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Review

Role of *myo*-inositol phosphate synthase and sucrose synthase genes in plant seed development

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ABSTRACT

The aim of this review is to highlight the role of *myo*-inositol phosphate synthase (MIPS), which catalyses the first step in inositol biosynthesis and of sucrose synthase (Sus), an enzyme involved in UDP-glucose formation, the principal nucleoside diphosphate in the sucrose cleavage reaction and in trehalose biosynthesis. These two enzymes are involved in various physiological processes including seed growth and resistance to biotic and abiotic stresses.

The study of mutated *MIPS* and *Sus* genes in some crops, such as soybean and cotton, has shown that these two proteins are directly involved in embryogenesis. They exhibit several isoforms that are essential for normal seed development.

The possible role of both genes in seed development is discussed in this review.

1. Introduction

The high nutritional value of seeds derives from the deposition of starch, lipids, proteins and essential minerals during their development. Alongside this process there is an accumulation of phytic acid and sugars which play a pivotal role in plants, acting as carbon currency and metabolic signals controlling many aspects of seed development, such as intracellular signaling regulation, signal transduction pathway, cell wall biogenesis, membrane formation and energy metabolism.

During the various stages of seed development, the availability of nutrients and the co-ordinated genetic program during embryo development are important in controlling the progression to seed completion.

In higher plants, embryogenesis plays a key role in establishing the correct pattern of cell division and orientation for embryo development and the accumulation of storage reserves. A better understanding of the molecular and physiological basis of this process is important in the search for ways of improving grain quality and yield.

In flowering plants, early seed development requires a co-ordinated interaction between embryo and maternal tissues, particularly endosperm, a tissue that supports embryo development through nutrient metabolism and use.

Analysis of the early stages of development, however, is often difficult because the embryo is too small and is embedded inside the maternal tissue. Although many morphological and histological processes in embryogenesis are well known, the molecular and physiological events leading to seed formation are still far from being completely understood.

A great number of genes demonstrated by embryo mutant obtention might be needed for normal embryogenesis, and seem to be key regulators of the process (Tzafrir et al., 2004).

Several mutations in the endosperm, discovered in *Arabidopsis thaliana* (Kinoshita et al., 1999) and *Ulmus minor* (Lopez-Almansa et al., 2004), showed that embryo abortion was due, at least in part, to a defect in endosperm function supporting embryo growth and development.

Recently, studies of *myo*-inositol phosphate synthase (MIPS) in *Arabidopsis* (Mitsuhashi et al., 2008) and *Glycine max* (Chiera and Grabau, 2007), as well as mutated sucrose synthase (Sus) of *Gossypium hirsutum* using an RNAi approach (Ruan et al., 2008) revealed the critical role of both genes in early seed development through the regulation of endosperm formation. MIPS and Sus are both expressed in endosperm tissues in early seed development and during embryo formation. Chiera and Grabau (2007) reported that MIPS ensures the primary inositol supply for the early embryo, whereas Sus is involved in the cell wall biosynthesis of the endosperm.

Abbreviations: EMS, ethyl methanesulfonate; EST, express sequences tag; IAA, indole-3-acetic acid; LPA, low phytic acid; MIPS, *myo*-inositol phosphate synthase; P-Sus, particulate Sus; SSH, suppressive subtractive hybridization; S-Sus, soluble Sus; Sus, sucrose synthase; T6P, trehalose-6-phosphate; TPP, trehalose-6-phosphate phosphatase; TPS, trehalose-6-phosphate synthase; UDP-glucose, uridine-5-diphosphoglucose.

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Interestingly, Lackey et al. (2003), using microscopic and biochemical analyses, detected MIPS expression in the cell wall and plasma membrane. Particulate Sus (P-Sus), the predominant form of Sus, has been shown to be localized in the plasma membrane and associated with the cellulose synthase for channeling UDP-glucose, the leading compound in cell wall biosynthesis during the developmental transition between primary and secondary wall synthesis. The relationship between MIPS and Sus in cell wall biosynthesis has not yet been reported.

In this review, we discuss the role of MIPS and Sus in normal embryogenesis and their possible functional role in plant biology, which includes cell wall biosynthesis, defense against biotic and abiotic stresses, and developmentally related processes.

2. Diversity, gene structure and cellular localization of MIPS and Sus

2.1. Myo-inositol phosphate synthase (MIPS)

The MIPS enzyme has been reported from a large number of living organisms, including higher plants and animals, parasites, fungi, green algae and bacteria. To date, more than 60 *MIPS* genes have been identified (Majumder et al., 2003). In higher organisms, the enzyme has two forms: cytosolic and organellar. Genes coding for cytosolic MIPS have been studied in detail and represents a multigene family in some plants. MIPS activity has been localized in cytosolic and plastidic compartments in pea (*Pisum sativum*) (Imhoff and Bourdu, 1973), *Vigna radiata* and *Euglena gracilis* (Adhikari et al., 1987), *Oryza sativa* (Hait et al., 2002; Ray Chaudhuri and Majunder, 1996), *Citrus paradisi* (Abu-abied and Holland, 1994), *A. thaliana* (Johnson and Sussex, 1995), *Mesembryanthemum crystallinum* (Ishitani et al., 1996), *Zea mays* and *Hordeum vulgare* (Keller et al., 1998), *Brassica napus* (Larson and Raboy, 1999), *G. max* (Iqbal et al., 2002), *Sesamum indicum* (Chun et al., 2003) and *Phaseolus vulgaris* (Johnson and Wang, 1996). Some experimental data have shown that chloroplastic MIPS activity increased when plants were exposed to strong light treatment and salinity stress (Ishitani et al., 1996).

The distribution and evolution of *MIPS* genes from a probable cenancestor to cyanobacteria, eubacteria and archaea, and ultimately to higher eukaryotes such plants and humans, indicate that MIPS started evolving from different sources (Majumder et al., 2003) and that MIPS enzyme activity is distributed in evolutionary diverse phyla, from eubacteria, archaebacteria, cyanobacteria, alguae, fungi to higher plants and animals.

The first MIPS reported from an archaebacterial source was from *Archaeoglobus fulgidus* (Chen et al., 2000). The characterization of *Archaeoglobus* MIPS showed that this enzyme was more active (by one order of magnitude) than other MIPS enzymes, and was functional at extremely high temperatures. Subsequently, MIPS proteins (with about 510 amino) acid were reported from other phylogenetically diverse organisms, such as red algae, bryophytes, gymnosperm and fungi. All the fungal MIPS proteins have an extended N-terminal end of about 28 amino acid residues, which is absent in the other MIPS. Phylogenetic analysis of fungal MIPS has shown a high degree homology with the *Saccharomyces MIPS* gene.

Analyses of the total protein sequences (Majumder et al., 2003; Bachhawat and Mande, 2000) indicate that the eukaryotic MIPS family is homogenous but not similar to the prokaryotic *MIPS* genes. This could be explained by a monophyletic origin of the eukaryotic *MIPS* genes. In contrast, prokaryotic *MIPS* genes show a lower sequence similarity.

The genomic structure and organization of MIPS has been determined in several organisms, including yeast, *A. thaliana*, *C. paradisi*, *Nicotiana tabacum*, and *Z. mays*. All these sequences have shown regions of high conservation at the nucleotide level (Hegeman et al., 2001).

An alignment (Fig. 1) of the amino acid sequences of selected *MIPS* genes from eubacteria, archaea, parasites, fungi, plant and animal sources reveals several domains; the amino acid stretch GWGGNNG (domain 1), LWTANTERY (domain 2), NGSPQNTFVPGL (domain 3) and SYNHLGNNDG (domain 4), a stretch of eukaryotic MIPS identified as 'highly conserved' (Majumder et al., 2003). These four domains are involved in MIPS protein binding and are essential for MIPS functions, such as cofactor NAD^+ binding and reaction catalysis (Majumder et al., 1997).

Cytosolic MIPS was also isolated and characterized from *Perilla frutescens* and *A. thaliana*, indicating their presence during normal seed growth (Chung et al., 1999; Jin et al., 2000; Baud et al., 2004). In *P. vulgaris*, only one gene was identified, but Western blot analysis of the soluble proteins detected two distinct polypeptides during embryonic and postembryonic development. A small 33 kDa protein was active during the globular stage of embryogenesis and in the mature roots. In contrast, another protein, with approximately 56 kDa, was present during the cotyledonary phase of embryogenesis and in the young roots (Johnson and Wang, 1996). Four genes were found in soybean (*G. max*), up-regulated in developing seeds and in flowers, leaves, roots and germinating cotyledons (Hegeman et al., 2001; Chappelle et al., 2006). In maize (*Z. mays*), seven homologous MIPS sequences were mapped on different chromosomes (Larson and Raboy, 1999). In *A. thaliana*, two distinct *MIPS* genes were identified (Johnson and Sussex, 1995). In sesame (*S. indicum*), two or three sequences were isolated, and the expression pattern showed accumulation in leaves, stems, roots and developing seeds, but no expression in mature seeds (Chun et al., 2003).

2.2. Sucrose synthase (Sus)

Sucrose is the primary transport carbohydrate and the predominant form of translocated carbon in most higher plants. It is actively channeled into the different plant organs and is subsequently converted into sugars, such as hexose sugars, and into starch through a series of enzymatic reactions. These enzymes involved in sucrose metabolism play a pivotal role in plant growth and development (Koch, 2004). The primary enzymes in sucrose metabolism include sucrose phosphate synthase (EC 2.4.1.14), sucrose synthase (EC 2.4.1.13) and invertase (EC 3.2.1.26).

In the species examined to date, Sus is encoded by a small multigene family, Sus occurs as isoforms and is encoded by at least two genes. In many dicotyledonous species such as *A. thaliana* (Chopra et al., 1992), *Solanum tuberosum* (Fu and Park, 1995), *Lycopersicon esculentum* (Anguenot et al., 1999), *Daucus carota* (Sebkova et al., 1995), *C. paradisi* (Komatsu et al., 2002) and *P. sativum* (Barratt et al., 2001), two or more sucrose synthase genes have been found. In *Vicia faba* and *G. max*, however only one Sus has been identified (Arai et al., 1992).

A comparison of deduced amino acid sequences of plant Sus showed that this gene is expressed in the various taxonomic branches of land plants. However, dicotyledonous genes have clear similarities in sequence and overall structure, which distinguish them from monocotyledons and suggest a different evolutionary origin for both plant types. However, monocot/dicot orthologous groups (Fig. 2) supported by statistical analyses (bootstrap values) suggested conservation of Sus function in different species.

The prokaryotic Sus was isolated and biochemically characterized from cyanobacterium *Anabaena* sp. (Porchia et al., 1999). A comparison of deduced amino acid sequences originated from prokaryotic and plant Sus showed that a long divergent region extends to approximately 100 amino acids. These data suggest that the prokaryotic enzyme clearly diverges from the plant Sus reported to date (Curatti et al., 2000). Prokaryotic Sus is characterized by an N-terminal amino acid stretch SELMQAILDS, whereas plant proteins are characterized by the following conserved region DTGGQVVY.

Sus can exist as homotetramers or heterotetramers. The molecular weight of each subunit is about 90 kDa, and the total native protein mass is 280–400 kDa (Koch et al., 1992). Substrates for sucrose cleavage by Sus can be UDP, ADP, dTDP or GDP (Romer et al., 2004).

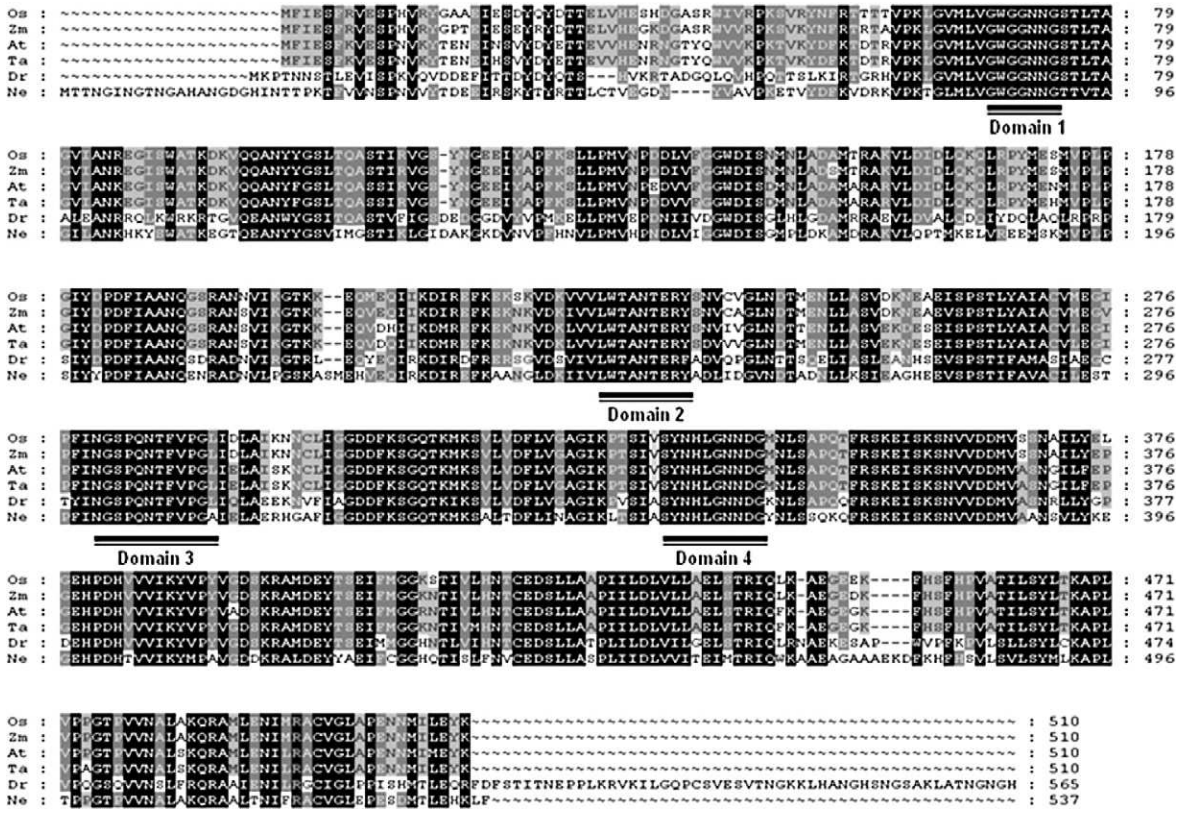

Fig. 1. Amino acid alignment of MIPS from different plant species; *Oryza sativa* (Os), *Zea mays* (Zm), *Arabidopsis thaliana* (At), *Triticum aestivum* (Ta), *Drosophila* (Dr) and *Neurospora* (Ne). Dark and/or grey background appeared to highlight the positions. Where more than half of the accessions show identical and/or similar residues, respectively explain about domain 1 (GWGGNNG), domain 2 (LWTANTERY), domain 3 (NGSPQNTFVPGL), domain 4 (SYNHLGNNDG).

Kinetic measurements have shown that UDP-glucose is the most favorable substrate for Sus.

Five genes encoding Sus-like proteins have been discovered in the *P. sativum* genome (Barratt et al., 2001); they have a distinct expression pattern in plant organs and in organ development. In rice, six *Sus* genes have been characterized and the expression analysis suggests that Sus is involved in different growth processes (Hirose et al., 2008). *Sus1* is expressed in elongating tissues, *Sus3* and *Sus4* are expressed mainly in caryopses, and *Sus2* is expressed in all vegetative tissues. The roles of the recently isolated *Sus5* and *Sus6* genes are not clear yet.

Baud et al. (2004) showed that the *A. thaliana* genome contains six *Sus* genes; the isoforms they encode could be classified into three distinct groups based on their amino acid sequences and each isoform might have a specific function. Sus5 and Sus6 are expressed in leaves, vascular cotyledons, petals, anthers and roots; Sus2 is expressed during seed development; SuS3 transcripts appear in mid seed development; SuS1 is widely expressed in vascular cotyledon, mature leaves and siliques; and the expression of SuS4 is clearly confined to stems, roots and germinating seeds (Bieniawska et al., 2007). In maize, three *Sus* genes have been identified (Carlson et al., 2002). Five Sus isoforms have been detected in *Sorghum bicolor* (Chourey et al., 1991). Silvente et al. (2003) indicated that there are at least two *Sus* genes in common beans (*P. vulgaris*). One of these genes was expressed almost exclusively in nodules and the other was expressed in all the tissues tested, including cotyledons, stems, leaves, and roots. In sugarcane, two genes were identified and their level of expression varied significantly in different parts of the internodes and at the various development stages. These results suggested different levels of Sus activity (Schafer et al., 2004). In *Pyrus pyrifolia* fruit, two isoforms of Sus were purified. One was present mainly in young fruits, and the other was expressed particularly in mature fruit (Tanaze and Yamaki, 2000). In *V. faba*, one enzyme was identified with a total molecular mass of 360 kDa in cotyledons (Heather and Howard, 1992).

Sus is a phosphor protein. The role of the status in plants was to regulate the proteolytic turnover of the centrally important enzyme. Initial data indicated that a phosphorylated Sus protein was associated with the plasma membrane, whereas the dephosphorylated form was located in the cytosol (Carlson et al., 2002; Komina et al., 2002; Nakai et al., 1997). Nevertheless, Hardin et al. (2003) showed that Sus phosphorylation occurred on serine 15 (Ser15) and serine 170 (Ser170) and was associated with the 20S and 26S proteasomes.

In maize phosphorylation and dephosphorylation at Ser15 might be one of the regulatory mechanisms for modulating of Sus activity and/or subcellular localization.

To investigate the molecular evolution and phylogenetic relationships among Sus in plants, the entire SuS protein sequences were aligned by ClustalX and tree calculated by neighbor-joining algorithms. Using the full length of the alignment of all plant Sus selected, the distance method resulted in one tree. The Sus could be divided phylogenetically into six major groups; A, B, C, D, E, and F (as shown in Fig. 2); out the 52 plant genes, 17 were classified into group A. Each group could be further subdivided into subgroups of related proteins. The dramatic expansion of dicotyledonous and, to a lesser extent, monocotyledonous *Sus* genes, such as *Z. mays* and *O. sativa* in the D and E group indicated that these two groups of genes should have expanded both before and after the monocot–dicot split. Interestingly, monocotyledonous *Sus* genes were clustered into one monocotyledon-specific group (C), suggesting a considerable divergence between monocotyledonous and dicotyledonous Sus. The recent expansion should be produced by polyploidy. In addition, the full-length Sus phylogeny showed that the evolution of dicotyledonous plant groups A and B was probably shaped by two duplication events: the first duplication produced group B and the common ancestor of group A;

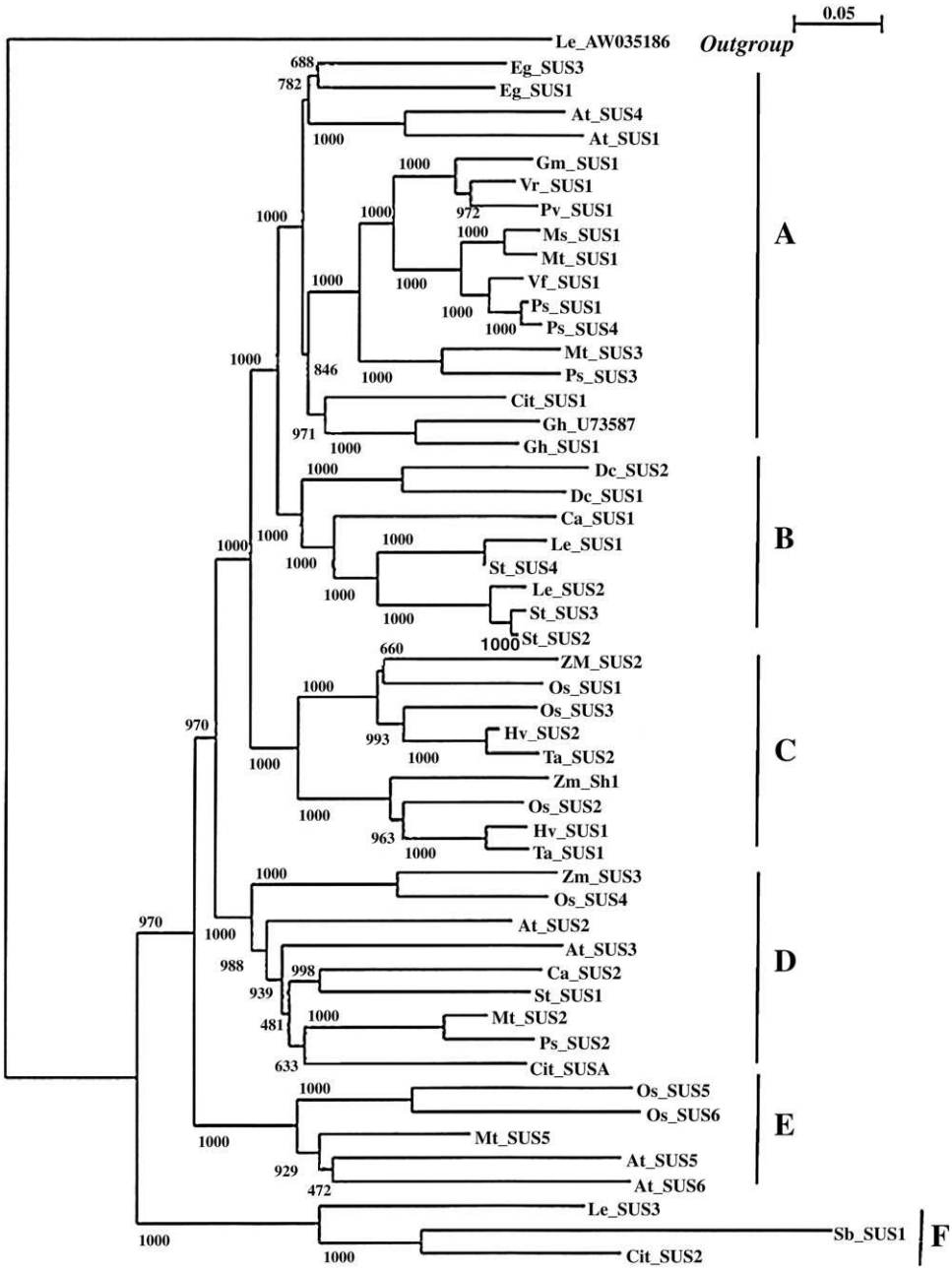

Fig. 2. Phylogenetic analysis of aligned amino acid sequences deduced from several cDNA *Sus* genes. The amino acid sequences of the ORFs were initially aligned using the program ClustalX-2.0.3-win. Distance analyses used the program Protdist and tree was calculated by neighbor-joing algorithms. To estimate phylogenetic relationships from the deduced amino acid sequences, a bootstrap majority-rule consensus tree was generated after 1000 cycles of bootstrap replicates using the parsimony method of the njplotWin95 software program. The numbers on the line denote frequency of occurrence of bootstrapping. The sequence sources were taken from the GenBank, TAIR, TIGR, EMBL-EBI and Gramene database: AY124703; *Zea mays* SUS1 (Zm_SUS1), L22296; *Z. mays* SUS2 (Zm_SUS2), X02400; *Z. mays* Sh1 (Zm_Sh1), At5g20830; *Arabidopsis thaliana* SUS1 (At_SUS1), At5g49190; *A. thaliana* SUS2 (At_SUS2), At4g02280; *A. thaliana* SUS3 (At_SUS3), At3g43190; *A. thaliana* SUS4 (At_SUS4), At5g37180; *A. thaliana* SUS5 (At_SUS5), At1g73370; *A. thaliana* SUS6 (At_SUS6), DQ227993; *Eucalyptus grandis* SUS1 (Eg_SUS1), DQ227994; *E. grandis* SUS3 (Eg_SUS3), AB022091; *Citrus unshiu* SUSA (Cit_SUSA), AB022092; *C. unshiu* SUS1 (Cit_SUS1), AB021745; *C. unshiu* SUS2 (Cit_SUS2), AJ012080; *Pisum sativum* SUS1 (Ps_SUS1), AJ001071; *P. sativum* SUS2 (Ps_SUS2), AJ311496; *P. sativum* SUS3 (Ps_SUS3), AF079851; *P. sativium* SUS4 (Ps_SUS4), Os03g0401300; *Oryza sativa* SUS1 (Os_SUS1), Os06g0194900; *O. sativa* SUS2 (Os_SUS2), Os07g0616800; *O. sativa* SUS3 (Os_SUS3), Os03g0340500; *O. sativa* SUS4 (Os_SUS4), Os04g0309600; *O. sativa* SUS5 (Os_SUS5), Os02g0831500; *O. sativa* SUS6 (Os_SUS6), X65871; *Hordeum vulgare* SUS1 (Hv_SUS1), Y15802; *H. vulgare* SUS2 (Hv_SUS2), AJ001117; *Triticum aestivum* SUS1 (Ta_SUS1), AJ000153; *T. aestivum* SUS2 (Ta_SUS2), X75332.1; *Daucus carota* SUS1 (Dc_SUS1), Y16091; *D. carota* SUS2 (Dc_SUS2), AY205302; *Solanum tuberosum* SUS1(St_SUS1), AY205084; *S. tuberosum* SUS2 (St_SUS2), U24088; *S. tuberosum* SUS3 (St_SUS3), AJ537575; *S. tuberosum* SUS4 (St_SUS4), U73588; *Gossypium hirsutum* SUS1 (Gh_SUS1), U73587; *G. hirsutum* U73587 (Gh_U73587), X69773; *Vicia faba* SUS1 (Vf_SUS1), TC67957; *Medicago truncatula* SUS1 (Mt_SUS1), TC62069; *M. truncatula* SUS2 (Mt_SUS2), TC67958; *M. truncatula* SUS3 (Mt_SUS3), TC65865; *M. truncatula* SUS5 (Mt_SUS5), AF049487; *Medicago sativa* SUS1 (Ms_SUS1), AJ853494; *Phaseolus vulgaris* SUS1 (Pv_SUS1), AF030231; *Glycine max* SUS1 (Gm_SUS1), L19762; *Lycopersion esculentum* SUS1 (Le_SUS1), AJ011319; *L. esculentum* SUS2 (Le_SUS2), AF011534; *L. esculentum* SUS3 (Le_SUS3), AW035186; *L. esculentum* AW035186 (Le_AW035186), D10266; *Vignia radiata* SUS1 (Vr_SUS1), AM087674; *Coffea arabica* SUS1 (Ca_SUS1), AM087675; *C. arabica* SUS2 (Ca_SUS2), EF089747; *Shorghum bicolor* SUS1 (Sb_SUS1).

the last group would result from the second duplication. Interestingly, some putatively orthologous and paralogous pairs could be identified from the tree, such as Ms_SUS1/Mt_SUS1, Hv_SUS1/Ta_SUS1, Mt_SUS3/Ps_SUS3, Os_SUS5/Os_SUS6, etc. Group B is a far more homogenous group than any of the other groups. In summary, there are two or more monocotyledon and dicotyledon subgroups, a consequence of the large variation of divergence in these groups. A lower limit of divergence would result in group F. It is known that *Sus*

genes were relatively well conserved over a long period of evolutionary time, and genes belonging to the same group might serve similar functions (Pramanik et al., 2005; Komatsu et al., 2002; Barratt et al., 2001; Sebkova et al., 1995). Therefore, the identification of orthologs and paralogs would greatly facilitate the functional annotation of uncharacterized Sus.

3. Expression and regulation of *MIPS* and *Sus* genes

3.1. Relationship between MIPS and phytic acid biosynthesis

In the higher organisms, as mentioned earlier, the MIPS enzyme has two forms, cytosolic and chloroplastic. The latter form, which is associated with thylakoid membranes, is regulated by light, whereas the former is involved in various important metabolic reactions (Majumder et al., 1997).

MIPS catalyses the conversion of glucose-6-phosphate (G6P) to *myo*-inositol 3-phosphate (Ins(3)P), the first product in the biosynthesis of inositol, phytic acid and other essential cellular components (Loewus and Murthy, 2000). *Myo*-inositol has a diverse biological role and participates in several cellular processes, including signal transduction, stress response, cell wall biogenesis, growth regulation, osmo-tolerance, IAA metabolism, membrane trafficking (Irvine and Schell, 2001) and in phytic acid synthesis.

Phytic acid is the predominant form in which phosphorus occurs in plants; it is a regulator of intercellular signaling and a phosphate storage form. This metabolite is accumulated mainly in seeds (up to 4–5% of dry weight) and pollen. Phytic acid, discovered in 1855, has an inositol ring with six attached phosphate groups and is the most stable form of phosphorus (Oatway et al., 2001). Phytic acid is found in cereals and legumes grains, nuts, oilseeds, tubers, pollen, spores and organic soils (Oatway et al., 2001).

The biosynthetic pathway to phytic acid is summarized in Fig. 3. The first committed step in inositol synthesis involves the synthesis of *myo*-inositol-3-phosphate (Ins(3)P) from D-glucose-6-phosphate (G6P) catalysed by the MIPS enzyme. Ins(3)P is converted directly to phytic acid via sequential phosphorylation by two or more kinases.

The generated Ins(3)P is then dephosphorylated by inositol-3-phosphatase to release free inositol. The inositol formed by this process from G6P can enter in different pathways and cellular compartments. There is evidence that the inositol backbone for phytic acid might be derived from MIPS activity. Inositol is crucial for normal plant growth and development. Plant cells metabolize *myo*-inositol via a number of pathways leading to phytate production.

3.2. Sus key enzymes involved in sugar synthesis

Sus is implicated in many aspects of plant metabolism. In various plants its activities play a major role in energy metabolism, controlling the mobilization of sucrose into various pathways that are important for the metabolic, structural and storage functions of the plant cell (Hess and Willmitzer, 1996).

A large part of the carbon produced during photosynthesis is channeled into synthesis of sugars, which play a key role as metabolic signals controlling many aspects of plant growth and development (Smeekens, 2000). Among the sugars, trehalose is a storage carbohydrate which protects the plant against stresses caused by various invertebrates and fungi (Goddijn and Van Dun, 1999).

The biosynthesis of trehalose (Fig. 4) involves the formation of trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose from the enzyme trehalose-6-phosphate synthase (TPS). The latter is dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP) (Cabib and Lenoir, 1958). Higher plants have two ways of forming UDP-glucose, one based on Sus and the other on UDP-

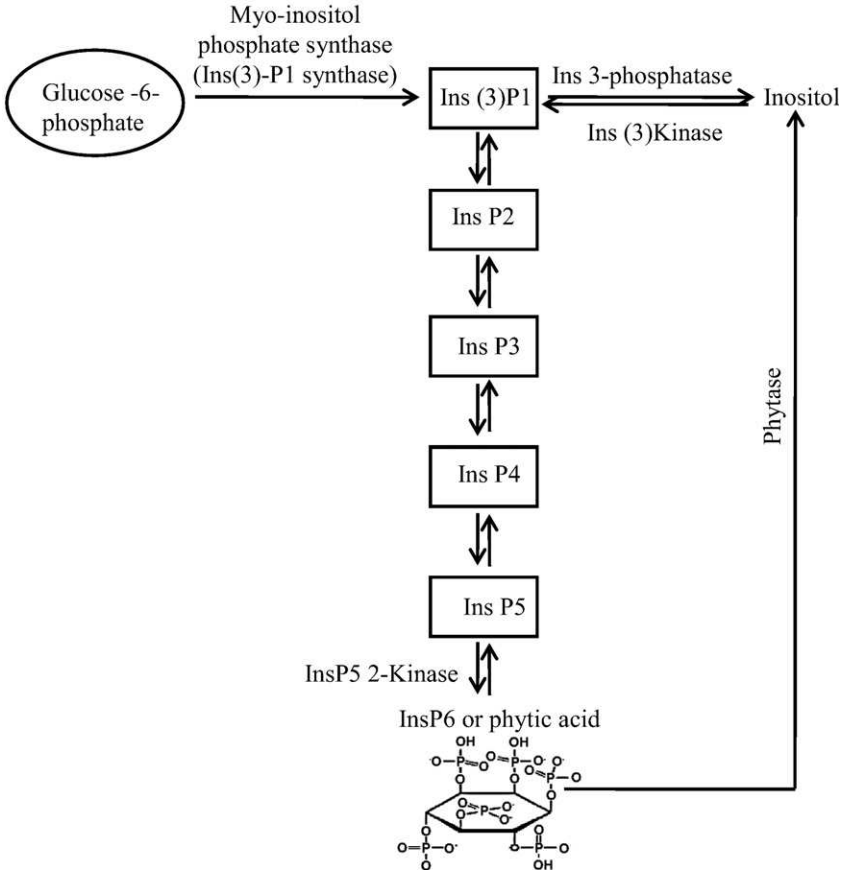

Fig. 3. Phytic acid metabolism in plants. Synthesis of phytic acid occurs from glucose-6-phosphate.

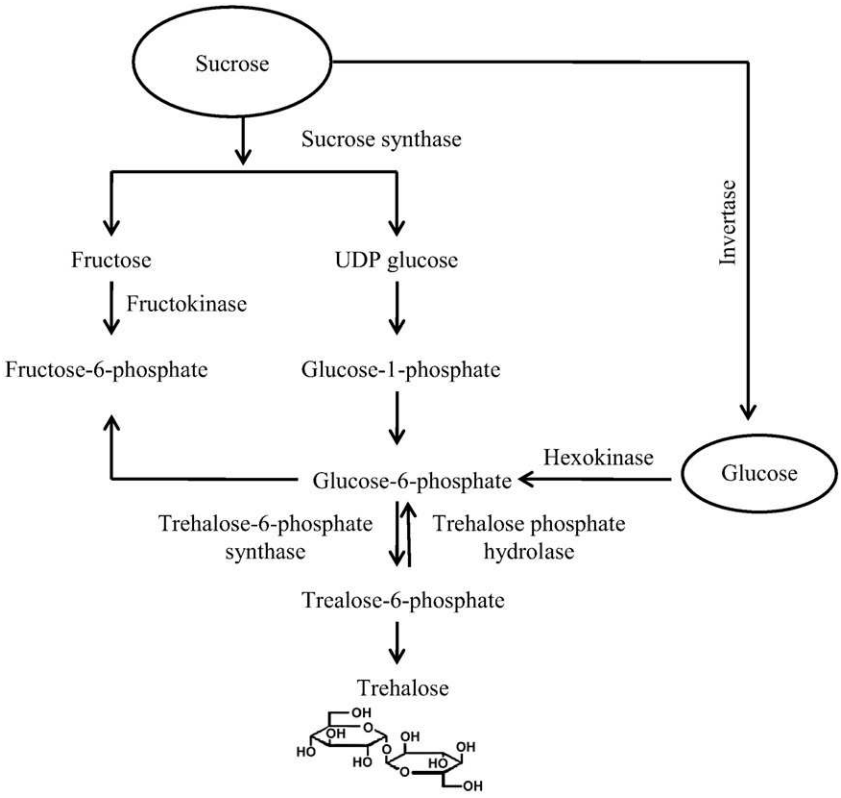

Fig. 4. Proposed degradation pathway for sucrose by sucrose synthase.

glucose pyrophosphorylase. T6P is of great importance in plant biology and has much potential in crop improvement (Eastmond and Graham, 2003). It is considered to be a central regulator of many cellular processes. Alternatively, trehalose metabolism could play a more direct role in regulating cell division as an integrator of nutritional status and growth (Gomez et al., 2006). It is thought to be responsible with invertase for sucrose mobilization in sink organs such as leaves, phloem, shoots, nodules, seed testas, embryos and fruits by converting sucrose and UDP into fructose and UDP-glucose (Craig et al., 1999).

Invertase is widely believed to be the main entry route of carbon as sucrose into the cellular metabolism in plants, and it is located mainly in the extracellular compartment (intercellular space and cell wall) and in the large central vacuole. This suggests an important physiological role of acid invertase as an entry point for carbohydrate into cellular metabolism, probably via control of sugar composition and metabolic fluxes, but also in sucrose portioning by regulating the cellular content of several major metabolites, including UDP-glucose, fructose, glucose, glucose-6-phosphate and fructose-6-phosphate. These metabolites regulate cell wall synthesis, the respiration rate, starch synthesis, storage in the vacuoles and other product synthesis. More interestingly, an optimal level of hexose sugars maintains cell division and expansion in the embryo (Borisjuk et al., 1998)

In general, Sus enzymes appear to be located primarily in sink tissues, where they facilitate sucrose import. Young leaves tend to have high Sus activity, but mature leaves do not (Hardin et al., 2003). In some plants, Sus activity is constant during leaf development (Baroja-Fernandez et al., 2001). In carrot (*Dacus carota*), Sus activity was highly correlated with both polypeptides and transcript levels, indicating that gene expression is regulated mainly at the mRNA level in the different tissues and organs of the developing carrot (Sebkova et al., 1995). In plants, the expression of *Sus* genes has been shown to be cell-specific, developmentally regulated or regulated by tissue carbohydrate status (Ruan et al., 1997). Sus is the major sucrose-cleaving enzyme involved in starch synthesis. According to Dejardin et al. (1997), Sus participates in the synthesis of sugars.

4. Putative function of *MIPS* and *Sus* genes in seed plants

4.1. Myo-inositol phosphate synthase (MIPS)

MIPS is a pivotal biosynthetic enzyme in the *myo*-inositol pathway, including the synthesis of phytic acid. It is found in uni and multicellular eukaryotes. A complete elimination of the *myo*-inositol pathway through *MIPS* gene mutation stops the formation of phytic acid, but it also disrupts the many other biochemical pathways needed for germination. Indeed, several mutant MIPS lines of maize (Raboy and Cook, 2000), barley (Larson et al., 1998; Ockenden et al., 2004), rice (Larson et al., 2000; Suzuki et al., 2007) and soybean (Wilcox et al., 2000; Hitz et al., 2002) show a reduction of phytic acid levels.

MIPS activity is halted by including deoxyglucose in growth media. Deoxyglucose is a substrate for MIPS and phosphorylated into the enzyme inhibitor deoxyglucose 6-phosphate resulting in a reduction in the capacity of the cell to synthesize *myo*-inositol as well as in cell division (Biffen and Hanke, 1990). This confirms that MIPS signalizations are necessary for maintaining cell division.

Phytic acid, a natural plant antioxidant, is the main storage form of phosphorus in many plant tissues, especially bran and seed. More phosphorus as phytate is generally, not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase, necessary to separate phosphorus from the phytate molecule. The bioavailability of phytate phosphorus can be increased by supplementing of the diet with phytase enzyme.

Viable low phytic acid mutant lines have been developed in several crop species in which the phytic acid levels drastically reduced in seeds. Low phytic acid (LPA) maize (*Z. mays*) mutant have been

obtained by pollen treatment with EMS (ethyl methanesulfonate). A reduction in *MIPS* gene expression has been shown using an antisense approach (RNAi) in rice (*O. sativa*), barley (*H. vulgare*) and soybean (*G. max*). These mutants have genetically reduced amounts of phytic acid (at least 70%) in the seed. Raboy (2001) reported that a 90% reduction in MIPS activity causes severe restriction of plant growth. Maize mutants with a phenotype of reduced phytic acid increased *myo*-inositol and reduced amounts of *myo*-inositol phosphate intermediates in the seeds had a minimal effect on plant growth and development (Shi et al., 2005). These facts suggest that a high level of stored phytate is not necessary for seed viability and germination or seedling growth. Such mutants were isolated in soybean (Wilcox et al., 2000) and the genetic lesion was a single base change, which causes a replacement from lysine to asparagines in MIPS. The amino acid substitution reduces the specific activity of the seed expressed MIPS by about 90% (Hitz et al., 2002). Nunes et al. (2006), however, showed that total knockout of the soybean *MIPS* gene (GmMIPS1) leads to the abortion of soybean immature seeds. The characterization of mutant soybean indicates that *MIPS* genes play a critical role in the early developmental stages of the embryo. Using an immunological technique, the expression of the soybean MIPS was clearly detected in early developing seed, particularly in maternal tissues and in the developing embryo. At the globular embryo stage, MIPS is expressed in the outer integumentary layer (micropylar complex). Recently, in soybean, the MIPS enzyme showed association with calcium oxalate crystal idioblast (Chiera and Grabau, 2007), a complex that modulates physiological calcium levels in plant tissues and requires *myo*-inositol as a precursor to biosynthesis. In potato (*S. tuberosum*), the suppression of MIPS activity using the RNAi approach showed a variety of morphological and physiological changes. The transgenic plants exhibited reduced apical dominance and altered leaf morphology, and provoked precocious leaf senescence and a decrease in overall tuber yield. These data indicate the essential role of *myo*-inositol in plant physiology and development (Keller et al., 1998). Several low phytic acid (*lpa*) mutants have been generated; mapping some of them to early steps in inositol metabolism might explain the pleiotropic phenotypes. The alteration of the *MIPS* gene is a potential approach in the development of these low phytic acids plant. The characterization of these genotypes suggested that phytate and *myo*-inositol, play a role as precursors to many compounds whose function in plants is linked to phosphorus storage, signal transduction, stress protection, hormonal homeostasis and cell wall biosynthesis (Hegeman et al., 2001). This MIPS role has a direct influence on plant growth and development. A complete elimination of the *myo*-inositol pathway would halt the formation of phytic acid, and it would disrupt many other biochemical pathways needed for germination, which in turn are fundamental in embryonic axis and cotyledon development (Stevenson et al., 2000).

MIPS activity has been localized in different organs (roots, leaves, suspensors and/or embryos, and cotyledons), and different forms of MIPS at different stages of development reflect regulatory controls at the transcriptional and translational levels (Adhikari et al., 1987). MIPS is also expressed during embryonic and postembryonic development in *P. vulgaris* (Johnson and Wang, 1996). These data indicate the important role of MIPS not only in basic metabolism, but also in the establishment of developmental programs. Over-expression in transgenic *A. thaliana* elevated the content of inositol and MIPS activity, but it did not affect plant growth or salt tolerance (Smart and Flores, 1997). The localization of three isoforms of the *MIPS* gene was investigated by Mitsuhashi et al. (2008) in developing *Arabidopsis* seeds. Immunolocalization, immunofluorescent microscopy and Western blot analysis indicated that AtMIPS proteins appear to be specifically located in the cytosol of the endosperm, but not in the embryo. Phytic acid is therefore accumulated during seed development. These data suggested, however a possible interaction between endosperm and embryo to ensure phytic acid synthesis.

4.2. Sucrose synthase (Sus)

Sus is important for the carbon metabolism used by storage tissues in many species and is located mainly in transport and sink tissues (Koch et al., 1996). It occupies a critical position in the cleavage of sucrose imported into developing plant structures, but members of *Sus* gene families can have diverse functions. Its substrate (sucrose) and product (UDP-glucose) are involved in carbon partitioning and signal sensing. For this reason, considerable effort has been made to study the biochemical properties of Sus, its localization, gene expression and regulation.

Sus provides the energy for sucrose channeling from the apoplast, in starch storage and in callose biosynthesis.

The level of soluble sugars, such as glucose and sucrose, is known to regulate developmental processes from embryo development to senescence. In embryo, storage reserve protein gene expression and accumulation such as 2S albumin and 12S globulin in *Arabidopsis* (Baud et al., 2002), are differentially induced at the transition from torpedo to early cotyledon phase. Interestingly, storage reserve gene induction and reserve accumulation have been reported occurring in embryo lethal mutants where growth stops as early as the globular stage of embryo development. Craig et al. (1999) studied Sus activities in pea *rug4* mutants. They showed that mutations at the *rug4* locus caused a considerable and specific reduction in Sus activity in the developing embryo, leading to a reduction in starch contents and a smaller reduction in Sus activity in testas and leaves. In transgenic potato (*S. tuberosum*) plants obtained with reduced Sus activity had a lower starch content than the control plants (Zrenner et al., 1995). In mutants of cotton (*Gossypium hirstum*), the suppression of Sus activity in only the maternal seed represses fiber development without affecting embryo development and seed size. Additional suppression in the endosperm and embryo inhibits their development, which blocks the formation of adjacent seed coat transfer cells and halts seed development entirely (Ruan et al., 2003). Recently cotton mutants have been obtained by silencing the expression of Sus in the endosperm using the RNAi approach, halting early seed development. The results provide new insight into the roles of Sus in controlling plant cell and early seed development through the regulation of endosperm formation (Ruan et al., 2008).

The expression of Sus has been manipulated by the antisense technique in transgenic carrot (*D. carota*). The results of phenotypical and biochemical examination of the transgenic plants demonstrated that Sus in carrots is a major determinant of plant growth (Tang and Sturm, 1999). In carrots, the two Sus isoforms have different expression patterns; one is expressed exclusively in flowers, and the other in stems, roots, flowers, and maturing seed (Sturm et al., 1999). The phenotype of the antisense plants clearly differed from that of the control plants. The leaves and roots were markedly smaller, and the antisense plantlets with the lowest Sus activity also developed into smallest plants.

In maize, three *Sus* genes have been identified: *SH1*, *SuS1* and *SuS3* (Carlson et al., 2002). The loss of their functions suggested their involvement in starch biosynthesis and cell wall formation during endosperm formation (Chourey et al., 1998).

The *A. thaliana* genome contains six *Sus* genes. An examination of knockout mutants of all the isoforms caused, in particular, a loss of sugar, starch, cellulose content and weight and lipid content in mature seeds (Martin et al., 1993).

In contrast, however a loss of both isoforms (SuS2 and SuS3) most highly expressed in developing *Arabidopsis* seeds had no effect on final seed weight and lipid content (Bieniawska et al., 2007). The expression of different isoforms Sus in *Arabidopsis* did not show strong tissue-specific expression. Fallahi et al. (2008) reported that *Arabidopsis* Sus were expressed in the same stages of seed development and in several tissues from plants including immature and mature rosettes, cauline leaves, stems, flowers, and developing

siliques. These data illustrated the potential role of Sus enzymes in carbon partitioning during the different stages of seed and plant development.

When plant growth occurs under controlled light conditions, the lack of any obvious phenotypes among the single and double knockout mutants showed a specific requirement for Sus activity in maize, pea, cotton and potato. In cotton and carrot species, mutant and antisense plants deficient in specific isoforms of SuS had marked phenotypes (Tang and Sturm, 1999; Ruan et al., 2003). Pea mutants (*rug4*) lacking an isoform very similar to Sus1 and Sus4 of *A. thaliana* were characterized by reduced seed mass and starch content, and they failed to assimilate N_2 in their *Rhizobium* root nodules (Craig et al., 1999).

It is known that sugar metabolism is affected by temperature stress. In cabbage (*Brassica oleracea*) seedlings, the starch, hexose and sucrose content and the Sus activities increased during cold treatment. These data suggest that Sus is regulated by cold acclimation and play an important role in sugar accumulation and the acquisition of freezing tolerance in the leaves of cabbage seedlings (Sasaki et al., 2001). The *Sus* gene, however, is also present in other tissues such as roots and shoots, and its expression is involved in the response to biotic and abiotic stress (Zeng et al., 1999).

4.2.1. Putative function of Sus in cell wall biosynthesis

Sus plays a major role in the degradation of sucrose (sucrose + UDP ↔ UDP − glucose + fructose). All these substrates constitute a start compound for cellulose biosynthesis. Cellulose is a compound with a critical role as the foundational polymer in plant cell walls. Studies of transformed carrot (Sturm et al., 1999) and maize (Chen and Chourey, 1989; Carlson and Chourey, 1996) plants showed Sus expression changes and cell wall synthesis disruption. These data strongly suggested a possible role of Sus in the biosynthesis of primary and secondary wall cellulose synthesis. During the formation of the primary cell wall, cellulose is not abundant. Walls contain 20–25% cellulose and non-cellulosic polymers such as xyloglucan and galacturonic acid (Meinert and Delmer, 1977). Towards the end of the development of the primary cell wall, secondary cell wall deposition begins via enhanced cellulose synthesis. In cotton (*G. hirsutum* and *Gossypium barbadense*), the rate of cellulose synthesis increases more than a hundredfold during the formation of the secondary cell wall (Basra and Malik, 1984). At this stage, about 80% of the imported carbon is channeled to cellulose. Amor et al. (1995) reported the presence of two forms of Sus: particulate Sus (P-Sus) associated with the plasma membrane, and soluble Sus (S-Sus). Using cryogenic electron microscopic methods and immunofluorescence, Amor et al. (1995) studied both forms of Sus activity during cotton fiber synthesis; the data showed that P-Sus channels UDP-glucose to cellulose synthesis in the plasma membrane. Interestingly, Pear et al. (1996) identified three genes involved in cellulose synthesis in cotton; one was up-expressed during primary cell wall synthesis, and the other genes were highly expressed in secondary cell wall synthesis. Subsequently, 10 genes in *Arabidopsis* (Turner and Somerville, 1997), eight in maize and only one in rice (Holland et al., 2000) were identified. The discovery and cloning of the gene that encodes cellulose synthase was carried out after searching for plant orthologs in the corresponding bacterial cellulose synthase gene from *Actobacter xilinum* and *Agrobacterium tumefaciens*. The characterization of the cellulose synthase complexes in the plasma membrane appears to play a key role in the shape of the cellulose molecules synthesized. Both forms of Sus (P-Sus and S-Sus) adjust intracellular carbon partitioning, although, P-Sus could be critical for partitioning carbon to other different compounds such as cellulose. In contrast S-Sus could partition carbon in response to other demands, such as respiration and deposition of storage. The predominant form of Sus is P-Sus, which channels of UDP-glucose directly into cellulose synthase for cellulose cell wall biosynthesis.

Sucrose synthesis and degradation with Sus can occur intensively during high-rate cellulose synthesis for secondary wall deposition and at a lower level during primary wall synthesis. It is clear however, that Sus, particularly the P-Sus form, plays an important role in primary and secondary wall cellulose biosynthesis in plants.

5. Conclusions

Essential genes are required for growth and seed development (Harada, 1999). MIPS, the pivotal biosynthetic enzyme in inositol metabolism, is the only enzyme known to catalyse the conversion of glucose 6-phosphate to inositol phosphate, a precursor for many compounds whose functions in plants are involved in phosphorus storage, signal transduction, stress protection, hormonal homeostasis and cell wall biosynthesis.

Sus occupies a central position in the cleavage of sucrose, which in turn might regulate the rate of carbon imported into developing seeds, including the supply of carbon to the developing embryo, and control gene expression in it. However, SuS is believed to play an important role in cell growth by providing UDP-glucose and fructose through sucrose cleavage for energy metabolism and cell wall biosynthesis. Some members of the *MIPS* and *Sus* gene families could have diverse functions.

MIPS and *Sus* genes are essential for the higher plants. They are considered to be central regulators of several metabolisms and physiological processes in plant growth and seed development.

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