Review

Tonoplast Inositol transporters: Roles in plant abiotic stress response and crosstalk with other signals

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Abstract: Inositol transporter (INT) is reputed as the pivotal transporter for vital metabolites like lipids, minerals, and sugars particularly. These transporters play important role in transitional metabolism and various signaling pathways in plants through regulating the transduction of messages from hormones, neurotransmitters, and immunologic and growth factors. Extensive studies have been conducted on animal INT with promising outcomes. However, few recent studies have highlighted the importance and the complexity of INT genes in the regulation of plant physiology stages including growth and tolerance to stress conditions. The present review sum-up the most recent findings on the role of INT or inositol genes in plant metabolisms and the responsive mechanisms that cope with external stressors. Moreover, we highlighted the emerging role of vacuoles and vacuolar inositol transporters in plant molecular transition and their related roles in plant growth and development. Inositol transporters are the essential mediator for the inositol uptake and its intracellular broadcasting for various metabolic pathways where they play crucial roles. Also, so far characterized only in animals, we reported evidence on Na+/inositol transporters H+/inositol symporters and suggested their roles and operating mode in plants. Thus, understanding the INT functioning system, the coordinated movement of inositol, and the relation between inositol generation and other important plant signaling pathways would be an excellent asset for advancement in researches on plant stress adaptation.

Keywords: inositol, inositol transporters, vacuole, stress tolerance

1. INTRODUCTION

Unlike animals, plants are sessile organisms equipped with an exceptional defense system that helps them to adapt and survive under environmental extremes. To cope with such stresses, plants have developed complex and well-coordinated molecular and metabolic networks involving signal receptions, signal transduction, gene expressions, and cell acclimation responses. This exceptional defense system also allows plants to regulate important physiological processes including photosynthesis, osmotic maintenance, carbohydrate homeostasis growth, and development [1]. Among these processes, carbohydrate homeostasis is of most importance in plant abiotic stress because of its crucial role in stress perception, signaling, osmotic adjustment, the modulation of plant carbon partitioning, and cellular energy status, subsequently [2]. Several studies conducted on sugar transporters have revealed their key roles in the perception and adaptation of plants under biotic and abiotic stresses. therefore, the multifunctional position supplied by *Myo-inositol* has emerged as a central feature in plant resistance to abiotic and biotic stresses [3].

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Plant vacuoles are principally grouped in three types: food vacuoles represent ursine of phagocytosing; central or sap vacuole mostly contains water and maintains the structure of the plant cell; then, contractile vacuoles or drain cells that serve to evacuate waste materials [4]. The number of plant vacuoles varies from one cell to another, and every typical plant cell possesses at least one central vacuole which is one of the biggest cell organelles with a crucial function in the maintaining of their structures. In addition to these central vacuoles, 10 to 50 more vacuoles can be found in each plant cell depending on their tissue localisations. These vacuoles could represent in term of volume 30% to 80% of plant cell total volume [5]. During the germination stage of plant development, some activated vacuoles named protein body or oil body provide essential nutrients (protein and lipids) while other vacuoles will absorb the higher quantity of water and accelerate cell growth. This conjugated reaction makes the initial plant growth stage faster than its maturation process. The sum-up of vacuoles roles in plants resumes in protecting other cell organelles from hurtful materials, drain and store cell rubbishes, store plant extra water and maintain intracellular osmotic pressure, maintain turgor within cells, keep balance the cell pH, store and provide important nutrients and so on [6].

Recent studies have described the mechanism of inositol transportation (inositol transporters) as mainly located in the tonoplast, and have less deepened their impacts on plant abiotic stress resistance. It is well known that D-glucose-6-P to 1l-*Myo-inositol*-1-P constitutes a pivotal initiator for this brief metabolic survey. Inositol, as an essential molecule for plant cell communication or cellular communication, plays an unavoidable function in plant signal transduction [7]. Increasing pieces of evidence have pointed generation of two important inositols in response to various plants' stressful events which are *myo*inositol (1,4,5) trisphosphate and phosphatidylinositol (4,5) bisphosphate [8, 9]. The first *Myo-inositol* (MI) was isolated from muscle by Scherer in 1850, and its cyclitol functions, its natural occurrence, properties, derivatives, and stereoisomers immediately captivated much attention. Later, the inositol emerged as an important growth factor for many microorganisms and was established as fundamental for the growth of certain forms of yeast [7, 10]. Many molecular entities containing or using *Myo-inositol* are involved in several plant structural and functional mechanisms. Meanwhile, few studies reported the specificity of MI metabolism in plants, as well as modes of transports that could lead to a better appreciation of this unique molecule and its position in plant science [11-13].

As for the animal, plant inositol transporters (INT) play important role in nutrient uptakes and intracellular. INT can be grouped into two distinct groups in which sodium ion coupled is the most studied than proton-coupled inositol transporter. Herein, we described the biosynthesis of the inositol, its pathway, and derived roles in plants. Then, summarized findings related to plant inositol transporters and their different functions in plant physiology and stress responses precisely. In addition, through bioinformatic analyzes, the review also depicted the structure and isoforms of inositol transporters as well their subcellular localizations and functions in plant vacuoles and tonoplast. Moreover, it resumes research progresses on plant inositol transporters, their signaling pathway, and crosstalk with other signals, and highlights new directions for research on plant stress resistance. Therefore, understanding the role of INT mechanisms in plant stress responses might open new avenues in plant research to promote their adaptation to their ongoing changing environment, and consequently, improve crop yields.

2. Biosynthesis of inositol

The inositol biosynthesis starts with the conversion of D-glucose-6-P to 1L-MI-1-P, and its metabolic processing is tightly linked to photosynthesis which includes important reactions in plant physiology such as; oxidation of free inositol to D-glucuronic, a principal generator of uronosyl and pentosyl units of pectin, hemicelluloses, and related structures in plant cell walls [10, 14] [15, 16]. Some inositol isoforms (O-methyl inositols) such as bornesitol, sequoyitol quebrachitol, ononitol, pinitol were reported to be essential in plant stress responses, storage of seed products, and production of inositol-glycoside [17]. Generation of Plant inositol can occur in several ways and the most used remain the glycolytic glucose-6-phosphate (Glc6P). *Myo*-inositol (MI) is a pivotal component of many vital plant metabolic pathways like lipid signaling pathway, sugar transport, carbohydrates partitioning, and PI signaling pathway. The biosynthesis pathway of MI is very special because it is ubiquitous among all organisms; it starts with the transformation of d-glucose-6-phosphate to l-inositol-1-phosphate through the enzymatic reaction of myoinositol-phosphate-synthase **(Fig.1)** [18]. MI also results from l-inositol-1-phosphate dephosphorylation by *Myo*-inositol-monophosphatases; besides *Myo-inositol*, there are nine different chaps of inositol stereoisomers: *Myo*, d-chiro-, l-chiro-, scyllo-, cis-, neo-, muco-, epi- and allo-inositol), but only eight of them occur naturally. However, *Myo*-inositol is the most frequent inositol stereoisomer identified as playing a crucial role in all organisms [19, 20].

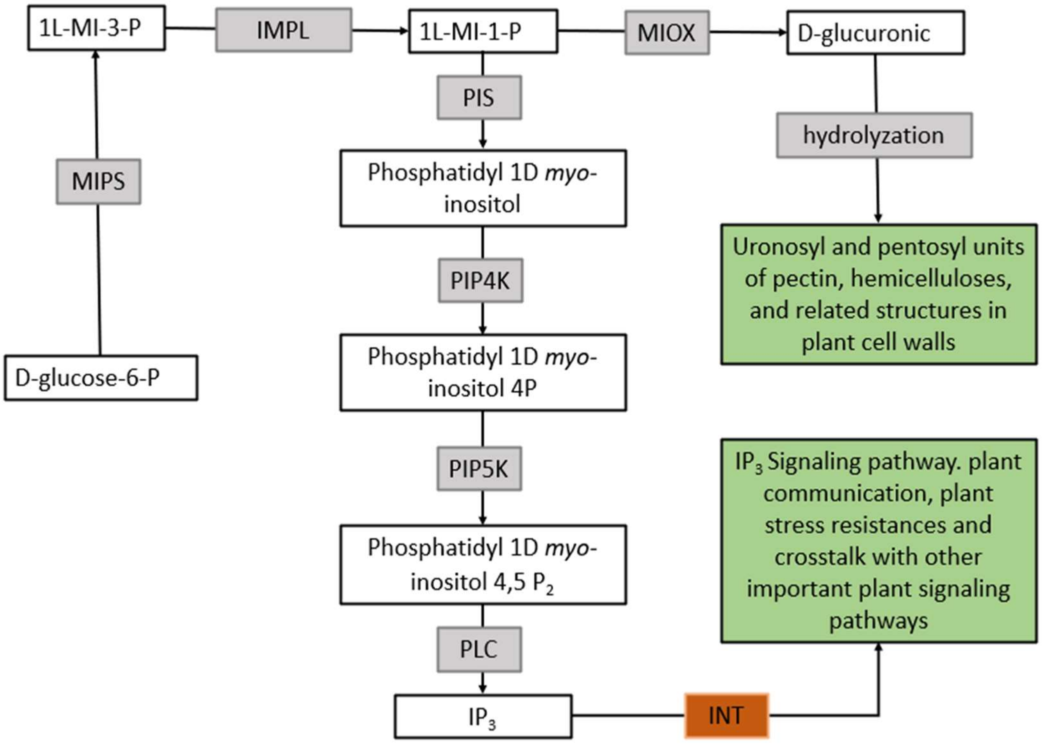

Figure.1 Schematic resume of inositol bio-generation and their derived roles in plant metabolisms. All metabolites are highlighted in white boxes, the corresponding enzymes are represented in gray boxes. Dotted arrows indicate reactions including more than one step. The green boxes displaying the derived roles of inositol.

3. Inositol pathway and derived in plant

Free inositol is capable of strong ligation with various molecules, nutrients, or hormones to generate an easier transitive polarized form like the synthesis of basic phosphatidylinositol (PtdIns) by phosphatidylinositol synthase (PIS)[8]. Plant PtdIns could produce PtdIns3P, PtdIns4P, PtdIns5P by phosphorylation at the positions 3, 4, and 5 carbon, respectively. Five inositol phosphorylated isomers are present in plants, including the mentioned PtdIns3P, PtdIns4P, PtdIns5P (inositol monophosphates); PtdIns(3,5)P_2 and PtdIns(4,5)P_2 inositol bisphosphates[21]. However, some inositol derivates like diacylglycerol [22] and inositol-1,4,5-trisphosphate (IP_3) are amongst the most active metabolites in plant physiology [23]. DAG and IP_3 are generated through the hydrolyzation of PtdIns(4,5)P_2 and PtdInsPs by phospholipase C (PLC); these MI derivates are important second messengers for plant signal transduction and are widely localized in almost all cell compartments. This suggests that their precursor's movements are most fluent and well organized to operate at the right place and the right moment [24]. To summarize, DAG is known as the first generator of phosphatic acid which importance is not questionable in plant stress tolerance reactions. The membrane-localized DAG can also activate protein kinase C (PKC) and spread soluble IP_3 into cytosol and outflow Ca^{2+} vacuoles through a ligand-gated Ca^{2+} channel [25]. In addition, the inositol-1,2,3,4,5,6-hexakisphosphate ($InsP_6$), a storage form of inositol in seeds and also named phytic acid is also widely identified in higher plants. $InsP_6$ results from the phosphorylation of inositol polyphosphates by inositol polyphosphate multi kinases (IPKs) are important as well [26, 27]. In another study, $InsP_6$ was revealed as a signaling molecule that can regulate Ca^{2+} outflow in cells and mentioned the existing crosstalks between phytohormones and the Ins signaling pathway under abiotic stress [26, 28, 29]. In this review, we briefly summarize the importance of tonoplast in vacuolar trafficking with a focus on tonoplast inositol transporters and their related roles in plant abiotic stress resistance.

4. roles of inositol transporters in plants

One important feature of plant cell wall biosynthesis is that it involves multiple cellular compartments. It is the single major determinant of plant structure and has fundamental functions in plant growth, development, and resistance to biotic abiotic stresses. polysaccharides (sugars) are the main component of Plant cell walls and Inositol is essential for their production. The oxidized form of inositol (D-glucuronic) represents the most prominent and important sugar during the production of plant cell wall polysaccharides. Besides its crucial role in cell wall biosynthesis, inositol biomechanism provides various sugars and components for other important networks involved in plant storage, transport, and development [10].

Unlike animals, plants can not escape to their environment cuds although they have developed an extraordinary mechanism to respond to various stressful events. Plants can use many molecular signaling pathways for establishing efficient communication among cells. Inositol transporters play an active role in the phosphatidylinositol (PI) signaling pathway which is one of the most important signaling pathways in physiology [30, 31]. PI signaling pathway has been demonstrated as a hub molecular network that is involved in a plethora of plant physiology reactions including the growth of the downward roots in response to gravity, regulation of pressure in leaf pores, and plant wilting consequently [32, 33].

MI molecule and related molecules play critical roles in plant protection against salt stress, accumulation, and movement of MI and MI derivatives in response to salinity have been widely demonstrated in *Mesembryanthemum crystallinum* (ice plant) [34] [35]. In response to the salt stress plant, MI and derived metabolites are known to operate in two principal ways. Firstly, protect cellular structures from reactive oxidizers like hydrogen peroxide, and secondly, the modulation of cell's osmolarity important for balancing water pressure inside cells. A previous study revealed that in Ice plants undergoes salt stress conditions, the production and accumulation of inositol occurred within a very short period following the stress application. Also, the MI accumulation in Ice plant was simultaneous to plant wilting subsidence. Meanwhile, the restoration of water pressure in cells was followed by an increased concentration of MI in the phloem and the accumulation of the couple Sodium ($Na+$) / inositol in the leaves[36]. Finally, the salt stress-responsive mechanism in ice plant seems to be engaging two important transporters; the sodium transporters controlling the proper uptake of sodium out of the roots, and the inositol transporters acting as a facilitator of sodium uptake and long-distance transport and confer salt tolerance to crop species [37].

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5. INT genes in plant

5.1. Structure and Isoforms of Plant inositol transporter genes

Expressions of many sucrose transporters have been investigated in diverse organisms including plants. plant carbohydrate partitioning includes important processes like initial fixation, metabolic conversion, and long-distance distribution of reduced carbon compounds throughout the plant. The carbohydrate partitioning is mainly base on sugars and/or sugar alcohol translocation from the light stage of photosynthesis in plant leaves to its dark stage (non-photosynthetic) in sink tissues [38, 39]. This carbohydrate partitioning is controlled by several sugar transporters involving the perception of stimuli (biotic and abiotic stress), the signal transduction, and the related responses and environmental adaption[40, 41]. Therefore, it becomes essential to understand the factors and genetic regulators influencing carbohydrate partitioning plant physiology under stressful conditions. This review section stands on biomolecular responses of the plant toward abiotic stresses through the action of inositol transporters on cell carbohydrate partitioning [2].

Many studies have been describing different families of sugar transporters and their prominent roles in plant physiology and adaptative mechanism to biotic and abiotic stresses. Among those studied sugar transporter MONOSACCHARIDE TRANSPORTERS (MSTs) SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER (SWEETs and SUCROSE TRANSPORTERS (SUTs). MSTs represents a large family of seven subgroups including EARLY RESPONSE TO DEHYDRATION (ERD6), SUGAR TRANSPORT PROTEINS (STP), PLASTID GLUCOSE TRANSPORTER (pGlcT), INOSITOL TRANSPORTERS (INT), VACUOLAR GLUCOSE TRANSPORTER (VGT), TONOPLAST SUGAR TRANSPORTERS (TST), and POLYOL/MONOSACCHARIDE TRANSPORTERS (PLT) [42-44]. Sucrose transporters' activities are strictly linked to the stimuli levels; their sensibilities enable plant adaptation to different external changes as small as it is such as temperature, drought, pathogen attack, saline, and other stresses. Here we focusing on the regulation of plant carbohydrate partitioning in response to abiotic stresses through inositol transporters [45].

Tonoplast INT as a sugar transporter represents the most described plant polyol transporter (PLT) until now [46]. Also named as sugar alcohols, polyols are devised in two principal groups including cyclic and acyclic compounds; these two groups of rehashed water-soluble sugars, the first representing a group of pinitol, ononitol, and *Myo*-inositol and the second consisting of sorbitol, mannitol, and inositol [46]. Four INT genes have been recently identified in Arabidopsis, among which three (*INT1*, *INT2,* and *INT4*) could encode for functional proteins and one (*INT3)* was classified as a pseudogene. Moreover, INT1, INT2, and INT4 were revealed as H^+/inositol symporters genes capable of mediating the uptake of inositol into the cytoplasm. INT2 and INT4 were first found in the plasma membrane and INT1 was later discovered in the tonoplast. So far, only INT1 has been well assessed and was established as the unique transporter able to mediate the transport of MI from the vacuole to the cytosol.

The distribution of INT isoforms in plants has been less studied and little is known about plant INT genes. Since INT genes were previously identified and characterized in Arabidopsis only, we used the soybean *Glycine max* as a sample of this study to mainly classify INT1 in soybeans, with Gorai and Glyso is also selected to assist. Therefore, a phylogenetic analysis was monitored to compare Arabidopsis and soybean using protein successions and we found that three clusters of *INT*s were distinguished and regrouped with the organization of the *INT* genes from *Arabidopsis* **(Fig.2)**.

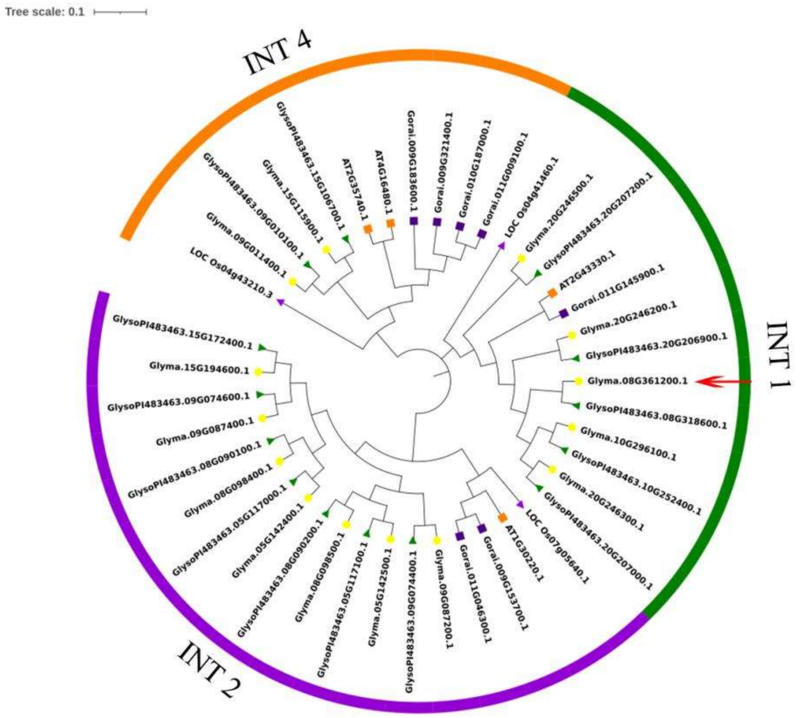
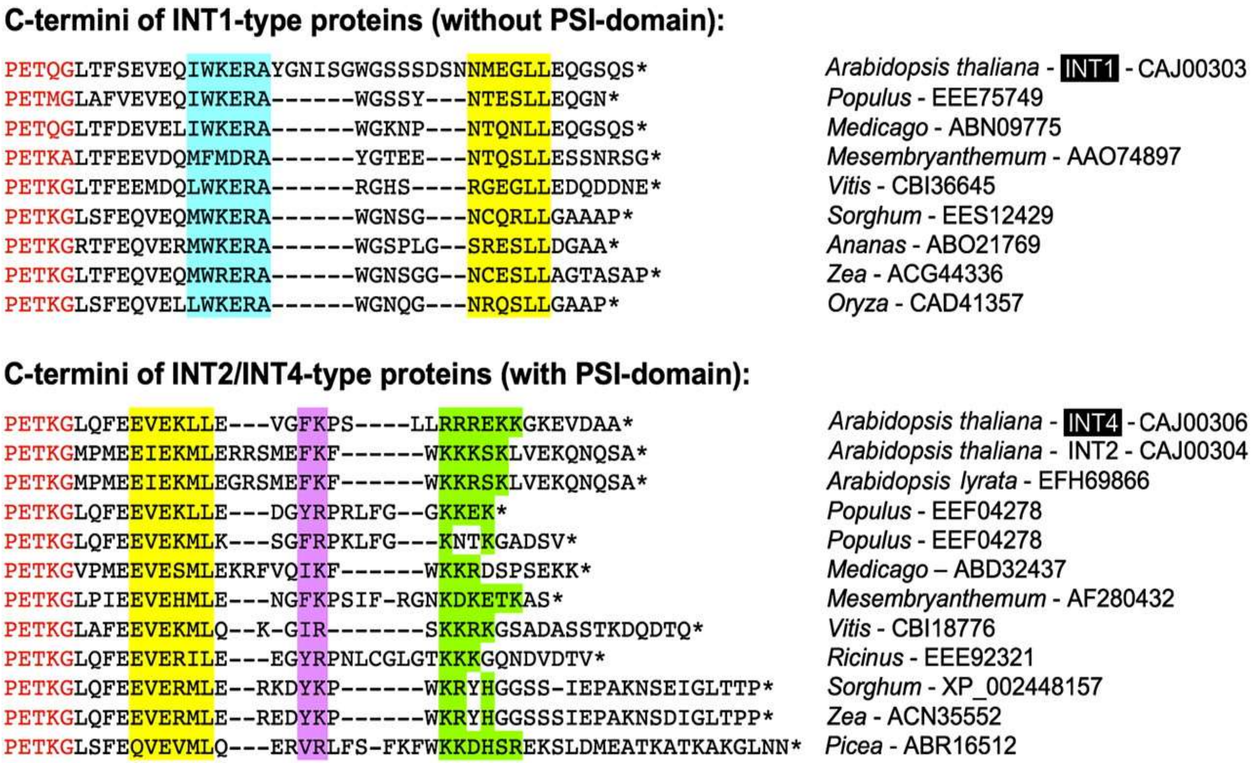


Figure 2. Phylogenetic analyses of inositol transporter (INT) genes in four plant models: Glycine max (Gm), Arabidopsis thaliana (At). The maximum likelihood tree was constructed using the MEGA 6.0 program and was based on the full-length amino acid sequences encoded by the INT-related genes, which were named according to their names in the Arabidopsis database.

5.2. INT conserved protein domains in plant

Myo-inositol [10] is a sugar-like carbohydrate produced by most plants; it has been assayed at half the sweetness of table sugar (sucrose). *Myo*-inositol [10] is an added ingredient in various root products because it is important for normal plant growth and development. *Myo*-inositol is a pivotal molecule in plant growth, development, and adaptive responses to abiotic stresses; it plays important roles in certain plant vital processes such as cell wall biosynthesis, phosphate storage, cell-to-cell communication, production of stress-related molecules, storage and transport of plant hormones [21, 47]. The transport of plant inositol is catalyzed by some HMIT-like H+-symporters. In *Arabidopsis thaliana*, three HMIT-like H+-symporters were mainly identified in the tonoplast (AtINT1) and the plasma membrane (AtINT2 and AtINT4)[47]. The localization of inositol in such an important cell compartment suggests that the modification of its concentration might directly affect certain essential signaling pathways and the plant cell physiology consequently. Altered cytoplasmic inositol concentrations might, therefore, affect the general metabolism as well as specific signaling pathways. In the brains of patients suffering from depression or bipolar disorders, increased inositol concentrations are thought to affect cellular signaling and behavior. As neurons cannot synthesize inositol [48, 49], increased neuronal inositol concentrations might be explained by altered HMIT-catalyzed inositol uptake; Similarly, in plants, a defect in a single inositol transporter causes severe developmental defects [49, 50].

Moreover, comparative studies were previously conducted with *arabidopsis thaliana* INT2 protein (AtINT2) and the human HMIT protein (hHMIT) regarding the function of plexin/semaphorin/ integrin (PSI) domain in the inositol transporters, revealing a shared sequence and thigh similarity between the two groups. Also, the author demonstrated the possibility of removing the PSI domain from its inositol transporter without affecting the related transport function or modifying its predicted substrate affinity. Therefore, it appears that the PSI domain of inositol transporters possessed cysteine knots supplemented by a small region of secondary structure that may play key roles in the modulation of plant INTs [51]. The localization of INT genes in the plasma membrane and vacuole and the relation with the H^+ translocation in eucaryotic cells ranged the INT amongst plant H+-inositol symporters. It is well-known that except for fungi or endomembranous plants, other plant plasma-membrane H+-inositol symporters hold a double transmembrane helices domain flanked by the CXXC consensus in which two cysteines (C) are surrounding amino acids (A). These membrane helices called IX and X also frame an impressive extracellular loop domain (IX/X-loop domains) but its characterization and function still not well understood On the other hand, investigations on the IX/X-loop conserved domains of inositol transporters located in both plant (AtINT2) and animal (hHMIT) plasma-membranes revealed that the INT IX/X-loops and the PSI domains displayed same sequences and similar structure with previous evidence for a role of this PSI which is target sites for Ni++ (**Fig.3**). Thus, the interaction between Ni++ ions and PSI domains inhibits the inositol uptake in plants [51, 52].



C-termini of INT1-type proteins (without PSI-domain):

```
PETQGLTFSEVEQIWKERAYGNISGWGSSSDSNNMEGLLEQGSQS*     Arabidopsis thaliana - INT1 - CAJ00303
PETMGLAFVEVEQIWKERA------WGSSY---NTESLLEQGN*       Populus - EEE75749
PETQGLTFDEVELIWKERA------WGKNP---NTQNLLEQGSQS*     Medicago - ABN09775
PETKALTFEEVDQMFMDRA------YGTEE---NTQSLLESSNRSG*    Mesembryanthemum - AAO74897
PETKGLTFEEMDQLWKERA------RGHS----RGEGLLEDQDDNE*    Vitis - CBI36645
PETKGLSFEQVEQMWKERA------WGNSG---NCQRLLGAAAP*      Sorghum - EES12429
PETKGRTFEQVERMWKERA------WGSPLG--SRESLLDGAA*       Ananas - ABO21769
PETKGLTFEQVEQMWRERA------WGNSGG--NCESLLAGTASAP*    Zea - ACG44336
PETKGLSFEQVELLWKERA------WGNQG---NRQSLLGAAP*       Oryza - CAD41357
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C-termini of INT2/INT4-type proteins (with PSI-domain):

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PETKGLQFEEVEKLLE---VGFKPS----LLRRREKKGKEVDAA*             Arabidopsis thaliana - INT4 - CAJ00306
PETKGMPMEEIEKMLERRSMEFKF------WKKKSKLVEKQNQSA*            Arabidopsis thaliana - INT2 - CAJ00304
PETKGMPMEEIEKMLEGRSMEFKF------WKKRSKLVEKQNQSA*            Arabidopsis lyrata - EFH69866
PETKGLQFEEVEKLLE---DGYRPRLFG--GKKEK*                      Populus - EEF04278
PETKGLQFEEVEKMLK---SGFRPKLFG---KNTKGADSV*                 Populus - EEF04278
PETKGVPMEEVESMLEKRFVQIKF------WKKRDSPSEKK*                Medicago – ABD32437
PETKGLPIEEVEHMLE---NGFKPSIF-RGNKDKETKAS*                  Mesembryanthemum - AF280432
PETKGLAFEEVEKMLQ--K-GIR-------SKKRKGSADASSTKDQDTQ*        Vitis - CBI18776
PETKGLQFEEVERILE---EGYRPNLCGLGTKKKGQNDVDTV*               Ricinus - EEE92321
PETKGLQFEEVERMLE--RKDYKP------WKRYHGGSS-IEPAKNSEIGLTTP*   Sorghum - XP_002448157
PETKGLQFEEVERMLE--REDYKP------WKRYHGGSSSIEPAKNSDIGLTPP*   Zea - ACN35552
PETKGLSFEQVEVMLQ---ERVRLFS-FKFWKKDHSREKSLDMEATKATKAKGLNN* Picea - ABR16512
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Figure3. Alignment of the C-Terminal Sequences from INT1-Type and INT4-Type Transporters. Sequences obtained from BLAST analyses (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) were divided into two groups based on the presence or absence of a PSI domain between their predicted transmembrane helices IX and X and listed as INT1-type transporters [53] or as INT4-type transporters (Bottom). A consensus sequence at the start of the C terminus of plant and animal transporters of the major facilitator superfamily is shown in red. Di-Leu motifs are highlighted in yellow, a conserved region found only in INT1-type transporters is highlighted in blue, and conserved regions found only in INT4-type transporters are highlighted in magenta and green. Gene names or genus names plus GenBank accession numbers are given. (Adapted from Susanne Wolfenstetter et al; plant cell 2012)

In addition, another study was applied on *Arabidopsis thaliana* using the tonoplast INT1 and plasma membrane INT4 revealed through swapping and mutational analyses the presence of the C-terminal di-leucine patterns that play crucial roles in high plants by activating inositol transporters, including INT1 tonoplast activities in *Arabidopsis* mesophyll, precisely. The authors demonstrated that those motifs potentially inverse the synthetic reactions of other proteins including, SUCROSE TRANSPORTER2 (SUC2) or INT4 to the tonoplast and that the location of consensus is linked to transmembrane helix was also pivotal[54, 55]. The functional activity of the reversed INT4 remains in the tonoplast and serves as a supplement to that of INT1 in the *int1* mutant. However, the SUC4 was found inhibited in *cis*-Golgi's tacks while both INT1 and SUC4 migration to the tonoplast was greatly activated and modulated by the brefeldin A. Thus, during plant metabolism various Golgi-dependent are activated to target the transduction of the newly generated inositol transporters to the tonoplast. The gene structure of INT genes showed many conservative domains between *Arabidopsis thaliana* and soybean *Glycine max;* interestingly, cis-motifs cis-regulatory units revealed many conservative elements that commonly convert essential abilities to plant to cope with both abiotic and biotic stresses (**Fig.4**).

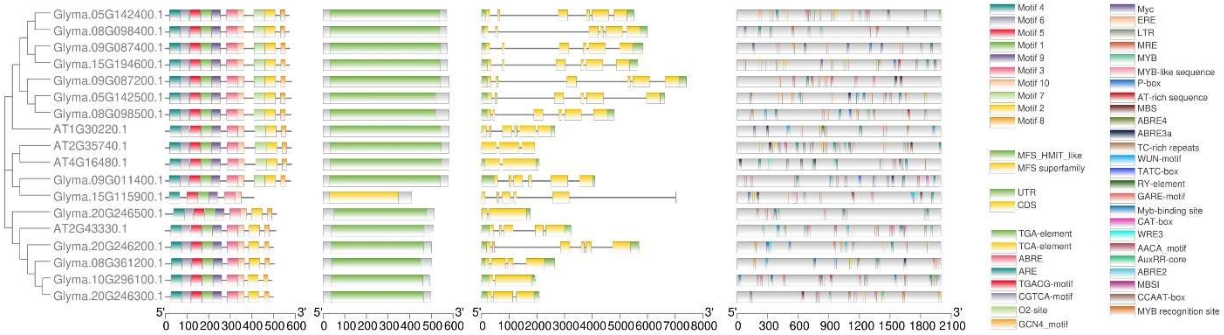

Figure 4. Phylogenetic intron/exon analysis coupled with functional domain analysis of INT protein and identification of cis-motifs cis-regulatory units in all *INT* genes from *Arabidopsis* and soybean.

6. Roles of INT genes in vacuoles and tonoplasts

For long-time considered as simple garbage of the immune system in eukaryotic cells and later valued as an excellent storage organelle for vital nutrients in plant cells, vacuole lumen and tonoplasts have finally become essential actors in the development, growth, and physiological responses of plants to various environmental stresses [56, 57]. Those vital nutrients stored in plant vacuoles are mainly constituted of sugars and proteins [57]. Understanding the mechanisms of vacuolar trafficking and molecule transport across the vacuolar membrane is of great importance in understanding basic plant development and cell biology and for crop quality improvement.

Vacuoles are the important compartment of plant cells' endomembrane system; they occupy a large percentage of the cell volume; finally, vacuole lumen is separated from the cytoplasm by a dynamic membrane named tonoplast. Vacuole's morphology is constantly modified in response to environmental stresses and differs from one stage of the plant developmental process [58-60]. Plant cell molecular transit is mainly dominated by the movement of water, metabolic products, and ions. However, these components require an extraordinary reaction from tonoplast proteins to cross the tonoplast, since it is practically impossible to freely move across the vacuole membrane (tonoplast). The modulation of vacuole volume and composition depends on the synchronization of different transporters and channels of the tonoplast. The role of lipids in vacuolar trafficking is critical in plant physiology but less understood. However, it is crucial for plant scientists to first investigate the molecular composition of plant vacuoles, molecular

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movement, and communication among them and finally elucidate their molecular trafficking mechanism. Therefore, it is essential to understand the function and mechanisms that regulate plant tonoplast transporters' actions on both organic compounds and inorganic ions (**Fig.5**). In the last decade, many proteomics assays were conducted on various plant species and the protein composition of vacuole lumens and tonoplasts emerged from their related data, providing certain gene candidates for studying the molecular transport mechanism in vacuoles and their targeted functions in plant biology [22, 61, 62].

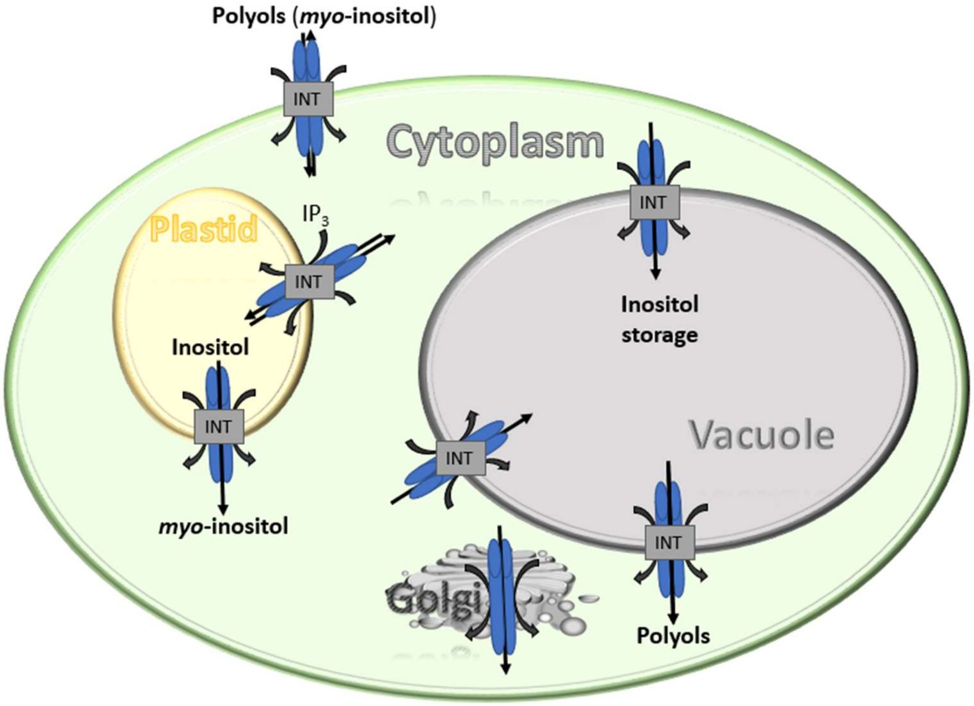

Figure.5 Overview of *Myo-inositol* traffic from vacuoles to cytosol and Golgi through the INT transmembrane transporters

Tonoplasts are constituted of various transporters, enzymes, and various channels which vary in response to cytoplasmic conditions and modulate molecular exchanges between the vacuole lumen and cytoplasm which directly maintain cellular homeostasis. The vacuole compartmentalization was also revealed as an essential factor of heavy metal detoxification in plants; however, its tonoplast potassium uptake regulation directly affected the stomatal aperture, and flower color was strongly related to the combined actions of two different tonoplast P-ATPases. [63, 64]. The activation of tonoplast transporters in response to cytoplasmic variations induced by various cellular signaling is of the most important factors in plant growth, nutrient distribution, and adaptation to biotic and abiotic stresses [65].

Plant cells controlling the composition and volume of vacuoles are linked to the coordinated reactions of different channels and transporters found in the tonoplast (vacuolar membrane). The tonoplast is mainly constituted of three components; the H^+-pyrophosphatase (V-PPase), H^+-ATPase (V-ATPase), and aquaporins which are the most abundant protein in the tonoplast in most plants. Moreover, recent studies have found many other kinds of tonoplast transporters like Ca^{2+}/H^+ antiporter Ca^{2+}-ATPase, Na^+/H^+ antiporter, and inositol transporters. Plant inositol signaling is very large and identification of signals that disturb either InsPs or PtdInsPs remains the prominent methodology in plants. Although, the understanding of plant inositol signaling is mainly based on gravitropism which is the best and the easiest factor that impacts the fluctuation of InsP in plants. In maize and oat pulvini (*Zea mays*), gravi-responsive maize displayed prompt raise of Ins(1,4,5)P_3 accumulation in preceding growth in *Avena sativa* [66].

7. Plant Inositol signaling and crosstalk with other signals

7.1. INT in plant vacuole lipid signaling

Plant lipids are generated under a plethora of forms including storage oils, components [67]. Lipids also play some critical roles in plant tonoplasts by providing physical support for proteins, and regulating tonoplast enzyme activities, and driving vacuole fusion. A study has recently evidenced the role of lipid tonoplast trafficking and its interaction with ribosomal RNA [68]. The presence of lipids in the vacuole membrane confers a filter system between the vacuole lumen and the cytoplasm; its structure and fluidity mainly linked to its lipids composition are fundamentals for the vacuolar physiology [69]. In yeast, the vacuole biogenesis and fusion reactions require various lipids including phosphatidylethanolamine (PE) ergosterol, diacylglycerol [22], phosphatidic acid (PA), and phosphoinositides (PI). The latter is vital in plant vacuole biogenesis while its mode of action demands more investigation [70-73]. The lipid ratio in tonoplast can change from one species to another. For example, phospholipids represent 51% of the total lipids in the hypocotyls of *Vigna radiata* L, 44.5% *Acer pseudoplatanus* [74]. Phosphatidylinositol is reputed for its structural role in most eukaryotic cell membranes[75]; it is a crucial and one of the most abundant phospholipids in plants. In addition, phosphatidylinositol transporters are essential in signal transduction in cells [76, 77]. Inositol also acts as the substrate for the generation accumulation and transportation of important methylated derivatives (cyclitols) such as the 1-D-4-Omethyl-myoinositol (D-ononitol) and 1-D-3-O-methylchiro-inositol (D-pinitol). These cyclitols are commonly found in *Mesembryanthemum crystallinum*, tropical legumes (*pigeonpea*), mangrove fern (*Acrostichum aureum L.*), maritime pine (*Pinus pinaster*) and *mistletoes* [78].

7.2. INT as a sugar transporter

Plant responsive mechanisms toward salt, drought and osmotic stress are characterized by a massive accumulation of these water-soluble sugars' derivatives important for various physiological roles in biotic and abiotic stresses such as movement of carbon from origin to the target organs, antioxidation, and osmoregulation [46, 79]. During their fight again osmotic stress saline stress, plant polyols prompt reaction is the surrounding important molecules and organelles with a sphere of hydration ensuring their metabolic inactivation in response to low osmotic variation [80]. Polyols are also known as source tissues that can be firstly transported within cellular organelles or compartments and secondary the long-distance flow by phloem loading. The identification and characterization of polyol transporters have been carried out from certain high plant species including *Plantago major, Prunus cerasus, Olea europaea, and Malus Domestica*, [81-84]. In the last decade, researchers have demonstrated that their transporters (PLT and INT) expressed differently under various abiotic stress conditions [79]. The expression patterns of four polyol transporters OsPLT3, OsPLT4, OsPLT13, and OsPLT14 were significantly increased in rice under salt osmotic and drought stresses [2, 85, 86]. Also, another study in *Lotus Japonicus* revealed high expression for three PLT1, PLT3, and PLT3 in response to salinity and/or osmotic stresses[87]. Inositol is the simplest cyclohexane, it is a ubiquitous component of plant cells. This important molecule is super active in plant physiology with a special PI signaling pathway. Since its transportation majorly occurs into a bound form, a good understanding of plant inositol distribution implies a perfect mastery of the inositol transporters family. INT plays different important roles in plants depending on its bound molecule and the involved signaling pathway consequently. In certain cases, MI can link to phosphorus during its cell translocation or cell-to-cell communication [88].

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7.3. INT in plant hormone distribution

Plant hormones are chemical compounds present in very low concentrations in plants; hormone production occurs in certain parts of the plant and their transmission to other parts constitute can be synergic, individual, antagonistic, and complementary [89, 90]. Plant hormones control all the growth and development activities like cell division, enlargement, flowering, seed formation, dormancy, and abscission[91, 92]. The plant hormonal system is mainly dominated by five groups of hormones including, Abscisic acid (ABA), Ethylene, Gibberellins Cytokinins, and Auxins, each group playing a key role in plant life. For example, Cytokinins [70] influence cell division; Ethylene is a ripening hormone; finally, ABA, Gibberellins, and Auxins are growth promoters [93-95]. Auxins were the first class of growth regulators discovered; they also play role in plant growth promotion under stress conditions by enhancing plant root growth [53].

Myo-inositol is reputed in storing processes and transport auxins in plants. Therefore, inositol transporters provide an extraordinary mode of transport to hormones (Auxin) in a non-reactive form to target tissues or cells. This explains the hypothesis of the key roles of *Myo-inositol* in plant auxin regulation [96]. The transport of inositol is linked up with that of auxins forming a complex of hormones that are momently silenced. These complexes of hormones make easier and safe the storage and/or transport of auxins implying the regulation of plant stress responses by controlling the availability of active auxins [97]. In addition, a recent study conducted on corn enzymes revealed linked auxin to *Myo-inositol* by isolating two enzymes; one that conjugates the two metabolites and another that may be involved in the separation of auxin from its inositol conjugate. Even though inositol is not considered a hormone, its fluent movement and ability to carry crucial hormones like auxins may help to ensure that function at the right time or place [98]. The role of inositol in plant hormone signaling has been driving more and more attention, and protecting the auxin to facilitated its phloem long-distance transport remains the most discussed role of inositol on auxin signaling [98]. Recent exploration of *Myo-inositol* phosphate (*MIPS*) roles in wheat growth under heat stress suggested the *MIPS* as essential for orchestrating the ethylene reaction and modulate plant growth and resistance to abiotic stress [99]. Additionally, *Myo-inositol* mediates the ROS programming cell death via ethylene-dependent and salicylic acid-dependent pathways. Thus, *Myo-inositol* can directly upgrade the cell wall polysaccharides' integrity and coordinate ROS-induced PCD via both salicylic acid and ethylene-dependent pathways in apples [3].

7.4. Hydrolysis of tonoplast PtdInsPs and second messenger production

In response to their environment changes, PtdInsPs are actively hydrolyzed in the plasma membrane through the enzymatic reaction of phospholipases resulting in the release of inositol in the cytoplasm. This free inositol will be uptake and distributed in different cell organelles. In animals, inositol transporters can operate in two ways: sodium ion coupled and proton-coupled inositol transporters, respectively. Although phospholipases are widely studied in plant lipid signaling, phospholipase C (PLC) remains the most affecting inositol signaling [100]. PLC is super active in plant physiology and can be encoded by many genes plants, while its production and circulation require various subtracts such as PtdIns, PtdInsP, and PtdIns (4,5)P_2.

PLC importance in plant signal transduction is well illustrated in many plant studies. Regarding the importance of inositol on PLC activities, in response to their environmental stress, plant PLC hydrolyses plasma membrane-localized PtdIns(4,5)P_2 to produce diacylglycerol [22] with remains fixed on the membrane, and a water-soluble Ins(1,4,5)P_3 molecule that can coordinate the intracellular diffusion of information or inositol notably[101, 102] (**Fig. 2**). However, phosphate is a vital nutrient stored in plant seeds as inositol hexaphosphate (IP6) to support the early growth of the new seedling stage of development. IP6 molecules are parked in the seeds in competent cellular organelles named globoids. During the germination stage, IP6 is separated into inositol and phosphorous through the phytase enzymatic reaction.IP6 can also affect protein production in plants by accelerating the translocation of messenger RNA. Plant seed phosphate storage is mainly generated and regulated by inositol [103].

8. Inositol transporter system

Known as one of the most studied cyclic polyols, *Myo-inositol* is widely spread in eukaryotic organisms, and movement is principally orchestrated by a group of transporters named inositol transporters. These transporters play vital roles in modulating the uptake and intracellular repartition of inositol. In animals, INTs are divided into two groups depending on the involved mechanism including the sodium ion coupled inositol transporters and proton-coupled inositol symporters[104, 105].

8.1. Na$^+$ coupled inositol transporters.

In higher plants, INTs play fundamental roles in various signaling pathways under different physiological conditions. In the ordinary Ice plant, two inositol transporters (Mitr1 and Mitr2) are described as similar to H+ [65] inositol symporters (ITRs)[106]. These ITRs and certain Na+ /H+ -antiporters are mainly activated in response to a brutal increase of cell osmolarity; in this vein, these transporters will actively participate in sodium transfer from plant root tissues to the leaf mesophyll. In addition, another study on *Medicago falcate* revealed that inositol transporter (MfINT-like) located plasma membrane was highly expressed in response to salt and cold stress while in Tobacco, the overexpression of the same gene conferred a transient cold stress tolerance to the transgenic plants[107]. To sum up, under certain stressful conditions (salt, cold, osmotic, etc.) plant inositol transporters present in the vacuole membrane call tonoplast are widely activate. Consequently, INT will manage the transfer of Na+ or/and H+ from cell cytoplasm to the vacuole lumen and induce the reduction or maintain of ion molecules in the cytoplasm. Once the abortion is completed, the vacuoles containing a huge quantity of ions will migrate from the root tissues to the leaf's mesophyll through the phloem long-distance transports. The destiny of ions enrolled into vacuoles still not well understood, but we believe that they could be useful or recycle in plant leaf's mesophyll by photosynthesis. The uptake of important nutrients like sugars and amino acids is of most important existing mechanism in the regulation of plant cell homeostasis. Several studies have been conducted on different co-transport in animal, bacterial and algal plants including sodium and hydrogen co-transport. Meanwhile, a similar process was revealed in higher plants, but the understanding of the plant cotransport mechanism remains unclear[107-109]. However, this mode of transport is mainly found in plant sugar transport, which is tightly linked with the movement of phloem sieve components mainly controlled by the proton co-transport system.

8.2. H$^+$ coupled inositol transporters.

The pyrophosphatase gene family (H+ -PPase) are fundamental proton transporters in plant development and stress resistance. Many studies have been targeting the physiological characterization functions of H+ -PPases are in plants under different conditions[110, 111]. In a recent study, 124 H$^+$-PPase gene members were identified from 27 different plant species through full genomic data from algae to angiosperms[112]. Primary identified as an extended extracellular bend connecting TM9 and TM10, H$^+$-*Myo*- inositol co-transporter [4] was described in the Human brain, where the functional characterization showed the presence of three *N*-glycosylation sites. The sequence analysis of HMIT revealed its closest structure with unknown proteins that mediated important functions in certain plants like *C.elegans* or *A.thaliana*. A similar extracellular loop was also present in five plants ESTs which conserved eight remaining cysteines including the 2–4 *N*-glycosylation bounds and CGFC motif. These revelations that the above loop could play critical roles in inositol transport activity or mediate recognition of proteins.

8.3. Transport into the phloem

Photosynthesis is a vital reaction in plants that produces almost all the required products in plant metabolisms. Plant growth and development are strictly linked to the distribution of photosynthesis-derived products, which are mainly synthesized into mature leaves. These products are needed in every part of the plant, and their distribution requires different mechanisms including the transport into the phloem notably. The phloem provides long-distance transport to many important nutrients in higher plants[113]. *Myo-inositol* generation in plants under stress conditions is related to the tissue's localization. For example, in response to abiotic stress, the accumulation of *Myo-inositol* is upgraded in leaf mesophyll (meso) and downregulated in the root tissues, creating a huge inositol imbalance between the two plant compartments and enhancing the translocation of inositol to the root tissues consequently. During the leaf to root translocation of inositol, as revealed for NaCl root uptake, when the NaCl was mostly located in the shoot, only *Myo-inositol* was necessary for the pinitol generation. This suggested the correlation between sodium uptake and *Myo-inositol* translocation in plants[113]. Therefore, translocation of inositol in higher plants is related to the photosynthetic mechanism and can serve as a leaf-to-root signaling pathway that mediates the sodium uptake, xylem transit, and accumulation in the vacuole of leaf mesophyll cells[113, 114]. Inositol transporters are crucial in plants; they can modify the flux of inositol and phosphoinositide metabolism consequently. By extending or smoothing the movements of inositol in plants, we could positively affect many important plant stress signaling pathways and, hence facilitated the distribution of vital products derived from the photosynthesis through long-distance transport provided by the phloem system.

9. Conclusion and perspective

The roles of inositol signaling in plants are now widely investigated but their mode of transport and distribution in plants requires deeper attention due to their ubiquitous participation in every compartment of cell biology. During the past decade, many studies have been performed to further depicted the inositol pathway, which is majorly stimulated in response to both biotic and abiotic stresses in plants. However, fewer studies have questioned the inositol transporter genes and operative mode in plants under stressful conditions. Although the inositol transporters and their model homologous have been extensively investigated in animal field studies and have reported their kinetic functions and the physiological role and great progress in the clarification of their roles and modes of action in mammalian cells is notable. Besides, the role of INT in plants is still not perplexing. Analysis of plant inositol transporters indicated a new strategy for plants to adapt to abiotic stress. Furthermore, inositol transport proteins are a suitable tool to study the targeting mechanisms of membrane proteins. In the future, it is indisputable that research on inositol transporters will bring further important insights into cell physiology in eukaryotic organisms. Thus, to better understand the mechanism of plant adaptation to environmental stress, it will be a good asset to combine metabolic, molecular, and genetic approaches to further clarify the protein-protein interactions around INT reactions in plant cells.

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