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SUGAR TRANSPORT DURING SEED DEVELOPMENT

Deepika[1], Vivek Kumar Saini[2*] , Mukesh R.Jangra[3], Naresh Kumar[4]

[1]Assistant Professor, Department of Botany, Government College, Hisar
[*2]Associate Professor, Department of Zoology, Government College, Hisar
[3]Assistant Professor, Department of Biotechnology, Government College, Hisar
[4]Assistant Professor, Department of Botany, Narnaund, Government College, Hisar

*Corresponding author: viveksaini25@rediffmail.com

Abstract:

Development of seed is a complex physiological and molecular process that involves transport of nutrient, metabolic regulation, and expression of genes in a coordinated Significant progress has been made in understanding seed maturation, nutrient flow in developing seeds, and metabolism of storage compounds through physiological and genomic studies. Transport of sugar play a vital role in supplying carbon to seed and this mainly occurs through symplastic compartments within maternal and filial tissue. Molecular studies have also revealed the importance of gene regulation in seed development, including genomic imprinting and differential expression of maternal and paternal genes in the endosperm. In controlling autonomous endosperm development through mechanism involving DNA methylation and chromatin remodelling, Fertilization Independent Seed (FIS) genes play a vital role. Furthermore, carbohydrate and nitrogen metabolic pathways interact closely during seed maturation, influencing assimilate partitioning between starch and protein synthesis. Identification of nutrient sensing systems and signaling pathways involving sugars and nitrogen compounds will enhance understanding of seed development and may contribute to future crop improvement strategies.

Keywords:

Seed development, maternal–filial interface sucrose transport, nutrient partitioning, , endosperm development, carbohydrate metabolism, nitrogen metabolism, nutrient signaling.

INTRODUCTION

Plants, as sessile life forms, have evolved diverse mechanisms to circumvent unfavorable growth conditions; among these interruptions of the life cycle is one of the most successful strategies. Seed formation, dispersal and germination are crucial stages in the life cycle of angiosperms. The unique virtues of seeds like tolerance to desiccation, their mobility/dispersal and their ability to schedule their germination to coincide with times when environment conditions are congenial for their survival as seedlings, have indeed contributed significantly to the success of angiosperms. Seeds are important for human beings as the main means of propagation of crop species and also as an important source of food, feed and raw material. Seeds are warehouse of starch, proteins and oils in different proportions depending on the species which are the determinant of seed yield and quality of crop plants. These products are synthesized in the storage organs, the endosperm or the cotyledons, mainly based on the imported sucrose and a variety of fatty and amino acids. In addition, these compounds fuel the establishment of seedling after germination (Bewley and Black,

1994). Seed development is a genetically programmed and includes transcriptional and physiological reprogramming mediated by sugar and hormone responsive pathway. Understanding sucrose transport activities involved in post phloem assimilate partitioning appears to be of major economic importance. Phloem unloading, post-phloem transfer and transport processes of assimilates within maternal and embryonic seed tissues have attracted attention but most attention has been paid on seed filling phase when the storage reserves are deposited whereas the earlier stages of seed development have not been given due attention. Cellular pathway and the physiology of post-phloem assimilate transport within seed has been examined in wheat (Fisher, 1995), legumes (Patric and Offler, 1995) and maize (Prioul, 1996). This chapter focuses on current morphological, physiological and molecular aspects of photoassimilate transfer and storage into the developing seeds.

SEED DEVELOPMNT-A REPRODUCTIVE PERSPECTIVE

Seed formation is an intricate process that comprises of proper embryogenesis followed by maturation phase characterized by storage compound accumulation, acquisition of desiccation tolerance, growth arrest and finally the entry into a dormancy of variable periods. Seeds, in general, consist of three genetically distinct components viz. embryo, endosperm and seed coat. In angiospermous plants male and female gametophytes are formed after meiosis of meiocytes in anthers and ovules, respectively The female gametophyte which is the site of fertilization is deep seated within the maternal sporophytic tissues of the egg like ovules. The most prevalent type of mature female gametophyte is 7-celled structure consisting of three antipodals at the chalazal end, three celled egg apparatus comprising of two synergids and an egg at the micropylar end and a diploid or binucleate central cell. Synergids are known to attract pollen tubes to the female gametophyte. The egg cell lies adjacent to the synergids and is the progenitor of embryo upon fertilization with male gamete (syngamy). The large central cell is the progenitor of the endosperm upon fertilization with the second male gamete (triple fusion). The male gametes are delivered to the synergid through a conduit like structure called pollen tube which is endowed with the unique trait of unidirectional tip growth. The resulting embryo and endosperm share many features concerning the physiological changes and underlying molecular mechanisms associated with maturation except for ploidy level. Fertilization also initiates changes in maternal tissues. The ovary develops into a fruit and the ovular integuments get differentiated into the protective seed coat. Seed coat is comprised of a vascular compartments embedded in ground tissues and these tissues serve as symplasmic compartment to deliver nutrients. Nutrients are released into the seed apoplasm. The endosperm, a terminally differentiated tissue nurtures the embryo during seed development. Endosperm development begins with the division of triploid primary endosperm nucleus, which precedes the division of zygote. During embryogenesis zygote repeatedly divides to form embryo, the embryo begins to absorb the endosperm when it is still free nuclear or upon cellularization depending upon the plant species. As the embryo matures after passing through different stages, the cotyledons functions as storage organs and the endosperm disappears completely in some plant species while in cereals the endosperm persists and constitutes major part of the grain. Based on the growth pattern, seed development can be divided into four distinct phases: i) cell division ii)

cell elongation, iii) dry matter accumulation and iv) cell maturation of variable durations.

CARBOHYDRATE STATUS –A CONTROL ELEMENT IN SEED DEVELOPMENT

Seeds represent a well defined system for sink metabolism, transport processes and plant development. Storage products like starch, proteins and oils in the seed are synthesized in the storage organ like cotyledons in legumes /endosperm in cereals based upon import of sugars and a variety of fatty acids and amino acids during later stages of development. To analyze how is seed metabolism connected with its development and how biosynthetic pathways are controlled by specific enzymes necessitates an integrated biochemical, physiological and molecular approach. Further seeds constitute an excellent system for studying fundamental processes in plant biology, as they develop from a single fertilized egg and central cell into an embryo and endosperm, respectively, in association with the surrounding maternal tissues. In the developing seeds, the embryo is isolated from the maternal seed coat, thus assimilate must pass at least two membranes on their way from the phloem to the embryo. Photoassimilates synthesized in the leaves (source) are transported through phloem bidirectionally either to growing apices or storage sites (sinks) depending upon their ability to unload major osmotic species from the importing phloem sieve elements. Phloem sap normally consists of 90% sucrose while the remainder 10% includes sugar alcohols (sorbitol, mannitol), hormones, viruses, alkaloids, amino acids and N-compounds (Turgeon and Wolf, 2009). Before sucrose is transported, it is loaded into sieve tubes of phloem which is an energy requiring process. This is so because sugars are pumped into sieve tube against a concentration gradient: sucrose concentration in the chlorenchyma cell is typically 10-50 mM while that in a sieve tube of minor vein of leaf is may be as high as 1M. ATP and H^+ carriers in the cell membranes are used to pump protons out of the sieve tubes. A proton gradient forms across the membrane with high H^+ concentration on the outside of the membrane; K^+ enters to keep the charge balance. Diffusion of proton back into sieve tube, through ATPase is coupled to a carrier and powers the transport of sugar. Loading of sucrose into the sieve tubes lead to decline in water potential and consequently water enters sieve tubes. After reaching the sink, sucrose is unloaded from the sieve tubes either symplastically or apoplastically. Removal of sucrose at sink increases water potential to move out of sieve tube. Solutes move to sink cell and water goes back to the xylem. Seed coats are known to possess vasculature beyond which distribution of unloaded sugars within the fertilized ovules has been a matter of controversies and speculations. Seed coat influences embryo development through assimilate supply which can effect cell division phase in wheat endosperm (Singh and Jenner, 1984) by nutritional effects on mitotic activity (Jenner *et al*. 1991). In addition to sucrose, spectrum of amino acids and amides largely account for biomass gain of seed filial tissues and sucrose contributes carbon skeletons for the biosynthesis of these storage compounds. Demand for sugars and amino acid-N is set by the biosynthetic capacities of processes responsible their sequestration in to fats, proteins and starch. How is this metabolic demand communicated from filial storage tissue to phloem import of nutrients in to seed maternal tissues is not fully understood? Studies indicate that nutrient demand by filial tissues in legumes seems to be sensed by osmotically controlled alterations in turgor pressures of nutrient release cells located in their coat (Zhang *et al*.

2007).This increased uptake of nutrients by filial tissues results into decline in the osmolarity of the seed apoplasmic sap concomitant with rise in turgor of seed coat cells. Under conditions of sustained nutrient demand, the turgor of seed coat declines to drive higher rates of phloem transport. Post sieve elements transport through a symplasmic compartment allows for imported nutrients to be sequestered into seed components for short term storage and to undergo metabolic interconversions. Legumes which are characterized by highly vacuolated cell provide greater scope for temporary nutrient storage. These vacuoles in maternal seed tissues provide a driving force for efflux by facilitated diffusion through carriers and facilitated diffusion from maternal seed tissues provides a mechanism specific for each nutrient to respond to filial demand. Photoassimilates release to seed apoplasm is considered to occur from cells located at, or near, the entire maternal/filial tissue or specific sites. Demand for sugars and amino nitrogen compounds are set by biosynthetic capacities of processes responsible for their sequestration into fats, proteins and starch (Borras *et al*. 2004). When seed coat turgor exceeds a set point, a regulatory system activates the efflux transporters in the seed coat in order to meet the demand of the growing embryo. Since there is a sustained demand, the turgor set point decreases to enable higher rates of phloem transport (deJong *et al*. 1996, Zhang *et al.* 1996). This process relies upon a set of intercellular (post sieve element) transport events which are integrated with growth or storage functions of the recipient sink tissues. Several aquaporins are reported to be expressed in the seed coats which represent potential candidates for controlling water transport and thus the control of turgor (Zhou *et al*. 2007, Offler *et al*. (1997). The intimate anatomical connections between cells in most sinks present technical difficulties for unambiguous experimental investigation of post sieve element transport. However, the morphology of developing seeds especially those of cereals and legumes obviates this experimental impasse. Absence of symplastic linkages between maternal and filial tissues permits independent investigation of nutrient transport and metabolism in these two tissues

Active transport systems are present in different parts of the seed (McDonald *et al*. 1996) and these serve as control points. In wheat, unloading is controlled at the level of transport through the maternal seed tissue (Fisher and Wang, 1995). The control of import is exercised by the rate of assimilate delivery through the chalazal plasmodesmata. In *Vicia faba* seeds, the sieve elements terminate in the seed coat and are symplastically connected with coat cells. Phloem unloading and subsequent transport through the seed coat is thought to be symplastic, with regulated transfer occurring through plasmodesmata. The unloading may be a passive process mediated by porin like transporters (pea) or carrier mediated (*Vicia)* even though passive unloading may account for 50%. Further this seed coat unloading is sensitive to turgor and a turgor –homeostat model has emerged for the regulation of efflux from the seed coat, by rates of sucrose utilization in the cotyledons (Patrick and Offler, 1995). Imported sucrose is then hydrolysed and used either in the metabolism or storage product synthesis. Among the enzymes involved in cleavage process the invertase pathway is directed towards growth and cell expansion, while the sucrose synthase pathway is associated with storage product biosynthesis (Quick and Schaffer, 1995). Invertase mediated cleavage which takes place in placenta-chalazal cells of developing embryo is considered as a necessary step

in either carbohydrate transport out of the vascular system or into the endosperm (Cheng *et al*.1996). Invertase activity, which generates high concentrations of hexoses in the embryo, appears to be crucial for early seed development in plants like sorghum, pea, *Vicia* etc. This contention is further supported by the studies in the miniature mutant (mn-1), characterized by absence of this enzyme; seed growth is inhibited in the early stages of development. During this phase of development, the final number of cells of the storage organs, cotyledons and/or endosperm is established. Exogenous feeding of high levels of sucrose stops seed growth (pea and faba bean) concomitant with starch accumulation and the transcription of storage protein genes (Wang and Hedley, 1993; Weber *et al.* 1996). Exogenous supply of hexose sugars to faba bean storage phase cotyledons under *in vitro* conditions is reported to increase sucrose: starch ratio by inducing sucrose-phosphate synthase whereas free amino acids and storage proteins legumin B mRNA levels are lowered (Weber *et al*. 1996). Therefore, it evinces that hexose status of the premature embryo, as controlled by cell wall bound invertase favours cell division. The transition from the cell division phase to cell maturation or storage phase in the embryo is accompanied by a decline in hexose to sucrose ratio and a dramatic increase in the fresh weight. When the enlarging embryo comes in contact with the seed coat, the inner cell rows of the thin walled parenchyma become degraded and invertase activity declines . This is associated with switch to storage function. Seed tissues actively engaged in storage often exhibit low acid invertase activity concomitant with high sucrose synthase activity, a marker of sink strength (Quick and Schaffer, 1996). Sucrose-phosphate synthase activity, generally associated with non photosynthetic tissue is thought to be involved in resynthesis of sucrose and this built up of sucrose leads to accumulation of dry matter in *V. faba* and pea cotyledons. Storage of starch is a function of the concentration of sucrose in the wheat endosperm. Thus, in a storage organ, storage product is synthesized via a sucrose synthase pathway. Some seeds do contain alkaline invertase. However, its activity is lower than the sucrose synthase and increases during the last stages of development (Ross *et al*. 1996). Though alkaline invertase is active, sucrose synthase appears to make greater contribution. Weber *et al*. (1995) reported expression of cell wall bound invertase gene in the thin walled seed coat-the region of phloem unloading. Such an accumulation of hexoses leads to differentiation of organs from the embryo.

Starch biosynthetic activity within seed is a major determinant of sink strength. ADP-glucose pyrophosphorylase (AGPase) is the rate limiting step in starch biosynthesis (Weber *et al*., 2005); an increase in AGPase activity within the cotyledons could increase the strength of the developing seeds. AGPase activity in turn depends upon the formation of glucose-1-phosphate by UGPAse (UDP- pyrophosphorylase). When sucrose concentration is built up in the developing cotyledons after the initial mitotic activity during the first few days of seed development, sucrose synthase (SuSy), AGPase and UGPase may become important in determining sink strength of developing seeds. The interdependence of these enzymes is evident from the following reactions they catalyse.

UDP + Sucrose---------→UDP-Glucose+ Fructose

UDP-Glucose+ PPi---------→Glucose-1 phospate + UTP

Glucose-1 phospate + ATP---------→ADP-glucose+ PPi

Although abovementioned reactions are reversible, continuous utilization of ADP-glucose for starch synthesis makes the reactions to proceed unidirectional.

Sugar species are known to have profound effects on seed development, with hexoses sustaining mitotic index while sucrose induces expression of the biochemical machinery responsible for storage product biosynthesis (Borisjuk *et al*. 2003). Sucrose released from seed coats is taken up from the seed apoplasm by the abaxial epidermal transfer cells, subepidermal cells and one or two layers of underlying storage parenchyma cells of pea cotyledons (Rosche *et al*., 2002). Culturing of cotyledons with their adaxial epidermal surface in contact with agar medium supplemented with sucrose level identical to those found in the seed apoplasm, yields growth rates comparable to *in planta* rates (McDonald *et al*., 1995). It evinces that nutrients enter through adaxial epidermis. Hayati *et al*., (1996) concluded that sugars are essential for cotyledon growth and amino acid metabolism is unable to compensate for their absence.

PHYTOHORMONES- REGULATORS OF SUGAR METABOLOISM AND TRANSPORTERS

Phytohormones have been implicated in the regulation of sugar metabolism and/or transport (Finkelstein and Gibson, 2002; Leon and Sheen, 2003). Among phytohormones, cytokinins (CKs) are well known to be regulator of source /sink interactions. CKs have been shown to induce expression of cell wall invertase and a hexose transporter (Ehnab and Roitsch, 1997) in *Chenopodium rubrum*. Similarly, ABA plus glucose treatment to the germinating grains of rice induces higher accumulation of sugars in the scutellum than treatment with glucose alone, suggesting stimulatory role of ABA in glucose uptake. Kashem *et al*. (1998) observed ABA mediated stimulation of sucrose formation. ABA and gibberellins are also known to regulate sugar concentrations by altering α-amylase levels, thereby affecting the rate at which sugars are produced from starch. The effect of ABA on mitotic activity appears to be opposite of glucose during early cotyledon development. As we know that glucose levels are highest in mitotically active cells while increase in the level of ABA promotes cell cycle arrest. On the other hand, ABA appears to promote accumulation of storage reserves in the later development. Early and like sucrose midphases of maturation are dominated by the action of ABA, initially synthesized in the maternal tissues and later on, to a lower extent, in the embryo and endosperm (Nambara and Marion-Pall, 2003). Transcription of major seed storage protein genes occurs mainly during this period. Subsequently, ABA level declines and late embrogenesis abundant (LEA) proteins associated to dehydration process and acquisition of desiccation tolerance. During this stage, accumulation of storage metabolites followed by accomplishment of quiescent stage and dormancy until the environmental conditions are congenial for growth. The mechanism by which sugars affect phytohormone response is by altering the levels, localization and/or transport of different phytohormones. Recent studies have convincingly proved that maturation is not determined solely by ABA but instead by the ABA/GA balance. Sucrose or glucose has long been known to inhibit gibberellin synthesis or release from barley scutellum

(Radley, 1969) and exogenous application of glucose has been shown to decrease gibberellins in rice also (Yu *et al*. 1996). In addition, sucrose has been postulated to affect gibberellins response by affecting gibberellin conjugation (Simko, 1994). Conjugated gibberellins contain glucosyl moieties that are obtained from UDP-glucose (Sembdner *et al*. 1991) and UDP-glucose is generated primarily from sucrose via sucrose synthase. Thus, sucrose may act by increasing the amount of UDP-glucose leading to increase in conjugated gibberellins and a decrease in bioactive free gibberellin (Simko, 1994).

The role of auxins in cell division and cell elongation is well documented. In pigeon pea, free IAA level remains low in the initial stages of seed development which increases gradually during dry matter accumulation phase and decline thereafter during seed maturation. IAA is known to cause loosening of the cell walls, thus resulting in increased water uptake and cell enlargement. The ratio of conjugated/free IAA decreases with seed maturation indicating that during cell division and cell elongation phase cells hydrolyzed conjugated IAA to free forms, while at later stages the IAA is stored in conjugated forms. Similarly, conjugated IAA exhibits strong correlation with seed water content. Generally, the major portion of IAA present in leguminous seeds is in amide linked forms (Walz *et al*. 2002). Bialek and Cohen (1989) also demonstrated disappearance of free IAA during seed maturation of bean seeds whereas amide-linked IAA increased and became the major part of IAA.

SUGAR TRANSPORT SYSTEM IN THE DEVELOPING SEED

As mentioned earlier, most nutrients imported into seed through the phloem reach the symplasmically isolated filial tissues (endosperm/embryo) following their release and accumulation in the seed apoplasm. These are retrieved from the apoplast by specialized cells located at the maternal/filial interface which then move to the storage site. Because of the absence of symplastic linkages between maternal and filial tissues, partitioning of post-phloem assimilate requires membrane located transport mechanisms. Transporters involved in retrieval of sucrose from the apoplast by the embryo or endosperm have been isolated and characterized in faba bean (Weber *et al*., 1997a,b), pea (Tegeder *et al*., 1999), rice (Hirose *et al*., 1997) and barley (Wescheke *et al*.,2000). Sucrose tranporter gene has been found to be expressed in expanding cotyledon using an H^+/sucrose symporter antisenseriboprobe. Sucrose/H^+ symport plays an important role in the accumulation of storage product and rates of gain in dry matter during seed development and transport activity in *V.faba* are strongly correlated (Harrington *et al.,* 1997a,b). Enhanced uptake of sugars by the filial tissues leads to a decline in osmomolatity of the apoplasmic sap leading to an increase in turgor of seed coat cells (Patrick and Offler, 2001). Strong evidences suggest that sucrose transporters (SUTs) are involved in import of sucrose present in the cell wall space of leaves into the phloem conduit (sieve element and companion cell complex, SECCC). Moreover, they appear to be responsible for import of sugars into developing seeds. What is unknown is how metabolic demand is communicated to cotyledon sucrose transporters responsible for withdrawing sucrose from the apoplasmic space? Zhou *et al* (2009) explored this question by employing rugosus mutant of pea (rrRbRb) for starch biosynthesis compared to its wild

counterpart (RRRbRb). Sucrose influx was found to account for 90% of developmental variations in the absolute growth of cotyledons and hence starch biosynthetic rates. Moreover, rr and RR cotyledons shared identical response surfaces thereby indicating that the control of transporter activity is likely to be identical for both lines.

Zhou et al. (2007) have shown that sucrose uptake by pea cotyledons is mediated by the combined activities a sucrose/proton transporter (PsSUT1) and two sucrose facilitators (PsSUF1 and PsSUF4). Expression of PsSUT1 was inhibited by sugars while those of PsSUF1 and PsSUF4 remained unaffected and enhanced, respectively. *In vitro* studies have clearly evinced that intracellular sucrose was the physiologically active sugar signal that communicated demand to sucrose influx and this transport function was primarily determined by PsSUT1 regulated at the transcriptional level. The positive relationship between transcript levels of PsSUT1 and sucrose transport suggests a primary role for PsSUT1 in sucrose uptake by cotyledons. Negative correlation between sucrose facilitators genes with sucrose uptake activities are related to sucrose binding proteins (a putative sucrose facilitator) expressed in French beans (Tegeder *et al*.,2000). The physiological significance of whole cotyledon expression depends upon cellular localization patterns, with cells proximal to the abaxial surface accounting for 70% of sucrose influx (Tegeder *et al*. 1999, Rosche *et al*., 2002). Transport activities of sucrose facilitators have been distinguished by the latter's selective sensitivity to membrane impermeant sulphydryl reagent, diethypyrocarbonate. Patrick group also identified a set of sucrose transporter homologues called SUFs (Sucrose facilitators) that has apparently lost the coupling of sucrose to protons, a feature common to all other members of SUT family. Therefore, SUFs are the potent candidates for facilitating efflux from seed coat along the concentration gradient into the space surrounding embryo (Zhou *et al*, 2007). Recently, Rosche *et al*. (2002) and Baud *et al*. (2005) provided evidences that these genes are also responsible for the import of sucrose into the developing seeds. Induction of cell wall ingrowths which increase the surface area of plasma membrane of the cotyledons is essential for nutrient flux into pea embryo and these ingrowths are induced by sugars (Harrington *et al*., 1997a, b, Patrick and Offler, 2001, Wardini *et al*. 2007a, b). Wall ingrowths formation is preceded by extensive vesiculation and laying down of a smooth wall. In all cases, a mitochondrial rich dense cytoplasm dominated by mitochondria is often aligned to the plasma membrane and an extensive network of rough endoplasmic reticulum. Different strategies are exhibited to enhancethe plasma membrane surface area required for nutrient exchange. In broad bean and wheat, plasma membrane surface area is amplified as a consequence of development of extensive wall ingrowth labyrinths in contrast release cells of French bean do not develop wall ingrowths. A plasma membrane bound isoform of sucrose synthase is known to play central role in supplying UDP-glucose units to cellulose rosettes embedded in plasma membrane for cellulose synthesis (Haigler, 2001) and this enzyme has been shown to be localized to transfer cells in developing seeds of cotton (Ruan *et al*., 2003). Sucrose is a substrate for sucrose synthase and thus sucrose provides a more direct biochemical route to cellulose biosynthesis than hexose, where a metabolic cost is exacted for sucrose synthesis through sucrose phosphate synthase induced by hexoses (Weber *et al*., 1996). Thus, sucrose could contribute to wall ingrowths formation both as a substrate for cellulose biosynthesis

and as a putative developmental signal controlling expression of sucrose synthase. Functional SUTs are detected in these epidermal transfer cells in another 24h. Continuous culture of cotyledons on a medium containing 6-methyl purine completely blocked wall ingrowth formation. Delaying of 6-methyl purine exposure by 1h at the start of culture period, the adaxial epidermal cells are reported to contain small ingrowths. Treating cotyledons for 1h with 6-methyl purine at 15h following cotyledon excision halted further wall ingrowths development. It thus vividly evince that transfer cell induction is rapid and that signaling and early events leading to wall ingrowths formation relied on gene expression. Moreover, these genes products have a very high turnover rate. Conversely, adaxial epidermal cells of cotyledons which do not form ingrowths can be induced to develop wall ingrowths upon culturing on 100 mM sucrose within 24 h (Farley *et al* 2000, Wardini *et al.,* 2007a). This *in planta* induction of their abaxial epidermal cells occurs during an extremely dynamic stage of seed development and Rosche *et al*. (2002) confirmed that SUT are crucial players for uptake of sucrose into developing seeds. At this stage , endosperm reserves are approaching depletion and the activity of an extracellular invertase is abolished by the expanding cotyledons crushing the abutting seed coat tissues in which invertase is expressed (Weber et al. 1995). Concurrently, rates of sucrose delivery to seed apoplasm increases and the sugar composition of the seed apoplasm changes from hexose to sucrose ratio of approx. 100: 1 to 3:1 (Offler *et al*. 1997). This altered sugar ratio is considered to be a signal for transfer cell formation

The dual response of sucrose transporter expression in faba beans (Weber *et al*., 1997a) or pea (Zhou *et al*., 2009) cotyledons to sucrose and hexoses is anticipated to result from a number of factors. Glucose could be the active signal (Rolland *et al*., 2002) and the link with the sucrose is simply as a substrate supplying glucose moieties through intracellular hydrolysis by invertase or sucrose synthetase. Alternatively, sucrose may be active signal and correlation with hexoses reflects sucrose pool size available for hydrolysis (Zhou *et al*., 2009) or externally supplied glucose is converted in to sucrose through storage product synthesis (SPS) pathway (Weber *et al*., 1997b). Moreover, sugars could be sensed at cotyledon plasma membrane or intracellularly (Rolland *et al*., 2006). *In vitro* studies of Zhou *et al*., (2009) in pea have evinced that enhance uptake of sucrose by cotyledons is linked with lowered intracellular sucrose concentrations which were corroborated with unaltered levels of hexoses. Further, sucrose functions as a regulatory signal of symporter activity which is mediated by repression of PsSUT1 transcription by intracellular sucrose concentration. Contradicting a potential role for sucrose-H+ symporters in the efflux from the phloem is evidence for nonspecific passive efflux of disachharides and other small solutes to the apoplast. Long distance transport of galactnol was limited upon its synthesis in minor veins of phloem and substantial quantities were present in the lamina and apoplast of mature leaves. It is opined that galactinol leaked from the phloem during transport and redistributed in the leaf by xylem transport (Ayre *et al.* 2003). Similarly the sucrose isomer palatinose, which is not recognized by sucrose transporters, demonstrated non specific transport across the membrane in transgenic tobacco expressing a sucrose isomerase enzyme (Bornke *et al*. 2002). However, culturing of cotyledons on medium containing palatinose, a non-

metabolizable and non-transportable analogue of sucrose (Atanassova *et al.,* 2003) did not exert any inhibitory effect on sucrose uptake or PsSUT1 expression by RR cotyledons. This clearly evinced that sucrose signal responsible for controlling sucrose transporter activity is not sensed by plasma membrane. Thus it is evident that metabolic demand for sucrose by starch and protein synthesis in developing pea cotyledons appears to be transmitted to cotyledon sucrose symporters as an intracellular signal that regulates sucrose symporter activity of PsSUT1 negatively at the transcriptional level.

The AtSUC family comprising of nine putative sucrose transporter genes is the largest SUC family identified to date. The question arises why so many carriers are present in this plant. Apart from substrate specificity, a possible explanation might be that genes are regulated differentially in specific cell types. Accordingly, these transporters have been assigned different roles ranging from control of osmotic driving force (Stadler et al. 1999), response to wounding (Meyer et al. 2004), loading of sugars into sieve elements (Stadler and Sauer, 1996) to sugar sensing (Barker *et al*. 2000). AtSUC5 is the only member of AtSUC family to exhibit a marked seed specific expression pattern. It has been shown to be involved in post phloem sucrose transport in the developing seeds while AtSUC2 *functions* in the loading of sugars from the apoplast in to the conductive sieve elements (Baud *et al.* 2005). Most of these energy dependent sucrose-H+ transporters are localized in companion cells.

From the foregone discussions, the whole process of sugar transport and control of seed development can be generalized and summarized as shown in Fig. 1 along with the transporters involved

CONCLUSIONS AND FUTURE PROSPECTS

From the above explained account, it is evident that important contributions have been made on different aspects of seed maturation, nutrient flow in developing seed, metabolism of storage compounds, identification of key regulatory elements or transcriptosome analysis of the process at the whole genome scale etc. Movement of sucrose occurs through extensive symplasmic compartments in both maternal and filial tissues with nutrient exchange between them localized to the maternal /filial interface. How does sucrose enters the apoplast in the source tissues in preparation for loading and in sink tissues during unloading is not clear, since a clear efflux carrier has not emerged (Lalonde et al. 2003; Sauer 2007). The mechanism of symplasmic transport is yet to be resolved. Transport and transfer processes in maternal symplasm both quantitatively and qualitatively modulate nutrient release to the filial tissues. The dynamic of buffering nutrient supplies from short term storage needs more attention. Walker *et al*. (1999) however, made some progress in identifying cellular compartments in which amino N metabolism occurs. Unequivocal elucidation of transport mechanism responsible for nutrient release to the seed apoplasm and their physiological significance will depend on physiological and molecular approaches adopted. Genetic and molecular studies have started to illuminate how products of genes interact to initiate seed development. Imprinting or differential expression of paternal and maternal genes seem to be involved in controlling seed development, presumably by controlling gene expression in developing endosperm. The discovery of FIS genes has

illuminated control of autonomous endosperm development which is an important developmental and agronomical trait. Fertilization Independent Seed Development (FIS) genes are targets of imprinting and the genes they control in developing endosperm are also regulated by DNA methylation and chromatin remodeling genes. Another key and recurrent issue concerning seed development is the differences in embryo and endosperm, linked to the still controversial origin of the later (Berger, 2003). Involvement of key regulators of maturation programmes is significantly similar in the embryo and aleurone tissue of the endosperm. A deeper insight of maturation in both systems and the influence of maternal and dosage effects is anticipated to shed more light on this query. Carbohydrate and nitrogen metabolic pathways are connected and therefore, the identification of branch points will help to clarify the changes of assimilates partitioning between starch and proteins. The identification of nutrient sensing mechanisms and signal transduction pathways will be another challenge. Sugars as well as nitrogen are signal molecules and it will be interesting to elucidate how these metabolic signals are involved in seed development. Apoplasmic hexoses could be sensed by by plasm membrane receptor, and it will be worthwhile to see whether a hexose transporter itself can serve a sensor as shown for glucose transporter in yeast (Ozcan *et al*. 1996). Cloning of sucrose and amino acid transporter genes provides opportunities to evaluate their role in regulating nutrient flows in to filial tissues of seed.

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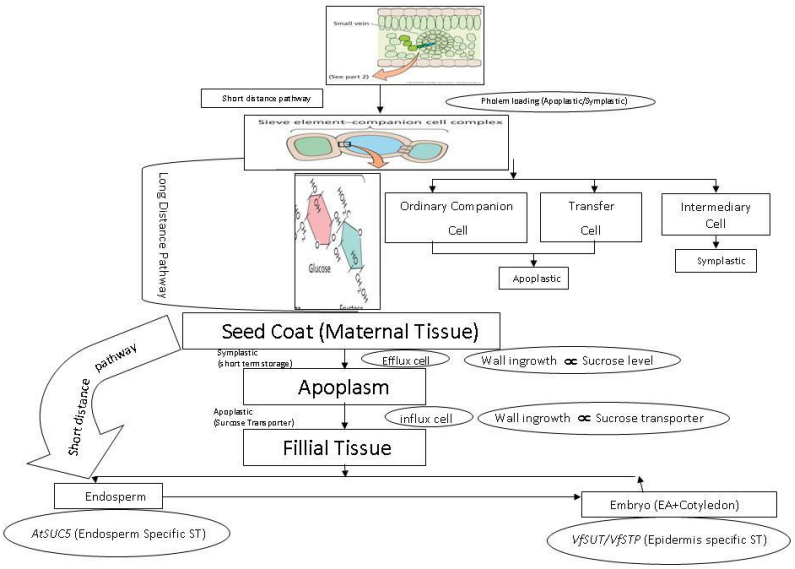

Fig.1 Summary information of sugar transport during seed development and associated transporters.