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REVIEW

Potential of engineering the myo-inositol oxidation pathway to increase stress resilience in plants

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Abstract

Myo-inositol is one of the most abundant form of inositol. The *myo*-inositol (MI) serves as substrate to diverse biosynthesis pathways and hence it is conserved across life forms. The biosynthesis of MI is well studied in animals. Beyond biosynthesis pathway, implications of MI pathway and enzymes hold potential implications in plant physiology and crop improvement. *Myo*-inositol oxygenase (MIOX) enzyme catabolize MI into D-glucuronic acid (D-GlcUA). The MIOX enzyme family is well studied across few plants. More recently, the MI associated pathway's crosstalk with other important biosynthesis and stress responsive pathways in plants has drawn attention. The overall outcome from different plant species studied so far are very suggestive that MI derivatives and associated pathways could open new directions to explore stress responsive novel metabolic networks. There are evidences for upregulation of MI metabolic pathway genes, specially *MIOX* under different stress condition. We also found *MIOX* genes getting differentially expressed according to developmental and stress signals in *Arabidopsis* and wheat. In this review we try to highlight the missing links and put forward a tailored view over *myo*-inositol oxidation pathway and MIOX proteins.

Keywords *Myo*-inositol · *Myo*-inositol oxygenase (MIOX) · Phytic acid · Plant stress

Introduction

Inositols are sugar-like carbohydrates that exist as seven isomeric forms in plant cells. Among these isomers, the most abundant *myo*-inositol (MI) could exist as free or bound form with phospholipids or inositol phosphate derivatives. The biosynthesis of MI generally starts with Glucose-6-phosphate; however, it could also synthesize from other inositol phosphates. Glucose-6-phosphate catalyzed into *myo*-inositol-1-phosphate by *myo*-inositol-1-phosphate synthase enzyme (MIPS), it is also first and rate-limiting step of biosynthesis of MI [1–3]. *Myo*-inositol is principally catabolized into D-glucuronic acid (D-GlcUA) by *myo*-inositol oxygenase (MIOX) enzyme (EC 1.13.99.1), which is the first step of this pathway [4]. There are various important functional role of D-GlcUA for MIOX is a unique monooxygenase encoded by *MIOX* gene, ubiquitous to all eukaryotic cells, including those in yeast, animals, humans and plants. Animal genome contains only single *MIOX* gene whereas it is a multigene family in plants. For instance, the genome of Thale cress *Arabidopsis thaliana* contains four *MIOX* genes (*AtMIOX 1, 2, 4,* and *5*), numbered according to their chromosomal location [5]. Isoforms of *A. thaliana* MIOX are 66–84% identical at the amino acid level and the same catalytic domain is present in all four proteins. In wheat, three *MIOX* homologs are mapped on chromosomes 7A, 7B, and 7D [6]. Recently, five MIOX homologs were identified from tomato, on chromosomes 6, 10, 11, and 12 [7].

MI oxidation pathway is well interconnected with other important metabolic pathways. These include, the biosynthetic pathway of MI polyphosphates, pyrophosphates, and

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phytic acid (PA) [8], cell wall biosynthetic molecules such as galactinol, raffinose, and pinitol etc., Ascorbic acid (AsA) biosynthesis [7], and Phosphoinositides and Ins(1,4,5) P3 [9] (Fig. 1). Additionally, it is also linked with various pathways related to signal transduction and transportation of sugars and auxin [10]. MIOX oxidize MI and therefore directly influence the MI homeostasis. The triple mutant *miox1/2/4/5* in *A. thaliana* has increased MI and MI containing metabolites. MI is the primary precursor for PA biosynthesis via lipid dependent and independent pathways. The PA content in seeds reduced in *CaMV35::AtMIOX4* over-expression line compared to wild type and *miox1/2/4/5* mutant [11].

During MI oxidation pathway, D-GlcUA is converted into uridine diphosphate (UDP) glucuronic acid by glucurono kinase enzyme. UDP-glucuronic acid (UDP-GlcUA) is an important sugar precursor for various nucleotide sugars need for synthesis of cell wall related polysaccharides. Though, the sugar nucleotide oxidation pathway is primarily used in biosynthesis of pectin (cell-wall polysaccharides), it is also important as substrate (UDP-GlcUA) for the production of cell-wall polysaccharides. Labeling ^{3}H-*myo* inositol and inositol-2-(^{14}C) in *AtMIOX1* and *AtMIOX2* mutants of *Arabidopsis* showed that approximately 70% of MI converted to D-GlcUA, which is further used in cell wall biosynthesis [5, 12–14]. Thus, cell wall engineering and stress adaption by altering tissue specific expression of MIOX is an open niche to explore as genetic engineering and synthetic biology approaches.

Ascorbic acid (AsA) biosynthesis via MI oxidation pathway is well characterized and active among mammals. This process involves, D-GlcUA reduced into L-gulonic acid through the action of glucuronic acid reductase, which then finally oxidized to AsA [15, 16]. However, whether AsA biosynthesis via MI oxidation pathway exists in plants is still debatable. Identification of D-galacturonate reductase and L-glucose in strawberry (*Fragaria ananassa)* indicate that MI may be an alternative precursor for AsA biosynthesis. Ectopic expression of *D-galacturonate reductase* gene in *Arabidopsis* improved vitamin C content by 2–3 folds [17, 18]. Moreover, ectopic expression of the *AtMIOX4* gene in *Arabidopsis* increased AsA content in the transgenic line by two to three times, suggesting a correlation between MI oxidation and AsA biosynthesis [19]. Conversely, Endres and Tenhaken (2009) showed that *AtMIOX4* did not increase the AsA content but, instead control the MI level in *AtMIOX* over-expressed plants [20]. In tomato plants, the over-expression of *MIOX* gene increased AsA content in leaves and ripe fruits compared to control plants [7]. All these finding suggests that engineering *myo*-inositol pathway could be a good target to improve fruits with higher AsA.

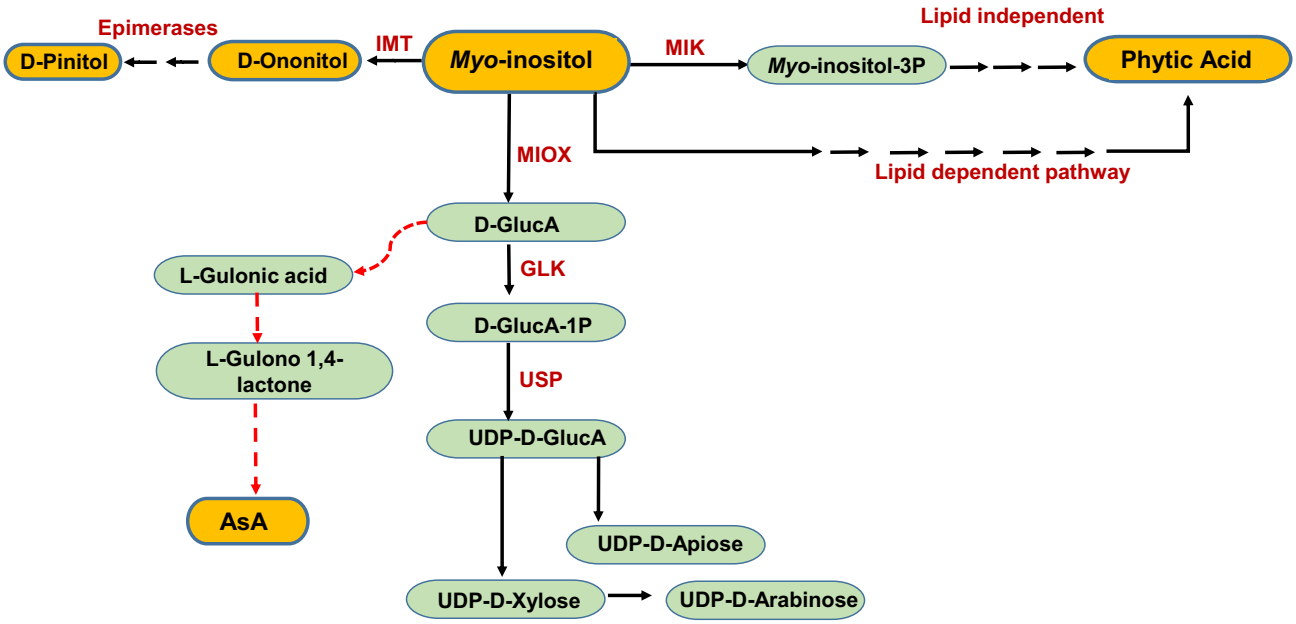

Fig. 1 Myo-inositol oxidation pathway and its interconnection to other pathways. The pathways towards L-ascorbic acid are represented with dot line, which is active in the animal system, whereas in plants it exists or not is in debate. The pathway towards cell wall biosynthesis is active in the plant system. The *myo*-inositol catalyzed into D-GlucA (D-glucuronic acid) by MIOX (*myo*-inositol oxygenase). D-GlucA converted into D-GlucA-1P (D-glucuronic acid-1-phosphate) with the help of GLK (glucuronokinase)., D-GlucA-1P further catalyzed into UDP-D-GlcA by USP (UDP-sugar pyrophosphorylase). UDP-D-GlcA is an important precursor of numerous cell wall polysaccharides i.e. UDP-D-GalA (UDP-D-galacturonic acid), UDP-D-xylose, UDP-D-apiose, and UDP-D-Arabinose. IMT—inositol methyltransferase. *Myo*-inositol kinase (MIK)

To this end, multifaced role of MIOX and *myo*-inositol oxidation pathway proved to be significant influencers on multiple aspect of plant physiology. Our current work will revisit various research reports to bring a practical perspective on possible aspects of this pathway across broaden areas includes PA biosynthesis, cell wall biosynthesis, ascorbic acid biosynthesis, abiotic, and biotic stresses.

Diversity, gene structure and chromosomal locations

The plant kingdom in general have very discreet genomic diversity among *MIOX* genes. In *Arabidopsis*, there are four homologs of the *MIOX* gene, named as *AtMIOX1*, *2*, *4,* and *5* according to their chromosomal location. All members of this family have 11 exons and 10 introns [11]. The soybean (*Glycine soja*) genome contains five homologs *GsMIOX1a, 1b, 2a, 2b*, and *4*, located on chromosome 1, 7, and 8, respectively [21]. In wheat (*Triticum aestivum*), homeologous *TaMIOX* genes (*Ta7AMIOX, Ta7BMIOX,* and *Ta7DMIOX*) are mapped on 7A, 7B, and 7D and have 11 exons and 10 introns [6]. In tomato (*Solanum lycopersicum*) five *MIOX* genes are identified; *SlMIOX1*, *2*, 3, *4,* and *5,* they are located on chromosomes 6, 10, 11, and 12 respectively. *SlMIOX1*, *2*, *3*, and *4*, contain ten, one, seven and eight introns, respectively, while *SlMIOX5* contains none [7]. Recently, two genes, *MhMIOX1 & 2* were identified in Chinese crab apple (*Malus hupehensis* Rehd.). *MhMIOX1* contain six exons and five introns, whereas the *MhMIOX-2*comprised nine exons and eight introns [22]. Diploid and tetraploid cotton contains 6 and 11 MIOX genes [23].

Transcriptional regulation of *MIOX* genes

MIOX promoters express differentially across plant tissues, developmental stages, and under abiotic stresses. Several drought associated transcription factors like MYB, MYB-like, MYC, and Myb-have putative binding sites on wheat *MIOX* promoter (*pro:TaMIOX*). Similar, MYB binding sites are also present in *Arabidopsis MIOX2* and *MIOX4* promoters. Drought, wound and water stress related transcription binding sites such as DRE2COREZMRAB17, T/GBOXATPIN2, and MYBCORE are present in wheat *MIOX* promoter. Dehydration, low temperature and ABA responsive transcription binding sites such as ABRELATERD1, DRECRTCOREAT, MYCATERD1, MYBCORE, ACGTATERD1, MYB1AT, MYCATRD22, LTRECOREATCOR15, CBFHV, RYREPEATBNNAPA, MYCCONSENSUAT, and DRE2COREZMRAB17 were also found on *TaMIOXpro* [24].

Arabidopsis AtMIOX2 promoter (*AtMIOX2pro*) express constitutively whereas, *AtMIOX4pro* and *AtMIOX5pro* are specific to reproductive stages [5]. *Arabidopsis* transgenic lines expressing *uidA* gene under the control of *AtMIOX2pro,* showed its activity in the roots, cotyledons, developing seeds, anthers, stigma, and stipules. While *AtMIOX4pro::uidA* expression is reported in the primary root tip, sepals, young petals, anthers, top of the pistil, and stipules. The *AtMIOX5pro::uidA* construct, displayed strong GUS activity in pollen grains and the stigma while lower was in petals, sepals, and filament [25]. In another report, microarray data showed *AtMIOX4* and *AtMIOX5* were highly expressed in pollen of *Arabidopsis*, whereas *AtMIOX2* expressed in roots and seedlings in normal condition [26].

Expression of *AtMIOX* genes are also correlated with nutrient status and energy sensing [27]. *AtMIOX2pro* have strong activity in cotyledons, hypocotyl, and roots under nutrient deficient conditions (0.8% agar without Murishige and Skoog salts) while promoter was moderately active in low nutrient condition (half MS salts with 0.8% agar). However, in the absence (only 0.8% agar) of nutrients, expression of *uidA* gene under *AtMIOX4pro* was not detected. GUS activity of *AtMIOX4pro* under low nutrient condition was observed in cotyledons and roots. The strength of*AtMIOX-2pro* and *AtMIOX4pro* were decreased in seedlings under optimal nutrient (half MS, 0.8% agar, and 3% glucose) [27]. The *AtMIOX* genes express differentially under biotic stress. When challenged with cyst nematode *Heterodera schachtii* for 10–15 days *proAtMIOX5::uidA* lines showed significant GUS activity in roots [28]. *In-silico* identification and mining of *cis*-regulatory elements present in all *AtMIOXpro*, positively correlates to expressions pattern related to abiotic and biotic stresses [29].

Rice *MIOX* promoter (*OsMIOXpro*) showed its activity across all tissues, including panicles, stems, leaves, and roots. *OsMIOXpro* was highly active under drought stress [30]. The promoter activity of *TaMIOXpro* was 5–sevenfold higher in transgenic *Arabidopsis* leaf under abiotic stresses such as heat, cold, and drought as compared to controls. Apart from this, *TaMIOXpro::uidA* lines showed lesser GUS activity during *myo*-inositol feeding assay of plants [24].

We identified that these *MIOX* genes express in very tissue specific manner. Though, the substantial level of *AtMIOX1* was higher in most tissues as compared to other isoforms in publicly available data (Fig. 2A). Similar pattern was also observed for *TaMIOX* genes during developmental stages. Additionally, we tested that wheat *MIOX* genes could express differentially in response to specific environmental triggers (Fig. 2B and C).

MIOX proteins and their homology

MIOX is a soluble protein and lacks any signal peptide and transmembrane helix [5, 31, 32]. Heterologous expression and purification of MIOX from yeast and *Arabidopsis*,

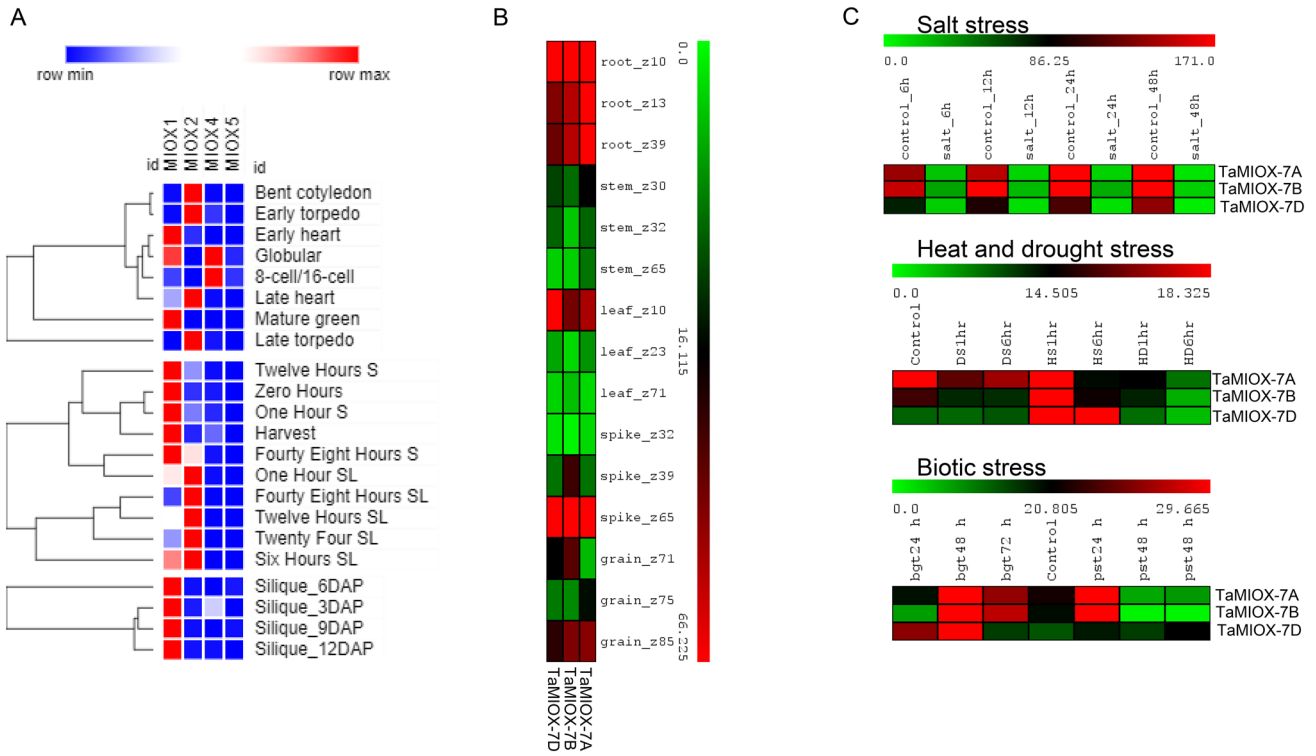

Fig. 2 Differential transcript level of **A** Arabidopsis, **B** wheat *MIOX* genes in different publicly available dataset from RNAseq experiments. Expression data for *AtMIOX* 1, 2, 4 and 5 genes in different stages of development in embryo development (Bent cotyledon, early torpedo, early heart, globular, 8/16 cell, late heart, mature green and late torpedo), Germination (zero hour to harvest stage), and Silique 3 to 12 DAP) were normalized for fold changes and hierarchical clustering was applied to group genes based on similarities in their expression, represented as a heat map. All the raw data taken from the https://www.ebi.ac.uk/gxa/home, in the form of TPM (transcript per million read). Wheat *TaMIOX* gene expression was analyzed on http://www.wheat-expression.com/. **C** Different stress samples were selected and expression was tested using default settings. Each *TaMIOX* gene represent chromosome they are located

showed MIOX enzymatic activity [19, 32]. The yeast and *Arabidopsis* MIOX have57% sequence similarity [5]. In wheat 77% of the protein sequence showed similarity with AtMIOX1, whereas this ranges between 68 to 94% with other plants [6]. Amino acids (aa) sequences of MIOX across all kingdom, approx 1–100 aa as variable region, 100–250 aa are highly conserved and 251–303 aa are less conserved on C-terminal. TaMIOX contain 3 substrate binding regions, two substrate binding sites, and six potential iron binding sites [6]. The occurrence of H_{139} and D_{140} aa in TaMIOX showed a strong affinity with metal ions and the HD-domain superfamily is well known for strong binding with metal ions [33]. The 3-D structure reveals that MIOX protein is a monomeric with a single-domain and helices. The 3-D crystal structure of MIOX was reported from animal source using crystallization X-ray diffraction. So far, the crystal structure of MIOX from plant has not been reported. In human MIOX proteins comprised eight α-helices and 2 antiparallel β-sheet [34]. Plant MIOX protein comprised nine α-helices, five forming the core and other four the protein surface [33].

Interconnection of MI oxidation pathways to phytic acid biosynthesis

PA is the hexa-*kis*-phosphate ester of MI, which accumulates in seeds and is stored in the embryo, endosperm and seed coat. All seed crops contain PA, which may vary from one plant to another but it usually accounts for 65–85% of total phosphorus in seeds [35]. PA strongly binds with iron and other divalent minerals such as Fe, Zn and Mg, there by limit their bioavailability. This correlation of PA and micronutrients loading to seed is well studied in crops like wheat [36]. There are high chances of possible link between MI and PA biosynthetic pathways as the *miox1/2/4/5* knockout *Arabidopsis* seeds showed higher PA content than wild type. While, overexpression of *AtMIOX4* also showed a decreased PA content in *Arabidopsis* seeds compared to wild type and non-functional mutants [11]. The *1-myo-inositol 1-P synthase* (*INO1*), *Inositol monophosphatase* (*IMP*), and *myo-inositol O-methyl transferase* (*IMT*) genes, which are linked with MI pathways, also demonstrated their potential for reducing PA content. Overexpression of *IMT* gene from ice plant (*Mesembryanthemum crystallinum*)

under seed-specific promoters into *Brassica napus,* showed 19–35% phytate reduction in seeds [37]. RNAi mediated suppression of wheat *inositol-pentakisphosphate 2-kinase 1* (*IPK1*) gene, leading to a 28–56% reduction in the phytic acid content of wheat seeds and an increase in iron and zinc content [38]. Similarly, in rice suppression of *myo-inositol 1-phosphate synthase* resulted in a decrease in PA content but a 1.3, 1.6 and 1.27-fold increase in calcium, iron and magnesium, respectively [39]. Overexpression of *MIOX* result in lower PA in different crops as the free pool of *myo*-inositol is greatly utilized in the conversion of D-Glucuronic acid (D-GlcUA). All these studied suggest that, MIOX overexpression might decrease PA content in seed and increase nutrient content in seeds.

Engineering downstream pathways: biosynthesis of cell wall precursors

The cell wall is a complex and dynamic structure that consists of four major components i.e. polysaccharides cellulose, non-cellulosic polysaccharides (hemicellulose and pectin) and proteins. Various glycosyltransferases promote the synthesis of hemicellulose and pectin in the golgi body. These glycosyltransferases transfer the sugar moiety from an activated nucleotide donor onto acceptors polysaccharide in the form of a UDP or GDP-sugar [40]. D-GlcUA is converted into D-GlcUA-1P through the action of Glucuronokinase (GlcAK), which is enzyme encoded by *Glucuronokinase* gene [3]. D-GlcUA-1P is further converted into UDP-GlcUA by UDP-sugar pyro-phosphorylase (USP). Conversion of sugars into nucleotide sugars is generally initiated by a substrate-specific sugar-1-kinase or USP (broad substrate specificity) [41]. UDP-GlcUA is a primary precursor to numerous nucleotide sugars involved in biosynthesis of hemicellulose and pectin. These nucleotide sugars are UDP-galacturonic acid, UDP-D-xylose, UDP-arabinose, and UDP-apiose [42]. Cell wall polysaccharides that have nucleotide sugars originated from UDP-GlcUA account for approximately 50% of the cell wall biomass [40, 43]. UDP-GlcUA is synthesized and utilized in cytoplasm that is further transported inside Golgi body for biosynthesis of UDP-galacturonic acid, UDP-D-xylose, UDP-arabinose, and UDP-apiose (Fig. 3). UDP-Uronic Acid Transporter1 (UUAT1) is a Golgi-localized nucleotide sugar transporter that transports UDP-GlcUA [40]. In *Arabidopsis* and sea onion (*Ornithogalum caudatum*), the enzyme UDP-GlcA 4-epimerase (UGlcAE) is characterized to convert UDP-GlcUA into UDP-D-galacturonic acid [44]. The genes

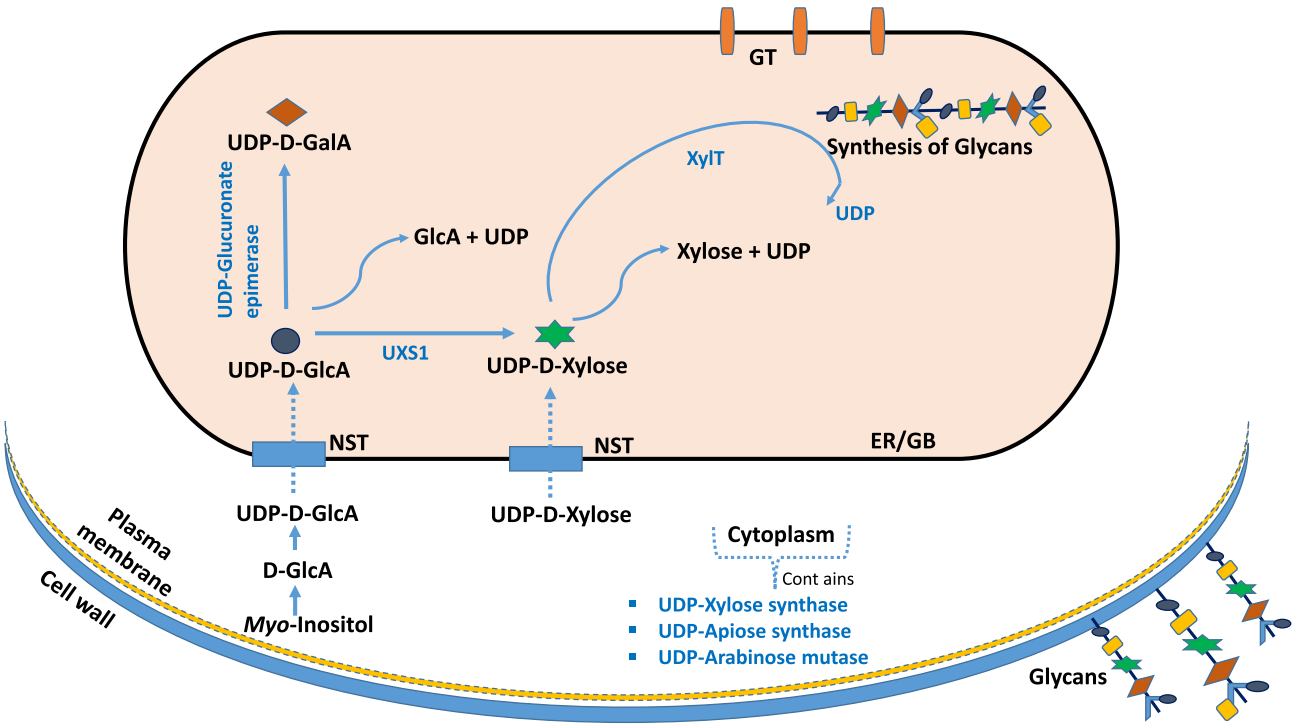

Fig. 3 UDP-D-GlcA biosynthesis and cellular transport. UDP-D-GlcA and UDP-D-Xylose are synthesized into cytoplasm. The NST (nucleotide sugar transporter) has been shown to transport UDP-GlucA over the Endoplasmic Reticulum (ER) membrane and to transport UDP-Xylose over the Golgi bodies (GB) membrane. The function NST transporter is still unclear. UXS1 (UDP-glucuronic acid decarboxylase) responsible for the conversion of UDP-D-GlcA to UDP-D-Xylose. UDP-D-Xylose is synthesized within the lumen of the ER or GB and further XylT (xylosyltransferases) incorporates xylose in the glycans. *GT* -glucosyltransferase

AtUGlcAE1, *2*, and *3* that are present in the *Arabidopsis* genome encodes UGlcAE enzyme [44]. Decarboxylation of UDP-GlcUA leads to production of UDP-xylose with the help of UDP-glucuronic acid decarboxylase (UXS) enzyme. Further, the enzyme UDP-D-xylose epimerase encoded by *MUR4* gene converts UDP-D-xylose into UDP-L-arabinose [45, 46] The ectopic expression of *AtMIOX2* in tobacco (*Nicotiana tobaccum*) and RNAi mediated down regulation of tobacco *MIOX* showed significant changes in arabinan and D-galacturonate content [47]. Recently, RNA sequencing and analysis of *AtMIOX4* over-expressing *Arabidopsis* line showed that the MIOX pathway is plays an important role in cell wall biosynthetic pathway [48].

Mysterious connection between MIOX and AsA biosynthetic pathways

L-ascorbic acid (AsA) is an antioxidant molecule crucial in various cellular functions across plants and animals. Because humans are unable to synthesize it, hence they must obtain it by eating different fruits and vegetables. In plants, however, AsA is synthesized by multiple pathways. Among them, L-galactose, D-glactouronate, and L-glucose are well known as initial precursors but the existence of a fourth pathway starting from MI is still questionable (Fig. 4). An earlier study showed that the overexpression of the *AtMIOX-4*gene in *Arabidopsis* resulted in a two to three times higher AsA content compared to the wild type [19]. However, other finding suggests that the AsA content does not increase in lines that overexpress *AtMIOX4* but does regulate the level of MI [20]. In rice *OsMIOX* regulates the MI content without affecting AsA content, with or without stress [30]. The ectopic expression of *AtMIOX4*, *glucuronate reductase* or *L-gulono-1,4-lactone oxidase* genes increased AsA 1.5–3 times in transgenic *Arabidopsis* [49]. While, knockout of *AtGlcAK1*, next successive enzyme of MI oxidation pathway did not alter AsA content [50]. The overexpression of *SlMIOX4* in tomato showed an increased content of total AsA in leaves and red fruits relative to wild type. *SlMIOX4* overexpressing lines and the controls showed an improved AsA content in leaves when fed MI. In contrast, the AsA content in mature green fruit did not show any significant difference among these genotypes. These finding suggest that the MI pathway contribute directly to AsA biosynthesis and accumulation in tomato leaves [7]. Low-vitamin C (*vtc*1-1 and *vtc*2-1) mutants in *Arabidopsis* are well characterized for producing less foliar AsA. Mutation in *GDP-d-mannose phosphorylase* and *GDP-L-galactose phosphorylase* resulted *vtc*1-1 and *vtc*2-1 in *Arabidopsis* accumulates only 20–30% of the AsA content. Recently, a study showed that *AtMIOX4* is able to restore the AsA level of the *vtc* mutants as well as increasing biomass and growth rate relative to controls [51].

The role of MIOX in relieving cope of abiotic stress

Inositol and its derivatives are well known to involve in alleviating various abiotic stresses such as salt stress, drought, and UV-B induced oxidative stress [30, 52–54]. MI and its derivatives are involved in combatting salt stress in plant

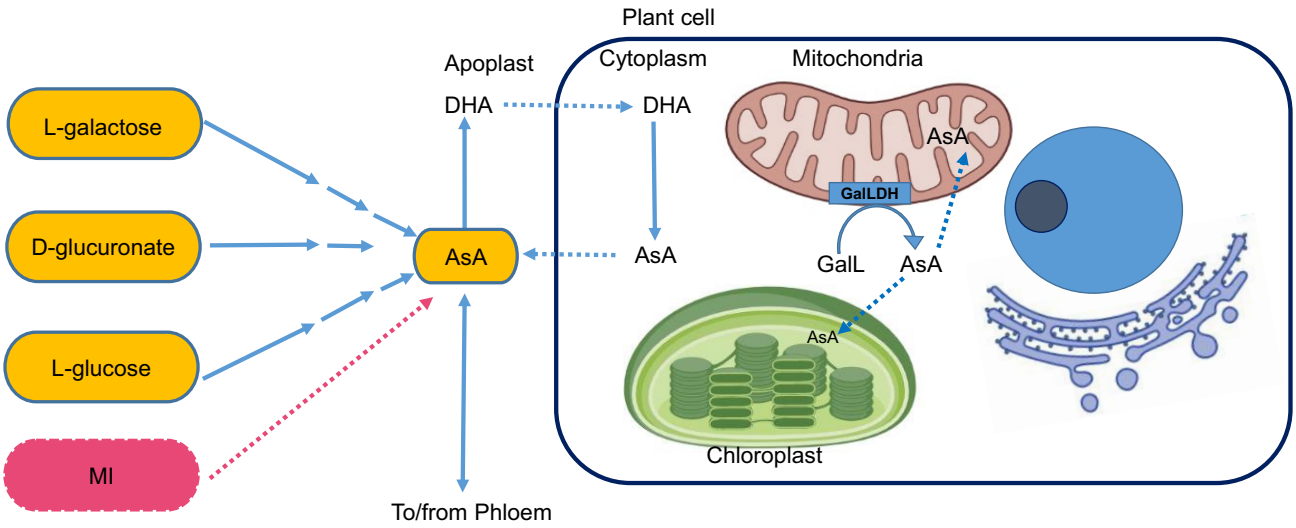

Fig. 4 Biosynthesis pathways of Ascorbic acid and its committed reactions in organelle. Galactose, D-Galacturonate, L-glucose, and MI are the initial precursors of AsA. Solid arrows symbolize the exist pathway in plants from its initial precursors. Dashed pink line represent the myo-inostiol oxidation pathway, which exist or not in plants are in debate. Dashed blue line symbolize the translocation of a metabolite from one cellular compartment to another. *GalL*, L-galactono-1,4-lactone; *AsA* ascorbic acid, *GLDH* L-galactono-1,4-lactone dehydrogenase; and *DHA* dehydroascorbate

in two key ways: (i) by protecting cellular and cytoplasmic structures from reactive oxygen species (ROS) i.e. and (ii) by controlling water pressure inside the plant cells [52]. Under salt stress, sodium is transported and accumulated in vacuoles whereas MI, d-ononitol, and d-pinitol accumulated in the cytoplasm of ice plant (*Mesembryanthemum crystallinum*) [54]. Rice *MIOX* (*OsMIOX*) was highly induced by drought, H_2O_2, salt, cold, and ABA (Abscisic acid). Overexpression of *OsMIOX* in rice significantly improved drought resistance [30]. Overexpression of *MIOX4* and *L-gulono-1,4-lactone oxidase* genes increased root and shoot biomass in *Arabidopsis*. Apart from this, phenotypic changes are reported as these lines had increased tolerance of cold, heat, and salt [49, 55]. *OsMIOX* is upregulated in transgenic rice lines those overexpress *PeaT1* gene during the drought treatment. Yeast two-hybrid assays suggested that PeaT1 interacts with OsMIOX, indirect evidences indicate that PeaT1 might promote the transcription of *OsMIOX* and also participate in some yet to explore protein complexes to play a role in drought tolerance. In soybean, *GsMIOX1a* is highly expressed in flowers and under alkaline stress. *Arabidopsis* lines that overexpressed *GsMIOX1a* were more alkaline stress-tolerant than *atmiox*1 mutant. *Vacuolar H + -pyrophosphatase, NADP-malic enzyme, KIN1, and RD29B*, like alkaline stress inducible markers genes were upregulated in *GsMIOX1a* overexpression plants [21]. *MIOX4* overexpressing tomato lines were tolerant to oxidative stress. The increased content of AsA in tomato might remove excess reactive oxygen species and protect tomato plants from oxidative damage [7]. Transcriptome analysis of *AtMIOX4* overexpressing lines upregulation of gene families that enhanced tolerance against cold, water, and heat stresses [48]. Apple (*Malus hupehensis* Rehd.) *MhMIOX1* and *MhMIOX2* were expressed under ABA and salt stress with NaCl treatment. Overexpression of *MhMIOX2* in *Arabidopsis* and poplar lines improved resistance to salt stress compared to the wild type. Overexpression of *MhMIOX2* in poplar lines under salt stress led to a decrease in H_2O_2 and MDA content and upregulated activity by POD, APX and CAT [22]. All aforementioned findings in *Arabidopsis*, soybean, tomato, rice and apple thus suggest that the MIOX pathway plays an important role in abiotic stress in plants.

Role of MIOX protein in combating biotic stresses

Transcriptome analysis of *Arabidopsis* and soybean syncytia induced by different nematodes showed up regulation of *MIOX* genes. One of 12 probe sets of *MIOX* genes was up-regulated by soybean root syncytia caused by *Heterodera glycines* [56]. In other study, probe set ID (Gma.17873.1.S1_s_at) corresponding to *AtMIOX2* gene was 20 times more expressed in syncytia five days post inoculation than it was in the control [56].Total RNA sequencing showed that two *AtMIOX* genes were highly up-regulated in root syncytia caused due to *Heterodera schachtii* on 5 and 15 days of infection [57]. The real-time expression of *AtMIOX2* gene was eight times higher in root syncytia made by cyst nematode. In another report, the expression analysis confirmed that *AtMIOX4* and *AtMIOX5* were highly up-regulated in root syncytia [28]. Wild type *Arabidopsis* and the double mutants *miox1/2* and *miox4/5* were infected with *H. schachtii* to examine the possible role of MIOX. Authors reported smaller syncytia and females per plant were less in the double mutant roots as compared to control root [28]. While, quadruple *miox1/2/4/5* mutant showed a substantial decrease in susceptibility to cyst nematode *H. schachtii*. Metabolite profiling of root syncytia of the quadruple *miox1/2/4/5* mutant showed that the MI and galactinol content was increased [58]. Galactinol is known to induce various defense-related genes, such as the antimicrobial *Thionin* gene in *Arabidopsis* [58]. Thus, the evidences we discussed so far strongly emphasize that MIOX plays an important role in growth and development of root syncytia and could be a potential target to reduce biotic stress impact caused by cyst nematodes. Transcriptome studies of the wild type and *AtMIOX4* over-expressing lines showed that various genes related to jasmonic acid biosynthesis were also up-regulated in the latter but not in the wild type. Genes related to parthenogenesis were less expressed in overexpressing lines whereas the opposite is true of genes such as o-methyl transferase that are related to the expression of phenolics in biosynthesis [48]. *AtMIOX4* over-expressing lines contain more glucoerucin and AsA than wild type, which suggests that overexpressing lines could be less prone to herbivore attack. It is interesting that relatively high amount of jasmonic acid in *Arabidopsis AtMIOX4* overexpressing lines indicates greater resistance to insects and fungi [48, 59].

Conclusive remarks

The MIOX pathways has great potential as novel player working at cellular level in response to environmental cues. The dispensable nature of pathway enzymes, while key roles of upstream and downstream regulators provide perfect environment to bio-engineer this pathway for stress resilience in plants. In present work, we tried to snap shot the cross talk of *myo*-Inositol oxidation pathway with some important biosynthetic pathways (Table 1). An important link we could recommend to explore further is Ins(1,4,5) P3 biosynthesis pathway. In near future, we hope to see some exciting outcomes from this crosstalk beyond just metabolic pathways. One such area could be tissue remodeling and developmental reprogramming in response to stress.

Table 1 Details of *MIOX* gene expression and their functional validation in different plants

Source plant	Gene name	Gene ID	Expression pattern	Function validation	References
Arabidopsis	*AtMIOX1*	At1g14520	Less in leaf & stem; high in flower		[5, 47]
	AtMIOX2	At2g19800	Root, stem & flower	Overexpression in tobacco changes arabinan and D-galacturonate	
	AtMIOX4	At4g26260	Higher in flower	Overexpression in tobacco changes arabinan and D galacturonate	
	AtMIOX5	At5g56640	Only in flower		
soybean	*GsMIOX1a*	Glyma07g01660	Higher in flower; In alkaline stress	Overexpression in *Arabidopsis* enhances tolerance to alkaline stress	[21]
	GsMIOX1b	Glyma08g21300	In alkaline stress		
	GsMIOX2a	Glyma01g00840	In alkaline stress		
	GsMIOX2b	Glyma07g15190	In alkaline stress		
	GsMIOX4	Glyma08g10690	In alkaline stress		
Wheat	*Ta7AMIOX*	TraesCS7A01G357800	High in spike & root; Under heat stress &*B. graminis* infection		[24]
	Ta7BMIOX	TraesCS7B01G269900	High in spike & root; Under heat stress &*B. graminis* infection		
	Ta7DMIOX	TraesCS7D01G364900	High in spike & root; Under heat stress &*B. graminis* infection		
Tomato	*SlMIOX1*	Solyc06g062430	Flower & fruit	NA	[7]
	SlMIOX2	Solyc10g005400	Leaf	NA	
	SlMIOX3	Solyc11g006570	Less in flower	NA	
	SlMIOX4	Solyc12g008650	High in flower & fruit, moderate in roots & leaves	Overexpression in tomato altered the expression of AsA related genes and AsA content in both leaves and fruits	
	SlMIOX5	Solyc12g098120	Higher in flower		
Rice	*OsMIOX*	AK103977	Under drought and cold stresses	Overexpression in rice improves tolerance to drought stress	[30]
Apple	*MhMIOX1*	MDO0000161309	Under salt stress	NA	[22]
	MhMIOX2	MDO0000285285	Under salt stress	Overexpression in *Arabidopsis* leads to increased growth under salt stress	

Table 1 (continued)

Source plant	Gene name	Gene ID	Expression pattern	Function validation	References
Cotton Gossypium ranmondii	*GrMIOX01*	XP_012472181.1			[23]
	GrMIOX02	XP_012435726.1			
	GrMIOX03	XP_012439315.1			
	GrMIOX04	XP_012447306.1			
	GrMIOX05	XP_012452593.1			
	GrMIOX06	XP_012455200.1			
Cotton *Gossypium Hirsutum*	*GhMIOX1*	Ghir_A03G000510.1			
	GhMIOX2	Ghir_A05G013690.1	Under heat, cold, drought, and salt		
	GhMIOX3	Ghir_A06G007270.1	Under cold		
	GhMIOX4	Ghir_A10G006080.1	Under heat, cold, drought, and salt		
	GhMIOX5	Ghir_A12G016630.1			
	GhMIOX6	Ghir_A12G022070.1			
	GhMIOX7	Ghir_D03G019230.1			
	GhMIOX8	Ghir_D05G013430.1	Under heat, cold, drought, and salt		
	GhMIOX9	Ghir_D06G007460.1	Under cold		
	GhMIOX10	Ghir_D10G006900.1	Under heat, cold, drought, and salt		
	GhMIOX11	Ghir_D12G016880.1			
	GhMIOX12	Ghir_D12G022100.1			

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Declarations

Conflict of interest All authors read the draft and approved. Authors declares no conflict of interest.

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