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Aldoximes: compounds at the crossroads of multiple metabolic pathways in plant

Doosan Shin · Veronica C. Perez · Jeongim Kim

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Abstract Aldoximes are amino acid-derivatives well recognized as precursors of defense compounds, such as glucosinolates and cyanogenic glycosides. However, recent studies have elucidated the multi-faceted roles of aldoximes in plant survival beyond defense, as they exert influence over multiple metabolic pathways, including auxin biosynthesis and the phenylpropanoid pathway. Aldoxime accumulation affects the homeostasis of auxin, an essential plant hormone that controls almost every aspect of plant growth and development. While auxin biosynthesis primarily occurs through the conserved TAA/YUC pathway, tryptophan-derived aldoxime and phenylalanine-derived aldoxime also serve as precursors of two major auxins, indole-3-acetic acid (IAA) and phenylacetic acid (PAA), respectively. Notably, this conversion process is not limited to Brassicales and is present in monocots like maize and sorghum. Furthermore, in Brassicales, the accumulation of aldoximes derived from aliphatic and aromatic amino acids represses the phenylpropanoid pathway that produces an array of specialized metabolites crucial for plant survival. These novel findings extend beyond the conventional understanding of aldoximes and shed light on their intricate involvement in enhancing plant fitness. In this review, we discuss the role of aldoximes as precursors for auxins and their inhibitory effect on phenylpropanoid biosynthesis. We also explore the mechanisms by which aldoximes influence these metabolic pathways. Finally, we discuss the implications of these findings for our understanding of plant biology.

Keywords Aldoxime · Glucosinolate · Cyanogenic glycoside · Auxin · Phenylpropanoid · Metabolic network

Introduction

Aldoximes (R–C=N–OH) are metabolites carrying an imine group at the end of a carbon chain and are found widely in the plant kingdom (Sørensen et al. 2018). They are derived from various amino acids, including phenylalanine, tyrosine, tryptophan, valine, leucine, isoleucine, and methionine (Fig. 1A). In most plants, the conversion of an amino acid to an aldoxime is catalyzed by cytochrome P450 monooxygenases

D. Shin · J. Kim (✉)
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA
e-mail: jkim6@ufl.edu

V. C. Perez · J. Kim
Plant Molecular and Cellular Biology Graduate Program, University of Florida, Gainesville, FL, USA

J. Kim
Genetic Institute, University of Florida, Gainesville, FL, USA

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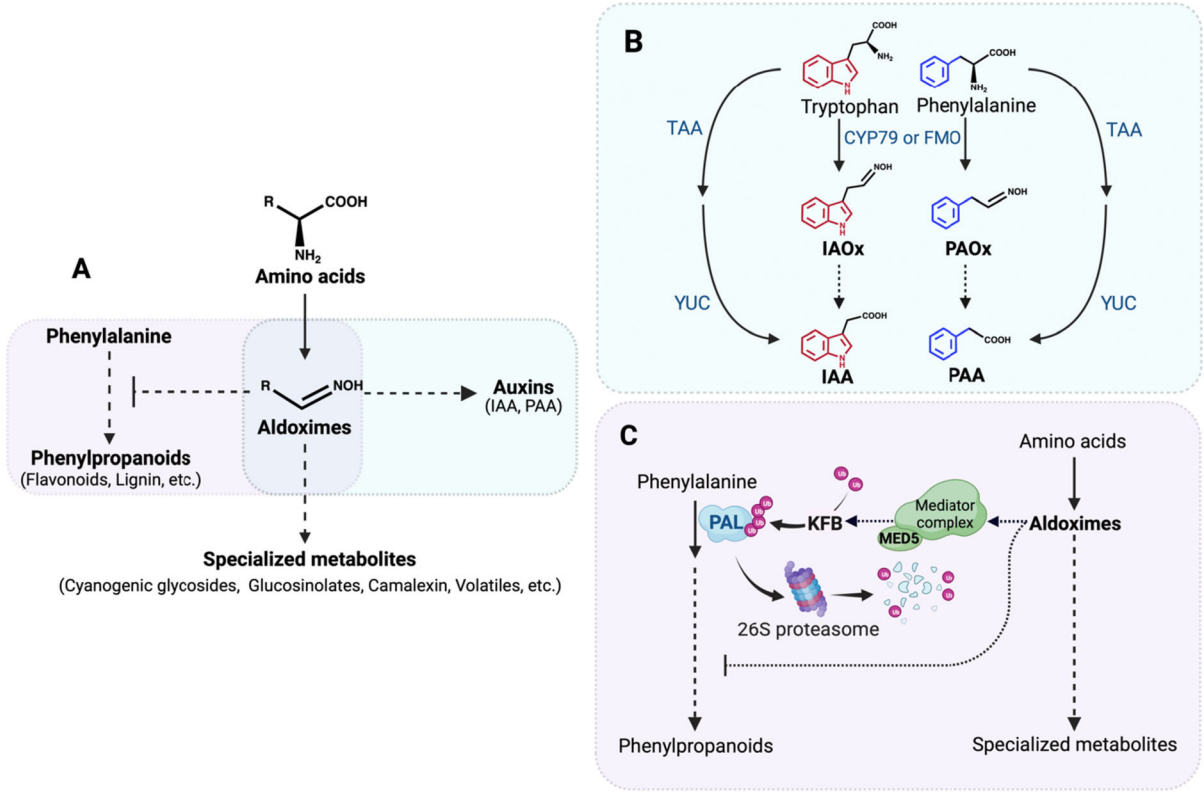

Fig. 1 Schematic illustration depicting the metabolic link centering on aldoxime metabolism, **A** Aldoximes derived from amino acids or chain-elongated amino acids, serve as precursors to specialized metabolites, including glucosinolates, camalexin, cyanogenic glycosides, and floral volatiles. The accumulation of aldoximes or their derivatives represses the phenylpropanoid pathway in plants (shaded in pink). and two aldoximes, IAOx and PAOx, serve as precursors to plant hormone auxins (shaded in blue). **B** The pathway involving TAA and YUC enzymes is a well-established major route for the biosynthesis of auxins, converting tryptophan to IAA and phenylalanine to PAA, respectively. Additionally, IAOx and PAOx are known as precursors of IAA and PAA. However, the detailed steps from aldoximes to auxins have not yet been fully elucidated. **C** Recent studies on Arabidopsis have demonstrated that the accumulation of aldoximes represses the phenylpropanoid pathway through accelerating the degradation of PAL via transcriptional activation of kelch repeat-containing F-box (KFB). Mediator subunit 5 (MED5) is in part involved in this process, and there may be additional, yet unidentified mechanism(s) that further repress the phenylpropanoid pathway. Acronyms in the diagram include: CYP79 for cytochrome P450 family 79, FMO for flavin-containing monooxygenase, TAA for tryptophan aminotransferase, YUC for YUCCA enzyme, PAL for phenylalanine ammonia-lyase, IAOx for indole-3-acetaldoxime, PAOx for phenylacetaldoxime, IAA for indole-3-acetic acid, PAA for phenylacetic acid, KFB for kelch domain-containing F-box protein, MED5 for mediator subunit 5, Ub for ubiquitin. (Color figure online)

belonging to the 79 family (CYP79) (Fig. 1B, Table 1). The CYP79 enzyme catalyzes the conversion of amino acids into aldoximes through *N,N*-dihydroxylation of the amino acid, followed by decarboxylation via a cyclic transition state (Vazquez-Albacete et al. 2017). In Arabidopsis, CYP79B2 and CYP79B3 convert tryptophan to indole-3-acetaldoxime (IAOx) while CYP79A2 can produce phenylacetaldoxime (PAOx) from phenylalanine (Wittstock and Halkier 2000; Mikkelsen et al. 2000; Zhao et al. 2002). CYP79F1 and CYP79F2 generate aliphatic aldoximes (AAOx) from chain-elongated methionine (Hansen et al. 2001; Chen et al. 2003). In some fern species, flavin-dependent monooxygenases produce aldoximes (Thodberg et al. 2020).

Aldoxime production is triggered by stress or stress-responsive hormones in plants. This phenomenon is observed in several species, including

Table 1 List of characterized CYP79 enzymes and their substrates

Species	Enzyme	Substrate	Reference
Arabidopsis thaliana	CYP79A2	Phenylalanine	(Wittstock and Halkier 2000)
	CYP79B2	Tryptophan	(Mikkelsen et al. 2000; Zhao et al. 2002)
	CYP79B3	Tryptophan	(Zhao et al. 2002)
	CYP79F1	Chain-elongated Methionine (2–6 carbon)	(Hansen et al. 2001; Chen et al. 2003)
	CYP79F2	Long-chain (5–6 carbon) Methionine	(Hansen et al. 2001; Chen et al. 2003)
White mustard (*Sinapis alba*)	CYP79B1	Tryptophan	(Naur et al. 2003)
Erythroxylum coca	CYP79D62	Tryptophan, Phenylalanine, Tyrosine, Leucine, Isoleucine	(Luck et al. 2016)
	CYP79D63	Tryptophan	(Luck et al. 2016)
Erythroxylum fischeri	CYP79D60	Tryptophan, Phenylalanine, Tyrosine, Leucine, Isoleucine	(Luck et al. 2016)
	CYP79D61	Tryptophan, Phenylalanine, Tyrosine, Leucine, Isoleucine	(Luck et al. 2016)
Lima bean (*Phaseolus lunatus*)	CYP79D71	Isoleucine, Valine	(Lai et al. 2020)
Eucalyptus cladocalyx	CYP79A125	Phenylalanine	(Hansen et al. 2018)
Almond (*Prunus dulcis*)	CYP79D16	Phenylalanine	(Thodberg et al. 2018)
White clover (*Trifolium repens*)	CYP79D15	Isoleucine, Valine	(Olsen et al. 2008, 2021)
Cassava (*Manihot esculenta*)	CYP79D1	Isoleucine, Valine	(Andersen et al. 2000)
	CYP79D2	Isoleucine, Valine	(Andersen et al. 2000)
Plumeria (*Plumeria rubra*)	CYP79D73	Phenylalanine	(Dhandapani et al. 2019)
Lotus japonicus	CYP79D3	Isoleucine, Valine	(Forslund et al. 2004)
	CYP79D4	Isoleucine, Valine	(Forslund et al. 2004)
Loquat (*Eriobotrya japonica*)	CYP79D80	Phenylalanine	(Yamaguchi et al. 2021)
Japanese apricot (*Prunus mume*)	CYP79D16	Phenylalanine	(Yamaguchi et al. 2014)
Balsam poplar (*Populus trichocarpa*)	CYP79D6	Phenylalanine, Leucine, Isoleucine, Tryptophan, Tyrosine	(Irmisch et al. 2013a)
	CYP79D7	Phenylalanine, Leucine, Isoleucine, Tryptophan	(Irmisch et al. 2013a)
Black poplar (*Populus nigra*)	CYP79D6v4	Phenylalanine, Tryptophan, Leucine, Isoleucine, Tyrosine	(Irmisch et al. 2013b)
Tea (*Camellia sinensis*)	CYP79D73	Phenylalanine	(Liao et al. 2020)
Barley (*Hordeum vulgare*)	CYP79A8	Leucine	(Knoch et al. 2016)
	CYP79A12	Leucine	(Knoch et al. 2016)
Seaside arrow grass (*Triglochin maritima*)	CYP79E1	Tyrosine	(Nielsen and Moller 1999)
	CYP79E2	Tyrosine	(Nielsen and Moller 1999)
Maize (*Zea mays*)	CYP79A61	Tryptophan, Phenylalanine	(Irmisch et al. 2015)
Sorghum (*Sorghum bicolor*)	CYP79A1	Tyrosine	(Sibbesen et al. 1995)
	CYP79A61	Phenylalanine	(Perez et al. 2023a)
European yew (*Taxus baccata*)	CYP79A118	Tryptophan, Phenylalanine, Tyrosine	(Luck et al. 2017)
Neotropical myrmecophyte tococa (*Miconia microphysca*)	CYP79A206 CYP79A207	Phenylalanine	(Müller et al. 2024)

poplar (*Populus trichocarpa*), maize, tea (*Camellia sinensis*), and Erythroxylum species (Irmisch et al. 2013a, b, 2015; Luck et al. 2016; Liao et al. 2020). The expression of *CYP79* genes often increases upon herbivory or pathogen attacks or exposure to jasmonic acid (JA), a hormone produced in response to stress (Mikkelsen et al. 2003). In Arabidopsis, the transcriptional activation of *CYP79B2* and *CYP79B3* is observed in response to temperature elevation (Franklin et al. 2011). These observations suggest that aldoximes play critical roles in plant adaptation to environmental stimuli/changes. Indeed, aldoximes are precursors of defense compounds such as glucosinolates, camalexin, and cyanogenic glycosides.

Glucosinolates are sulfur-containing defense compounds found in Brassicales. Over 130 distinct structures of glucosinolates have been discovered in the plant kingdom, with Arabidopsis capable of producing over 30 of them (Blažević et al. 2020). The composition and content of glucosinolates within a species can vary organ-to-organ, among developmental stages as well as depending on environmental factors (Brown et al. 2003). The biosynthesis of the core structure of glucosinolates starts from the formation of an aldoxime. IAOx, PAOx, and AAOx are precursors of indole glucosinolates, benzylglucosinolate, and aliphatic glucosinolates respectively.

Under normal conditions, glucosinolates and myrosinases, the enzymes that degrade glucosinolates, are spatially segregated within cellular and subcellular compartments. Upon herbivore or pathogen attack, however, these compartments are compromised, resulting in glucosinolate hydrolysis by myrosinases and the rapid release of toxic metabolites such as isothiocyanates, nitriles and epithionitriles, which deter herbivory and inhibit the growth of pathogenic microorganisms (Blažević et al. 2020).

Cyanogenic glycosides are another class of defense compounds. A total of 25 cyanogenic glycosides have been identified. Well-recognized cyanogenic glycosides include dhurrin from sorghum (*S. bicolor*), amygdalin from almond (*Prunus dulcis*), and linamarin from lima bean (*Phaseolus lunatus*), which are derived from tyrosine, phenylalanine, and valine respectively (Cressey and Reeve 2019). Like glucosinolates, cyanogenic glycosides are harmless in their intact state. However, when degraded by β-O-glucosidases, they release toxic substances such as hydrogen cyanide, which are lethal to pathogens and pests and serve to deter generalist herbivores (Cressey and Reeve 2019). A recent study with tococa (*Miconia mycrophysca*) showed accumulation of PAOx and its glucoside upon herbivore infestation, suggesting their roles in defense (Müller et al. 2024). Since aldoximes are precursors of aldoxime-driven defense compounds, altered aldoxime production may directly affect plant defense. Indeed, studies have shown that the overexpression of *CYP79* genes results in the increased production of related aldoxime-derived defense compounds in Arabidopsis, leading to enhanced defense responses (Mikkelsen et al. 2000; Perez et al. 2023a).

While plants produce compounds such as glucosinolates and cyanogenic glycosides accumulate under normal conditions to prepare for defense, there are other defense compounds produced under attack, but typically not accumulated in normal growth conditions, known as phytoalexins. Camalexin is a phytoalexin found in Brassicales and it is synthesized from IAOx (Zhao et al. 2021). Although camalexin is an IAOx-derived defense compound similar to indole glucosinolates, its biosynthesis is activated only in response to specific stressors, and precisely at the site of infection or stress (Zhao et al. 2021).

Besides acting as precursors of these classical defense metabolites, aldoximes serve as intermediates of other specialized metabolites. PAOx is a precursor of floral volatiles, which act as floral scent components and participate in plant–insect interactions. Examples of floral-related PAOx derivatives include (2-nitroethyl)benzene and 2-phenylethanol (Irmisch et al. 2014; Dhandapani et al. 2019). In poplar, giant knotweed (*Fallopia sachalinensis*) and tea (*C. sinensis*), aldoximes produced from leucine, isoleucine, valine, and phenylalanine, as well as nitriles generated from the metabolism of these aldoximes, can be detected in the volatile mixture released from herbivore-damaged leaves (Sørensen et al. 2018; Liao et al. 2020). Similarly, isoleucine- and leucine-derived aldoximes are also volatiles and *Erythroxylum* species and common bean (*Phaseolus vulgaris*) produce them upon treatment with JA (Luck et al. 2016). These volatile aldoximes may not directly function as defense compounds, but the emission of volatile aldoximes may indirectly protect plants from biotic stresses through deterring herbivores or attracting the predators of herbivores (Sørensen et al. 2018; Liao et al. 2020). Indeed, the release of volatile aldoximes

has been shown to effectively mitigate the level of damage caused by herbivory (Sørensen et al. 2018).

The roles of aldoximes in plant defense are relatively well known. However, recent studies have uncovered unexpected interactions of aldoximes with multiple metabolic pathways. This review explores the multifaceted roles of aldoximes, focusing on their significance as precursors to the plant hormone auxins and the impact of altered aldoxime metabolism on phenylpropanoid production. These insights, extending beyond the conventional understanding of aldoximes, illuminate their pivotal role in enhancing plant fitness under stress conditions (Fig. 1).

Aldoximes are precursors of auxins

Auxins regulate numerous aspects of plant growth and development, including cell division, elongation, and differentiation, in response to both external and internal stimuli (Teale et al. 2006; Simon and Petrášek 2011). Indole-3-acetic acid (IAA) and phenylacetic acid (PAA) are two major auxins in plants. IAA is the most potent auxin and controls various biological processes through the dynamic alteration of its cellular concentration via polar auxin transport (Teale et al. 2006). Conversely, PAA is less potent than IAA in most plant systems, despite its endogenous concentrations sometimes being 10- to over 100-fold greater than IAA across various plant species (Perez et al. 2023b). Multiple studies suggest that PAA may not engage in polar auxin transport, unlike IAA (Simon and Petrášek 2011). Overall, IAA and PAA have both common and distinctive physiological roles as auxins in plants.

The primary pathway for the biosynthesis of IAA from tryptophan in plants is the YUCCA pathway (Fig. 1B) (Teale et al. 2006). The initial step involves the conversion of tryptophan into Indole-3-pyruvate (IPA) by enzymes from the Tryptophan Aminotransferase of Arabidopsis (TAA) family (Zhao 2010). Subsequently, flavin-containing monooxygenases from the YUCCA (YUC) family transform IPA into IAA (Zhao 2010). This pathway is also considered to play a role in PAA biosynthesis. The TAA and YUC enzymes can respectively convert phenylalanine into phenylpyruvate (PPA) and PPA into PAA in vitro (Tao et al. 2008). Furthermore, YUCCA overexpression enhances both endogenous PAA and its conjugate content (Sugawara et al. 2015). However, it's noteworthy that plants with defects in TAA and YUC still have normal levels of PAA despite significant changes in IAA content (Cook and Ross 2016). This implies the potential involvement of different genes or pathways in the biosynthesis of PAA.

Several genetic studies have revealed that IAOx-derived IAA production occurs in Arabidopsis. Multiple independent forward screens have identified alleles of CYP83B1, the major IAOx catalyzing enzyme, as well as SUR1 functioning in downstream of IAOx because of their characteristic high auxin morphological phenotypes (Mikkelsen et al. 2004). Indeed, these mutants, *sur2, red1, rnt1, ref5,* which are allelic variants of the REF5*,* along with *sur1*, display an accumulation of IAA, attributed to the redirection of IAOx towards the synthesis of IAA (Mikkelsen et al. 2004; Kim et al. 2015). Moreover, significant ^{13}C labeling of indole-3-acetonitrile (IAN), indole-3-acetamide (IAM) and IAA when fed with $^{13}C_6$-IAOx to *cyp79b2 cyp79b3* mutants, demonstrating the metabolic conversion of IAOx to these compounds (Sugawara et al. 2009). However, considering that IAN is a byproduct of indole glucosinolate hydrolysis, further study is necessary to determine the route of IAN from IAOx. A recent study demonstrated that IAOx is a precursor of nitric oxide (NO) production, catalyzed by peroxidase (POD), and that indole-3-acetaldehyde (IAAld) is produced from this reaction (López-Gómez et al. 2024). IAAld has been suggested as an IAA precursor in bacteria and plants through an indole-3-acetaldehyde dehydrogenase-dependent manner (Quittenden et al. 2009; McClerklin et al. 2018).

In Arabidopsis, the enzyme CYP79A2 is responsible for converting phenylalanine to phenylacetaldoxime (PAOx) and the overexpression of *CYP79A2* results in elevated levels of PAA and its conjugates and morphological alterations, including epinasty leaves and elongated hypocotyls—characteristics reminiscent of those observed in plants with elevated IAA levels (Perez et al. 2021a). It is notable that the production of auxins from aldoximes, initially identified in the Brassicales order, has been recognized in recent years as not exclusive to this group. Monocots like maize and sorghum can convert aldoximes to auxins, evidenced by deuterium labeled (D_5)-aldoximes being converted into D_5-labeled auxins in

these species (Irmisch et al. 2015; Perez et al. 2021a, b).

The aldoxime-derived auxin pathways, unlike the YUCCA pathway, are not the primary avenue for auxin biosynthesis during normal growth conditions. For instance, the IAOx-deficient Arabidopsis mutant, *cyp79b2 cyp79b3*, is indistinguishable with wild type under these normal conditions (Kim et al. 2015). However, under stress conditions such as high temperatures and salt stress, the *cyp79b2 cyp79b3* mutant shows the low auxin growth phenotype, indicating a significant role of these pathways in auxin homeostasis under stress (Zhao et al. 2002; Franklin et al. 2011). In a similar vein, the gene expression of *CYP79A2* encoding PAOx production enzyme in Arabidopsis is induced under pest attack, leading to an increased level of PAA content (Perez et al. 2021b). Considering these facts, along with the regulation of aldoxime-producing enzymes by stresses or stress hormones such as jasmonic acid (Luck et al. 2016; Mikkelsen et al. 2003), it can be inferred that aldoximes potentially play a dual role in both the defense response and plant growth and development by coordinating the production of auxins and defense compounds. However, the biosynthesis pathway from aldoximes to auxins remains largely uncharted. Although the stable isotope labeling assay revealed the presence of IAOx-derived compounds (Sugawara et al. 2009), further investigations are necessary to identify the genes and intermediates responsible for the conversion of IAOx into IAA, which remains mostly unknown. Given that IAOx and PAOx serve as precursors for their corresponding glucosinolates as well as IAA and PAA, it is also conceivable that the production of both auxins from aldoximes takes place via a shared pathway. Indeed, benzyl cyanide has been identified as an intermediate of PAOx-derived PAA in maize and sorghum (Perez et al. 2021a, 2023a), suggesting that the aldoxime-derived auxin pathway may include the production of nitrile intermediates.

Auxin, as a potent growth hormone, exerts substantial influence on plant growth and development. Overproduction of auxin, therefore, has the potential to be toxic to plants, especially when it exceeds the optimal physiological concentration. There is likely a metabolic control mechanism that directs IAOx primarily towards glucosinolate production rather than IAA. Improper regulation of this metabolic shift could lead to detrimental effects on plants. Maintaining the intricate balance between hormone synthesis and defense compound production may serve as a survival strategy under unfavorable conditions. The elucidation of the mechanisms by which plants regulate this metabolic flux of IAOx would broaden our understanding of these complex metabolic networks.

Metabolic link between aldoxime metabolism and the phenylpropanoid pathway

Phenylpropanoids are specialized metabolites derived mainly from phenylalanine and include various phenolic compounds such as lignin and flavonoids essential for plant growth and stress responses. The unexpected discovery of an intricate metabolic interplay linking aldoxime metabolism and phenylpropanoid biosynthesis emerged from a forward mutant screen which specifically targeted phenylpropanoid-deficient Arabidopsis mutants, named *'reduced epidermal fluorescence'* (*ref*) mutants (Ruegger and Chapple 2001). Several *ref* mutants having reduced phenylpropanoid contents were found to have mutations in *CYP83A1/REF2* and *CYP83B1/REF5* (Hemm et al. 2003; Kim et al. 2015). REF2 and REF5 function redundantly to convert various aldoximes into their respective hydroxy nitriles, intermediates of glucosinolates (Bak and Feyereisen 2001). However, REF2 and REF5 are not completely interchangeable due to their different substrate specificities. REF5 has a higher activity towards indole-3-acetaldoxime (IAOx), a tryptophan-derived aldoxime, compared to REF2, while REF2 shows a preference for aliphatic (i.e., chain-elongated methionine-derived) aldoximes (Bak and Feyereisen 2001). Consistently, *ref5* produces reduced indole glucosinolates and increases IAA due to the redirection of IAOx to IAA. Additionally, it reduces the levels of phenylpropanoids such as sinapoylmalate and flavonoids (Kim et al. 2015). The deficiency of phenylpropanoids in *ref5* is completely restored upon removal of the IAOx production enzymes CYP79B2 and CYP79B3 (Kim et al. 2015). Overexpression of *CYP79B2* decreases phenylpropanoid production, whereas the aldoxime-deficient *cyp79b2 cyp79b3* double mutant accumulates more phenylpropanoids than the wild type (Kim et al. 2015). These findings collectively suggest that an accumulation of IAOx or its derivatives negatively impacts

phenylpropanoid biosynthesis. The IAOx-mediated phenylpropanoid repression was also shown in *Camelina sativa* (Zhang et al. 2020).

Consistent with CYP83A1/REF2 function, *ref2* mutants display reduced aliphatic glucosinolates and increased indole glucosinolates, yet all have diminished phenylpropanoid production (Hemm et al. 2003). It was assumed that reduced phenylpropanoids in *ref2* resulted from the accumulation of aliphatic aldoximes as *ref2* does not show any phenotypes related to increased IAOx, such as the high auxin morphological phenotypes observed in *ref5*. However, a recent study by Shin et al. uncovered the elevated level of IAOx in *ref2*, which is unexpected as *ref2* has functional CYP83B1/REF5 with high activity toward IAOx (Shin et al. 2023). The disruption of IAOx producing enzymes, CYP79B2 and CYP79B3, restores phenylpropanoids in *ref2* substantially but not to the wild-type level, which further confirms the repressive roles of IAOx in the phenylpropanoid pathway and suggests the presence of additional repressive factors in *ref2*, likely AAOx (Shin et al. 2023). Indeed, the co-suppression of AAOx producing enzymes, CYP79F1 and CYP79F2, completely restores phenylpropanoid production in *ref2*, suggesting that AAOx also play a role in aldoxime-mediated repression of phenylpropanoid biosynthesis. Given that the accumulation of PAOx represses phenylpropanoid production in Arabidopsis (Perez et al. 2021b), the metabolic link between aldoxime metabolism and phenylpropanoid biosynthesis may not be limited to specific aldoxime structure.

One mechanism underlying this aldoxime-mediated phenylpropanoid repression is in part via accelerated degradation of Phenylalanine Ammonia Lyase (PAL) the first enzyme of the phenylpropanoid pathway. Transcriptome analysis using a set of glucosinolate mutants identified that both *ref5* and *ref2* contain increased expression of a group of Kelch domain-containing F-Box protein (KFB), KFB1, KFB20, KFB39 and KFB50 (Kim et al. 2020). These KFBs are subunits of the ubiquitin E3 ligase complex targeting PAL for ubiquitination and consequent degradation (Zhang et al. 2013). As PAL functions at the entry point of the phenylpropanoid pathway, the transcriptional activation of these KFBs leads to increased PAL turnover and reduced flux toward phenylpropanoid production (Kim et al. 2020). When all four KFBs were disrupted, phenylpropanoid contents in *ref5* and *ref2* were substantially restored in Arabidopsis (Kim et al. 2020). Camelina transgenic lines with increased IAOx contain reduced phenylpropanoids as well as increased expression of KFB homologs targeting PAL in *Camelina sativa*, suggesting its conserved mechanism at least in Brassicales (Zhang et al. 2020). Similarly, Arabidopsis plants overproducing PAOx showed increased expression of PAL-targeting KFBs (Perez et al. 2021b). Moreover, the transcription activation of at least two of them, KFB39 and KFB50, requires functional Mediator subunit 5 (MED5), a subunit of the Mediator complex that is a transcriptional coregulator in eukaryotes (Kim et al. 2015, 2020). These findings indicate that the aldoxime-mediated phenylpropanoid repression occurs through sophisticated transcriptional regulation, which links the production of defense compounds and phenylpropanoids.

Interestingly, accumulation of aldoximes does not exert an impact on the transcript level of PAL. Since, PAL is positioned at the gate of entire phenylpropanoid production pathway, any perturbations in aldoxime metabolism can potentially affect the overall flow of phenylpropanoids to some degree. Indeed, the Arabidopsis mutants that accumulate high levels of IAOx show a reduced level of overall phenylpropanoids, including flavonol glycosides, sinapoylmalate, and overall lignin monomers (Kim et al. 2015). Interestingly, the *ref2* mutant displays a significantly reduced level of S-unit lignin content but not G-unit content, and no change in flavonoid production (Hemm et al. 2003). This variation could be due to the organ or tissue-specific expression of aldoxime production and/or consumption enzymes, leading to different aldoxime accumulation patterns. Alternatively, different aldoximes may utilize different repression mechanisms.

Disruption of all four KFBs in the *ref5* background restores phenylpropanoids, but not to the levels of the *kfb1/20/39/50* although it restores PAL activity completely (Kim et al. 2020). This result suggests that there are additional mechanisms underlying repressed phenylpropanoid production in high IAOx condition other than PAL repression (Fig. 1C).

Aldoximes have a wide variety of structures, with methionine-derived aldoximes exhibiting even more structural diversity due to the number of elongated carbon chains. It remains uncertain how plants sense different aldoximes, and how this signal is transduced

to repress phenylpropanoid production. Therefore, questions remain as to where their precise regulatory points lie and any specificity of different aldoximes on phenylpropanoid repression to clarify these intriguing aspects of aldoxime metabolism.

Perspectives

Recent findings suggest that metabolisms centered around aldoximes in plants represents more than just the production of stress-resistant compounds. It forms a critical junction linking the biosynthesis of auxins derived from aldoximes and the suppression of phenylpropanoid biosynthesis. Furthermore, the perturbation of methionine-derived aliphatic aldoxime production induces distinctive morphological phenotypes, including cup-shaped leaves and bush-like growth patterns (Shin et al. 2023). Arabidopsis plants with disrupted CYP79F1 and F2 demonstrated elevated levels of methionine accumulation. Remarkably, similar phenotypic outcomes were reproducible through exogenous methionine feeding to Arabidopsis, which suggests the morphological abnormalities associated with CYP79F1 and F2 disruption may primarily be caused by methionine accumulation (Shin et al. 2023). These findings make the aldoxime-mediated metabolic networks even more complicated by linking primary and specialized metabolism. It is noteworthy that aliphatic aldoxime synthesis from methionine by CYP79F1/F2, including chain elongation of methionine, is the multistep process and it remains unclear how the feedback regulation occurs.

These metabolic links might play a key role in enabling plants to intricately fine-tune their responses and adapt more efficiently to environmental challenges. Despite these advancements, significant knowledge gaps persist in our understanding of these vital metabolic interconnections. 1. How are auxins made from aldoximes? What are intermediates and genes responsible for converting IAOx and PAOx into IAA and PAA? 2. Aldoximes repress phenylpropanoid biosynthesis. Do different aldoximes have specificity of repression? What are other mechanisms of aldoxime-mediated phenylpropanoid repression in addition to PAL degradation? 3. Aldoximes affect multiple metabolic pathways, including specialized metabolites and primary metabolites, directly and indirectly. What are the mechanisms mediating multiple fates or impacts of aldoximes? Further exploration of these connections is crucial to deeply unravel how plants integrate diverse signals and responses, thereby optimizing their growth and survival under varying environmental conditions.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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