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REVIEW

Bridging the Knowledge Gap: Utilization of Mediator Subunits for Crop Improvement

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ABSTRACT

The Mediator complex is a multisubunit transcription coregulator that transfers regulatory signals from different transcription factors to RNA polymerase II (Pol II) to control Pol II-dependent transcription in eukaryotes. Studies on Arabidopsis Mediator subunits have revealed their unique or overlapping functions in various aspects of plant growth, stress adaptation and metabolite homeostasis. Therefore, the utilization of the plant Mediator complex for crop improvement has been of great interest. Advances in genome editing and sequencing techniques have expedited the characterization of Mediator subunits in economically important crops such as tomato, rice, wheat, soybean, sugarcane, pea, chickpea, rapeseed and hop. In this review, we summarize recent progress in understanding the molecular mechanisms of how the Mediator complex regulates crop growth, development and adaptation to environmental stress. We also discuss the conserved and diverse functions of the Mediator complex in different plant species. In addition, we propose several future research directions to deepen our understanding of the important roles of Mediator subunits and their interacting proteins, which would provide promising targets for genetic modification to develop new cultivars with desirable agronomic traits.

1 | Introduction

Transcription is a highly orchestrated process that requires essential factors, including RNA polymerase II (Pol II), general transcription factors (GTFs) and gene-specific transcription factors (TFs) (Allen and Taatjes 2015; Freytes, Gobbini, and Cerdán 2024) (Figure 1). Mediator is a multisubunit transcription coregulator that transfers regulatory signals from transcription factors to Pol II to regulate nearly all Pol II-dependent transcription in eukaryotes (Allen and Taatjes 2015; Yang, Li, and Qu 2016) (Figure 1). As a bridge between the general transcription machinery and gene-specific transcription factors, the Mediator complex is vital for decoding genetic information stored in DNA to govern biological processes. Although it functions as a complex, some subunits have unique functions and their alteration impacts specific organs or biological processes. The Mediator subunits can be prominent targets for altering the expression of genes that control specific traits in any organism.

The Mediator complex was identified in yeast and humans in the 1990s, revealing its necessity for activating Pol II-dependent transcription (Fondell, Ge, and Roeder 1996; Kelleher, Flanagan, and Kornberg 1990). The plant Mediator subunits

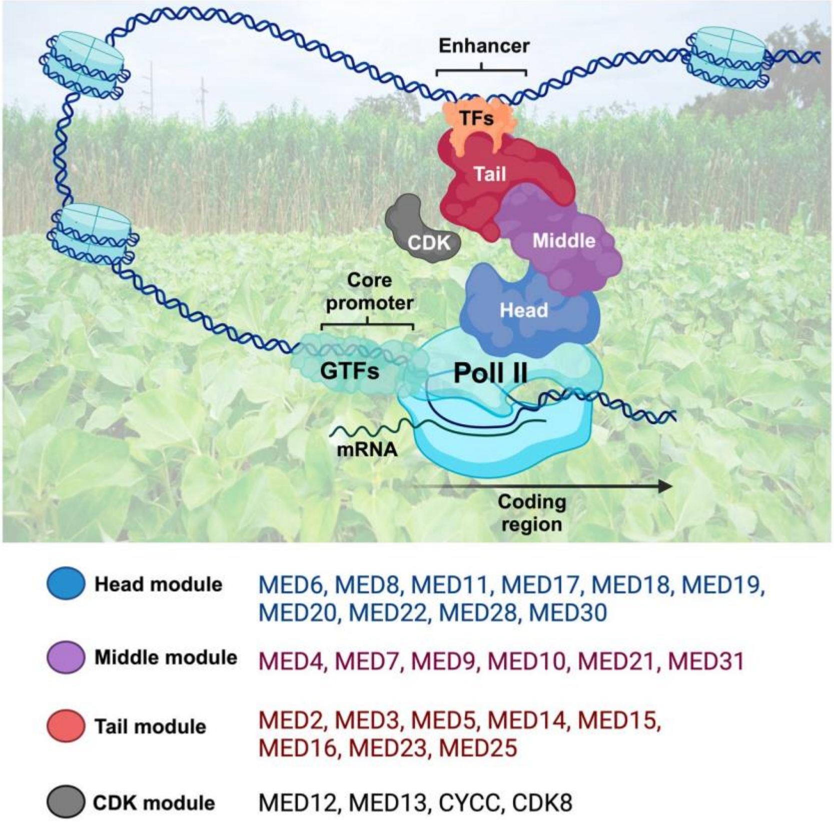

FIGURE 1 | A schematic diagram of the Mediator complex-dependent transcriptional regulation in plants. The Mediator complex transfers regulatory signals from gene-specific transcription factors (TFs) to RNA polymerase II (Pol II) to regulate Pol II-dependent transcription. GTFs, general transcription factors. The plant Mediator complex is structurally divided into four modules: head (blue), middle (purple), tail (brick red) and a dissociable cyclin-dependent kinase (CDK) module (grey). The Mediator subunits of each module are listed at the bottom. The figure was created with BioRender.com. [Color figure can be viewed at wileyonlinelibrary.com]

were first identified in the model species *Arabidopsis Thaliana* (Bäckström et al. 2007), wherein 19 Mediator subunits shared with yeast and metazoans (AtMED4, AtMED6-AtMED22, AtMED31), three subunits conserved in metazoans (AtMED23, AtMED27, AtMED28) and six plant-specific subunits (MED32-MED37) were isolated. Later, AtMED32 and AtMED33 were found to be orthologs of yeast MED2 and yeast MED5, respectively (Bourbon 2008). AtMED1 and AtMED26 were not copurified with other plant Mediator subunits in the initial study (Bäckström et al. 2007). A recent Arabidopsis study copurified 28 Mediator subunits with AtMED8, AtMED11, AtMED18, AtMED4, AtMED31 and AtMED25, yet AtMED26 and other plant-specific subunits (AtMED34-AtMED37) were not isolated in this study (Guo, Wei, et al. 2021). Thus, it is still debatable whether these subunits are indeed part of the Mediator complex in plants.

The Mediator complex is structurally divided into three core modules: head, middle and tail, and a dissociable cyclin-dependent kinase (CDK) module among all eukaryotes (Verger, Monté, and Villeret 2019) (Figure 1). In *Arabidopsis thaliana*, the head module includes AtMED6, AtMED8, AtMED11, AtMED17, AtMED18, AtMED19, AtMED20, AtMED22, AtMED28 and AtMED30. AtMED4, AtMED7, AtMED9, AtMED10, AtMED21 and AtMED31 belong to the middle module. The tail module contains AtMED2, AtMED3, AtMED5, AtMED14, AtMED15, AtMED16, AtMED23 and AtMED25. AtMED12, AtMED13, AtCDK8 and AtCYCC comprise the CDK module (Dolan and Chapple 2017; Freytes, Gobbini, and Cerdán 2024) (Figure 1). Studies suggest that each module and each subunit of the Mediator complex may have distinct functions. In general, the primary function of the head module is interacting with Pol II; the middle module can transfer transcription signals from the tail to the head; the tail module interacts with TFs; and the CDK module either activates or inhibits the initiation of Pol II transcription, as well as to control the elongation of Pol II (Richter et al. 2022; Yang, Li, and Qu 2016).

It is noteworthy that disruption of several yeast and mammal Mediator subunits leads to lethality (Soutourina 2018; Yin and Wang 2014). Similarly, some plant Mediator subunits are indispensable as several Arabidopsis Mediator subunit mutants, including *med4*, *med8*, *med13* and *med21*, are embryonic lethal (Dhawan et al. 2009; He et al. 2021; Ito et al. 2011; Li et al. 2015), and *med14* and *med15* mutants are sterile (Autran et al. 2002; Wang, Du, and Mou 2016). On the other hand, altering some Arabidopsis Mediator subunits does not affect fertility but influences plant stress adaptation and growth. With recent advances in genome editing and sequencing technologies, several plant Mediator subunits have been characterized not only in Arabidopsis but also in various crops. In this review, we summarize recent progress on plant Mediator subunits characterized in crops, emphasizing the physiological functions of these subunits in plant growth and development and stress adaptation. We also note that several comprehensive reviews focusing on the plant Mediator complex in Arabidopsis are available (Dolan and Chapple 2017; Freytes, Gobbini, and Cerdán 2024; Yang, Li, and Qu 2016).

2 | Mediator Complex Plays a Key Role in Plant Growth and Development

Alteration of some Arabidopsis Mediator subunits affects various aspects of plant growth and development (Table 1). *atmed8* mutant exhibited smaller flowers (Xu and Li 2012); *atmed14* mutant showed a dwarf stature with aberrant architecture (Autran et al. 2002); *atmed18* mutant displayed altered floral organ number and decreased silique size (Pérez-Martín et al. 2018); *atmed25* mutant displayed hypocotyl-length inhibition under red and far-red light conditions (Cerdán and Chory 2003); *atcdk8* mutants exhibited disordered flower organ morphology as well as reduced pollen viability, shorter siliques and decreased seed numbers (Xu, Chong, and Zhu 2024). Given that the Mediator complex functions as a transcription coregulator, bridging the general transcription machinery and transcription factors, these complex and pleiotropic phenotypes of Mediator subunit mutants are not unexpected. However, some Mediator subunit mutants do not display obvious morphological changes under normal growth conditions. For example, *atmed5a/5b* double mutants, which have defects in both *AtMED5A* and *AtMED5B*, significantly increase phenylpropanoids but look wild type (Bonawitz et al. 2012). Similarly, AtMED16 functions in cold acclimation, but its mutant is fertile and does not display radical morphological changes under well-controlled conditions (Hemsley et al. 2014).

Similar to Arabidopsis Mediator subunits, the alteration of several Mediator subunits in crops, including MED4, MED5, MED8, MED14, MED15, MED16, MED18, MED25 and CDK8, also affects their growth and development at various stages (Figure 2). In tobacco (*Nicotiana tabacum*), knockdown of *NtMED8* resulted in disordered development of both vegetative and floral organs, such as the increased number of leaves with thicker blades, reduced lateral root formation, enlarged flowers, less germinable pollens and early flowering when grown under long-day conditions but late flowering under short-day conditions (Wang et al. 2011). SlMED18 functions in pollen development and hormone-signalling responses in tomato (*Solanum lycopersicum*) (Pérez-Martín et al. 2018; Wang et al. 2018). Tomato *SlMED18-RNAi* lines produced smaller flowers with less viable pollen and parthenocarpic fruits with reduced fresh weight compared to control plants. The expression of some essential genes involved in anther and pollen development significantly downregulated in the *SlMED18* silenced lines, indicating that SlMED18 is required to express the genes related to pollen formation and fruit development (Pérez-Martín et al. 2018). Another study revealed that SlMED18 positively regulates the biosynthesis and signal transduction of gibberellin (GA) in tomato (Wang et al. 2018). *SlMED18-RNAi* lines displayed short internodes, a reduced level of GA_3 and downregulated expression of some GA biosynthesis and signal transduction genes (Wang et al. 2018). However, it remains unknown whether the defects in pollen development in *SlMED18-RNAi* lines are related to GA biosynthesis or signalling.

Another tomato Mediator subunit, SlCDK8, also contributes to pollen development (Xu, Chong, and Zhu 2024). *slcdk8* mutants have reduced atypical, collapsed and shrunken pollen grains, and the pollen viability of *slcdk8* mutants was significantly diminished compared to their control counterparts, suggesting that SlCDK8 plays an essential role in pollen development. Biochemical assays revealed that transcription factor TEOSINTE BRANCHED1-CYCLOIDEA-PCF15 (SlTCP15) can interact with SlCDK8, which phosphorylates serine 187 of SlTCP15 to enhance SlTCP15 stability and phosphorylated TCP15 directly binds to the promoters of DYSFUNCTIONAL TAPETUM 1 (SlDYT1) and MYB DOMAIN PROTEIN 103 (SlMYB103) to regulate pollen development (Xu, Chong, and Zhu 2024) (Figure 3).

SlMED25 functions in tomato fruit ripening. *SlMED25-antisense* (*MED25-AS*) tomato transgenic lines exhibited delayed fruit ripening and decreased ethylene content in fruits (Deng et al. 2023). The expression of ethylene biosynthetic genes and several essential transcription factors required for ripening was reduced in *MED25-AS* fruits, and SlMED25 interacted with the master transcription factor of the ethylene signalling pathway, SlEIL (ETHYLENE-INSENSITIVE 3 (EIN3)/EIN3-LIKE) protein. The formation of a transcriptional complex of SlMED25 with SlEIL1-4 is likely involved in regulating ripening-related genes and ethylene homeostasis during fruit ripening (Deng et al. 2023) (Figure 3).

In rice (*Oryza sativa*), OsMED25 also functions in hormone signalling and plant growth and development. *OsMED25-RNAi* plants showed erect leaves, a typical morphologic phenotype of brassinosteroid (BR)-deficient and BR-signalling impaired mutants. Similarly, the *osmed25* mutants resemble *OsMED25-RNAi* plants in morphological phenotypes and decreased BR sensitivity. OsBZR1 (Brassinazole-resistant 1) is a critical transcription factor in BR signalling, and its mutant exhibits increased BR sensitivity. It was shown that OsMED25 physically interacts with OsBZR1 in vivo, and OsMED25 mainly functions as a corepressor of OsBZR1 in regulating BR signalling (Ren et al. 2020) (Figure 3). Besides BR signalling response, *OsMED25* is involved in jasmonic acid (JA)-mediated root growth and leaf senescence (Suzuki et al. 2021). *OsMED25-RNAi* and *osmed25* mutants displayed enlarged panicles with increased branching and spikelet numbers, suggesting that MED25 can be an ideal target for acquiring high-yield cultivars in monocots. Subsequent biochemical analyses revealed that OsMED25 interacts with the zinc finger transcription factor DROUGHT AND SALT TOLERANCE (DST) at the promoter region of *cytokinin oxidase/dehydrogenase 2* (*OsCKX2*) and subsequently recruits Pol II to activate *OsCKX2* transcription to control spikelet number (Lin et al. 2022).

OsMED4, OsMED14 and OsMED15 are also required for proper growth and development in rice. *OsMED14_1-RNAi* lines showed lower plant height, reduced lateral root formation, narrower leaves and culms with decreased vasculature, fewer panicle branches, impaired microspore development and smaller seed size than control plants. The authors also found that OsMED14_1 physically interacts with several organ-specific transcription factors, including OsYABBY5, OsTDR and OsMADS29 (Malik et al. 2020). The non-synonymous SNPs in

TABLE 1 | Plant Mediator subunits function in growth and development, stress adaption and other processes.

Module	Subunit	Function	Species	References
Head	MED6	Unknown		
Head	MED8	Organ development; floral transition; pathogen defence; oxidative stress; JA signalling	Arabidopsis, tobacco, tomato	Feng et al. (2021), He et al. (2021), Wang et al. (2011), Xu and Li (2012), Zhang, Song, et al. (2021) and Zhang and Guo (2020)
Head	MED11	Unknown		
Head	MED17	Floral transition; root development; JA and auxin signalling; thermomorphogenesis; noncoding RNA production; DNA repair	Arabidopsis	Agrawal et al. (2022, 2023), Giustozzi et al. (2022) and Kim et al. (2011)
Head	MED18	Organ development; GA, ABA, SA, JA and auxin signalling; floral transition; pathogen defence; heavy metal stress; noncoding RNA production; m^6A RNA modification	Arabidopsis, tomato	Kim et al. (2011), Lai et al. (2014), Li et al. (2024), Pérez-Martín et al. (2018), Raya-González et al. (2018), Ruiz-Aguilar et al. (2020), Wang et al. (2018), Zhai and Li (2019) and Zhang, Shi, et al. (2021)
Head	MED19	Pathogen defence; ABA signalling; nitrogen deficiency-induced senescence	Arabidopsis	Cheng et al. (2022), Li, Yang, Gong, et al. (2018) and Seo et al. (2017)
Head	MED20	Pathogen defence; floral transition; noncoding RNA production	Arabidopsis	Kim et al. (2011), Zhai and Li (2019) and Zhang and Guo (2020)
Head	MED22	Unknown		
Head	MED28	Root development; senescence	Arabidopsis	Shaikhali et al. (2016)
Head	MED30	Embryo development; floral transition	Arabidopsis	Jaskolowski et al. (2019)
Middle	MED4	Organ development; pathogen defence	Rice, tobacco	Li et al. (2015), Malik et al. (2016) and Wu et al. (2023)
Middle	MED7	Organ development; Pathogen defence; JA signalling; SA response; heavy metal stress; cold stress; salt stress; drought stress	Arabidopsis, sugarcane, tobacco	Kumar, Blomberg, and Björklund (2018), Wu et al. (2023) and Zhang et al. (2017)
Middle	MED9	Heat stress; pathogen defence	Arabidopsis, tobacco	Crawford et al. (2020) and Wu et al. (2023)
Middle	MED10	Pathogen defence; JA signalling	Tobacco, tomato	Wu et al. (2023)
Middle	MED21	Pathogen defence	Arabidopsis, tobacco	Dhawan et al. (2009) and Wu et al. (2023)
Middle	MED31	Organ development; pathogen defence	Arabidopsis, tobacco	Wu et al. (2023) and Zhang et al. (2018)
Tail	MED2	Root development; floral transition; senescence; ABA response; phenylpropanoid metabolism; cold stress	Arabidopsis	Dolan et al. (2017), Dolan and Chapple (2018), Hemsley et al. (2014) and Shaikhali et al. (2016)
Tail	MED3	Unknown		

(Continues)

TABLE 1 | (Continued)

Module	Subunit	Function	Species	References
Tail	MED5	Phenylpropanoid metabolism; organ development; floral transition; pathogen defence	Arabidopsis, hop, chickpea	Awasthi et al. (2023), Bonawitz et al. (2012), Dolan and Chapple (2018), Malik et al. (2023) and Wang, Du, and Mou (2016)
Tail	MED14	Organ development; cold stress; ABA, auxin, SA and JA/ET signalling; thermomorphogenesis	Arabidopsis, rice	Autran et al. (2002), Bajracharya et al. (2022), Hemsley et al. (2014) and Wang, Du, and Mou (2016)
Tail	MED15	Organ development; lipid metabolism; floral transition; ABA, SA and JA/ET signalling; drought stress; pathogen defence	Arabidopsis, rice, rose, wheat	Hiebert et al. (2020), Kim, Jang, and Chua (2016), Shang et al. (2024) and Wang, Du, and Mou (2016)
Tail	MED16	Organ development; nodulation regulation; cold stress; osmotic stress; ABA, SA, auxin and JA/ET signalling; iron homeostasis; phenylpropanoid metabolism; floral transition; pathogen defence; phosphate deficiency response	Arabidopsis, barrel medic, rapeseed, rice, wheat, tobacco, soybean	Boyce et al. (2003), Chaulagain et al. (2023), Dolan et al. (2017), Guo, Chong, et al. (2021), Hemsley et al. (2014), Hu et al. (2021), Huerta-Venegas et al. (2022), Raya-González et al. (2021), Wang, Du, and Mou (2016), Wathugala et al. (2012), Xue et al. (2019), Yang et al. (2014), Zhang and Guo (2020) and Zhang et al. (2023)
Tail	MED23	Phenylpropanoid metabolism; floral transition; organ development	Arabidopsis, chickpea	Dolan et al. (2017), Dolan and Chapple (2018) and Malik et al. (2023)
Tail	MED25	Organ development; auxin, ABA, JA and BR signalling; iron homeostasis; floral transition; pathogen defence; shade stress; cold stress; drought stress; salt stress; phytochrome signalling; thermomorphogenesis; JA-mediated leaf senescence; root development upon density	Arabidopsis, wheat, tomato, rice, rapeseed	Cerdán and Chory (2003), Deng et al. (2023), Guo, Chong, et al. (2021), Guo et al. (2023), Hu et al. (2021); Lin et al. (2022), Liu et al. (2016), Luo et al. (2023), Muñoz-Parra et al. (2017), Ren et al. (2020), Shapulatov et al. (2023), Sun et al. (2020), Suzuki et al. (2021), Xia et al. (2024), Yang et al. (2014), Zhai and Li (2019) and Zhang and Guo (2020)
CDK	MED12	Organ development; floral transition; auxin and ABA signalling; pathogen defence; sucrose response	Arabidopsis	Gillmor et al. (2014), Ito et al. (2016), Raya-González et al. (2023) and Zhu et al. (2014)
CDK	MED13	Organ development; floral transition; auxin and ABA signalling; pathogen defence; sucrose response	Arabidopsis	Gillmor et al. (2014), Ito et al. (2016), Raya-González et al. (2023) and Zhu et al. (2014)
CDK	CDK8	Organ development; floral transition; pathogen defence; SA accumulation; ABA and auxin signalling; drought stress; heat stress	Arabidopsis, tomato, pea	Crawford et al. (2020, 2024), Hasan et al. (2020), Ito et al. (2016), Xu, Chong, and Zhu (2024) and Zhu et al. (2014, 2020)
CDK	CYCC	Organ development; floral transition; pathogen defence	Arabidopsis, pea	Hasan et al. (2020) and Zhu et al. (2014)

Abbreviations: ABA, abscisic acid; BR, brassinosteroid; CDK, cyclin-dependent kinase module; ET, ethylene; GA, gibberellin; JA, jasmonic acid; SA, salicylic acid.

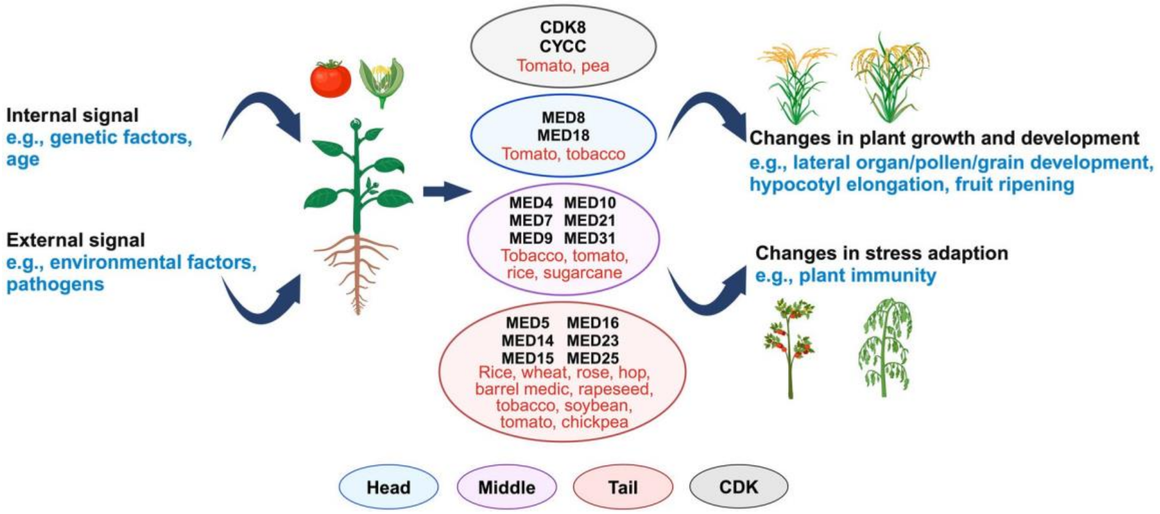

FIGURE 2 | Several Mediator subunits are identified to be involved in growth and development and stress adaptation in crops. Internal and external signals affect gene expression, leading to changes in plant growth and development and stress adaptation. Characterized Mediator subunits and crop species are listed. The figure was created with BioRender.com. [Color figure can be viewed at wileyonlinelibrary.com]

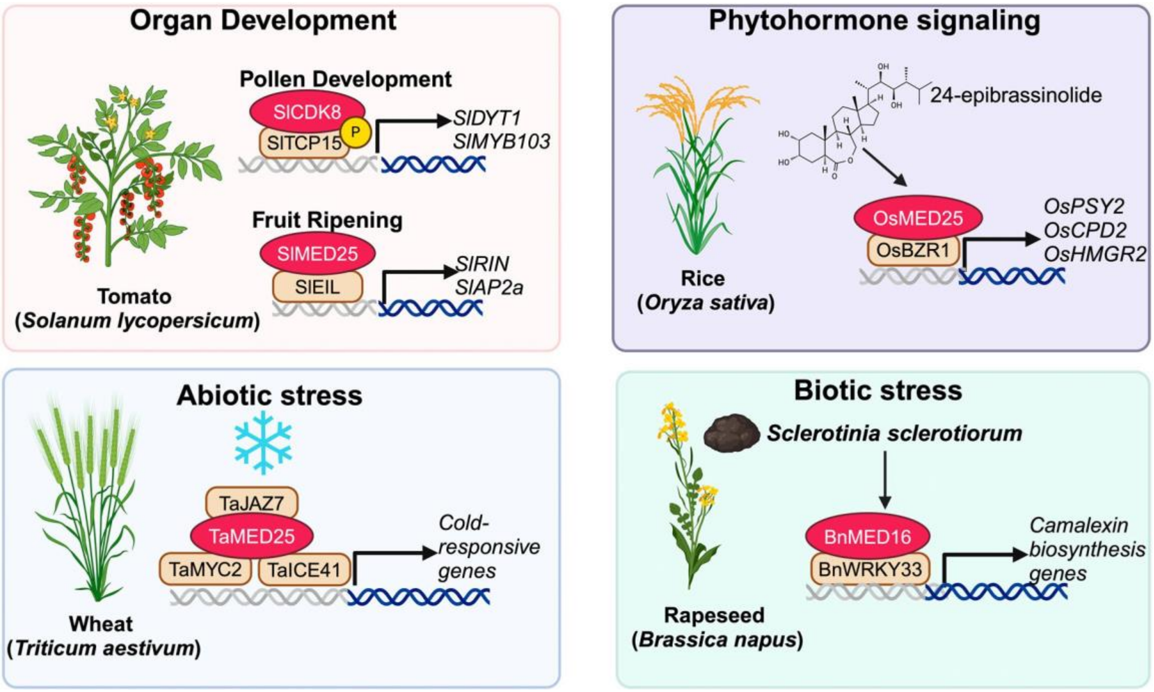

FIGURE 3 | Mediator-interacting proteins have been identified in crops. The interaction of the Mediator complex with vital proteins affects the expression of downstream genes in crops. Some examples are shown. Tomato CDK8 and MED25 interact with TCP15 and EIL to regulate pollen development and fruit ripening, respectively. Rice MED25 interacts with BZR1 to respond to brassinosteroid signalling. Wheat MED25 interacts with TaMYC2, TaICE41, and TaJAZ7 in cold tolerance. Rapeseed MED16 interacts with WRKY33 to regulate the resistance to *Sclerotinia sclerotiorum*. The figure was created with BioRender.com. [Color figure can be viewed at wileyonlinelibrary.com]

OsMED4 and *OsMED15* were associated with grain size, width and weight in rice (Malik et al. 2016). It was previously shown that OsMED4 physically interacts with OsSAD1 to regulate tiller number, affecting the grain yield (Li et al. 2015).

MED5 has been identified in hop (*Humulus lupulus*) and chickpea (*Cicer arietinum*). In Arabidopsis, two MED5 paralogs, AtMED5A and AtMED5B, require the repression of an array of phenylpropanoid biosynthesis genes. The *atmed5a/5b* mutants show increased expression of phenylpropanoid biosynthesis genes and phenylpropanoid content without affecting their growth phenotypes (Bonawitz et al. 2012; Dolan et al. 2017). The hop *med5a/5b* double mutant showed distortion in lupulin gland morphology (Awasthi et al. 2023). Interestingly, the expression of phenylpropanoid biosynthesis genes was decreased in *hlmed5a/5b* lines compared to control plants, which is contradictory to the results observed in Arabidopsis *med5a/5b* mutants. However, lignin contents in the stems and leaves of the *hlmed5a/5b* mutants were increased substantially compared to controls (Awasthi et al. 2023). It remains unclear how lignin content increases while its biosynthesis genes are downregulated in the mutants. These contradictory phenotypes of Arabidopsis and hop *med5* mutants raise the question of whether MED5 function is conserved across species. A chickpea study utilizing a combination of genomic approaches, including QTL/fine mapping and map-based cloning, identified *CaMED5* and *CaMED23* and their natural alleles associated with plant height trait in chickpea (Malik et al. 2023). They concluded that the chickpea height trait is associated with altered expression of phenylpropanoid biosynthesis genes, but whether CaMED5 or CaMED23, or both, regulate the phenylpropanoid biosynthesis genes remains unknown (Malik et al. 2023).

MED16 in barrel medic (*Medicago truncatula*), and CDK8 and CYCC in pea (*Pisum sativa*) have been shown to function in specialized nodulation, and flowering. *mtmed16a* mutant inhibited nodulation and increased arbuscular density (Chaulagain et al. 2023). LATE BLOOMER3 (LATE3) and LATE4 are orthologs of CDK8 and CYCC1, respectively. The pea mutants, *pslate3* and *pslate4*, displayed a late-flowering phenotype with defects in the formation of flower and inflorescence, flower fertility, pod development and seed content. LATE3 and LATE4 physically interacted with each other, which contributes to the transcriptional regulation of some critical flowering genes, such as *FTa1* and *LF* (Hasan et al. 2020).

Some important agronomic traits, including grain yield, developmental transition, fruit ripening and metabolism homeostasis, are likely governed by the Mediator complex or specific Mediator subunits, as shown in various crops. Interestingly, most Mediator subunit mutants displaying morphological changes show altered transcription in the corresponding genes, which further confirms the Mediator complex as an essential hub in the transcription process.

3 | Mediator Complex Exerts a Crucial Role in Plant Adaptation to Environmental Stress

In Arabidopsis, several Mediator subunits function in biotic and abiotic stress responses (Table 1). AtMED8 regulates oxidative stress responses and plant immunity to *Botrytis cinerea* (*B. cinerea*) (He et al. 2021; Li, Yang, and Chen 2018); AtMED15 and AtMED16 act as key players in plant defence signalling crosstalk (Wang, Du, and Mou 2016); AtMED25 is a crucial integrative hub in the transcriptional regulation of jasmonate signalling (Zhai and Li 2019). Their homologs have been identified and characterized in several crops, including tomato, rice, wheat, rapeseed, soybean and rose (Table 1 and Figure 2).

Both SlMED8 and SlMED25 are positive regulators in *B. cinerea* resistance in tomato. Tomato transgenic lines overexpressing *SlMED8* or *SlMED25* showed increased resistance to *B. cinerea*, whereas disruption of *SlMED8 or SlMED25* made tomato plants more susceptible to *B. cinerea* (Luo et al. 2023; Zhang et al. 2021). Additionally, overexpression of *SlMED8* rescued the hypersensitivity of Arabidopsis *atmed8* mutant to *B. cinerea* infection (Zhang et al. 2021).

SlMED25 is a coactivator of PHYTOCHROME INTERACTING FACTOR 4 (PIF4) to control shade-induced hypocotyl elongation. SlPIF4 acts as a positive regulator of shade-induced hypocotyl elongation by promoting the expression of auxin biosynthesis (*SlYUC8* and *SlYUC9*) and auxin-responsiveness (*SlIAA19*) genes under shade conditions by directly binding to their promoters. SlMED25 directly interacted with SlPIF4 in tomato (Sun et al. 2020). *SlMED25-antisense* seedlings showed a similar morphological phenotype to the *slpif4-c* mutant under shade, and the expression levels of *SlYUC8*, *SlYUC9* and *SlIAA19* also decreased in *SlMED25-antisense* lines (Sun et al. 2020).

In wheat (*Triticum aestivum*), MED25 is involved in cold stress response and pathogen defence. Overexpression of *TaMED25* in Arabidopsis increased low-temperature tolerance and the expression of some cold-responsive genes (Xia et al. 2024). Further biochemical analysis suggested that TaMED25 may control cold resistance, potentially through the JA pathway by interacting with TaJAZ7, TaMYC2 and TaICE41 (Xia et al. 2024) (Figure 3). Additionally, the wheat *TaMED25* knockdown lines were more resistant to powdery mildew. It turned out that TaMED25 interacting with ethylene signalling transcription factor TaEIL1 synergistically activated the transcription of *TaERF1* (ETHYLENE RESPONSE FACTOR1), a negative regulator of resistance to powdery mildew, to modulate the basal defence of bread wheat against powdery mildew (Liu et al. 2016).

TaMED15b.D functions in the resistance to stem rust a major disease of wheat. Nonsense mutations in *MED15b.D* significantly disrupted the transcriptional response to the fungi responsible for causing stem rust. Notably, wheat *med15b.D* mutants do not exhibit any obvious morphological changes, whereas Arabidopsis *med15* mutants show chlorosis, growth retardation and sterility (Hiebert et al. 2020). MED15 was also characterized as responding to drought stress in ornamental crop rose (*Rosa hybrida*). *RhMED15A* is drought-inducive, and its promoter has four cis-acting motifs that interact with ABA and MeJA. Indeed, ABA increased the expression of *RhMED15A* in both leaves and roots, while MeJA treatment suppressed *RhMED15A* expression in leaves. Silencing of *RhMED15A* impaired drought tolerance (Shang et al. 2024).

Interestingly, MED16 plays an opposite role in pathogen response in dicots and monocots. OsMED16 is a negative regulator of rice immunity. A rice lesion mimic mutant (LMMs), *spotted leaf 38* (*osspl38*), has a point mutation in the 14th exon of *OsMED16*. *OsMED16*-Crsipr/Cas9 lines exhibited the lesion mimic phenotype. *osspl38* plants exhibited a significantly enhanced resistance to both bacterial and fungal rice pathogens. *OsMED16-overexpression* plants showed an enhanced susceptibility when challenged with different virulent isolates. Similarly, the *TaMED16* knockdown lines displayed increased resistance to fungal infection in wheat (Zhang et al. 2023). However, MED16 in several dicot plants is a positive regulator in pathogen tolerance. AtMED16 promotes defence against pathogens in Arabidopsis (Wathugala et al. 2012). Knockdown of *NbMED16A* and *NbMED16B* in tobacco resulted in reduced resistance to fungi, as indicated by increased fungal biomass (Zhang et al. 2023). BnMED16 and GmMED16 positively regulate the resistance to *Sclerotinia sclerotiorum* (*S. sclerotiorum*) in a vital oil crop *Brassica napus* (*B. napus*) and to *Phytophthora* *sojae* (*P. sojae*) in soybean (*Glycine max*), respectively (Hu et al. 2021; Xue et al. 2019). Overexpressing *BnMED16* in a susceptible *B. napus* line reduced lesion size compared to the control. It turned out that BnMED16 physically interacted with BnMED25 and BnWRKY33, and BnMED25 interacted with BnMYC2, BnCOI1, and BnEIN3 in the JA/ethylene signalling pathway. BnMED16 enhances resistance to *S. sclerotiorum* in *B. napus* by facilitating BnMED25-mediated JA/ET defence pathways and activating BnWRKY33-mediated defence signalling (Hu et al. 2021) (Figure 3). *GmMED16-1* is induced in response to *P. sojae* infection in soybean, and the expression levels of some stress response genes, such as *GmNPR1*, *GmPR1a*, and *GmPR5*, were significantly downregulated in the *GmMED16-1* silencing lines (Xue et al. 2019).

Although little is known about the function of AtMED7 and AtMED10 in plant defence, their homologs in sugarcane, tobacco and tomato were reported to function in stress adaption (Table 1). In sugarcane, *ScMED7* is transcriptionally induced by multiple stresses such as heavy metal, low temperature and salicylic acid and methyl jasmonate treatments but suppressed by osmotic stresses of NaCl and polyethylene glycol. Overexpressing *ScMED7* increased H_2O_2 production and cell death in tobacco leaves and the expression of several defence-related marker genes was induced after infiltrating *ScMED7* in tobacco. ScMED7 acts as a negative regulator during pathogen infection, such as *Fusarium solani* var. *coeruleum* (Zhang et al. 2017). MED10 and MED7 in tobacco negatively affect the immune response to Tomato spotted wilt orthotospovirus (TSWV) mediated by Sw-5b (Wu et al. 2023). Knockout or knock-down of *MED10B* in tomato and tobacco led to immune activation. As silencing *NbMED4*, *NbMED7*, *NbMED9*, *NbMED21* and *NbMED31* in tobacco also led to reduced accumulation of TSWV replicon, these subunits in the middle module of the Mediator complex may function in plant defence against TSWV infection. NbMED10B directly interacts with NbMED7 but not with the other middle subunits in tobacco, and NbMED7 directly interacts with JAZ proteins (Wu et al. 2023).

Although limited crop Mediator subunits in stress response have been characterized, some of them, such as SlMED25 and TaMED25, play a critical role in responding to both biotic and abiotic stress. However, detailed mechanisms underlying the altered response of Mediator mutants upon stresses remain mostly understudied. Developing crops with enhanced stress resilience is essential to secure crop yields in the face of recent climate changes. Thus, investigating the underlying mechanisms would reveal additional targets for gene editing to develop stress-resilient crops.

4 | Future Perspectives

Given that the Mediator complex, as a transcriptional coregulator, interacts with general transcription machinery, such as Pol II, it is not surprising that disruption of one or more subunits results in pleiotropic phenotypes or lethality. However, some Mediator subunits are dispensable for survival as their loss-of-function mutants are fertile, and some exhibit specific phenotypes such as metabolite changes, altered biotic stress susceptibility and flowering time, which are attractive traits for crop engineering. Although significant progress has been made in plant Mediator research, there are still knowledge gaps in the identity of some subunits and functions of the plant Mediator complex and its subunits. Here are some future perspectives on plant Mediator research.

1. Do plant Mediator subunits have conserved functions across species?

   Some Mediator subunits have conserved functions in different species, while some function differently or even oppositely across the species. MED18 has conserved functions in organ development and flowering time in tomato and Arabidopsis. On the other hand, OsMED16 and TaMED16 are negative regulators in rice and wheat immunity, while MED16 in dicots such as Arabidopsis, tobacco and soybean, positively regulates immune response. Similarly, SlMED25 acts as a positive regulator of *B. cinerea*, whereas TaMED25 negatively controls powdery mildew. Despite low sequence similarities of Mediator subunit orthologs across species, in silico analyses have predicted Mediator subunits in several crops, such as rice, tomato, soybean and asparagus bean (Liang et al. 2024; Mathur et al. 2011; Wang et al. 2019; Xue et al. 2019). However, whether the putative subunits are indeed parts of the Mediator complex remains to be explored. Since Mediator subunits contain no predicted functional motif and their functions are not always conserved across species, the function of each subunit should be examined within the context of each species.

2. Utilizing Mediator subunits to improve crop traits.

   Upon recent global climate change, it is crucial to identify breeding targets that balance a plant's response to various stresses while maintaining growth to acquire more climate-resilient plant cultivars (Leisner, Potnis, and Sanz-Saez 2023). Several studies have demonstrated that altering one or more Mediator subunits affects agronomically important traits in crops, such as grain size and number, fruit ripening and resistance to stresses, placing Mediator subunits as reasonable targets to obtain desirable traits in crops. However, other aspects should also be considered when utilizing them for crop engineering. As most physiological studies have been conducted under controlled conditions, whether the engineered crops display the expected phenotypes in the field should be further explored. While some Mediator subunit mutants display specific phenotypes in plant growth and development and others show altered responses in plant stresses, there are no clear boundaries among them, and these traits can be linked directly or indirectly, as exemplified with the trade-off between growth and defence. Thus, the impact of altered Mediator complex on various aspects of crop traits should be examined comprehensively when engineering Mediator subunits for crop improvement.

3. Investigating the involvement of phase separation in plant Mediator complex.

   The transcription requires multiple biomolecule interactions, often forming biomolecular condensates via phase separation, which play a vital role in gene expression

(Wang, He, and Fang 2023). Although the number of studies on phase separation in plants keeps increasing (Yang, Huang, and Xia 2024), research on Mediator-mediated phase separation in plants is still in its infancy. In Arabidopsis, AtMED8 and AtHAC1 form liquid-like droplets, which is required for AtMED8 to interact with Pol II (Guo, Wei, et al. 2021). Another Arabidopsis study found that the crucial regulator of shoot meristem SHOOT MERISTEMLESS (STM) contains a phase separation-inducing prion-like domain in its N-terminal region, which stimulated STM to form nuclear condensate and this condensation of STM facilitated its interaction with MED8 to enhance its transcriptional activity (Cao et al. 2023). Arabidopsis MED19A undergoes liquid-liquid phase separation (LLPS) under nitrogen scarcity. The C-terminal mixed-charged intrinsically disordered region of MED19A is required for LLPS and ORESARA1 interaction to govern senescence triggered by nitrogen deficiency (Cheng et al. 2022). Further study on the involvement of the Mediator complex in dynamic control of transcriptional condensates will enhance our knowledge of how the Mediator complex effectively and efficiently regulates transcription.

4. Exploring the functions of crop Mediator subunits beyond transcription regulation.

   This review focuses on the functions of crop Mediator subunits in plant stress responses and growth mainly through interacting with transcription factors, but the plant Mediator complex indeed plays versatile roles. Several plant Mediator studies revealed the roles of Mediator subunits in microRNA (miRNA) and noncoding RNA production, RNA modification, and DNA repair (Giustozzi et al. 2022; Kim et al. 2011; Li et al. 2024). AtMED17 is engaged in miRNA production by recruiting Pol II to promoters of miRNA genes (Kim et al. 2011). It also functions in DNA repair. The expression levels of some genes associated with UV-B response and DNA repair were significantly altered in *atmed17* mutants, and AtMED17 is required for the proper expression by interacting with several DNA-repaired proteins (Giustozzi et al. 2022). Moreover, a recent study identified OsMED18 as a novel posttranscriptional regulator of N^6-methyladenosine (m^6A) RNA modification in rice (Li et al. 2024). CRISPR-Cas9 edited *osmed18* mutants had a significantly increased m^6A level, which is conserved in the *atmed18* mutants (Li et al. 2024). The investigation of crop Mediator function beyond transcription regulation is necessary and will help a comprehensive understanding of the plant Mediator complex, which will provide more opportunities to utilize Mediator for crop improvement.

5. Adapting advanced technologies for Mediator complex study.

   Despite advances in plant Mediator studies, there is still debate about the exact number of Mediator subunits present in plants, and the structure of the plant Mediator complex remains unresolved. Adapting improved technologies such as cryo-electron microscopy single-particle analysis, a powerful tool to resolve the high-resolution structure of macromolecules (Yip et al. 2020), may enable elucidation of the structure of the plant Mediator complex. Most Mediator research focuses on the characterization of Mediator subunits. Several biochemical and genetic studies identified proteins interacting with specific Mediator subunits, and physiological outcomes verified their functions. The interaction between the Mediator subunits and their interacting proteins might be stress-specific and dynamically controlled depending on internal and external signals. The single-molecule technique is a revolutionary and trending approach in plant research (Cui et al. 2024; Su et al. 2021). Employing a range of single-molecule techniques, such as single-particle tracking and single-molecule imaging along with labelling technology, enables the precise visualization of the Mediator subunits' spatiotemporal dynamic trajectories and the identification of dynamic interacting proteins under diverse environmental conditions in living cells. Also, proximity labelling assay using the complex would reveal dynamically interacting partners' identities. In addition to the classical labelling approach, advanced label-free imaging techniques such as Stimulated Raman scattering microscopy, a noninvasive method for generating images, can more efficiently capture highly dynamic processes (Zhao et al. 2019). This capability may significantly accelerate the exploration of how the Mediator complex naturally responds to various signals. Multi-omic approaches such as transcriptomics, proteomics and metabolomics will also uncover how the Mediator complex integrates multiple signals to orchestrate diverse biological processes beyond gene expression networks.

5 | Conclusion

The past decades of extensive studies with Arabidopsis have illuminated the function of the plant Mediator complex and its subunits in diverse biological processes, suggesting them as suitable targets to acquire desirable traits in other plant species. Recently, several Mediator subunits have been identified and characterized in some economically important crops. The outcomes, however, are not as straightforward as expected. While some subunits appear to have conserved functions across species, others function differently from species to species. Nevertheless, utilizing the Mediator complex for engineering crops remains promising. Further functional characterization of Mediator subunits from diverse species and a holistic understanding of plant Mediator complex upon various environmental conditions would provide better opportunities to exploit them for crop improvement.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no data sets were generated or analysed during the current study.

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