Chapter 4

The Transcriptional Modulation of Inositols and Raffinose Family Oligosaccharides Pathways in Plants — An (A)Biotic Stress Perspective

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Additional information is available at the end of the chapter

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Abstract

Among the multifunctional molecules that participate in processes of plant tolerance/resistance to stresses, inositol (Ins) and its derivatives (phosphorylated, methylated, oxygenated, and Raffinose Family Oligosaccharides) have attracted the attention of researchers. These compounds represent versatile and dynamic signaling molecules and osmolytes in all eukaryotes. Due to the impacts related to Ins and its derivatives in a plant cell, assays have been conducted to understand how these biomolecules affect plant physiology. Thus, overexpression or knockout of Ins-related genes has been shown as interesting strategies for generating more efficient plants capable of growing under stress conditions. In this chapter, studies using molecular tools are presented, and the impacts of their results are discussed based on the plant stress tolerance/resistance. Furthermore, an informative panel is provided with transcriptional modulation of genes related to Ins and its derivatives expressed in plants under stress. There is a gap involving about two dozen enzymes associated with the synthesis of Ins-related compounds that have not been adequately studied, and they represent an area of high biotechnological potential.

Keywords: Transgeny, tolerance, resistance, biotechnology

1. Introduction

To survive and integrate in the niche in which they germinate, plants constantly regulate their internal environment to external fluctuations encompassing soil, climate, and biological interactions. Thus, along its evolutionary processes, plants were selected through the need of

molecular mechanisms for physiological adjustments to inadequate conditions for development, resulting from adverse conditions. In this way, plants have a diverse and active cellular machinery at different stratified levels, covering perception, signaling, transcriptional control of key metabolic pathways and synthesis of molecules responsive to stresses [1].

Among the molecules functioning in more than one of the aforementioned levels, inositol (Ins; $C_6H_{12}O_6$) is a biomolecule of great interest. It is a cyclic carbohydrate (polyalcohol) that anchors in each of the six carbons forming the ring, a hydroxyl group. Along with their derivatives, Ins has multiple effects on plant metabolism. They act from the production of secondary messengers to the synthesis of osmolytes and antioxidants (more details in the reviews of [2, 3]). Phosphorylated Ins-derivatives [(poly)phosphoinositides and inositol (poly)phosphates] are versatile and dynamic signaling molecules in all eukaryotes, particularly in plants [4]. These two classes of compounds [highlighted in red and orange respectively, in Figure 1] are interdependent. While (poly)phosphoinositides are used in the synthesis of inositol (poly)phosphates through the action of phospholipases; the breaking of inositol (poly)phosphates produces inositol, which is a substrate for the synthesis of (poly)phosphoinositides. Moreover, according to Ins metabolism, shown in Figure 1, another branch realizes the synthesis of methylated derivatives (highlighted in green). These compounds act as important osmoregulators during periods of unfavorable conditions [5]. Additionally, oxygenated Ins-derivatives are observed (highlighted in yellow in Figure 1), which are involved in increasing plant tolerance to stresses by decreasing oxidative damage [6]. Still associated with Ins is the metabolism of the Raffinose Family Oligosaccharides (RFOs) [7]. In this biosynthetic pathway, the galactinol synthase (GolS; EC 2.4.1.123) uses myo-inositol and UDP-galactose to produce galactinol, which serve as galactose donors for subsequent synthesis of RFO members ([8, 9]; highlighted in red in Figure 2). Recent reports indicate that RFOs may assist in the reactive oxygen species (ROS) cleaning process. In periods of stress, ROS accumulation favors the physiological imbalance of plants [10].

Due to the impacts related to the Ins and its derivatives in a plant cell, assays have been conducted to understand how these biomolecules affect the physiology of plants. Thus, overexpression or knockout of genes present in these pathways has been shown as interesting strategy for generating more efficient plants capable of growing under abiotic stress conditions. In this chapter, studies using molecular genetic tools will be presented, which affect the above-mentioned metabolic pathways and the studied organisms.

2. The use of Ins and phosphorylated Ins-derivatives in plant cells under stress

Although there are several articles addressing the involvement of Ins and its derivatives in plant stress responses, so far no report has described the overall transcriptional orchestration of these components covering the metabolic pathways related to them. Information is available only in particular assays covering few genes and their expression modulations, some individual gene knockout analyses or a specific GMO (genetically modified organism) assay. In most

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cases, Ins and its phosphorylated derivatives have positive impact in plant tolerance/resistance to several stresses, promoting a biotechnological interest in these compounds.

Among the Ins-derivatives, those that are phosphorylated [(poly)phosphoinositides (highlighted in red in Figure 1) and inositol (poly)phosphates (highlighted in orange)] are the most discussed in the literature. This fact reflects the importance of these compounds in plant physiology in signaling activities. An example is a work developed by Hunt et al. [11] with transgenic tobacco plants (*Nicotiana tabacum* cv. Wisconsin 38) expressing reduced levels of a gene encoding phospholipase C (PI-PLC; EC 3.1.4.11). This enzyme catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to InsP3 [inositol (1,4,5)–trisphosphate; (Figure 1)], affecting from this point on the rest of the pathway. The obtained transgenic plants showed a partial inhibition of stomatal opening due to the action of ABA (abscisic acid phytohormone). A possible role of PI-PLC enzyme and InsP3 in ABA-dependent signaling pathway was also reported, thus suggesting that a complete response to ABA phytohormone in guard cells requires PI-PLC. However, other calcium-mobilizing pathways could also help in ABA guard cell signaling. The stomatal movement is a critical process for the plant under drought conditions by enabling better use of its water supply.

Mills et al. [12] provide more details on this ABA-mediated stomatal regulation in transgenic plants obtained by Hunt et al. [11]. A three-day assay under drought and in dark-adapted conditions, to reopen the stomata in response to light, was carried out. The results showed that transgenic plants with reduced PI-PLC as compared to control plants (with the empty vector without the transgene insert) have a greater increase in stomatal conductance. Thus, there is a strengthening of the role of inhibition due to PI-PLC in ABA-mediated stomatal opening. Further analysis indicated tobacco PI-PLC acting on the inhibition of stomatal opening by ABA, but not in promoting ABA-induced stomatal closure.

There are also reports of the involvement of Ins-derivatives in ABA-independent mechanisms during periods of drought. Perera et al. [13] obtained *A. thaliana* plants transformed with human type I gene for inositol polyphosphate 5-phosphatase (InsP5-ptase; EC 3.1.3.56; Figure 1). This enzyme hydrolysis InsP3, which is an essential element of the signal transduction pathway in general response to stresses. Looking at the impact on plant response to drought, the authors observed that transgenic plants showed increased stress tolerance after 12 days of watering suspension. After this period of stress, wild and controls plants (with the empty vector without the transgene insert) became brown and dry, while InsP5-ptase transgenic lines remained green and turgid. Furthermore, transgenic plants under drought conditions showed reduced levels of ABA compared with wild plants in the same condition as well as no induction of several genes regulated by the phytohormone. The analyses of stomatal responses in transgenic plants observed that guard cells are less responsive to the inhibition of opening stomata promoted by ABA. Nevertheless, there is an increase in sensitivity to the closing of the stomata, induced by the phytohormone. The transgenic plants showed irregular behavior in coordinated processes via ABA. Despite this, these plants showed a compensatory overexpression of an ABA-independent pathway involving the transcription factor (TF) DREB2A (dehydration-responsive element-binding protein 2A) and a subset of genes regulated by this TF. In this way, the drought tolerance of Ins5-ptase plants was mediated in part via DREB2A-

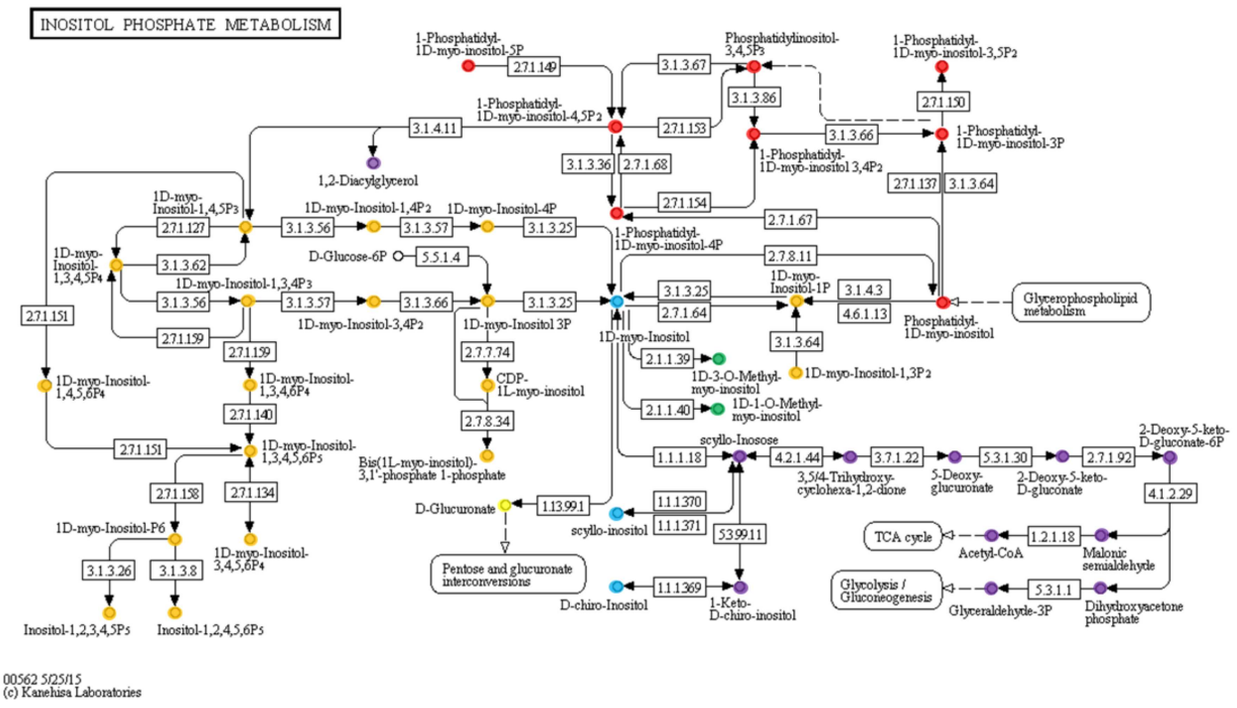

Figure 1. Inositol phosphate metabolism from the KEGG Pathway database. The stereoisomers (highlighted in blue); the phosphorylated derivatives [inositol (poly)phosphates (highlighted in orange) and (poly)phosphoinositides (highlighted in red)]; the methylated derivatives (highlighted in green); the oxygenated derivatives (highlighted in green); and others (highlighted in purple).

dependent and that constitutive dampening of InsP3 signal revealed unforeseen interconnections between signaling pathways.

In a similar assay, Khodakovskaya et al. [14] reported physiological consequences in transgenic lines of *Solanum lycopersicum* (cv. Micro-Tom) overexpressing a human type I InsP5-ptase gene. The transgenic lines presented a content of 15–30% of InsP3 observed in the wild-type plants. This reduction led to increases in: (1) the total vegetative biomass (two- to fourfold) with an increased ratio of root:shoot dry weight; (2) lycopene levels (in fruit); and (3) the hexose concentration (on fruits and leaves). After 13 days of water stress, the leaf water potential in transgenic plants was about -0.4 MPa higher than in control plants. Drought tolerance in transgenic lines was associated with increased hexoses in the leaves. This would contribute to maintaining a greater potential for water in transgenic leaves under drought. Furthermore, increases in the number of root biomass may have contributed to this improved performance.

The involvement of InsP3 in other stress tolerance processes, beyond drought, has also been demonstrated. Alimohammadi et al. [15] obtained transgenic tomato plants (*Lycopersicon esculentum* cv. MicroTom,) overexpressing a human type I InsP5-ptase gene. These plants presented a decreased level of InsP3 and supported a continuous exposure to light longer than wild plants. Prolonged exposure to light causes oxidative stress in plant cells and can result in irreversible damage. However, the molecular mechanism involved in this tolerance process was not reported, but these transgenic lines were characterized in more detail by Alimoham-

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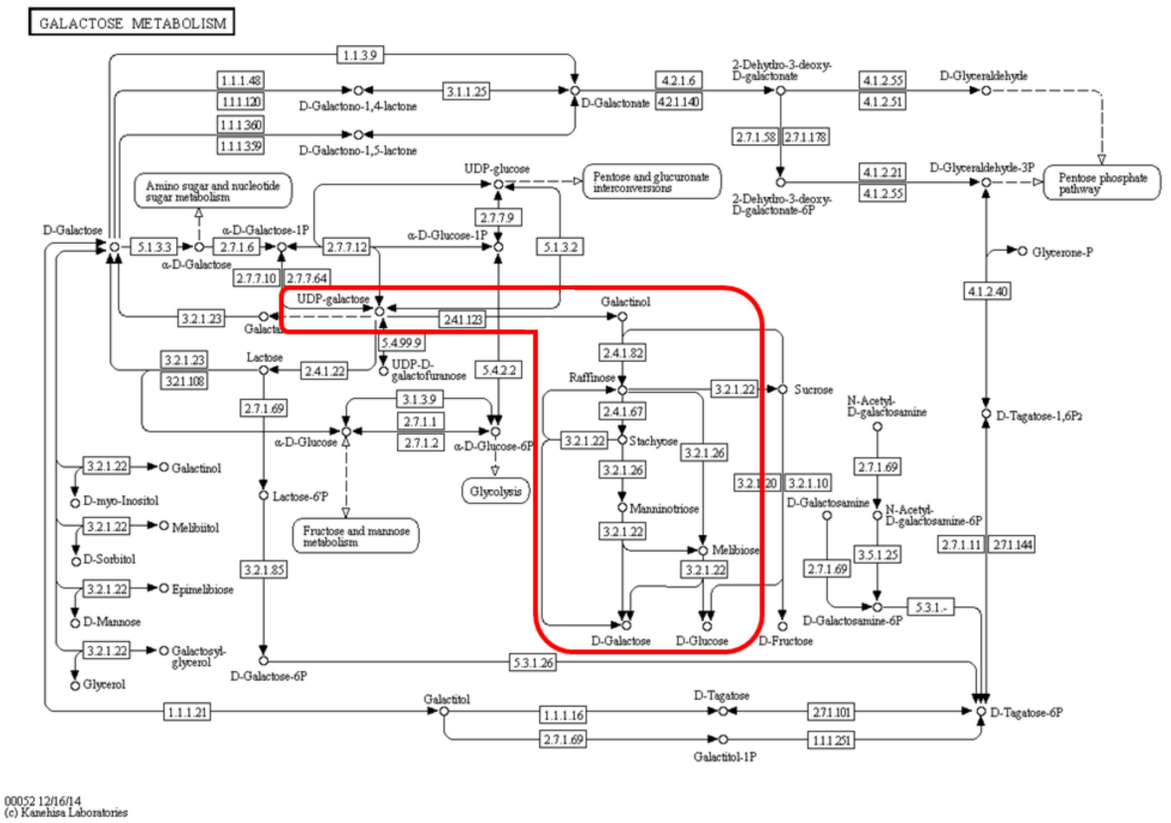

Figure 2. The Galactose metabolism from the KEGG Pathway database. Highlighted in red are the enzymatic reactions associated with the metabolism of Raffinose Family Oligosaccharides.

madi et al. [16]. These authors observed that under stress conditions, the transgenic plants maintained high chlorophyll content and accumulated low levels of hydrogen peroxide. This fact was attributed to the induction of genes related to multiple antioxidants [LeAPX1 (*L. esculentum* ascorbate peroxidase 1), SICAT2, LeSOD (*L. esculentum* superoxide dismutase)] during continuous exposure to light. Other effects included overexpression of the LePHYB photoreceptor (*L. esculentum* phytochrome B) and a key enzyme [LeCHS1 (*L. esculentum* chalcone synthase)] in the biosynthesis pathway of flavonoids, which are plant nonenzymatic antioxidants. There was also an overexpression of the SIMYB12 transcription factor, leading to an increase in flavonoids in tomato plants by up-regulation of the LeCHS1 expression. A relationship was established between change in phosphoinositol signaling pathway and increases tolerance to continuous exposure to light, through the activation of ROS-scavenging enzymes, and up-regulation of molecular activators of non-enzymatic antioxidants.

The biotechnological potential through the manipulation of compounds shown in Ins-related metabolic pathways may also be seen in the work of Ahmad et al. [17]. These authors performed a comprehensive analysis of *A. thaliana* genome, using the activation tagging technique in dedifferentiated calli, to identify salt-tolerant mutants (NaCl 150 mM). To this end, plants were modified with pRi35ADEn4 binary vector. Such vector contains four copies of the 339 bp long cauliflower mosaic virus (CaMV) 35S enhancer in the construct that induces the expres-

sion of adjacent genes after proper insertion. Of the 18 potential tolerant mutants (150 mM NaCl), a line (stc1; salt-tolerant callus 1) presented the gene for myo-inositol-1-P-synthase-1 [At4g39800; MIPS1; EC 5.5.1.4; Figure 1] with induced expression in callus, with or without salt. This gene expressed 45 and 15 times higher compared to wild-type under the control condition. MIPS catalyzes the first step in the biosynthesis of inositol from glucose-6-P (Figure 1). The referred induction was greater in the mutant line than in the wild type (approximately, 260 times higher), both under stress. The *mips* gene transcription in the wild type reduced dramatically under stress condition. The tolerance analysis revealed that the mutant plants regenerated from calli showed salt tolerance in germination and growth. However, the mechanism involved was not disclosed, but the authors suggested that MIPS protect the calli and the plants from salt stress as osmolytes or by providing a precursor in the regulation of signal transduction pathways.

Kusuda et al. [18] went beyond the study of transformed lines overexpressing enzymes from the Ins-related pathways. They analyzed the differences among wild type and transformants lines in regard to salt tolerance in 3.5 days in medium with concentrations up to 250 mM NaCl. They also sought for differences by mining the metabolomes (the fourth leaf tissue harvested at 0, 6, and 12 h after NaCl stress induction) of the studied plants. To this end, a rice cultivar (*Oryza sativa* cv. Kitaake) was transformed with the construction Act::RINO1. The RINO1 gene encodes an MIPS (EC 5.5.1.4, Figure 1). It has been demonstrated that the constitutive overexpression of rice MIPS when compared to a wild type, results in greater tolerance to salt stress. Furthermore, it leads to a range of metabolic changes, with increased production of various metabolites (such as inositol, raffinose, ascorbate, amino acids). These handle the protection of plants from abiotic stresses. Additionally, activation of basal metabolisms such as glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle has been observed during induction of the Ins metabolism in those plants overexpressing MIPS.

Ins metabolism and phosphorylated Ins-derivatives are also associated with response to biotic stresses in plants. This fact shows the plurality of actions of these compounds. Murphy et al. [19] report evidence in this direction. They obtained transgenic potato [*S. tuberosum* L. (cv. Desiree)] and *A. thaliana* lines, synthesizing low levels of phytic acid (1-D-myo-inositol-P6 or InsP6, Figure 1). The transgenic potato lines were obtained by: (1) constitutive expression of an antisense sequence of the myo-inositol 3-phosphate synthase gene. This enzyme (IPS, EC 5.5.1.4, Figure 1) catalyzes the first step in the InsP6 biosynthesis; (2) plants expressing the *Escherichia coli* polyphosphate kinase (PPK, EC 2.7.4.1; Figure 1). PPK inserts inorganic phosphate into chains of phosphate residues linked by phospho-anhydride bonds, and this decreases the phosphate available to InsP6 biosynthesis. Genetically modified potatoes presenting both (1) and of (2) showed increased susceptibility to avirulent pathogen potato virus Y and the virulent pathogen tobacco mosaic virus (TMV). In relation to *A. thalianai*, the authors obtained three loss-of-function mutants [two (atips1 and atips2) involving the gene for IPS (EC 5.5.4.1, Figure 1); and one (atipk1) involving the gene encoding inositol polyphosphate kinase (IPK1; EC 2.7.1.158, Figure 1)]. IPK1 catalyzes the final step in InsP6 and without this enzyme, very little InsP6 is done. The disruption of InsP6 biosynthesis in *A. thaliana* resulted in an increased susceptibility to viruses (tobacco mosaic virus), bacteria [*Pseudomonas syringae* pv tomato (Pst) DC3000 and Pst DC3000 AvrB], and fungus (*Botrytis cinerea*). The

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increased susceptibility only occurred for atips2 and atipk1 mutants. For atips1 mutants showing also InsP6 depletion, the resistance to pathogens was not compromised. This suggests either that a particular pool of InsP6 regulates defense against pathogens in *A. thaliana*, or IPS1 and IPs2 are differentially regulated, and one cannot compensate for the other. It was observed with regard to the typical responses of both species that levels of salicylic acid (SA), a key molecular signal for establishing local and systemic acquired resistance, were not diminished. Therefore, SA was not responsible for increased susceptibility to pathogens analyzed. Furthermore, a relationship between the inositol phosphate metabolism and basal resistance to pathogens (fungi, bacteria, and viruses) has been established. This indicates that InsP6 is also required as an essential operation signal for regulation and defense in plants, together with several other well-known defensive signals (NO, cADPR, SA and $Ca2^{+}$).

Recently, Meng et al. [20] found that *A. thaliana* ips1 loss-of-function mutant (atips1; IPS; EC 5.5.4.1, Figure 1) exhibits spontaneous cellular death and increased resistance to oomycete *Hyaloperonospora arabidopsis* pathogen. This result, together with the above, supports the premise that a particular InsP6 pool may regulate defense pathways, since atips1 mutants did not show increased susceptibility to the variety of testes pathogens (Tobacco mosaic virus; *Pseudomonas syringae* pv. tomato (Pst) DC3000 and Pst. DC3000 AvrB; and *Botrytis cinerea*). Additionally, it was observed that spraying the atips1 mutant plants with Ins could suppress the formation of spontaneous lesions, indicating that Ins production and not AtIPS1 protein is required to prevent the lesion formation. The authors also found evidence for the role of Ins (or Ins-derivatives) in the regulation of programmed cell death.

Reports presented in Table 1 also show the broad roles of Ins and its phosphorylated derivatives in plant cells. From the wide spectrum of analyzed genes, a range of effects on plants at different levels was observed. These effects have shown associations with hormone signaling pathways, such as ABA [12], influences in photosystems [21, 22], with reactive oxygen species (ROS; [23]), with relative water content, with osmotic adjustment [24], among others (Table 1).

3. Methylated Ins-derivatives in plant cell and the biotechnological use to increase stress tolerance

Some plants use Ins as precursor of compatible solutes such as D-ononitol and D-pinitol, which act as osmoprotectants (small molecules that act as osmolytes and help organisms survive in extreme osmotic stress [35]). In halophyte ice plant (*Mesembryanthemum crystallinum*), which is considered highly tolerant to drought, salinity, and cold, Ins is methylated to D-ononitol and subsequently epimerized to D-pinitol [36]. The myo-inositol O-methyl transferase gene (IMT1; EC 2.1.1.40; Figure 1) is transcriptionally induced by osmotic stress, whereas neither transcriptional nor enzyme activities is detectable in ice plants under normal growth conditions [37, 38]. Despite the positive influence of these metabolites in plant physiology under abiotic stress conditions, there are less available data for these compounds compared with phosphorylated Ins-derivatives.

EC† Number	Gene origin (specie)	Transformant (specie)	Gene Modulation	Analysed Condition	Impact on Tolerance	Authors	Notes
5.5.1.4	*Pc*	*Os and Bj*	Overexp.	HS	Raise	[25]	a.
5.5.1.4	*Pc*	*Nt*	Overexp.	HS	Raise	[21]	b.
5.5.1.4	*As*	*At*	Overexp.	HS	Raise	[22]	c.
3.1.3.25	*Ca*	*At*	Overexp.	HS, P, PEG, and HT	Raise	[26]	d.
2.7.1.140; 2.7.1.151	*At*	*Nt*	Overexp.	HS and OS	Raise	[27]	e.
2.7.1.140; 2.7.1.151	*Th*	*Bn*	Overexp.	HS, D, and OS	Raise	[28]	f.
3.1.4.11	*Zm*	*Zm*	Overexp.	D	Raise	[29]	g.
2.7.1.159; 2.7.1.134	*Os*	*Nt*	Overexp.	HS	Decrease	[24]	h.
3.1.3.57	*At*	*At*	Knockout	HS, F, and D	Decrease	[30]	i.
2.7.1.137	*At*	*At*	Knockout	HS	Decrease	[31]	j.
2.7.1.67	*At*	*At*	Overexp.	HS and ABA	Raise	[23]	l.
2.7.8.11	*Zm*	*Zm*	Overexp.	D	Raise	[32]	m.
3.1.3.8	*At*	*At*	Overexp.	HS and OSM	Raise	[33]	n.
2.7.1.149	*At*	*At*	Overexp.	HS, D, and ABA	**	[34]	–

†Enzyme Commission; *According to KEGG Database Pathway; − Not observed; Overexp. (Overexpression); **Legend:** HS (high salinity); P (paraquat); PEG (polyethylene glycol); HT (high temperature); OS (oxidative stress); D (drought); DH (dehydration); ABA (ABA hormone); OSM (osmotic stress); F (freezing). **a**. Albeit to a variable extent, overexpression of this gene confers salt-tolerance to diverse evolutionary organisms (from prokaryotes to eukaryotes), including crop plants; **b**. Transgenic individuals presenting retention of approximately 40–80% of the photosynthetic competence under analyzed stress condition; **c.** Transgenic individuals retained more chlorophyll and carotenoid by protecting the photosystem II; **d**. Improving seed germination and seedling growth in transgenic individuals under stress conditions; **e**. Expression patterns of various stress responsive genes were enhanced, and the activities of antioxidative enzymes were elevated in transgenic plants; **f**. The transcripts of various stress-responsive genes are increased in ThIPK2 transgenic plants under salt stress condition; **g**. The sense transgenic plants had higher relative water content, better osmotic adjustment, increased photosynthesis rates, lower percentage of ion leakage and less lipid membrane peroxidation, higher grain yield than the wild type; **h**. The 1,3,4-trisphosphate 5/6-kinase is a negative regulator of osmotic stress signaling in tobacco; **i**. The genetic evidence indicating that phosphoinositols mediate ABA and stress signal transduction in plants, and their turnover is critical for attenuating ABA and stress signaling; **j**. Salt stress responses, such as increased plasma membrane endocytosis and the intracellular production of ROS, are coordinated by phospholipid-regulated signaling pathways; **l**. AtPI4Kγ3 is activated by DNA demethylation and regulates the ROS accumulation induced by high salt treatment or ABA treatment; **m**. ZmPIS regulates the plant response to drought stress through altering membrane lipid composition and increasing ABA synthesis in maize; **n**. AtPAP15 (3-PHYTASE) may modulate AsA levels by controlling the input of myoinositol into this branch of AsA biosynthesis in *Arabidopsis thaliana*. *At*: *A. thaliana; Pc: P. coarctata; Sa: S. alterniflora; Ca: C. arietinum; Th: T. halophile; Zm: Z. mays; Os: O. sativa*; *Nt: N. tabacum; Bn: B. napus; Bj: B. juncea.*

Table 1. Transgenic and knockout plant assays available in the literature related to (poly)phosphoinositides and inositol (poly)phosphates. Relevant information: EC number of the enzyme coded by the studied gene, plant donor species, the genetically modified organisms (transformants), the modulation of the studied gene, the analyzed stress condition, the impact on plant tolerance and physiology (additional details, please see the legends).

Sheveleva et al. [39] were one of the first to report the biotechnological potential of methylated Ins-derivatives. In their work, the authors superexpressed O-methyltransferase (IMT1; EC

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2.1.1.40; Figure 1) of *Mesembryanthemum crystallinum* in tobacco (*Nicotiana tabacum* cv. SRl). The transgenic plant increased its tolerance to abiotic stresses [drought and high salinity (50–250 mM NaCl)] when compared to the wild control line. An accumulation of methylated inositol D-ononitol in amounts of fresh weight exceeding 35 μmol g^{-1} in the transformed lines was observed. Besides, the photosynthetic CO_2 fixation was less inhibited in those plants during drought and salt stress. Further, transformed plants recovered faster than the wild type after rehydration. In turn, Sengupta et al. [40] observed an increase of the D-pinitol synthesis in a wild-type rice (*Porteresia coarctata*) with halophilic characteristics when subjected to high salt environment (400 mM NaCl). An increment also occurred in both transcriptional and proteomic level of IMT1, not observable in domesticated rice under the same condition. The authors also reported an increase in the expression of L-myo-inositol 1-phosphate synthase (PcMIPS1; EC 5.5.1.4; Figure 1), along with the expression of IMT1. According to the authors, this suggests that the accumulation of D-pinitol would be a mechanism regulated by salt stress.

Recently, Zhu et al. [41] used a similar strategy as the one developed by Sheveleva et al. [39] to express in *A. thaliana*, a gene coding IMT1, from *Mesembryanthemum crystallinum*. The transformed plants showed higher growth compared to the wild control line and increased tolerance to cold stress (4°C). This increment in tolerance was attributed to different factors: (1) the electrolyte leakage content in the transgenic plants was significantly lower than that of the wild-type plants after freezing stress, showing less damage to the membranes of those plants; (2) transgenic plants showed lower MDA content than wild-type plants, not only in normal conditions but also after stress; and (3) a higher proline content presented in transgenic lines than in wild type, after application of stress.

4. Oxygenated Ins-derivatives in plant cell and the plant strategy to tackle stress

So far, the myo-inositol oxygenase (MIOX; EC 1.13.99.1; Figure 1) is the only enzyme known by the oxidation of Ins [42]. Its importance in plants stood out from the statement in the Ins metabolism as a precursor in Ascorbic Acid (AsA) biosynthesis in Arabidopsis. In this sense, Lorence et al. [43] observed the expression of a myo-inositol oxygenase (miox4) increasing the content of AsA in leaves (approximately two- to threefold). By that, they anticipated a potential use of the gene by genetic engineering, enhancing levels of this important antioxidant in plants. Further analysis indicated that D-GlcUA (Figure 1), a derivative from MIOX reaction (EC 1.13.99.1, Figure 1), plays a negligible role for AsA biosynthesis [44]. However, MIOX can control the metabolite level of myo-inositol in plants [44].

Nevertheless, the metabolic consequences of MIOX action are still unclear. In order to contribute with information from gene regulation and catalytic activity of this enzyme, Duan et al. [6] performed a functional characterization in rice (*Oryza sativa*), observing its predominant expression in root, with induced transcription under drought stress (20% PEG6000 solution), H_2O_2, high salt (200 mM NaCl), cold (4°C) and Abscisic Acid (100 μM). Transgenic rice lines overexpressing an MIOX gene showed a higher survival rate than a wild line, when in contact with 20% PEG6000 solution. In the same way, the authors also reported for the

transgenic lines, transcription levels significantly induced for genes coding enzymes associated with ROS scavenging, suggesting an MIOX activity in reducing oxidative stress.

Moreover, Alford et al. [45] reported MIOX enzymes responding to growing conditions of *A. thaliana* in low energy/nutrient environment. Their data supported the hypothesis that MIOX2 and MIOX4 enzymes would be encoded by multiple genes. Furthermore, on plants growing in low energy conditions/nutrients environments, MIOX2 enzyme would have a significant role in providing Ins to many different tissues, whereas MIOX4 would act in supplemental form at some tissues. An analysis of promoters was presented, considering multiple lines of MIOX2p:GUS and MIOX4p:GUS seedlings growing on: (I) no nutrients (agar); (II) low nutrients (agar + 0.5× MS salts); and (III) optimal nutrients (agar + 0.5× MS salts + 3% glucose) in low light (40 μE) during seven days. As a result, only MIOX2p:GUS was expressed abundantly in condition I, whereas in condition II, both MIOX2p:GUS and MIOX4p:GUS showed moderate expression, having been more expressed previously.

5. Raffinose Family Oligosaccharides (RFOs) and plant strategy to address stresses

RFOs are a class of compatible solutes coming from Ins metabolism ramifications. As mentioned before, the enzyme GolS (EC 2.4.1.123, highlighted in red in Figure 2) connects the metabolism of these compounds, producing galactinol (highlighted in red in Figure 2), which serves as galactose donor for further synthesis of RFO members [8, 9]. To date, structural genomics data and global transcriptome analysis concerning RFOs are only available for corn [46]. For this crop, the authors have performed a genomic identification of genes associated with raffinose metabolism, together with an expression analysis using data-mining from GEO (http://www.ncbi.nlm.nih.gov/geo) and PLEXdb databases (http://www.plexdb.org). Additionally some transgenic lines overexpressing specifics gene isoforms related to RFO pathway, under particular growth conditions, are available for some species. These studies showed a positive impact in some crops.

Taji et al. [47], for example, analyzed the expression of seven genes encoding GoIS in *A. thaliana* under different stresses. From those genes, only three (AtGolS1, 2 and 3) were stress-responsive. AtGolS1 and AtGolS2 were induced by drought and high salt (250 mM NaCl), but not by low temperature (4°C). On the other hand, AtGolS3 was induced by low temperatures, but not high salinity or drought. The AtGolS2 overexpression in *A. thaliana* was associated with an increase of raffinose and galactinol and resulted in a reduction of leaf transpiration with a greater drought tolerance. In turn, Dos Santos et al. [48] analyzing three *Coffea arabica* galactinol synthase isoforms (CaGolS1, CaGolS2, CaGolS3) observed a mainly tissue-specific expression but differentiated regulation depending on the applied stress (drought, heat, and high salinity). This reinforces the observation by Taji et al. [47], indicating that different galactinol sets can be necessary for response to various stresses. The GOLS (BnGOLS-1) activity was also positively correlated with desiccation tolerance in cabbage seeds (*Brassica napus*) during the vegetative growth period [8]. The tolerance was observed around 21-24 days after flowering cabbage, coinciding with the accumulation of raffinose and stachyose. The

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BnGOLS-1 transcripts accumulation was concomitant with the formation of these two RFO members [8].

In *Arabidopsis thaliana* leaves overexpressing HsfA2 (Heat-Shock Transcription Factor A2; [49]) was also found highly induced GolS1, -2, -4 and Raffinose Syntase 2 (RS2; EC 2.4.1.82, highlighted in red in Figure 2) transcriptions. The galactinol and raffinose levels in the transgenic plants were higher compared to the wild-type lines, both in the control condition. These higher levels were positively correlated with an increase in plant tolerance when exposed to the studied stresses [mevalonate (50 mM), high salt (100 mM NaCl) and low temperatures (4°C)].

Latter, Pennycooke et al. [50] studied the expression of α-galactosidase gene (EC 3.1.2.22 highlighted in red in Figure 2) from petunia (Petunia x hibrida "Mitchell"), monitoring acclimated plants to low temperatures (4°C) and in response to increasing temperature (25°C). Transcripts induction were observed after one hour of desacclimation occurring together with an increase in enzymatic activity and decreased raffinose content, suggesting that the rise in temperature can regulate the RFO catabolism of certain members, through gene regulation that encoding α-galactosidase.

Thus, the diversity of functions performed by compounds presenting in the Ins metabolism was shown in the described works. Also, studies of distinct isoforms showed positive correlations with plant responses to various abiotic stresses. In this way, the identification of new transcripts, as well as the understanding of its regulation (spatial and temporal) in plants under unfavorable conditions for the development may lead to the discovery of new genes with biotechnological potential.

According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database (http://www.genome.jp/kegg/pathway.html), which provides diagrams of various metabolic processes, at least 45 enzymes are associated with the metabolic pathways described here. Of these enzymes, 21 (highlighted in green boxes in Figures 3A and 3B) have been studied in previous works addressing the transcriptional expression of their genes or effects on plants under stress. Therefore, there are at least 24 enzymes (in red boxes in Figures 3A and 3B) that have not been targets of these analyses, with significant potential for further research in biotechnology.

6. Ins and its derivatives in humans: Antinutrients *versus* disease prevention

Once Ins and its derivatives are present in vegetables and these are part of the daily diet of large populations around the world, it is essential to analyze their potential effects on consumers. The Ins and related metabolites play a heterogeneous physiological role, depending on the concerned organism, plant or animal (including human). In plants, as already mentioned, such compounds help regulate plant homeostasis during periods of stress. In animals (including humans), their influence has very diverse physiological repercussions. Initially, they were only seen as harmful agents because some representatives when present in certain

plants could act as antinutritional factors, thereby reducing the bioavailability of important nutrients and the nutritional value of the food. According to Kokhar and Apenten [51], this effect is present a result of a selected adaptive mechanism due to a "chemical warfare" between higher plants and herbivorous pests.

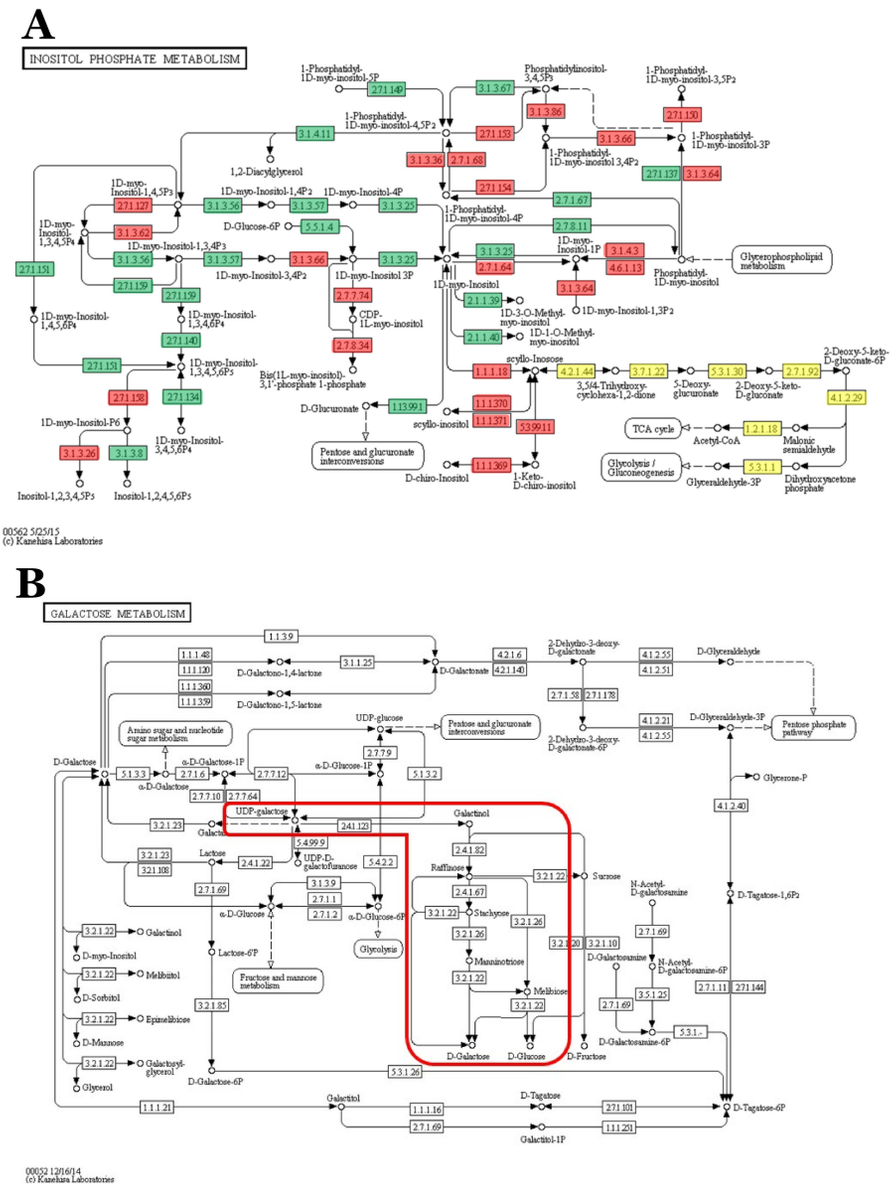

Figure 3. KEGG Pathway database diagrams presenting: (A) Inositol Metabolism; (B) Galactose Metabolism, showing the Raffinose Family Oligosaccharides (highlighted in red). Green Box: enzymes with transcriptional modulation data available from stress assays. Red Box: enzymes without transcriptional modulation data available in the literature. Yellow Box: enzymes not covered in this review.

Among the various Ins-derivatives, phytic acid (1-D-myo-inositol-P6; Figure 1) is the most studied, concerning the impacts on human and animal health. Its unique structure provides the ability to chelate cations such as iron, zinc, potassium, magnesium, and copper, forming

http://dx.doi.org/10.5772/61341

insoluble salts denominated phytate. These salts adversely affect animal's ability to absorb and digest nutrients [52, 53]. Phytates can form complexes with proteins, changing their structures and their enzymatic activities and characteristics of solubility and proteolytic digestibility [54]. However, there are reports that show positive aspects in phytates consumption. The presence of these in the diet of patients with diabetes has positive effects in reducing the level of blood glucose due to decreased starch digestion rate and slowing of gastric evacuation [55]. There are also reports of activity against HIV replication, kidney stones prevention, reduction of cholesterol and triglycerides levels, as well as assistance in prevention of heart diseases (for review see [54]). Studies also indicate that both Ins [56] and phytic acid [56, 57] have anticancer properties. With regard to RFOs, besides the fact that they are potential antinutritional factors, there are indications that they may act as important immunostimulants in animals (including humans). Also, RFOs' involvement is suggested in universal mechanisms of oxidative balance in several taxa [58].

7. Concluding remarks and perspectives

Experimentally, mutants and transgenic analyses are being successfully carried out to uncover the various roles played by Ins-related compounds. It is known today that some phosphorylated derivatives of inositol are connected with a large number of signaling procedures which are regulated by both abiotic and biotic stress. Methylated and oxygenated Ins-derivatives, including RFOs, have also proven to be active agents in the process of plant acclimatization to unfavorable conditions, involved in a number of functions. However, there is a gap to be filled. About two dozen enzymes associated with the synthesis of these compounds have not been adequately studied and they represent an area of high biotechnological potential.

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