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

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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 the human diet but also as renewable feedstocks for industrial use. 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. We summarize 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.

Keywords: 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 generated and stored mainly 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 about one-fourth of 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 increasing rapidly and is 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 (Chapman and Ohlrogge, 2012; Bates et al., 2013; Li-Beisson et al., 2013; Allen, 2016; Bates, 2016; Xu and Shanklin, 2016; Kong et al., 2020a; Song et al., 2021b; Miray et al., 2021). 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
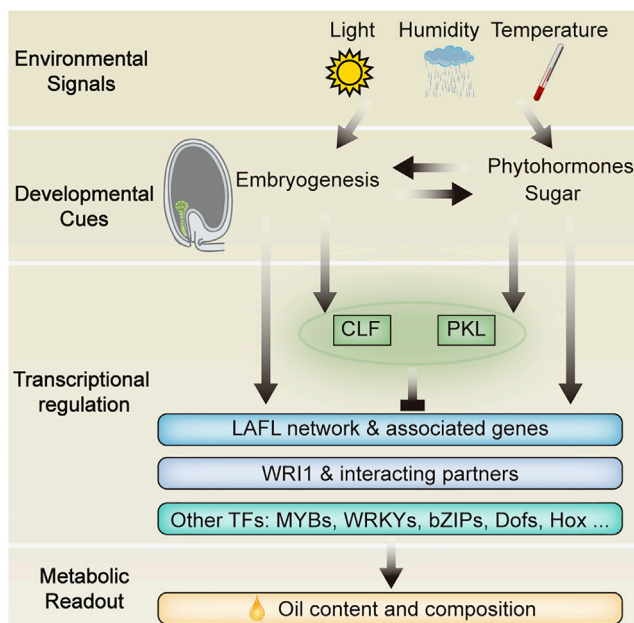
Figure 1. Environmental and developmental cues mediate gene expression and transcriptional activity during seed development.
Environmental signals affect embryogenesis as well as phytohormones and sugar levels, which also control embryogenesis. Developmental signals significantly affect the LAFL network (which includes *LEC1*, *LEC2*, *L1L*, *ABI3*, and *FUS3*), WRI1 and its interacting partners, and other transcription factors, leading to dynamic transcriptional regulation of plant oil biosynthetic pathways. Epigenetic regulators such as CURLY LEAF (CLF) and PICKLE (PKL) also determine the transcription levels of several key regulators in the LAFL network and WRI1. The combined transcriptional regulation leads to responsive metabolic readout, resulting in alteration of oil content and composition.

malonyl-ACP. After the first cycle of FA synthesis produces an acetoacetyl-ACP, additional cycles, facilitated by the fatty acid synthase complex, elongate the FA chain in a two-carbon increment, whereby 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 initiates 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 export to the endoplasmic reticulum. Currently, there are still some ambiguities regarding the specific steps and the sequence of events that occur during fatty acyl activation and export out of plastid envelopes (Bates et al., 2013; Bates, 2016). The pathway to TAG assembly from glycerol-3-phosphate and acyl-CoAs consists of multiple enzymatic steps (Chapman and Ohlrogge, 2012; Li-Beisson et al., 2013; Allen, 2016; Bates, 2016). First, glycerol-3-phosphate acyltransferase generates lysophosphatidic acid by transfer of an FA from acyl-CoA to the *sn*-1 hydroxyl of glycerol-3-phosphate. Then lysophosphatidic acid acyltransferase (LPAAT) esterifies a second FA to the *sn*-2 hydroxyl of lysophosphatidic acid to produce phosphatidic acid. Third, phosphatidic

acid phosphatase 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 used for biosynthesis of membrane lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine, 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 through a phosphocholine headgroup exchange mechanism. In the final stage, diacylglycerol acyltransferase (DGAT) or phospholipid:diacylglycerol acyltransferase (PDAT) catalyzes the conversion of DAG to TAG (Zou et al., 1999; Dahlqvist et al., 2000; Zhang et al., 2009). TAG is then coated with oleosins and stored as lipid droplets (Chapman et al., 2012).

TAG biosynthesis is a complex biological process that involves three main metabolic pathways: glycolysis, FA *de novo* synthesis and elongation, and TAG assembly. Glycolysis provides carbons that can be esterified with glycerol to produce TAG. Hence, seed oil accumulation requires the balanced transcriptional regulation of multiple branch pathways (Chapman and Ohlrogge, 2012; Lu et al., 2018; Kong et al., 2019). Transcription factors are proteins that 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 participate in regulating the expression of TAG biosynthetic genes (Cernac and Benning, 2004; Baud et al., 2007; Mu et al., 2008; Yamamoto et al., 2010; Ma et al., 2013; Li et al., 2017; Pelletier et al., 2017; Kong and Ma, 2018b; Tian et al., 2020). Among them, the APETALA2 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., 2014, 2019). Other transcriptional regulators also participate in regulation of the FA biosynthetic pathway by mediating the expression of *WRI1* (Baud et al., 2007; Yamamoto et al., 2010; Li et al., 2017; Kong et al., 2020a; Tian et al., 2020). Unlike 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 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 understanding some of the key regulators in these 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; Parcy et al., 1997; Lotan et al., 1998; Luerssen et al., 1998; Stone et al., 2001; Verdier and Thompson, 2008; Yamamoto et al., 2010; Pelletier et al., 2017; Jo et al., 2019;

Song et al., 2021a). Recent evidence shows that LEC1, LEC2, FUS3, and ABI3 are also involved in the transcriptional regulation of seed oil production (Baud et al., 2007; Mu et al., 2008; Shen et al., 2010; Tan et al., 2011; Zhang et al., 2016; Manan et al., 2017; Tian et al., 2020; Yang et al., 2021). Genes encoding these regulators are expressed predominantly during seed development and have a considerable effect on 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 the 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 the seed developmental process, including seed maturation (Song et al., 2021a). *LEC1* expression occurs in the endosperm, followed by LEC1 trafficking to the 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 its essential function in controlling the developmental process, LEC2 is involved in transcriptional regulation of FA biosynthesis and has therefore been used in engineering plants to enhance oil production (Santos Mendoza et al., 2005; Baud et al., 2007; Vanhercke et al., 2014, 2019; Kim et al., 2015). 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; Mu et al., 2008; Pelletier et al., 2017; Kong et al., 2019). *WRI1* expression is elevated in an *Arabidopsis* gain-of-function mutant of *LEC1*, *tnp*, as well as 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 with 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 (Shen et al., 2010; Manan et al., 2017).

The B3 domain transcription factor FUSCA3 (FUS3) triggers the expression of various FA biosynthetic genes, such as *FATTY ACID DESATURASE3* (*FAD3*), *KAS I*, and *FATTY ACID ELONGATION1* (*FAE1*) (Yamamoto et al., 2010; Wang and Perry, 2013). FUS3 also binds directly 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 with the WT (Yamamoto et al., 2010). *WRI1* was identified as a direct target of FUS3 in a genome-wide ChIP-chip analysis (Wang and Perry, 2013). The *fus3* mutant displays fewer oil droplets in the cotyledons of developing seeds, as well as reduced seed oil content compared with the WT (Meinke et al., 1994; Tiedemann et al., 2008; Roscoe et al., 2015). Transgenic overexpression of *FUS3* in *Arabidopsis* significantly triggers TAG accumulation in vegetative tissues, suggesting a useful role for FUS3 in the bioengineering of vegetable oil production (Zhang et al., 2016).

The B3 domain transcription factor ABI3 plays an important role in the abscisic acid (ABA) signaling pathway that mediates physiological processes such as seed maturation and dormancy (Rohde et al., 2000; Monke et al., 2012; Boulard et al., 2017; Tian et al., 2020). Recent ChIP-chip experiments identified direct targets of ABI3; they included *WRI1* and other genes encoding FA biosynthetic genes, including *FAD3* and *FATTY ACID BIOSYNTHESIS 2* (*FAB2*) (Tian et al., 2020). The combined results of ChIP-chip and comparative transcriptomic analysis of developing *Arabidopsis* seeds of *abi3* and WT showed that 317 genes, including *WRI1*, were directly activated by ABI3 (Tian et al., 2020). Furthermore, a recent study indicated that transgenic tobacco BY2 cell lines or transgenic *Arabidopsis* seedlings expressing an inducible *ABI3* showed activation of oil production, further supporting a role for ABI3 in the 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 transcription factor (Thakare et al., 2008; Zheng et al., 2009; Serivichyaswat et al., 2015; Cosio et al., 2017; Joshi et al., 2021). 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*) was significantly increased compared with the WT (Chen et al., 2018b). *BABY BOOM* (*BBM*), encoding an APETALA2 transcription factor, is essential for transcriptional regulation of plant cell totipotency (Horstman et al., 2014, 2017; Lutz et al., 2015). Functional analysis revealed that BBM binds directly 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 for BBM in the 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 with the WT (Focks and Benning, 1998). Transcriptomic analysis of developing *wri1-1* seeds showed that most genes with decreased expression encoded 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 binds directly to the promoter region and activates the expression of the *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, a recent discovery showed that *carboxyltransferase interactor* (*CTI*), encoding an envelope membrane regulator of ACCase, is also positively regulated by WRI1 (Ye et al., 2020). In contrast to the positive regulation by LEC1/LEC2/FUS3, the *Arabidopsis* R2R3-MYB transcription factor MYB89 represses *AtWRI1* expression (Li et al., 2017) (Figure 2A). It should be noted that alternative transcriptional regulators may mediate *WRI1* expression in other plant species. In oil palm mesocarp (where most palm oil is generated), *EgWRI1* was highly repressed during fruit ripening. However, no transcripts of orthologs of known WRI1-regulator genes (e.g., *LEC1*, *LEC2*, *ABI3*, *FUS3*) were 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, participate in the 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 has been unfolded, 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 AtWRI1 degradation by the 26S proteasome (Chen et al., 2013). A recent study showed that *BPM* mRNA stability is mediated by *Arabidopsis PUMILIO PROTEIN24* (*APUM24*, encoding a Pumilio homology domain-containing protein that is involved in 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 and bimolecular fluorescence complementation 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 hypothesis that 14-3-3 interacts with AtWRI1 at the expense of the AtWRI1–BPM interaction (Ma et al., 2016; Kong and Ma, 2018a). 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 abolished KIN10-mediated phosphorylation and improved AtWRI1 stability (Zhai et al., 2017). Trehalose 6-phosphate is involved in stabilizing AtWRI1 and boosting FA biosynthesis by suppressing KIN10 activity (Zhai et al., 2018).

Furthermore, interacting partners may modulate the assembly of the WRI1 transcriptional complex. In eukaryotic cells, recruitment of 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 to physically interact with WRI1 both *in vitro* and *in vivo* (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 that overproduces 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, protein complexes formed by members of a transcription factor family are well documented; nonetheless, the importance of cross-family interactions 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 properties, transactivation activities (e.g., functions as repressors or activators), and responses to environmental/developmental stimuli. As such, cross-family transcription factor interactions are 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 reduces AtWRI1-regulated oil accumulation by repressing AtWRI1 transactivation activity. The *tcp4* loss-of-function mutants exhibit elevated seed oil content compared with the WT (Kong et al., 2020c). Until recently, little was 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 (Ma et al., 2015; Kubota et al., 2017; Zhai et al., 2017;
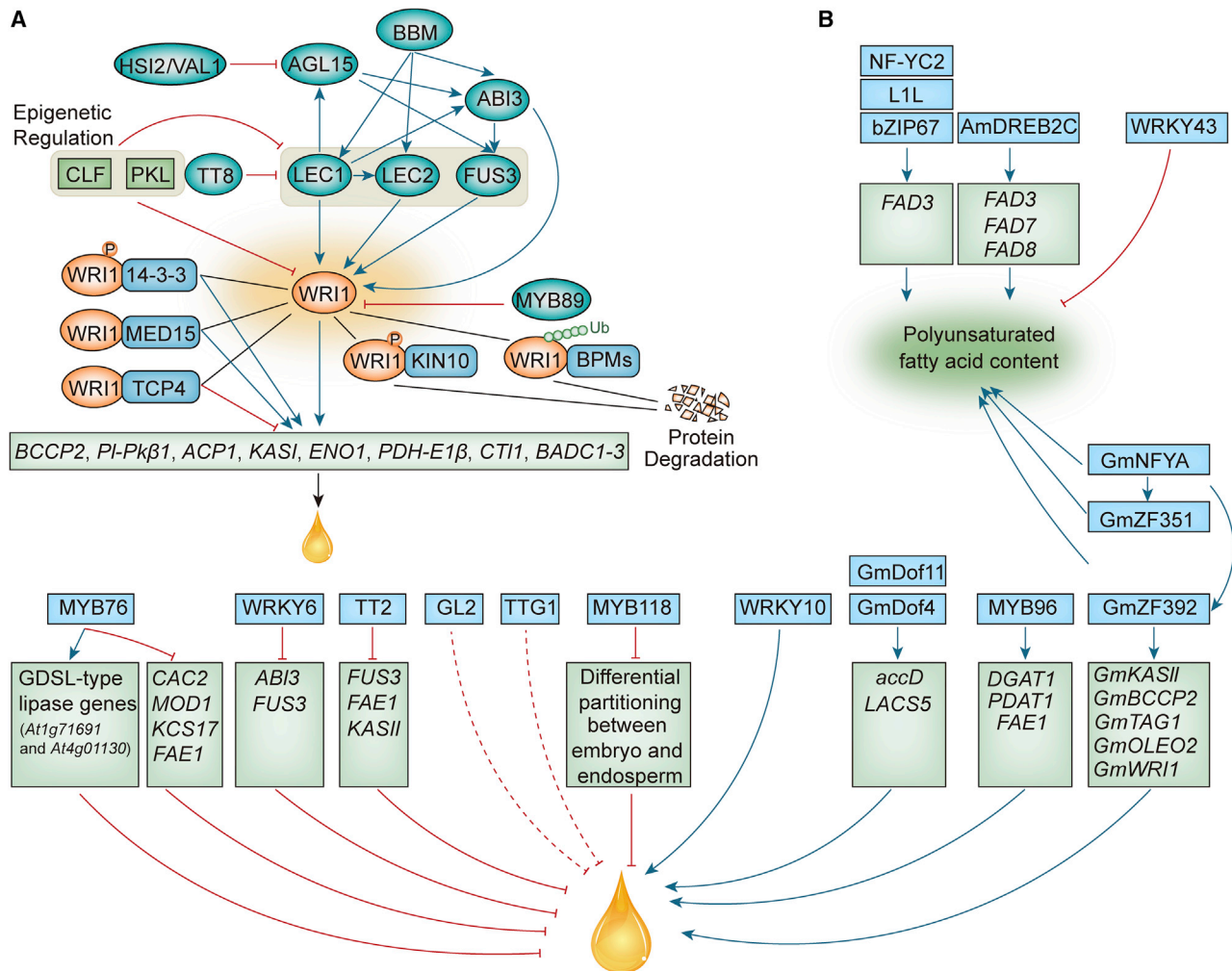
Figure 2. The complex and multi-level regulatory mechanisms for the control of plant seed oil accumulation.
(A) LEC1, LEC2, FUS3, and ABI3 are the major upstream transcriptional regulators that mediate embryo development, seed maturation, and metabolism. Together with HSI2/VAL1, AGL15, BBM, TT8, MYB89, 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, such as phosphorylation (circled P) and ubiquitination (Ub), that result in protein degradation. The targets of WRI1 include genes involved in the FA biosynthetic pathway and late glycolysis. The positive (arrows) and negative (T-bars) regulatory mechanisms, together with post-translational modifications, form a network that fine-tunes oil biosynthesis.
(B) Other transcriptional regulators that participate in the regulation of plant oil biosynthesis by upregulating 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 affect seed oil accumulation. The crosstalk among the positive and negative regulatory modules enables balanced and fine-tuned responses to 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 complexes. Genes in light blue shaded circles are downstream targets of the transcription factors.

Kong et al., 2020b, 2020c); 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, and it will be interesting to investigate the interactions between WRI1 and other TCP members and their roles in the regulation of FA biosynthesis.

Although 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 for At-WRI1 in auxin homeostasis has been suggested (Kong et al., 2017). This hypothesis is supported in soybean, as overexpression of *GmWRI1* elevates, and knockdown of *GmWRI1* decreases, the expression of 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). Yeast two-hybrid screening identified TCP20 as an interacting regulator of AtWRI1 (Kong et al., 2022). TCP20 was found to regulate the expression of *GH3.3* by binding to a *cis*-acting element. Intriguingly, AtWRI1 physically interacts with TCP20 to mitigate *GH3.3* expression, highlighting the role of 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 affected, but rather several sucrose transport-related genes are upregulated, suggesting that GmbZIP123 mediates FA biosynthesis by regulating sucrose metabolism (Song et al., 2013).

In addition to MYB89, other MYB transcription factors regulate seed oil biosynthesis. MYB96 regulates seed oil accumulation by controlling the expression of *DGAT1* and *PDAT1* (Lee et al., 2018) and controls the biosynthesis of very long chain fatty acids by regulating the expression of *FAE1* by directly binding to its promoter (Lee et al., 2015). MYB76, MYB118, and MYB123 (TRANSPARENT TESTA2 [TT2]) also affect seed oil biosynthesis (Chen et al., 2012a; Barthole et al., 2014; Duan et al., 2017). However, their functions seem to be distinct from that of MYB96. For example, MYB118 acts as a repressor of FA biosynthesis in the endosperm by repressing maturation-associated genes (Barthole et al., 2014). TT2, known for regulating proanthocyanidin production (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 that 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 by 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 production, and formation of trichomes and root hairs in *Arabidopsis* (Walker et al., 1999; Debeaujon et al., 2000; Western et al., 2001; Tsuchiya et al., 2004; Ramsay and Glover, 2005; Tominaga-Wada et al., 2011). The seeds of the *ttg1* mutant contain higher total proteins, starch, and fatty acids (Chen et al., 2015). Further analysis revealed that TTG1 indirectly represses the expression of various genes involved in seed developmental processes 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) proteins, are also involved in controlling seed oil accumulation. *GL2* encodes a homeobox protein that 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 with the 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 increased seed oil phenotype as the *gl2* mutant, possibly because the *gl2* mutant generates more oil with elevated carbon allocation to the embryo at the expense of seed coat mucilage production (Shi et al., 2012). WRKY6 was 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 study revealed a new role of WRKY6 in mediating plant seed oil accumulation. The *wrky6* loss-of-function mutant displays enhanced seed oil content compared with the 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 the *MINISEED3* gene, encoding WRKY10, which 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 for WRKY43 in modulating seed FA desaturation (Geilen et al., 2017). Dofs are plant-specific transcription factors that regulate diverse plant physiological processes, such as light response, seed germination, plant defense, vascular development, and phytohormone signaling (Yanagisawa, 2002; Le Hir and Bellini, 2013; Ruta et al., 2020). Overexpression of two soybean *Dofs* (*GmDof4* and *GmDof11*) in *Arabidopsis* increased seed oil content (Wang et al., 2007). The two GmDofs activate the expression of *ACCase* and *long-chain-acyl CoA synthetase* (*LACS*) genes while repressing *CRA1* (encoding a seed storage protein) by binding to the Dof *cis*-element in their promoters (Wang et al., 2007). Plant TZF proteins participate in a variety of phytohormone signaling events and stress responses to regulate gene expression (Kim et al., 2008; Lin et al., 2011; Bogamuwa and Jang, 2013). A recent work revealed 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 by 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 by altering the regional state of chromatin. Recent evidence suggests that

Environmental and developmental cues	Effect on seed oil biosynthesis	Species	Reference(s)
High light intensity	Increases seed oil content Increases 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	Reduces seed oil content Reduces unsaturated fatty acid 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	Reduces seed oil content Increases oleic acid content, reduces linoleic acid content	*Helianthus annuus, Glycine max, Brassica napus*	Singer et al., 2016
Salinity	Reduces seed oil content Increases oleic acid content, reduces 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 acid composition	*Arabidopsis thaliana*	Cao et al., 2006; Chen et al., 2012b; Zentella et al., 2007
Auxin and jasmonates	Alter seed oil content Modify fatty acid composition	*Brassica napus*	Niu et al., 2009

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

epigenetic regulators participate in controlling seed oil accumulation (Figure 2A). CURLY LEAF (CLF), a histone methyltransferase of Polycomb Repressive Complex 2 (PRC2), which modulates 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 with the 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 for CLF in the transcriptional repression of FA biosynthesis (Liu et al., 2016). PICKLE (PKL) is a nuclear-localized ATP-dependent chromatin remodeler from the chromodomain helicase DNA-binding domain (CHD3) family (Ogas et al., 1999; Dean Rider et al., 2003; Zhang et al., 2012; Park et al., 2017; Zha et al., 2020). PKL represses the expression of *LEC1*, *LEC2*, and *FUS3*. Expression levels of *LEC1/LEC2/FUS3* significantly increased in roots of the *pkl* mutant compared with the WT (Ogas et al., 1999; Dean Rider et al., 2003), correlated with the upregulation of storage lipid biosynthesis (Ogas et al., 1997, 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 activity (Eastmond et al., 1996; Eastmond and Rawsthorne, 1998). The light passing through the silique wall is an important driving force for FA production (Ruuska et al., 2004; Schwender et al., 2004;

Goffman et al., 2005). 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 (Li et al., 2006; Karki and Bates, 2018). Other studies have also indicated that reduced sunlight during seed filling leads to decreased oil content 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, increasing the 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 affects seed oil accumulation and composition. The influence of higher global temperatures due to climate change on FA biosynthesis may change crop oil yield and/or FA composition. For example, when Canada experienced a season of abnormally warm temperatures in 2012, *B. napus* seed oil yield was significantly reduced from the normal average of 44.4% to 43.5% (Singer et al., 2016). For certain *B. napus* varieties, cooler growing temperatures, particularly during the seed filling period, tend to result in greater oil contents (Harris et al., 1978; Carrera et al., 2009; Singer et al., 2016). Similar to seed oil content, seed FA composition is also affected by temperature. The FA unsaturation level tends to be inversely correlated with increasing environmental temperature. Plant species such as *B. napus* and sunflower show reduced levels of linoleic acid and α-linolenic acid when grown at higher temperatures, and the decrease in these polyunsaturated FAs is compensated by an increase in oleic acid (Canvin, 1965; Wolf et al., 1982; Schulte et al., 2013; Singer et al., 2016). The molecular

mechanism that underlies temperature effects 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 production, consistent with the inverse correlation between FA and protein yields under heat stress (Canvin, 1965; Singer et al., 2016). Lower FA desaturation at elevated temperatures can also be due to lower oxygen solubility, as has been demonstrated in *Acanthamoeba* (Jones et al., 1991; Rutter et al., 2002), although such a mechanism has not been reported in higher plants.

Drought generally leads to a substantial reduction in 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 FA biosynthesis (Singer et al., 2016). In addition, salinity stress causes reductions in seed oil and changes in FA composition (Singer et al., 2016). Salinity generates reactive oxygen species, which damage cellular redox homeostasis and trigger oxidative stress, negatively affecting metabolic enzymes, including those involved in FA biosynthesis. Interestingly, overproduction of DGAT1 in *B. napus* reduces the adverse effects of drought stress on seed oil production (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, the expression of *DGAT1* is upregulated under stresses such as salinity (Lu and Hills, 2002; Kong et al., 2013), suggesting a wider role for DGAT1 in response to environmental stresses. Transgenic plants overexpressing *DGAT1* are ideal materials for gaining 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 processes, including stem elongation, germination, dormancy, flowering, flower development, and leaf and fruit senescence (Sasaki et al., 2003; Fleet and Sun, 2005; 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 (Thomas and Sun, 2004; Jiang and Fu, 2007; 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 caused alterations in total seed FA content, modification of FA composition, and various morphological changes 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 (Woodward and Bartel, 2005; Zhao, 2010; Lavy and Estelle, 2016; Huang et al., 2017; Guo et al., 2018). Transcriptomic analysis of developing *B. napus* seeds revealed that multiple auxin *de novo* biosynthesis-associated genes and a JA-inducible gene are co-expressed 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 relationships among FA biosynthesis, phytohormone signaling, and seed development; however, the detailed molecular mechanisms by which phytohormone signaling modulates FA metabolism during embryogenesis remain to be fully elucidated.

Earlier studies provided evidence for the involvement of WRI1 in ABA and sugar signaling, particularly in developing seedlings (Masaki et al., 2005; Cernac et al., 2006). The interaction between ABA and sugar signaling pathways fine-tunes gene expression during plant developmental processes (Finkelstein and Gibson, 2002; Dekkers et al., 2008; 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 interactions have been crucial for initial land plant colonization and vascular plant evolution. The arbuscular mycorrhizal (AM) symbiosis is a close evolutionary relationship between land plants and Glomeromycotina fungi (Remy et al., 1994; Bonfante and Genre, 2010). Recent studies demonstrated that WRI-like proteins, including CTTC MOTIF-BINDING TRANSCRIPTION FACTOR1 (CBX1) and WRI5a (from *Lotus japonicus* and *Medicago truncatula*, respectively), play essential roles in FA production and phosphate uptake that are necessary for the AM symbiosis (Jiang et al., 2018; Xue et al., 2018). These findings shed light on the molecular mechanism underlying bidirectional nutrient exchanges during the AM symbiosis, suggesting that, evolutionarily, WRI-like proteins are ancient transcriptional regulators. A recent study showed a conserved transcriptional change in land plants during the AM symbiosis. A WRI-like transcriptional regulator identified from *Marchantia paleacea* functions in the 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 for 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 these transcriptional regulators to engineer plant oil production. Choosing appropriate promoters to drive *WRI1* expression is crucial for oil bioengineering. For instance, transgenic maize showed considerably elevated oil accumulation when *ZmWRI1* expression was 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, aiming to extend the oil accumulation period during the mid-phase of seed development, is an efficient strategy for improving seed oil accumulation (Kanai et al., 2016) (Table 2).

Transient overexpression of *AtWRI1* or other *WRI1* orthologs effectively activates TAG production in tobacco leaves

Promoter	Gene	Host species	Phenotype	Reference(s)
AtWRI1 and WRI1 orthologs				
CaMV 35S	AtWRI1	Arabidopsis thaliana	Increases seed oil content	Cernac and Benning, 2004
SiW6 promoter	AtWRI1	Camelina sativa	Enhances seed oil content and seed mass	An and Suh, 2015
CaMV 35S	GmWRI1a/GmWRI1b	Arabidopsis thaliana	Enhance total TAG content	Chen et al., 2020
Napin promoter	GmWRI1a	Glycine max	Increases the content of total oil and total fatty acids in seeds	Chen et al., 2018a
CaMV 35S	BnWRI1	Arabidopsis thaliana	Increases seed oil content, seed weight, and size	Liu et al., 2010
OLE promoter	ZmWRI1	Zea mays	Increases seed oil content with no significant difference in embryo size	Shen et al., 2010
EnP2 promoter	CoWRI1	Oryza sativa	Increases seed oil content and alters lipid composition	Sun et al., 2017
EnP2 promoter	CoWRI1	Arabidopsis thaliana	Increases seed oil content and alters lipid composition	Sun et al., 2017
ZmUBI1 promoter	BdWRI1	Brachypodium distachyon	Increases grain dry weight and leads to higher TAG content in endosperm and leaf blades	Yang et al., 2015
Glycinin promoter	HaWRI1	Arabidopsis thaliana	Increases seed oil content, seed weight, and size	Lim et al., 2022
CaMV 35S	JcWRI1	Jatropha curcas	Elevates seed lipid content and increases seed mass	Ye et al., 2018a
CaMV 35S	StWRI1/PtWRI1/AsWRI1/CeWRI1	Nicotiana benthamiana leaves	Cause significant increases 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	$AtWRI1^{1-397}$	Nicotiana benthamiana leaves	Deletion of the 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 multiple 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	The lysine to arginine mutation in the dilysine motif ($AtWRI1^{K2R/K3R}$) in AtWRI1 results in increased stability and TAG accumulation	Zhai et al., 2017

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

(Continued on next page)

Promoter	Gene	Host species	Phenotype	Reference(s)
WRI1-interacting partners				
CaMV 35S	14-3-3κ or 14-3-3λ	Nicotiana benthamiana leaves, Arabidopsis thaliana	Enhancement of AtWRI1 stability and transcriptional activity leads to increased oil production	Ma et al., 2016
CaMV 35S	MED15	Arabidopsis thaliana	Increases total fatty acid content in seeds	Kim et al., 2016
CaMV 35S	KIN10	Nicotiana benthamiana leaves	Significantly reduces 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	Increases total fatty acid content in seeds; AtWRI1 interacts with E3 ligase adaptor BPMs for degradation through the 26S proteasome	Chen et al., 2013
Combination of WRI1 and other genes				
CaMV 35S	AtWRI1, DGAT1	Nicotiana benthamiana leaves	Increase 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	Lead to 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)	Elevate 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			

Table 2. *Continued*

(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 with the expression of *AtWRI1* alone, indicating a synergistic effect 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 stabilize AtWRI1, results in enhanced oil production compared with expression of the native *WRI1* (Ma et al., 2015, 2016; Zhai et al., 2017). In an effort to improve oil biosynthesis in vegetative tissues, transgenic *Arabidopsis* were designed to overexpress *AtWRI1* and repress *AGPase* (the small subunit of ADP-glucose pyrophosphorylase) by RNAi, and they showed increased TAG accumulation in the leaves (Sanjaya et al., 2011). In sugarcane, co-expression of *WRI1*, *DGAT1*, and *oleosin 1* (*OLE1*), together with the repression of *AGPase* and *PXA1* (en-

coding a subunit of the peroxisomal ABC transporter) by RNAi, considerably increased TAG generation in vegetative tissues (Zale et al., 2016) (Table 2). The engineering scheme of "push, pull, package, and protect" involves concurrently augmenting (pushing) *de novo* FA biosynthesis by activating a key transcriptional regulator, pulling the precursors into the final products by rate-limiting enzymes, packaging TAG molecules into oil droplets, and shielding the TAG from decay by deactivating lipases (Vanhercke et al., 2014, 2019).

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 storage and carbohydrate metabolism). Transgenic *Arabidopsis* overproducing the soybean transcription factors GmbZIP123, GmDof4, and GmDof7 showed increased

seed oil content, possibly because of the upregulation of genes involved in sucrose metabolism and seed storage (Wang et al., 2007; Song et al., 2013).

Plants produce diverse unusual fatty acids, such as hydroxy fatty acids (HFAs), that are highly valuable for industrial applications because of their unique physical and chemical characteristics. Ectopic expression of a hydroxylase gene in *Arabidopsis*, while producing a small amount of HFAs, significantly decreases seed oil content (Bates and Browse, 2011; Bates et al., 2014), perhaps because of feedback inhibition of *de novo* FA synthesis in the plastids (Bates et al., 2014). Using an improved strategy in 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 that consistently face environmental challenges. Plants have developed elegant systems to respond to environmental signals and modify their metabolic activities to overcome these stresses. The available evidence clearly shows that an integrated regulatory network balances environmental responses, seed development, and FA biosynthesis. Although it has been recognized that various environmental cues affect seed oil biosynthesis and FA composition (Li et al., 2006; Singer et al., 2016; Karki and Bates, 2018), the molecular mechanisms that orchestrate these responses require further investigation. For instance, although 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 is poorly understood.

Manipulation of transcriptional regulators is a proven strategy for improving vegetable oil yield compared with 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 15 years ago, a wealth of information suggests that WRI1 plays an essential role in the transcriptional control of plant oil biosynthesis. However, how the key seed development regulators (LEC1, LEC2, FUS3, and ABI3) mediate *WRI1* expression remains to be fully elucidated. Whether additional upstream transcriptional regulators modulate *WRI1* expression, 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 transcription factors in oil palm that mediate *EgWRI1* expression beg the question of whether 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 *WRI1* promoters from diverse plant species; the resulting identification of transcription factor binding sites may 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, 2015, 2016; Zhai et al., 2017). The missing "VYL" motif in AsWRI1c, RcWRI1-B, and OsWRI1-1 casts doubt on 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 dual roles of phosphorylation in modulating the activity of WRI1 have been proposed (Ma et al., 2016; Kong and Ma, 2018a), future work will focus on illustrating the molecular mechanisms by which WRI1 activity is fine-tuned through phosphorylation and dephosphorylation by diverse kinases and phosphatases in response to a variety of developmental and environmental stimuli. The 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.

Alternative targets of WRI1 beyond the oil biosynthetic pathway might be factors to consider in translational application of WRI1 for oil bioengineering in crops. Nevertheless, numerous studies have demonstrated that WRI1 is one of the most effective transcriptional regulators for the metabolic engineering of vegetable oil production. The use of protein engineering and genome editing approaches to improve WRI1 binding specificity to genes in the oil biosynthetic pathway should be considered in future work on 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 chains for cutin production (To et al., 2012). No obvious changes in plant growth were observed in the *wri1 wri3 wri4* triple mutant, and the expression of FA biosynthetic genes remained at a basal level in the triple mutant, leading to speculation that alternative transcriptional regulators may control FA biosynthetic genes in vegetative tissues (To et al., 2012; Marchive et al., 2014). Dissecting the distinct and overlapping roles of the WRI1-like proteins will contribute to our 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 development remains to be explored at the whole-genome and transcriptome levels. The development of population transcriptome analysis in recent years has led to the exploration of genes that regulate lipid metabolism. Using population transcriptome data from maize, expression

quantitative trait locusi analysis revealed an intergenic locus that affected 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 expression quantitative trait locus 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 used to dissect oil biosynthesis in *B. napus*, and 690 genes, including *LPAAT*, *TT4*, and *C4H*, were identified as being associated with seed oil content (Tang et al., 2021). Thus, bioinformatics analysis based on transcriptome data from multiple tissues and multiple time series at the population level may provide more insights into the coordination of seed oil biosynthesis.

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 require a profound understanding of the regulatory mechanisms that impact critical steps in the FA biosynthetic pathway and how these regulatory mechanisms are connected to environmental and developmental signaling. The knowledge gained in such an endeavor will guide our design to enhance vegetable oil yield in crops with integrated approaches that include genome editing, protein engineering, and metabolic engineering.

AUTHOR CONTRIBUTIONS
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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