Transcriptional regulation of oil biosynthesis in seed plants: current understanding, applications and perspectives

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Running title: Transcriptional regulation of plant oil biosynthesis

Short Summary: This review encapsulates the progress on understanding the molecular mechanisms underlying the control of oil biosynthesis in seed plants, as well as oil biosynthesis responds to developmental and environmental cues. The translational applications of the oil regulators in bioengineering plant oil production have also been discussed.

ABSTRACT

Plants produce and accumulate triacylglycerol (TAG) in their seeds as an energy reservoir to support the processes of seed germination and seedling development. Plant seed oils are vital not only for human diets but also as renewable feedstock for industrial uses. TAG biosynthesis consists of two major steps: *de novo* fatty acid biosynthesis in the plastids and TAG assembly in the endoplasmic reticulum. The latest advances in unraveling transcriptional regulation have shed light on the molecular mechanisms of plant oil biosynthesis. This review summarizes the recent progress in understanding the regulatory mechanisms of well-characterized and newly discovered transcription factors and other types of regulators that control plant fatty acid biosynthesis. The emerging picture shows that plant oil biosynthesis responds to developmental and environmental cues that stimulate a network of interacting transcriptional activators and repressors, which, in turn, fine-tune the spatiotemporal regulation of the pathway genes.

Key words: plant oil biosynthesis, oil accumulation, seed development, environmental and developmental signals, transcription factor, transcriptional regulation

INTRODUCTION

Plant oils (often referred to as vegetable oils) are mainly generated and stored in the form of triacylglycerol (TAG) in seeds. For plants, TAG serves as a major carbon and energy source to support seed germination and seedling development. For humans, plant oils are not only essential as a component of the diet, providing around one-fourth of the dietary calories in developed countries, but also important as a source of carbon-neutral fuels and raw materials for industrial products, such as lubricants, detergents, and nylon (Chapman and Ohlrogge, 2012; Covas et al., 2015; Kong et al., 2020a; Song et al., 2021b). Global demand for vegetable oils is elevating rapidly and projected to double by 2030 (Chapman and Ohlrogge, 2012), incentivizing a tremendous amount of research effort to enhance plant oil biosynthesis.

The mechanism of fatty acid (FA) biosynthesis has been extensively studied and reviewed (Allen, 2016; Bates, 2016; Bates et al., 2013; Chapman and Ohlrogge, 2012; Kong *et al.*, 2020a; Li-Beisson et al., 2013; Miray et al., 2021; Song *et al.*, 2021b; Xu and Shanklin, 2016). In brief, FA synthesis starts with the provision of carbon from glycolysis. After glycolysis, pyruvate dehydrogenase converts pyruvate into acetyl-CoA, the initial substrate for *de novo* FA biosynthesis. Acetyl-CoA carboxylase (ACCase) catalyzes the first committed step converting acetyl-CoA and bicarbonate into malonyl-CoA. A key co-factor, acyl-carrier protein (ACP), then accepts the malonyl group to form malonyl-ACP. After the first cycle of FA synthesis producing an acetoacetyl-ACP, additional cycles, facilitated by the fatty acid synthase (FAS) complex, elongate the FA chain at a 2-carbon increment, where acetyl units provided from malonyl-CoA are added successively to a lengthening acyl chain esterified to an ACP. During this process, 3-ketoacyl-ACP synthase (KAS) catalyzes the condensation reaction to connect new carbons to the growing acyl chain. KAS III starts FA biosynthesis by combining acetyl-CoA and malonyl-ACP. KAS I subsequently extends the acyl chain to C12-C16. Eventually, KAS II completes the biosynthesis to C18. The acyl chains are released from acyl-ACPs by hydrolysis reactions catalyzed by thioesterases. The products of thioesterases are activated to acyl-CoA molecules prior to exporting to the ER. Currently, there are still some ambiguities on the specific steps and the sequence of events regarding fatty acyl activation and export out of plastid envelops (Bates, 2016; Bates *et al.*, 2013). The pathway to TAG assembly from glycerol-3-phosphate (G3P) and acyl-CoAs consists of multiple enzymatic steps (Allen, 2016; Bates, 2016; Chapman and Ohlrogge, 2012; Li-Beisson *et al.*, 2013). First, glycerol-3-phosphate acyltransferase (GPAT) generates lysophosphatidic acid (LPA) by transferring of a FA from acyl-CoA to the sn-1 hydroxyl of G3P. Then lysophosphatidic acid acyltransferase (LPAAT) esterifies a second FA to the *sn*-2 hydroxyl of LPA to produce phosphatidic acid (PA). Third, PA phosphatase (PAP) mediates the removal of the *sn*-3 phosphate to produce *de novo* diacylglycerol (DAG). The *de novo* DAG pool is vital, as it can be utilized for biosynthesis of membrane lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), or for TAG production. The DAG for TAG biosynthesis can also be obtained from PC. Acyl flux of the *de novo* DAG into PC, and the ensuing acyl flux of PC-derived DAG out of PC that generates the DAG substrate for producing TAG may occur via a phosphocholine headgroup exchange mechanism. At the final stage, diacylglycerol acyltransferase (DGAT) or phospholipid:diacylglycerol acyltransferase (PDAT) catalyzes the conversion of DAG to TAG (Dahlqvist et al., 2000; Zhang et al., 2009; Zou et al., 1999). TAG is then coated with oleosins and stored as lipid droplets (Chapman et al., 2012).

TAG biosynthesis is a complex biological process, involving three main metabolic pathways, including glycolysis, FA *de novo* synthesis and elongation, and TAG assembly. Glycolysis provides carbons which can be esterified with glycerol to produce TAG. Hence, seed oil accumulation requires a balanced transcriptional regulation of multiple branch pathways (Chapman and Ohlrogge, 2012; Kong et al., 2019; Lu et al., 2018). Transcription factors are proteins which recognize and bind, directly or indirectly, to specific DNA sequences in gene promoters to control gene expression (Lambert et al., 2018; Zhu et al., 2018). Numerous transcription factors have been shown to be involved in regulating the expression of the TAG biosynthetic genes (Baud et al., 2007; Cernac and Benning, 2004; Kong and Ma, 2018b; Li et al., 2017; Ma et al., 2013; Mu et al., 2008; Pelletier et al., 2017; Tian et al., 2020; Yamamoto et al., 2010). Among them, the APETALA2 (AP2) transcription factor, WRINKLED1 (WRI1) directly regulates the expression of various genes involved in glycolysis and FA biosynthesis, functioning to “push” FA *de novo* synthesis from upstream (Vanhercke et al., 2019; Vanhercke et al., 2014). Other transcriptional regulators also participate in regulation of the FA biosynthetic pathway through mediating the expression of *WRI1* (Baud *et al.*, 2007; Kong *et al.*, 2020a; Li *et al.*, 2017; Tian *et al.*, 2020; Yamamoto *et al.*, 2010). Different from animals, plants are sessile organisms that are incapable of escaping from various environmental hazards. Plants have therefore evolved sophisticated systems to translate environmental cues into metabolic modulation to mitigate the environmental impacts. FA biosynthesis is part of seed development which responds to various environmental signals, including light, humidity, and temperature. Transcriptional and other regulatory networks integrate the balanced regulation of environmental signal perception/transduction, seed development, and FA biosynthesis (Figure 1). In this review, we focus on our understanding on some of the key regulators in the networks.

The Essential Roles of Key Regulators of Seed Development in Transcriptional Regulation of Seed Oil Accumulation

Numerous studies suggest that four transcriptional regulators, LEAFY COTYLEDON1 (LEC1), LEAFY COTYLEDON2 (LEC2), FUSCA3 (FUS3), and ABSCISIC ACID INSENSITIVE3 (ABI3), play central roles in the process of seed development and maturation (Giraudat et al., 1992; Jo et al., 2019; Lotan et al., 1998; Luerssen et al., 1998; Parcy et al., 1997; Pelletier *et al.*, 2017; Song et al., 2021a; Stone et al., 2001; Verdier and Thompson, 2008; Yamamoto *et al.*, 2010). Recent evidence shows that LEC1, LEC2, FUS3 and ABI3 are also involved in transcriptional regulation of seed oil production (Baud *et al.*, 2007; Manan et al., 2017; Mu *et al.*, 2008; Shen et al., 2010; Tan et al., 2011; Tian *et al.*, 2020; Yang et al., 2021; Zhang et al., 2016). Genes encoding these regulators are expressed predominantly during seed development and considerably impact seed oil accumulation.

LEC1 encodes a HAP3 subunit of the CCAAT binding transcription factor. Inducible transgenic lines of Arabidopsis overexpressing *AtLEC1* (AtLEC1-OXi) showed enhanced FA production (Mu *et al.*, 2008). Global transcriptomic analysis of AtLEC1-OXi lines revealed that LEC1 regulates the expression of genes involved in FA metabolism, including key reactions of condensation, chain elongation, and desaturation of FA biosynthesis (Mu *et al.*, 2008). Elevation of *LEC1* enhanced seed oil content. Overexpression of *AtLEC1*, *BnLEC1* (*Brassica napus LEC1*), and *ZmLEC1* (maize *LEC1*) increased oil content in transgenic plants (Mu *et al.*, 2008; Shen *et al.*, 2010; Tan *et al.*, 2011). LEC1-LIKE (L1L), a close homolog of LEC1, displays a similar function as LEC1 in regulation of FA biosynthesis in Arabidopsis and *B. napus* (Mu *et al.*, 2008; Tan *et al.*, 2011). Notably, a recent study illustrated the significance of the endosperm-produced AtLEC1 in facilitating seed developmental process including seed maturation (Song *et al.*, 2021a). *LEC1* expression occurs in the endosperm, followed by LEC1 trafficking to embryo to regulate seed maturation, indicating the complex roles of LEC1 in mediating seed development and controlling metabolic activities, such as FA production (Song *et al.*, 2021a).

LEC2 is a B3 domain transcription factor vital for embryo development (Stone *et al.*, 2001). In addition to the essential function in controlling the developmental process, LEC2 is involved in transcriptional regulation of FA biosynthesis and therefore has been used in engineering plants to enhance oil production (Baud *et al.*, 2007; Kim et al., 2015; Santos Mendoza et al., 2005; Vanhercke *et al.*, 2019; Vanhercke *et al.*, 2014). Transgenic Arabidopsis seedlings expressing an inducible *LEC2* showed oil accumulation in leaves upon induction, accompanied by increased expression of genes encoding key transcription factors, such as LEC1, ABI3 and FUS3 (Santos Mendoza *et al.*, 2005).

In the transcriptional network, LEC1 and LEC2 positively regulate the expression of *WRI1* (Baud *et al.*, 2007; Kong *et al.*, 2019; Mu *et al.*, 2008; Pelletier *et al.*, 2017). *WRI1* expression is elevated in Arabidopsis gain-of-function mutant of *LEC1*, *tnp*, as well as the inducible *LEC1* overexpression lines (Casson and Lindsey, 2006; Mu *et al.*, 2008). Inducible *LEC2* overexpression after cycloheximide treatment (to block translation) showed the activation of *AtWRI1* without the participation of other intermediate proteins, and *AtWRI1* expression is decreased in the *lec2* mutant compared to that in the wild-type (WT) control (Baud *et al.*, 2007). Chromatin immunoprecipitation (ChIP) experiments confirmed *WRI1* as a direct target of LEC1 (Pelletier *et al.*, 2017). Effects of LEC1 and LEC2 on *WRI1* expression are conserved across other plant species, such as maize and soybean (Manan *et al.*, 2017; Shen *et al.*, 2010).

The B3 domain transcription factor FUSCA3 (FUS3) triggers the expression of various FA biosynthetic genes, e.g., *FATTY ACID DESATURASE3* (*FAD3*), *KAS I*, and *FATTY ACID ELONGATION1* (*FAE1*) (Wang and Perry, 2013; Yamamoto *et al.*, 2010). FUS3 also directly binds to the promoters of *LEC1*, *L1L*, and *ABI3* to mediate their expression (Wang and Perry, 2013). Transcriptomic analysis revealed that the expression of *WRI1* is decreased in the *fus3* mutant compared to WT (Yamamoto *et al.*, 2010). *WRI1* is a direct target of FUS3 in a genome-wide ChIP-chip analysis (Wang and Perry, 2013). The *fus3* mutant displays fewer number of oil droplets in cotyledons of developing seeds, as well as reduced seed oil content compared to WT (Meinke et al., 1994; Roscoe et al., 2015; Tiedemann et al., 2008). Transgenic Overexpression of

FUS3 in Arabidopsis significantly triggers TAG accumulation in vegetative tissues, suggesting a useful role of FUS3 in bioengineering vegetable oil production (Zhang *et al.*, 2016).

The B3 domain transcription factor ABI3 plays an important role in abscisic acid (ABA) signaling pathway that mediates physiological processes, such as seed maturation and dormancy (Boulard et al., 2017; Monke et al., 2012; Rohde et al., 2000; Tian *et al.*, 2020). Recent ChIP-chip experiments identified the direct targets for ABI3, among which is *WRI1* along with other genes encoding FA biosynthetic genes, including *FAD3* and *FATTY ACID BIOSYNTHESIS 2* (*FAB2*) (Tian *et al.*, 2020). The combined results of the ChIP-chip and comparative transcriptomic analysis from developing Arabidopsis seeds of *abi3* and WT show that 317 genes, including *WRI1*, are directly activated by ABI3 (Tian *et al.*, 2020). Furthermore, a recent work indicated that transgenic tobacco BY2 cell lines or transgenic Arabidopsis seedlings expressing an inducible *ABI3* results in the activation of oil production, further signifying the role of ABI3 in regulation of oil biosynthesis. In the *fus3* mutant, induction of *ABI3* triggers strong TAG production in transgenic seedlings, suggesting that ABI3 activates oil biosynthesis in a FUS3-independent manner . Further transcriptomic analysis showed that *LIPID DROPLET PROTEIN (LDP*) genes and *WRI1* are activated by ABI3 in transgenic *fus3* plants (Yang *et al.*, 2021).

AGAMOUS-Like15 (AGL15) is a well-characterized MADS transcriptional factor (Cosio et al., 2017; Joshi et al., 2021; Serivichyaswat et al., 2015; Thakare et al., 2008; Zheng et al., 2009). ChIP-chip analysis detected various direct targets of AGL15, including *LEC2*, *FUS3*, and *ABI3* (Zheng et al., 2009). Recent ChIP analysis revealed that HIGH-LEVEL EXPRESSION OF SUGAR INDUCIBLE GENE2 (HSI2)/VP1/ABI3-LIKE1 (VAL1) directly binds to the *AGL15* promoter via the RY element to repress the expression of *AGL15*. In the *hsi2-2* mutant, the expression of *AGL15* (as well as *LEC1*, *LEC2*, *ABI3*, and *FUS3*) significantly increased compared to WT (Chen et al., 2018b). *BABY BOOM* (*BBM*), encoding an AP2 transcription factor, is essential for transcriptional regulation of plant cell totipotency (Horstman et al., 2017; Horstman et al., 2014; Lutz et al., 2015). Functional analysis revealed that BBM directly binds to the promoters of *LEC1*, *LEC2*, and *ABI3*. In BBM overexpressing transgenic plants, expression of *LEC1*, *LEC2*, *FUS3* and *ABI3* was upregulated, suggesting a positive role of BBM in activation of these FA-biosynthesis related genes *in planta* (Horstman *et al.*, 2017). The direct involvement of AGL15 or BBM in oil production remains to be explored.

The Pivotal Role of WRI1 in Transcriptional Control of Plant Oil Biosynthesis

The *AtWRI1* loss-of-function mutant, *wri1-1*, displays a near 80% reduction in seed oil content compared to WT (Focks and Benning, 1998). Transcriptomic analysis of developing *wri1-1* seeds showed that most genes showing decreased expression encode key enzymes in late glycolysis and the FA pathways (Ruuska et al., 2002). Subsequent studies confirmed that AtWRI1 directly regulates many of these genes (Baud *et al.*, 2007; Maeo et al., 2009), including *pyruvate kinase beta subunit 1* (*PKP-β1*), *biotin carboxyl carrier protein* (*BCCP2*, encoding a subunit of the ACCase), *ACP1*, and *KAS I*. WRI1 directly binds the promoter region and activates the expression of *GLB1* gene, which encodes a PII protein, a key integrator of central metabolism (Baud et al., 2010). PII displays inhibitory effect on ACCase activity and is involved in fine-tuning FA biosynthesis in developing seeds (Baud *et al.*, 2010). Several *BIOTIN ATTACHMENT DOMAIN-CONTAINING* (*BADC*) genes that repress FA biosynthesis are activated by AtWRI1 (Liu et al., 2019), suggesting another level of mediation of WRI1-regulated oil biosynthesis. Furthermore, the latest discovery showed that *carboxyltransferase interactor* (*CTI*), encoding an envelope membrane regulator of ACCase, is also positively regulated by WRI1 (Ye et al., 2020). Contrast to the positively regulation by LEC1/LEC2/FUS3, the Arabidopsis R2R3-MYB transcription factor MYB89 represses *AtWRI1* expression (Li *et al.* 2017a) (Figure 2A). It should be noted that other plant species might have alternative transcriptional regulators mediating *WRI1* expression. In oil palm mesocarp (where most palm oil is generated), *EgWRI1* is highly repressed during fruit ripening. However, no transcripts of orthologs of known WRI1-regulator genes (e.g., *LEC1, LEC2, ABI3,* and *FUS3*) are detected, suggesting that additional upstream transcriptional regulators (possibly fruit-specific factors) control the expression of *EgWRI1* (Bourgis et al., 2011). Transcriptional regulators, including EgNF-YA3, EgNF-YC2, and EgABI5, are involved in transcriptional control of *EgWRI1* by binding to the *EgWRI1* promoter (Yeap et al., 2017).

To prevent hyperactivation of essential biological processes, which might result in metabolic imbalance, the activities of transcriptional regulators are frequently calibrated through interactions with other regulators (Hafner et al., 2019; Memelink and Gantet, 2007; Mouchiroud et al., 2014; Pireyre and Burow, 2015; Sui et al., 2018). As the protein-protein interaction network of WRI1 unfolds, various interacting partners of AtWRI1 have been found to modulate its activity (summarized in Figure 2A). The CULLIN3 (CUL3)-based E3 ligase adaptor BTB/POZMATH (BPM) interacts with AtWRI1 to bridge AtWRI1 to CUL3, which mediates the AtWRI1 degradation by the 26S proteasome (Chen et al., 2013). A recent study found that *BPM* mRNA stability is mediated by *Arabidopsis PUMILIO PROTEIN24* (*APUM24*), encoding a Pumilio homology domain-containing protein which is involved in processes of rRNA processing and mRNA degradation), subsequently fine-tuning the function of the BPM-WRI1 module (Huang et al., 2021). The 14-3-3 proteins, a family of phosphopeptide-binding proteins, physically interact with AtWRI1 in yeast-two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) assays (Ma et al., 2016). Transient overproduction of 14-3-3 with AtWRI1 results in increased oil biosynthesis in *Nicotiana benthamiana* leaves by enhancing the protein stability and transcriptional activity of AtWRI1 (Ma *et al.*, 2016). BPM1 and 14-3-3 independently interact with AtWRI1 protein in an overlapping region, leading to the postulation that 14-3-3 interacts with AtWRI1 at the expense of the AtWRI1-BPM interaction (Kong and Ma, 2018a; Ma *et al.*, 2016). The SNF1-related protein kinase KIN10 is another AtWRI1 interacting partner that phosphorylates AtWRI1, resulting in the degradation of AtWRI1 protein (Zhai et al., 2017). In addition, phosphorylation deficient mutagenesis at amino acid residues T70 and S166 of AtWRI1 resulted in the abolishment of KIN10-mediated phosphorylation and improvement of AtWRI1 stability (Zhai *et al.*, 2017). Trehalose 6-phosphate (T6P) is involved in stabilizing AtWRI1 and boosting FA biosynthesis by suppressing KIN10 activity (Zhai et al., 2018).

Furthermore, the interacting partners may modulate the assembly of the WRI1 transcriptional complex. In eukaryotic cells, recruiting the mediator (MED) subunits by transcription factors to initiate transcription is a conserved regulatory mechanism (Taatjes, 2010). The MED15 subunit of the Arabidopsis Mediator complex has been shown, both *in vitro* and *in vivo,* to physically interact with WRI1 (Kim et al., 2016). Transgenic Arabidopsis overexpressing *MED15* displays upregulation of the FA biosynthetic genes targeted by AtWRI1. ChIP experiments also revealed that MED15 binds to the promoters of the AtWRI1 target genes. However, the transgenic *wri1* mutant overproducing MED15 also displays enhanced expression of AtWRI1 target genes, suggesting that alternative transcription factors interact with MED15 to modulate the expression of the oil biosynthetic genes (Kim *et al.*, 2016).

Intra- and inter-family interactions of transcriptional regulators significantly increase the complexity of combinatorial transcriptional regulation. In plants, a protein complex formed by members of a transcription factor family is well documented; nonetheless, the importance of cross-family interaction of transcriptional regulators in gene regulation is increasingly recognized

(Bemer et al., 2017). Members of a transcription factor family are generally a group of *cis*-element specific DNA-binding factors with overlapping or divergent functions. Different transcription factor families vary in their DNA binding property, transactivation activity (e.g., functioning as a repressor or activator), and response to environmental/developmental stimuli. As such, cross-family transcription factor interaction is a key molecular mechanism for fine-tuning gene expression in response to diverse environmental and developmental cues. Screening of an Arabidopsis transcription factor library led to the identification of TCP4 as a cross-family interacting regulator of AtWRI1 (Kong et al., 2020c). Functional analyses revealed that TCP4 decreases AtWRI1-regulated oil accumulation through repressing the AtWRI1 transactivation activity. The *tcp4* loss-of-function mutants exhibit elevated seed oil content compared to WT (Kong *et al.*, 2020c). Until now, little is known about the transcriptional repressors of WRI1 activity. This work uncovered the potential function of TCP4 as a transcriptional repressor of AtWRI1-regulated oil biosynthesis. However, the molecular mechanisms of the WRI1-TCP4 regulatory module remain to be elucidated. For instance, phosphorylation of WRI1 or TCP4 individually affects their activities and functions (Kong *et al.*, 2020c; Kong et al., 2020b; Kubota et al., 2017; Ma et al., 2015; Zhai *et al.*, 2017); however, it is unclear how phosphorylation affects the DNA binding, subcellular localization, protein stability, protein complex formation, and transcriptional activity of the WRI1-TCP4 module. In addition, TCPs are a plant-specific transcription factor family with diverse functions, it will be interesting to investigate the interaction between WRI1 and other TCP members for their roles in FA biosynthesis regulation.

While the WRI1 binding sequence, AW-box, has been established as a key cis-regulatory element (Maeo *et al.*, 2009), AtWRI1 is capable of binding to the promoter of *GH3.3* (involved in auxin degradation) at a non-AW-box element (Kong et al., 2017). Because AtWRI1 recognizes and binds to the AW-box in the promoters of the auxin carrier-protein *PIN* genes, a role of AtWRI1 in auxin homeostasis has been suggested (Kong *et al.*, 2017). This hypothesis is supported in soybean as overexpression of *GmWRI1* elevates, while knockdown of *GmWRI1* decreases, the expression of the auxin-related genes (Chen et al., 2020). How WRI1 modulates alternative targets to affect plant growth and development is currently being explored. Recent interesting work showed that AtWRI1 itself neither activates nor suppresses the activity of the *GH3.3* promoter (Kong et al., 2022) . Y2H screening resulted in identifying TCP20 as an interacting regulator of AtWRI1 (Kong *et al.*, 2022). TCP20 was found to regulate the expression of *GH3.3* through binding to a cis-acting element. Intriguingly, AtWRI1 physically interact with TCP20 to mitigate *GH3.3* expression, highlighting a WRI1-TCP20 regulatory module in fine-tuning auxin homeostasis (Kong *et al.*, 2022).

The Complex Transcriptional Network Involved in Controlling Seed Oil Accumulation

In addition to LEC1, LEC2, FUS3, ABI3, and WRI1, significant progress has been made in understanding other transcriptional regulators that control seed oil biosynthesis (Figure 2B). The transcription factor bZIP67 binds to the G-box of the *FAD3* promoter and controls the biosynthesis of α-linolenic acid in Arabidopsis seeds in a L1L and NF-YC2 dependent manner (Mendes et al., 2013). In addition, overproduction of a soybean bZIP transcription factor (GmbZIP123) elevates seed oil content in transgenic Arabidopsis plants (Song et al., 2013). In this case, the expression of genes involved in *de novo* FA biosynthesis is not impacted, but rather, several sucrose transport-related genes are upregulated, suggesting that GmbZIP123 mediates FA biosynthesis via regulating sucrose metabolism (Song *et al.*, 2013).

In addition to MYB89, other MYB transcription factors regulate seed oil biosynthesis. MYB96 regulates seed oil accumulation through controlling the expression of *DGAT1* and *PDAT1* (Lee et al., 2018), and controls the biosynthesis of very-long-chain fatty acids (VLCFAs) by regulating the expression of *FAE1* by directly binding to its promoter (Lee et al., 2015). MYB76, MYB118, and MYB123 [TRANSPARENT TESTA2 (TT2)] also impact seed oil biosynthesis (Barthole et al., 2014; Chen et al., 2012a; Duan et al., 2017). However, their functions seem to be distinct from that of MYB96. For example, MYB118 is a repressor of FA biosynthesis in the endosperm by repressing maturation-associated genes (Barthole *et al.*, 2014). TT2, known for regulating proanthocyanidin generation (Nesi et al., 2001), also modulates seed oil content. The *tt2* mutant displays increased seed oil content and increased expression of genes involved in FA biosynthesis, such as *FUS3*, *FAE1*, and *KAS II*, suggesting TT2 negatively regulates seed oil accumulation (Chen *et al.*, 2012a). Moreover, TT8 (bHLH42) and TRANSPARENT TESTA GLABRA1 (TTG1), known to mediate the biosynthesis of proanthocyanidin and anthocyanin, respectively (Baudry et al., 2004), play negative roles in regulating seed FA biosynthesis. TT8 represses seed FA production through binding to the promoters of *LEC1*, *LEC2*, and *FUS3* (Chen et al., 2014). TTG1, encoding a WD40 protein, is involved in various physiological processes, including anthocyanin biosynthesis, embryogenesis, seed coat mucilage generation, and formation of trichomes and root hairs in Arabidopsis (Debeaujon et al., 2000; Ramsay and Glover, 2005;

Tominaga-Wada et al., 2011; Tsuchiya et al., 2004; Walker et al., 1999; Western et al., 2001). The seeds of *ttg1* mutant contains higher total proteins, starch, and fatty acids (Chen et al., 2015). Further analysis revealed that TTG1 indirectly represses the expression of various genes engaged in seed developmental process and FA biosynthesis (Chen *et al.*, 2015). Overproduction of soybean GmMYB73 resulted in increased lipid accumulation in seeds of Arabidopsis and Lotus, possibly through downregulation of *GLABRA2* (*GL2*) expression (Liu et al., 2014).

Other transcriptional regulators, including GL2, WRKY, Dof (DNA binding one finger), and tandem CCCH zinc finger (TZF) protein, are also involved in controlling seed oil accumulation. *GL2* encodes a homeobox protein which plays key roles in root development, trichome formation, and seed coat mucilage biosynthesis (Cheng et al., 2014; Hung et al., 1998; Masucci et al., 1996; Rerie et al., 1994; Western *et al.*, 2001). The *gl2* loss-of-function mutant displays an 8% increase in seed oil content compared to WT (Shen et al., 2006). Further study revealed that *GL2* is expressed in both embryo and seed coat, and its target gene *MUCILAGE MODIFIED 4* (*MUM4*) is involved in mucilage biosynthesis. The *mum4* mutant displays a similar seed oil increase phenotype as the *gl2* mutant, possibly due to the *gl2* mutant generating more oil with an elevated carbon allocation to embryo at the expense of seed coat mucilage production (Shi et al., 2012). WRKY6 is previously reported to play diverse roles in leaf senescence and responses to low phosphate stress (Chen et al., 2009; Ye et al., 2018b; Zhang et al., 2018). A recent work found a new role of WRKY6 in mediating plant seed oil accumulation. The *wrky6* loss-of-function mutant displays enhanced seed oil content compared to WT (Song et al., 2020). The expression levels of *FUS3* and *DGAT1* are upregulated in the seeds of *wrky6*; however, how WRKY6 controls the expression of the oil biosynthetic genes remains unclear (Song *et al.*, 2020). Mutation of *MINISEED3* gene, encoding WRKY10 that controls seed development in Arabidopsis, leads to reduced total FA content, as well as decreased seed size and seed weight (Fatihi et al., 2013). The disruption of *WRKY43* in Arabidopsis did not change total FA content in seeds, but significantly altered the ratio of polyunsaturated fatty acids (including linoleic acid and linolenic acid), suggesting a potential role of WRKY43 in modulating seed FA desaturation (Geilen et al., 2017). Dofs are plant-specific transcription factors which regulate diverse plant physiological processes, such as light response, seed germination, plant defense, vascular development, and phytohormone signaling (Le Hir and Bellini, 2013; Ruta et al., 2020; Yanagisawa, 2002). Overexpression of two soybean *Dofs* (*GmDof4* and *GmDof11*) in Arabidopsis increased seed oil content (Wang et al., 2007). The two Dofs activate the expression of *ACCase* and *long-chain-acyl CoA synthetase* (*LACS*) genes, while repressing *CRA1* (encoding a seed storage protein) via binding to the Dof *cis*-element in the gene promoters (Wang *et al.*, 2007). Plant TZF proteins participate in a variety of phytohormone signaling events and stress responses to regulate gene expression (Bogamuwa and Jang, 2013; Kim et al., 2008; Lin et al., 2011). A recent work found that a soybean TZF protein, GmZF392, functions as a transcriptional activator in a seed-specific manner (Lu et al., 2021). Overexpression of *GmZF392* enhanced seed oil accumulation in transgenic Arabidopsis and soybean plants. GmZF392 controls the expression of FA biosynthetic genes through binding to the bipartite elements in their promoters (Lu *et al.*, 2021).

Epigenetic Regulators Involved in Mediating Seed Oil Accumulation

Epigenetic regulators affect transcriptional regulation through amending the regional state of chromatin. Recent evidence suggests that epigenetic regulators participate in controlling seed oil accumulation (Figure 2A). CURLY LEAF (CLF), a histone methyltransferase of Polycomb Repressive Complex 2 (PRC2) modulating the trimethylation of histone H3 Lys 27 (H3K27me3), functions as a key regulator in repressing gene expression during embryonic development (Liu et al., 2016). The *clf* mutant produces larger seeds with increased seed mass, greater seed oil content, and modified long-chain FA composition compared to WT (Liu *et al.*, 2016). Genes encoding transcriptional regulators involved in controlling FA biosynthesis, such as LEC1, LEC2, FUS3, WRI1, as well as various FA biosynthetic genes are upregulated in developing seeds of the *clf* mutant, suggesting a role of CLF in transcriptional repression of FA biosynthesis (Liu *et al.*, 2016). PICKLE (PKL) is a nuclear-localized ATP-dependent chromatin remodeler, belonging to the chromodomain-helicase-DNA-binding domain (CHD3) family (Dean Rider et al., 2003; Ogas et al., 1999; Park et al., 2017; Zha et al., 2020; Zhang et al., 2012). PKL represses the expression of *LEC1*, *LEC2* and *FUS3*. Expression levels of *LEC1/LEC2/FUS3* significantly increased in roots of the *pkl* mutant compared to WT (Dean Rider *et al.*, 2003; Ogas *et al.*, 1999), correlated to the upregulation of storage lipid biosynthesis (Ogas et al., 1997; Ogas *et al.*, 1999).

Environmental and Developmental Cues that Affect Seed Oil Biosynthesis

Light potentially affects seed oil accumulation (Table 1). Most Brassicaceae family members, including Arabidopsis, produce green seeds with photosynthetic activities (Eastmond et al., 1996; Eastmond and Rawsthorne, 1998). The light passing through the silique wall is an important driving force for FA generation (Goffman et al., 2005; Ruuska et al., 2004; Schwender et al., 2004).

Analysis of Arabidopsis plants grown under different light conditions revealed a positive correlation between seed oil content (as well as seed yield and weight) and light intensity (Karki and Bates, 2018; Li et al., 2006). Other studies also support that reduced sunlight during seed filling leads to reduction of oil contents in oil crops (Andrade and Ferreiro, 1996; de la Vega et al., 2001; Singer et al., 2016). Increased light intensity also alters seed FA composition, with an increased oleic acid to linoleic acid ratio (Seiler, 1986). Although the molecular mechanism by which light affects seed lipid metabolism remains to be elucidated, it is rational to hypothesize that photosynthesis affected by light conditions modifies carbon supply which eventually influences FA biosynthesis.

Temperature impacts seed oil accumulation and composition. The influences of global temperature, due to climate change, on FA biosynthesis may change crop oil yield and/or FA compositions. For example, in 2012 when Canada experienced a season of abnormally warmer temperature, the *B. napus* seed oil yield significantly reduced, from the normal average of 44.4% to 43.5% (Singer *et al.*, 2016). For certain *B. napus* varieties, cooler growing temperature, particularly during the seed filling period, tends to result in greater oil contents (Carrera et al., 2009; Harris et al., 1978; Singer *et al.*, 2016). Similar to seed oil content, seed FA composition is also impacted by temperature. The unsaturation of FA level is generally inversely correlated with increasing environmental temperature. Plant species, such as *B. napus* and sunflower, grown under higher temperature show reduced levels of linoleic acid and α-Linolenic acid, and the decrease of these polyunsaturated FAs is compensated by an elevation of oleic acid (Canvin, 1965; Schulte et al., 2013; Singer *et al.*, 2016; Wolf et al., 1982). The molecular mechanism underlying the temperature impacts on seed oil yield and composition remains unclear. Nonetheless, it has been proposed that higher temperature increases nitrogen bioavailability, which leads to a competition for the carbon backbone required for storage lipid generation, consistent with the inverse correlation between FA and protein yield under heat stress (Canvin, 1965; Singer *et al.*, 2016). Lower FA desaturation at elevated temperature can also be due to lower oxygen solubility as has been demonstrated in *Acanthamoeba* (Jones et al., 1991; Rutter et al., 2002), although such mechanism has not been reported in higher plants.

Drought generally leads to substantial reduction of seed oil content and modification of FA composition in plants (Singer *et al.*, 2016) (Table 1). It is proposed that drought leads to reduced seed filling but faster embryo development, which affects the FA biosynthesis (Singer *et al.*, 2016).

In addition, salinity stress causes seed oil reduction and FA composition alteration (Singer *et al.*, 2016). Salinity generates reactive oxygen species (ROS) which damages cellular redox homeostasis and triggers oxidative stresses, negatively impacting metabolic enzymes including those involved in FA biosynthesis. Interestingly, overproduction of DGAT1 in *B. napus* reduces the adverse effects on seed oil production under drought stress (Weselake et al., 2008). Transgenic camelina co-producing CsMYB96A and CsDGAT1C also shows increased resistance to drought stress with improved seed oil content (Kim et al., 2019). Furthermore, expression of *DGAT1* is upregulated under stresses such as salinity (Kong et al., 2013; Lu and Hills, 2002), suggesting a wider role of DGAT1 in response to environmental stresses. The transgenic plants overexpressing *DGAT1* are ideal materials for an in-depth understanding of the role of DGAT1 under various stresses.

FA biosynthesis is associated with phytohormone signaling. Gibberellins (GAs) are important phytohormones involved in regulating numerous plant developmental process, including stem elongation, germination, dormancy, flowering, flower development, and leaf and fruit senescence (Fleet and Sun, 2005; Sasaki et al., 2003; Swain and Singh, 2005). GAs potentially affect FA metabolism in plant cells. The DELLA proteins are essential players in the complex GA signal transduction cascade (Jiang and Fu, 2007; Thomas and Sun, 2004; Zentella et al., 2007). A transcriptomic analysis showed that DELLAs mediate the downregulation of various *GDSL*-type lipase genes in seeds and flower buds, indicating a possible function of DELLAs in affecting seed FA metabolism (Cao et al., 2006). Further study showed that exogenous GA treatment or DELLA mutations cause alterations of total seed FA content, modification of FA composition, as well as various morphologic variations in seeds (Chen et al., 2012b). A global transcriptomic analysis revealed that GA3 treatment of *ga1–3* (a severe GA deficient mutant) significantly changes the expression of *WRI1* (Zentella *et al.*, 2007). As essential phytohormones, auxin and jasmonate (JA) participate in mediating various developmental processes (Guo et al., 2018; Huang et al., 2017; Lavy and Estelle, 2016; Woodward and Bartel, 2005; Zhao, 2010). Transcriptomic analysis of *B. napus* developing seeds revealed that multiple auxin *de novo* biosynthesis-associated genes and a JA-inducible gene co-express with FA biosynthetic genes, implying a potential role for auxin and JA in FA biosynthesis in *B. napus* seed development (Niu et al., 2009) (Table 1). These studies shed light on the relationship between FA biosynthesis, phytohormone signaling, and seed development; however, the detailed molecular mechanisms, by which phytohormone signaling modulates FA metabolism during the embryogenesis, remain to be further elucidated.

Earlier studies provided evidence of the involvement of WRI1 in ABA and sugar signaling, particularly in developing seedlings (Cernac et al., 2006; Masaki et al., 2005). The interaction between ABA and sugar signaling pathways fine-tunes the gene expression during plant developmental processes (Dekkers et al., 2008; Finkelstein and Gibson, 2002; Sakr et al., 2018). ABA broadly participates in various stress-related responses and developmental regulation. Unraveling the position of WRI1 in phytohormone crosstalk, particularly related to seed filling under stress conditions, is of agricultural importance. Plant-fungi interaction is crucial for initial land plant colonization and vascular plant evolution. Arbuscular mycorrhizal (AM) symbiosis is a close evolutionary relationship between land plants and Glomeromycotina fungi (Bonfante and Genre, 2010; Remy et al., 1994). Recent studies identified that WRI-like proteins, including CTTC MOTIF-BINDING TRANSCRIPTION FACTOR1 (CBX1) and WRI5a (from *Lotus japonicus* and *Medicago truncatula,* respectively), play an essential role in FA production and phosphate uptake that are necessary for AM symbiosis (Jiang et al., 2018; Xue et al., 2018). The findings shed light on the molecular mechanism underlying bidirectional nutrient exchanges during AM symbiosis, suggesting that, evolutionally, WRI-like proteins are ancient transcriptional regulators. A recent study showed a conserved transcriptional change in land plants during AM symbiosis. A WRI-like transcriptional regulator, identified from *Marchantia paleacea,* functions in transcriptional regulation of FA biosynthesis and transport, which are vital for mutualistic interactions in *M. paleacea* symbiosis (Rich et al., 2021).

Translational Applications of Plant Transcriptional Regulators in Bioengineering Plant Oil Accumulation

The function of WRI1 seems to be conserved across various angiosperms, both dicots and monocots. The orthologs of WRI1 have been characterized in a variety of plant species. Transgenic plants overproducing AtWRI1 or WRI1 orthologs display substantially enhanced seed oil content (An and Suh, 2015; Cernac and Benning, 2004; Chen *et al.*, 2020; Chen et al., 2018a; Liu et al., 2010; Shen *et al.*, 2010; Sun et al., 2017; Yang et al., 2015; Ye et al., 2018a) (Table 2). The shared regulatory network controlling both development and FA biosynthesis should be considered when using the transcriptional regulators to engineer plant oil production. Choosing appropriate promoters to drive the *WRI1* expression is crucial for oil bioengineering. For instance, transgenic maize showed considerably elevated oil accumulation when *ZmWRI1* expression is driven by the embryo-preferred *OLEOSIN* (*OLE*) promoter, but not by the starch endosperm-specific *19 KD ZEIN* promoter (Shen *et al.*, 2010). Another study showed that use of the *FUS3* promoter to drive *AtWRI1* expression, aimed to stretch oil accumulation period during the mid-phase of seed development, is an efficient strategy to improve seed oil accumulation (Kanai et al., 2016) (Table 2).

Transient overexpression of *AtWRI1* or other *WRI1* orthologs effectively activate TAG production in tobacco leaves (Grimberg et al., 2015; Ma *et al.*, 2015; Vanhercke et al., 2013) (Table 2). Transient co-expression of *AtWRI1* and *DGAT1* in tobacco leaves leads to markedly elevated oil production compared to expressing *AtWRI1* alone, indicating a synergistic impact of the two genes (Vanhercke *et al.*, 2013). Transient overproduction of the more stable AtWRI1 variants, or transient co-expression of At*WRI1* with AtWRI1-interacting regulators that stabilizes AtWRI1, result in enhanced oil production compared to expressing the native *WRI1* (Ma et al., 2015; Ma et al., 2016; Zhai et al., 2017). In efforts to improve oil biosynthesis in vegetative tissues, transgenic Arabidopsis overexpressing *AtWRI1,* while repressing *AGPase* (the small subunit of ADP-glucose pyrophosphorylase) by RNAi, increased TAG accumulation in leaves (Sanjaya et al., 2011). In sugarcane, co-expression of *WRI1*, *DGAT1*, *oleosin 1* (*OLE1*), together with repression of *AGPase* and *PXA1* (encoding a subunit of the peroxisomal ABC transporter) by RNAi, considerably increased TAG generation in the vegetative tissues (Zale et al., 2016) (Table 2). The engineering scheme of “push, pull, package, and protect” is to concurrently augment (push) the *de novo* FA biosynthesis via activating a key transcriptional regulator, pull the precursors to the final products by rate-limiting enzymes, package TAG molecules into oil droplets, and shield the TAG from decay by deactivating lipases (Vanhercke *et al.*, 2019; Vanhercke *et al.*, 2014).

Enhancement of seed oil accumulation can also be achieved by the manipulation of other transcriptional regulators that mediate the expression of genes involved in alternative metabolic pathways, e.g., seed protein and carbohydrate metabolism. Transgenic Arabidopsis overproducing the soybean transcription factors, GmbZIP123, GmDof4, and GmDof7, increased seed oil content, possibly due to the upregulation of genes involved in sucrose metabolism and seed storage (Song *et al.*, 2013; Wang *et al.*, 2007).

Plants produce diverse unusual fatty acids, such as hydroxy fatty acids (HFAs), that are highly valuable for industrial applications due to their unique physical and chemical characteristics. Ectopic expression of a hydroxylase gene in Arabidopsis, while producing small amount of HFAs, significantly decreases seed oil content (Bates and Browse, 2011; Bates et al., 2014), perhaps due to a feedback inhibition of *de novo* FA synthesis in the plastids (Bates *et al.*, 2014). Using an improved strategy, by which *AtWRI1* and *RcFAH12* (a castor fatty acid hydroxylase) are co-expressed, both HFA and seed oil content are considerably increased, indicating that AtWRI1 efficiently overcomes the feedback inhibition by the FA hydroxylase (Adhikari et al., 2016).

Concluding Remarks and Perspectives

Plants are sessile organisms consistently facing environmental challenges. Plants have developed elegant systems to respond to the environmental signals and modify their metabolic activities to overcome the stresses. The available evidence clearly shows an integrated regulatory network balancing the environmental response, seed development, and FA biosynthesis. Although it has been recognized that various environmental cues affect the seed oil biosynthesis and FA compositions (Karki and Bates, 2018; Li *et al.*, 2006; Singer *et al.*, 2016), the molecular mechanisms orchestrating the responses require further investigations. For instance, while many individual transcription factors in the regulatory network have been characterized, how they work in concert to perceive, transduce, and respond to environmental and developmental signals are poorly understood.

Manipulation of transcriptional regulators is a proven strategy to improve vegetable oil yield compared to the single-enzyme approach (Broun, 2004; Grotewold, 2008; Shen *et al.*, 2010). Although various degrees of success have been achieved, a deeper understanding of the transcriptional regulation of plant FA biosynthesis is required to overcome the remaining challenges and obstacles. Since the identification of *AtWRI1* more than fifteen years ago, a wealth of information suggests that WRI1 plays an essential role in the transcriptional control of plant oil biosynthesis. However, how do the key seed development regulators (LEC1, LEC2, FUS3, and ABI3) mediate *WRI1* expression remains to be further elucidated. Whether additional upstream transcriptional regulators modulate the expression of *WRI1*, particularly in response to different environmental and developmental cues, is an important question to be addressed in the future. The recently discovered non-seed specific transcriptional factors in oil palm that mediate *EgWRI1* expression begs the question whether the similar regulators exist in oil-producing tissues of other plant species (Yeap *et al.*, 2017). To tackle this question, it is necessary to conduct more thorough analyses of the *WRI1* promoters from diverse plant species, and the resulting identification of transcription factor binding sites might shed light on additional regulators of *WRI1*. Recent functional and structural analyses of different WRI1 orthologs and variants have concluded that the phosphorylation residues, IDR3-PEST motif, interacting motif of BPMs, and 14-3-3s are pertinent to the stability and transcriptional activity of WRI1 that affect plant oil accumulation (Ma *et al.*, 2013; Ma *et al.*, 2015; Ma *et al.*, 2016; Zhai *et al.*, 2017). The missing "VYL" motif in AsWRI1c, RcWRI1-B, and OsWRI1-1 questions the functional significance of "VYL" (Ji et al., 2018; Mano et al., 2019; Yang et al., 2019). Therefore, solving the three-dimensional structure, together with computational modeling and simulation, will facilitate our understanding of the structure/function relationship of WRI1.

Recent advances suggest that the crosstalk between WRI1-interacting partners is regulated at the post-translational level, underlining the collaborative nature of the WRI1 transcriptional apparatus. Although the dual roles of phosphorylation in modulating the activity of WRI1 have been proposed (Kong and Ma, 2018a; Ma *et al.*, 2016), future work will focus on illustrating the molecular mechanism of fine-tuning the activity of WRI1, by phosphorylation and dephosphorylation via diverse kinases and phosphatases, in response to a variety of developmental and environmental stimuli. Recently established WRI1 interactome has contributed to the discovery of novel WRI1-interacting regulators (Figure 2A), opening new doors to the investigation of the molecular action of the regulatory network.

The alternative targets of WRI1 beyond the oil biosynthetic pathways might be factors to be considered in translational application of WRI1 for oil bioengineering in crops. Nevertheless, numerous studies have well demonstrated WRI1 as one of the most effective transcriptional regulators for metabolic engineering of vegetable oil production. Utilization of approaches of protein engineering and genome editing to improve WRI1 binding specificity to the genes involved in oil biosynthetic pathway should be considered to future work of oil bioengineering.

The *AtWRI* gene family remains to be explored. *AtWRI3* and *AtWRI4* are *WRI1*-like genes, capable of complementing the phenotype of the *wri1* mutant (To et al., 2012). The functions of AtWRI3 and AtWRI4 in transcriptional control of seed oil accumulation are not certain as their genes are preferentially expressed in non-seed tissues (e.g. stems and flowers), despite their overlapping roles with AtWRI1 in supplying acyl chain for cutin production (To *et al.*, 2012). No obvious changes of plant growth were observed in the *wri1 wri3 wri4* triple mutant, and the expression of FA biosynthetic genes retained at basal level in the triple mutant, leading to a speculation of alternative transcriptional regulators in controlling FA biosynthetic genes in vegetative tissues (Marchive et al., 2014; To *et al.*, 2012). Dissecting the distinctive and overlaying roles of the WRI1-like proteins will contribute to understanding of the complex transcriptional regulation of FA biosynthesis, particularly in vegetative tissues.

Seed oil biosynthesis involves hundreds of genes in multiple regulatory and metabolic pathways. How this dynamic process is regulated during seed developmental process remains to be explored at whole genome and transcriptome levels. The development of population transcriptome analysis in recent years has led to the exploration of genes regulating lipid metabolism. In maize, using population transcriptome data, expression quantitative trait locus (eQTL) analysis revealed an intergenic locus affecting three oil-related genes, *long-chain fatty alcohol dehydrogenase* (*FADD*), *glycosylphosphatidylinositol* (*GPI*) and *glycolipid transfer protein 1* (*GLTP1*) , resulting in variation in kernel oil concentration (Liu et al., 2017). Combining eQTL with co-expression networks helped to identify some key regulatory genes of lipid metabolism. The transcription factor *BrMD-2* was identified using transcriptomic data from a *B. rapa* doubled haploid population (Basnet et al., 2016). Recently, transcriptome-wide association studies were applied in the dissection of oil biosynthesis in *B. napus* and 690 genes, including *LPAAT*, *TT4* and *C4H,* were identified to be associated with seed oil content (Tang et al., 2021). Thus, bioinformatics analysis based on transcriptome data from multiple tissues and multiple time series at population level may provide more insights into how seed oil biosynthesis is coordinated.

In summary, identification and characterization of essential regulatory factors that play roles in improving oil yield and agricultural traits are vital missions in vegetable oil research. To reach this goal, we need to have profound understandings of the regulatory mechanisms which impact the critical steps in the FA biosynthetic pathway and how these regulatory mechanisms are connected to environmental and developmental signaling. The knowledge gained in such endeavor will guide our design to enhance vegetable oil yield in crops with integrated approaches that include genome editing, protein engineering, and metabolic engineering.

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Y.Y., Q.K., A.R.Q.L., L.G, L.Y. and W.M. conceived the ideas, wrote the first draft and prepared the figures and tables. Q.K., S.L., H.Z., L.G., L.Y., and W.M. reviewed and edited the manuscript, figures, and tables. All authors have read and approved the final version of the manuscript.

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Figure 1. Environmental and developmental cues mediate gene expression and transcriptional activity during seed development.

The environmental signals affect embryogenesis as well as phytohormones and sugar levels, which also controls embryogenesis. The developmental signals significantly impact the LAFL network, which includes *LEC1, LEC2, L1L*, *ABI3*, and *FUS3,* as well as WRI1 and its interacting partners, and other transcription factors, leading a dynamic transcriptional regulation of plant oil biosynthetic pathways. Epigenetic regulators, such as CURLE LEAF (CLF) and PICKLE (PKL), also determine the transcriptional levels of several key regulators in LAFL network and WRI1. The combined transcriptional regulation leads to responsive metabolic readout, resulting in alteration of oil content and composition.

Figure 2. The complex and multi-level regulatory mechanisms for the control of plant seed oil accumulation.

A) LEC1, LEC2, FUS3 and ABI3 are the major upstream transcriptional regulators that mediate embryo development, seed maturation, and metabolism. Together with HSI2/VAL1, AGL15, BBM, TT8, MYB89, they form a network that tightly controls the expression of *WRI1*. These upstream regulators also mutually regulate one another. The upstream regulators are affected by epigenetic factors including CLF and PKL. WRI1 physically interacts with several transcription factors, such as BPMs, 14-3-3s, MED15, and TCP4, as well as post-translational modifiers, such as KIN10 kinase. WRI1 is subject to post-translational modifications, e.g., phosphorylation (circled P) and ubiquitination (Ub) that results in protein degradation. The targets of WRI1 include genes involved in FA biosynthetic pathway and late glycolysis. The positive (arrows) and negative (T-bars) regulations, together with post-translational modification, form a network that fine-tunes oil biosynthesis. B) Other transcription regulators that are involved in regulation of plant oil biosynthesis by upregulating the oil pathway genes. MYB96, DOF4, DOF11, TZF, WRKY10, WRKY43, DREB2C and bZIP67 positively regulate FA accumulation in seeds. Other factors, including TT2, TTG1, GL2, MYB76, MYB118 and WRKY6 negatively affects seed oil accumulation. The crosstalk among the positive and negative regulatory modules allows balanced and fine-tuned responses to the environmental and developmental signals during seed oil accumulation. The green arrows indicate positive regulation. The red T-bars represent negative regulation. Black lines indicate the formation of protein-protein interacting complex. Genes in light blue shaded circles are downstream targets of the transcription factors.

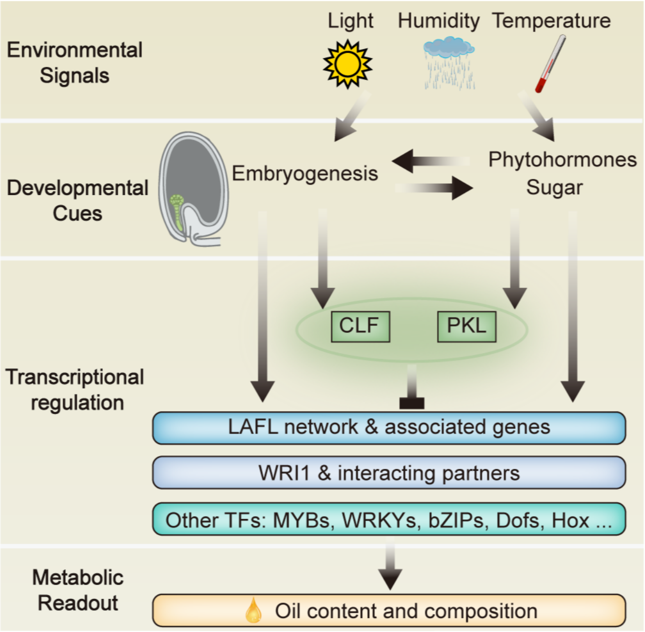
Table 1. Effects of environmental and developmental cues on seed oil biosynthesis.

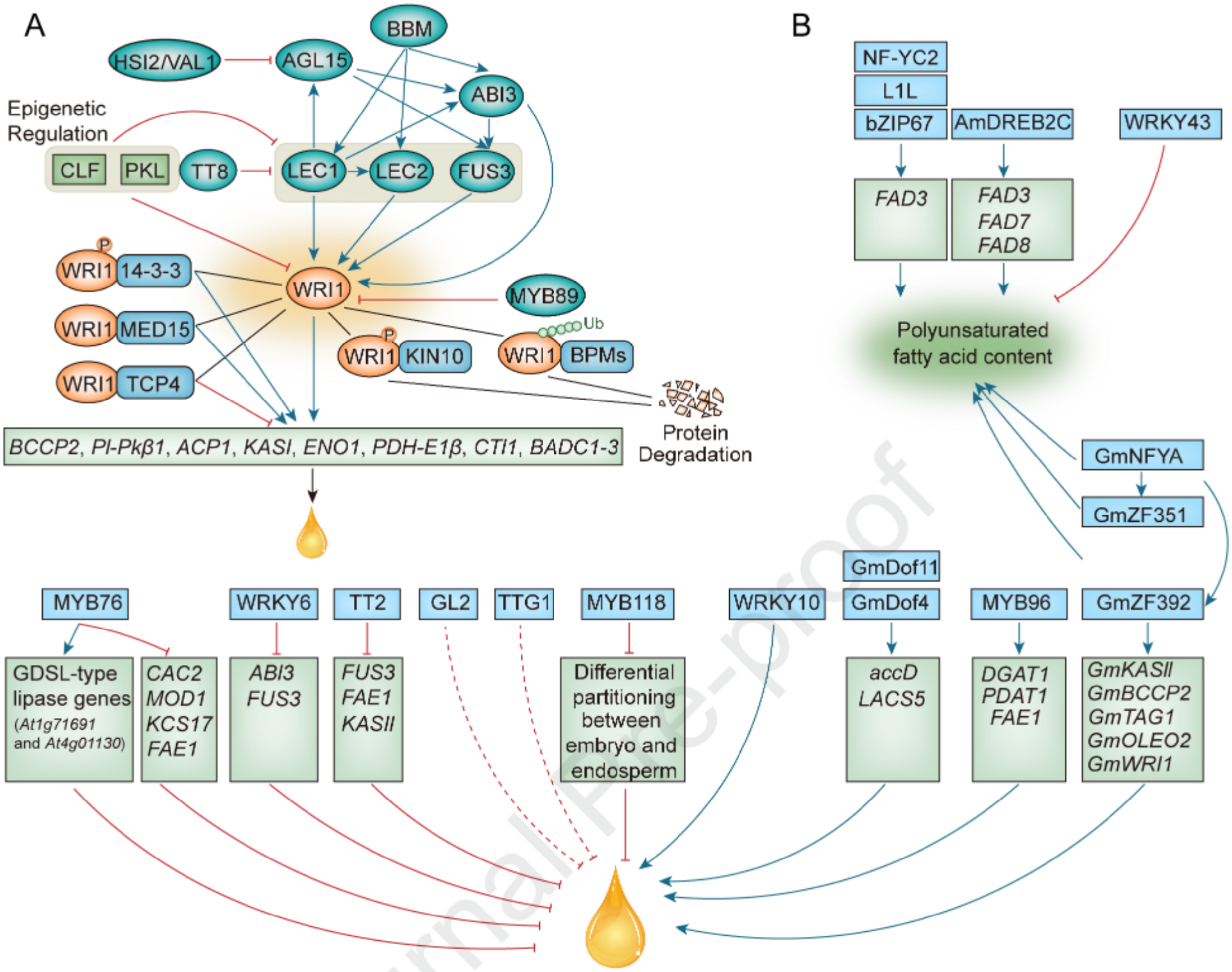
Environmental & developmental cues	**Effect on seed oil biosynthesis**	**Species**	**Reference**
High light intensity	Increase seed oil content Increase oleic acid content	*Brassica napus, Arabidopsis thaliana, Zea mays, Helianthus annuus*	Goffman et al. (2005); Ruuska et al. (2004); Schwender et al. (2004); Karki and Bates (2018); Li et al. (2006); Andrade and Ferreiro (1996); de la Vega et al. (2001); Singer et al. (2016); Seiler (1986)
High temperature	Reduce seed oil content Reduce unsaturated fatty acids content	*Glycine max, Helianthus annuus, Brassica napus, Linum usitatissimum, Glycine max*	Canvin (1965); Carrera et al. (2009); Harris et al. (1978); Singer et al. (2016); Schulte et al. (2013); Wolf et al. (1982)
Drought	Reduce seed oil content Increase oleic acid content, reduce linoleic acid content	*Helianthus annuus, Glycine max, Brassica napus*	Singer et al. (2016)
Salinity	Reduce seed oil content Increase oleic acid content, reduce linoleic acid and/or linolenic acid content	*Helianthus annuus, Carthamus tinctorius L., Gossypium hirsutum*	Singer et al. (2016)
Gibberellins	Alter seed oil content Modify fatty acids compositions	*Arabidopsis thaliana*	Cao et al. (2006); Chen et al. (2012b); Zentella et al. (2007)
Auxin and jasmonates	Alter seed oil content Modify fatty acids compositions	*Brassica napus*	Niu et al. (2009)

Table 2. Translational applications of plant transcriptional regulators in bioengineering plant oil.

Promoter	**Gene**	**Host species**	**Phenotype**	**Reference**
AtWRI1 and WRI1 orthologs				
CaMV 35S	*AtWRI1*	*Arabidopsis thaliana*	Increased seed oil content	Cernac and Benning (2004)
SiW6 promoter	*AtWRI1*	*Camelina sativa*	Enhanced seed oil content and seed mass	An and Suh (2015)
CaMV 35S	*GmWRI1a*/ *GmWRI1b*	*Arabidopsis thaliana*	Enhanced total TAG content	Chen et al. (2020)
Napin promoter	*GmWRI1a*	*Glycine max*	Increased the content of total oil and total fatty acids in seeds	Chen et al. (2018)
CaMV 35S	*BnWRI1*	*Arabidopsis thaliana*	Increased seed oil content, seed weight and size	Liu et al. (2010)
OLE promoter	*ZmWRI1*	*Zea mays*	Increased seed oil content with no significant difference in embryo size	Shen et al. (2010)
EnP2 promoter	*CoWRI1*	*Oryza sativa*	Increased seed oil content and altered lipid composition	Sun et al. (2017)
EnP2 promoter	*CoWRI1*	*Arabidopsis thaliana*	Increased seed oil content and altered lipid composition	Sun et al. (2017)
ZmUBI1 promoter	*BdWRI1*	*Brachypodium distachyon*	Increased grain dry weight, higher TAG content in endosperm and leaf blades	Yang et al. (2015)
Glycinin promoter	*HaWRI1*	*Arabidopsis thaliana*	Increased seed oil content, seed weight and size	Lim et al. (2022)
CaMV 35S	*JcWRI1*	*Jatropha curcas*	Elevated seed lipid content and increased seed mass	Ye et al. (2018a)
CaMV 35S	*StWRI1*/ *PtWRI1*/ *AsWRI1*/ *CeWRI1*	*Nicotiana benthamiana* leaves	Significant increase in oil accumulation	Grimberg et al. (2015)
FUS3 promoter	*AtWRI1*	*Arabidopsis thaliana*	Prolonged expression of *AtWRI1* during seed development leads to increased oil content in seeds	Kanai et al. (2016)
AtWRI1 variants				
CaMV 35S	*AtWRI1*$^{1\text{-}397}$	*Nicotiana benthamiana* leaves	Deletion of IDR-PEST motif of AtWRI1 leads to increased AtWRI1 protein stability and higher TAG content.	Ma et al. (2015)
CaMV 35S	*AtWRI1*$^{PEST/AA}$	*Nicotiana benthamiana* leaves, *Arabidopsis thaliana (wri1-1)*	Mutation of mutiple putative phosphorylation sites in PEST motif of AtWRI1 (AtWRI1$^{S398A/S401A/S402A/S407A/S415A/S416A/T420A/T421A/T422A/S423A}$) leads to increased stability of AtWRI1 and higher TAG content.	Ma et al. (2015)
CaMV 35S	*AtWRI1*^{4SA}	*Nicotiana benthamiana* leaves	Mutation of four residues (AtWRI1$^{S398A/S401A/S402A/S407A}$) in PEST motif of AtWRI1 leads to increased stability and higher TAG content.	Ma et al. (2015)

CaMV 35S	*AtWRI1*^{RR}	*Nicotiana benthamiana* leaves	Lysine to arginine mutation at the dilysine motif (AtWRI1$^{K2R/K3R}$) in AtWRI1 results in increased stability and TAG accumulation.	Zhai et al. (2017)
WRI1 interacting partners				
CaMV 35S	*14-3-3κ or 14-3-3λ*	*Nicotiana benthamiana* leaves; *Arabidopsis thaliana*	Enhancement of AtWRI1 stability and transcriptional activity, leading to increased oil production.	Ma et al. (2016)
CaMV 35S	*MED15*	*Arabidopsis thaliana*	Increased total fatty acid content in seeds.	Kim et al. (2016)
CaMV 35S	*KIN10*	*Nicotiana benthamiana* leaves	Significant reduction in TAG production. KIN10 negatively regulates AtWRI1 by triggering phosphorylation of sites in the AP2 DNA binding domains, leading to its degradation.	Zhai et al. (2017)
CaMV 35S	*TCP4*	*Nicotiana benthamiana* leaves	TCP4 negatively regulates the activity of AtWRI1, leading to reduced oil biosynthesis.	Kong et al. (2020b)
CaMV 35S	*BPM-microRNA*	*Arabidopsis thaliana*	Increased total fatty acid content in seeds. AtWRI1 interacts with E3 ligase adaptor BPMs for degradation through 26S proteasome.	Chen et al. (2013)
Combination of WRI1 and other genes				
CaMV 35S	*AtWRI1*, *DGAT1*	*Nicotiana benthamiana* leaves	Increased oil accumulation due to the upregulation of late glycolysis and fatty acid biosynthesis by WRI1 and TAG synthesis by DGAT1.	Vanhercke et al. (2013)
CaMV 35S	*AtWRI1*	*Arabidopsis thaliana*	Significant accumulation of oil in vegetative tissues due to the downregulation of starch formation by APS1 and directing carbon to fatty acid synthesis mediated by AtWRI1.	Sanjaya et al. (2011)
Patatin B33 promoter	*AtAPS1-RNAi*			
Rubi3 promoter	*AtWRI1*	Sugarcane (*Saccharum* spp. Hybrids)	Elevated TAG accumulation in leaves and stems.	Zale et al. (2016)
Mubi promoter	*ZmDGAT1-2*			
CaMV 35S	*AtOLE1*			
CaMV 35S	*AGPase-RNAi*			
CaMV 35S	*PXA1-RNAi*			


Environmental Signals
Light
Humidity
Temperature
Developmental Cues
Embryogenesis
Phytohormones
Sugar
Transcriptional regulation
CLF
PKL
LAFL network & associated genes
WRI1 & interacting partners
Other TFs: MYBs, WRKYs, bZIPs, Dofs, Hox ...
Metabolic Readout
Oil content and composition


A
HSI2/VAL1
AGL15
BBM
ABI3
Epigenetic Regulation
CLF
PKL
TT8
LEC1
LEC2
FUS3
P
WRI1
14-3-3
WRI1
MED15
WRI1
TCP4
WRI1
MYB89
P
WRI1
KIN10
Ub
WRI1
BPMs
BCCP2, PI-Pkβ1, ACP1, KASI, ENO1, PDH-E1β, CTI1, BADC1-3
Protein Degradation
B
NF-YC2
L1L
bZIP67
AmDREB2C
WRKY43
FAD3
FAD3
FAD7
FAD8
Polyunsaturated fatty acid content
GmNFYA
GmZF351
GmDof11
MYB76
WRKY6
TT2
GL2
TTG1
MYB118
WRKY10
GmDof4
MYB96
GmZF392
GDSL-type lipase genes (At1g71691 and At4g01130)
CAC2
MOD1
KCS17
FAE1
ABI3
FUS3
FUS3
FAE1
KASII
Differential partitioning between embryo and endosperm
accD
LACS5
DGAT1
PDAT1
FAE1
GmKASII
GmBCCP2
GmTAG1
GmOLEO2
GmWRI1