid content has garnered interest because of its association with human health and nutrition, including inflammatory responses, blood pressure regulation, blood clotting, and cell signaling (Berger et al., 2002; Niu et al., 2011; Tanaka et al., 2001).

Although sciadonic acid biosynthesis has not been fully elucidated, it is known that the synthesis of sciadonic acid requires the existence of a Δ^5-desaturase that would use 9,12-C18:2 and their elongation

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* Corresponding authors.
E-mail addresses: wujs@zafu.edu.cn (J. Wu), lilisong@zafu.edu.cn (L. Song).

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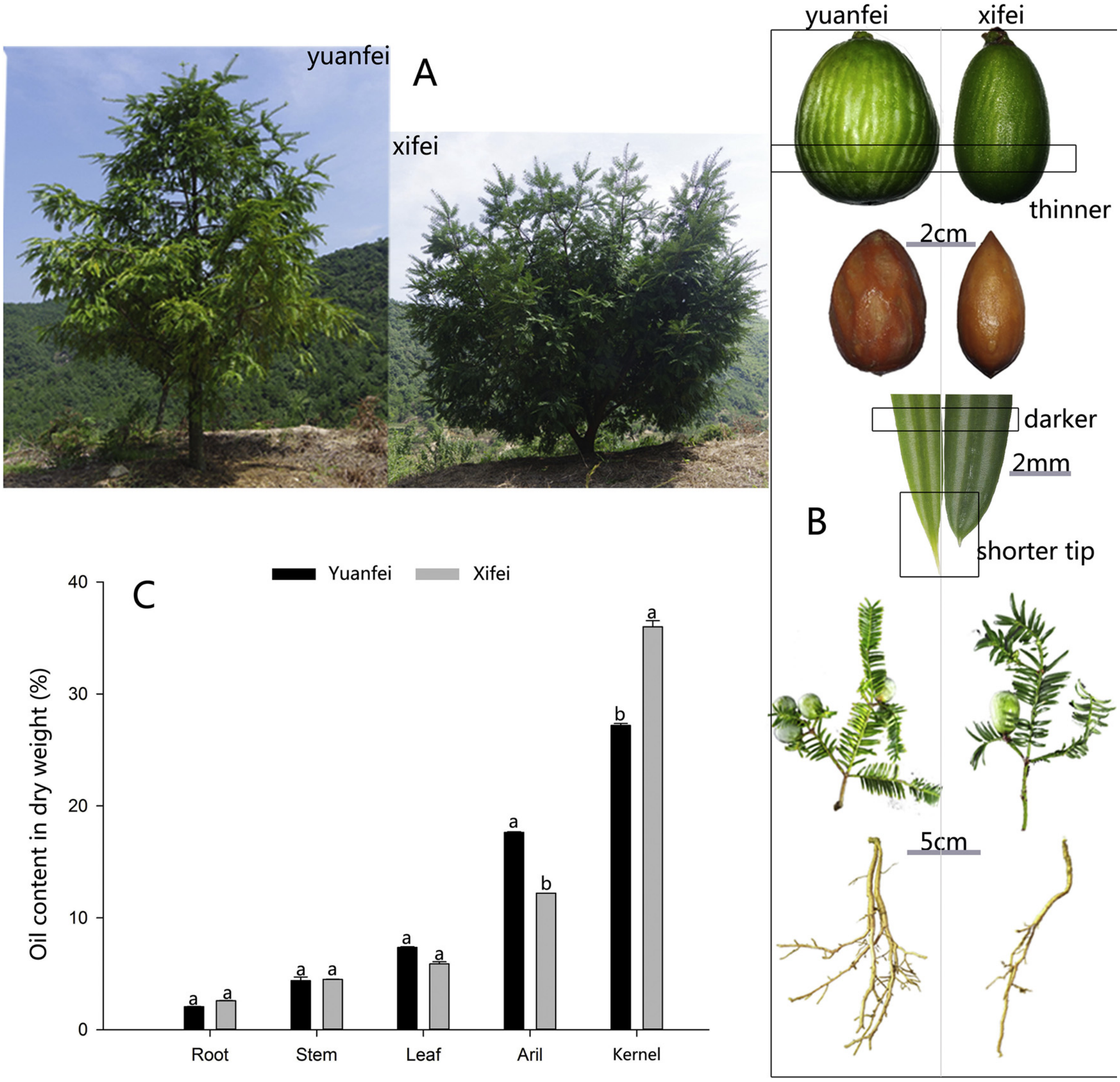

Fig. 1. The oil contents, appearance and different tissues of *T. grandis* cv. Yuanfei and Xifei. (A) The plants. (B) Root, stem, leaf, matured seed with or without a fleshy covering. (C) Oil contents of root, stem, leaf, aril and kernel tissue in 2015.

products, 11,14-C20:2 acids, as substrates (Wolff et al., 1999a,b). Furthermore, two candidate cDNAs for C20 Δ^5-desaturase from the developing seeds of *Anemone leveillei* were functionally characterized in transgenic Arabidopsis plants (Sayanova et al., 2007), in which the Δ^5-desaturase was biochemically demonstrated to use acyl-CoA as a substrate. The molecular mechanism of sciadonic acid biosynthesis in gymnosperms species, particularly with respect to the full set of genes involved in this pathway, remains unexplored.

Torreya, a large, evergreen coniferous tree, is a primitive member of the gymnospermous yew family (Taxaceae). *Torreya grandis* (*T. grandis*) is a common ornamental plant in China and has been listed as a national key protected wild plant (second group) (Saeed et al., 2007; Shen et al., 2014). Due to the influence of the environment and cultivation management, *T. grandis* is morphologically variable in China and exhibits different seed shapes and qualities, including Yuanfei, Xifei, Xiangyafei, Zhimafei, *etc*. Yuanfei (also named as Mufei) is the seedling of *T. grandis* and Xifei is a commercially important grafted variety that is widely cultivated for nut production (Li et al., 2005). In practice, the two cultivars are significantly different in external appearance (Fig. 1A and B). Furthermore, both Yuanfei and Xifei seed oils have rich unsaturated fatty acids with healthy and nutritional values, preventing angiosclerosis and coronary heart disease. Interestingly, siadonic acid has been found in abundance in *T. grandis* seeds including Yuanfei and Xifei, representing 8.3–16.4% of the oil, while Yuanfei has been established to be higher in sciadonic acid content in the seed oil than Xifei (Wang et al., 2016). Other gymnosperm species such as *Pinus* spp. seeds have sciadonic fatty acid content less than 1.24% of the oil (Wolff et al., 2001), although the presence of sciadonic acid in seed lipids of all coniferophyte families is a common biochemical feature. Thus, *T. grandis* species that contain high levels of this fatty acid constitute a useful and reliable model for identifying and determining sciadonic acid biosynthesis. For the past years, considerable research has been focused on the fatty acid composition, distribution and extraction in the seeds over *T. grandis* cultivars (Niu et al., 2011). Unfortunately, little information is available on the genomic analysis of *T. grandis* regarding the molecular mechanisms of fatty acid biosynthesis, especially associated with sciadonic acid biosynthesis.

Given that most gymnosperm species have a large genome, high proportions of repetitive elements and numerous pseudogenes, genetic information currently available for these species is limited. In this study, we generated the first tree transcriptome using high-throughput Illumina sequencing technology to uncover genes related to sciadonic acid biosynthesis, to observe gene expression patterns and characterize candidate genes/proteins responsible for sciadonic acid synthesis in *T. grandis*. More efforts has been done to complement and enhance the study, mainly by analyzing fatty acid composition of different tissues