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# Plant Responses to Sugar Starvation

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Additional information is available at the end of the chapter

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## 1. Introduction

The production of carbohydrates via photosynthesis is the most fundamental activity in plant life. Carbohydrate synthesis, transport, utilization, and storage are dynamic processes, strongly dependent on cell physiology, plant organ, environmental conditions, and developmental stage of the plant. The plant's ability to monitor and respond to the level of carbohydrates may act as a controlling mechanism, integrating the influence of environmental conditions (e.g. light, nutrients, biotic and abiotic stress factors) with internal developmental programs, controlled directly by hormones (Koch 2004, Rolland et al. 2006, Hammond and White 2008, Loreti et al. 2008, Ramon et al. 2008, Agulló-Antón et al. 2011). Studies conducted in recent years have provided an extensive body of information on the participation of carbohydrates in metabolic reactions of plant cells. In plants, sugars are essential as respiratory substrates for the generation of energy and metabolic intermediates that are then used for the synthesis of macromolecules. Binding to sugar is required for proper functioning of many proteins and lipids. Moreover, carbohydrates have important hormone-like functions as physiological signals, which cause activation or repression of many plant genes, and this in turn leads to specific metabolic effects. Sugar-signaling networks have the ability to regulate directly the expression of genes and to interact with other signaling pathways. The progress in research on molecular mechanisms of sugar sensing and signaling in plants shows that signal molecules include glucose, fructose, sucrose, and trehalose (Jang and Sheen 1994, Koch 1996, Müller et al. 1999, Rolland et al. 2002, Koch 2004, Gibson 2005, Gonzali et al. 2006, Rolland et al. 2006, Ramon et al. 2008, Rosa et al. 2009, Cho and Yoo 2011). Plants have developed effective mechanisms of perception and transduction of sugar signals. Perception of the sugar signal may take place already in the apoplast during transport across membranes or within the cell, in the cytosol. Sugar signal perception and transduction may involve cell wall invertases (CW-INV), sucrose and glucose transporters (and specific sugar receptors), and hexokinase (HXK) (Sheen et al. 1999, Smeekens 2000, Loreti et al. 2001, Rolland et al. 2002, Harrington and Bush 2003, Moore et al. 2003, Sherson et al. 2003, Koch 2004, Rolland and Sheen 2005,

Rolland et al. 2006, Ramon et al. 2008, Cho et al. 2009, Hanson and Smeekens 2009, Smeekens et al. 2010). Additionally, biochemical studies provide evidence for the involvement of a variety of protein kinases, i.e. Snf1-related kinases (SnRKs) (Rolland et al. 2006, Smeekens et al. 2010), calcium-dependent protein kinases (CDPKs), mitogen-activated protein kinases (MAPKs), and protein phosphatases, 14-3-3 proteins, Ca<sup>2+</sup> ions as a second messenger, and G-proteins (Rolland et al. 2006) in sugar signal transduction (Rolland et al. 2002, Sinha et al. 2002). However, it must be stressed that HXK plays a significant role as a component of the sugar sensing machinery. Genetic analyses have revealed a central role for HXK as a conserved glucose sensor (Moore et al. 2003, Harrington and Bush 2003, Rolland and Sheen 2005, Rolland et al. 2006, Ramon et al. 2008, Cho et al. 2009, Hanson and Smeekens 2009, Smeekens et al. 2010). HXK sensing and signaling functions are probably dependent on HXK's subcellular localization, translocation, and/or interactions with downstream effectors. The HXK sugar sensor, as a cytosolic protein or associated with mitochondria or other organelles, then could activate a signaling cascade through HXK-interacting proteins (HIPs) or affect transcription directly after nuclear translocation (Rolland et al. 2002, Rolland et al. 2006, Hanson and Smeekens 2009). Various sugar signals activate many HXK-dependent and HXK-independent pathways and use different molecular mechanisms to control transcription, translation, protein stability, and enzymatic activity. It has been shown that in 7-day-old *Arabidopsis* seedlings, glucose (3%) during 6 h increased the expression of 983 genes and decreased the expression of 771 genes (Li et al. 2006). Sucrose (3%) in *Arabidopsis* cell suspension during 48 h modulated the expression of 243 genes in light and 193 genes in darkness (Nicolai et al. 2006). Glucose-regulated transcription factors (TFs) account for 8.3% (82 factors among 978) of all glucose-regulated genes, where most of TFs showed repression (Price et al. 2004).

Genes encoding the enzymes of the phenylpropanoid biosynthesis pathway (Hara et al. 2003, Solfanelli et al. 2006, Morkunas et al. 2011) as well as sink-specific enzymes, such as sucrose synthase (Salanoubat and Belliard 1989), granule-bound starch synthase (Visser et al. 1991), and extracellular invertase (Roitsch et al. 1995), are induced by sucrose or glucose. Sugar-induced gene expression has also been detected for enzymes involved in pathogen and stress response, such as proteinase inhibitor II of potato (Johnson and Ryan 1990), chalcone synthase (Tsukaya et al. 1991), and flavonoid biosynthetic enzymes (Morkunas et al. 2011). In contrast, sugar repression of photosynthetic genes was observed. For example, genes encoding photosynthetic proteins, e.g. the small subunit of the Calvin cycle enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) and the chlorophyll *a* binding protein, are repressed by carbohydrates (Sheen 1990, Krapp et al. 1993).

Due to their regulatory and signal function, sugars affect all phases of the plant life cycle by controlling the number of essential metabolic processes. In animals, the level of sugar in cells is strictly controlled, but in plants the level and composition of carbohydrates varies widely, depending on tissue type and environmental conditions. An excess or loss of carbohydrates or their derivatives triggers various reactions in plants and significantly affects the metabolism, growth, and development. Moreover, all abiotic and biotic stress responses are regulated, at least in part, by sugars (Koch 1996, Rolland et al. 2002). Plants are

generally considered to be autotrophs but sometimes they can be heterotrophs, e.g. at some stages of development (such as seed germination, as long as the seedling grows in darkness, before it emerges above the soil surface) and in non-photosynthetic organs, such as most roots, stems, and flowers. In germinating seeds, in periods of unfavorable environmental conditions or after too deep sowing, mobilization of storage materials in cotyledons may be delayed or there may be some disturbances in distribution of carbohydrates. This may lead to a decrease in available carbohydrates in embryo axes (i.e. sugar starvation) and in a lower seed germination rate. Additionally, high carbohydrate losses can be observed in most plant species under the influence of environmental conditions, such as water or temperature, attack of pathogens or herbivores. These factors may lead to a remarkable decrease in photosynthetic rate in donor tissues (i.e. leaves that synthesize and export carbohydrates), and this reduces the supply of carbohydrates to acceptor tissues (i.e. non-photosynthetic tissues importing carbohydrates for respiration, growth, and development). Besides, in some conditions, e.g. during dormancy or leaf shedding, photosynthesis is switched off or slowed down. In such conditions, the stored carbohydrates must be used, so their reserves may be greatly diminished in non-photosynthetic tissues then. Knowledge of the response to sugar starvation and of adaptive mechanisms in plants is both fundamental and agronomically important.

## 2. Materials and methods of research on sugar starvation

Carbohydrate starvation has been studied in many of plant species, e.g. in common wheat (Wittenbach 1977, Wittenbach et al. 1982), maize (Saglio and Pradet 1980, Pace et al. 1990), barley (Farrar 1981), pearl millet (Baysdorfer et al. 1988), pea (Webster and van't Hof 1973, Sahulka and Lisa 1978, Webster and Henry 1987, Morkunas et al. 1999, 2000), soybean (Kerr et al. 1985, Walsh et al. 1987), sycamore (Journet et al. 1986, Dorne et al. 1987, Roby et al. 1987, Genix et al. 1990), tobacco (Moriyasu and Ohsumi 1996), lupine (Morkunas et al. 1999, Borek and Ratajczak 2002, Morkunas et al. 2003, Borek et al. 2006, Borek and Nuc 2011, Borek et al. 2011, 2012a, 2012b), *Arabidopsis thaliana* (Rose et al. 2006). Metabolic changes caused by sugar starvation are usually analysed in cell cultures, callus tissue cultures, and in isolated plant organs cultured *in vitro*. In all types of the cultures it is easy to control nutrition of the plant material by manipulating the medium composition. Cell cultures clearly react by metabolic changes when sugar is omitted from the nutrient medium, e.g. in sycamore (*Acer pseudoplatanus*) cells (Journet et al. 1986, Gout et al. 2011), but similar sensitivity is observed also in excised root tips (Brouquisse et al. 1991, 1992). *In vitro* cultured embryo axes (isolated from seeds), cut off from the natural source of nutrients, i.e. cotyledons, are a good model for research on the effect of sugars on seedling development and metabolism (Borek et al. 2001, Borek and Ratajczak 2002, Borek et al. 2003, 2006, Borek and Nuc 2011, Borek et al. 2012a, 2012b), or even for research on the role of sugar in plant resistance to pathogens (Morkunas et al. 2005, Morkunas and Gmerek 2007, Morkunas et al. 2007, Morkunas and Bednarski 2008, Morkunas et al. 2008, 2011). The influence of sugar on leaf senescence was assessed on excised leaves and on leaves of intact plants. The effect of sugar starvation was achieved by keeping the plant material in darkness or by its spraying

with photosynthetic inhibitors (Mohapatra et al. 2010). To examine the effects of sugar on senescence of various flower parts, cut flowers were treated with various sucrose concentrations in the vase solution (Azad et al. 2008, Arrom and Munné-Bosch 2012). Brouquisse et al. (1998) show that in roots (the main sink organs of maize plants) the consequences of carbon depletion induced by extended darkness are identical to those observed in the excised root tip, but some aspects of sugar starvation (e.g. effect on translocation of assimilates, links with the metabolism of nitrogen and phosphorus, interactions with plant hormones) were investigated in intact plants (Ciereszko et al. 2005). By spraying of sugar-starved plants with various sugars, it can be determined which of them are signal molecules and which of them are sugar sensors (Lothier et al. 2010).

Particularly valuable information is obtained as a result of research on various types of mutants, which sometimes supplies surprising information about the role of sugars as signal molecules (Gibson 2005, Ramon et al. 2008, Usadel et al. 2008, Hanson and Smeekens 2009).

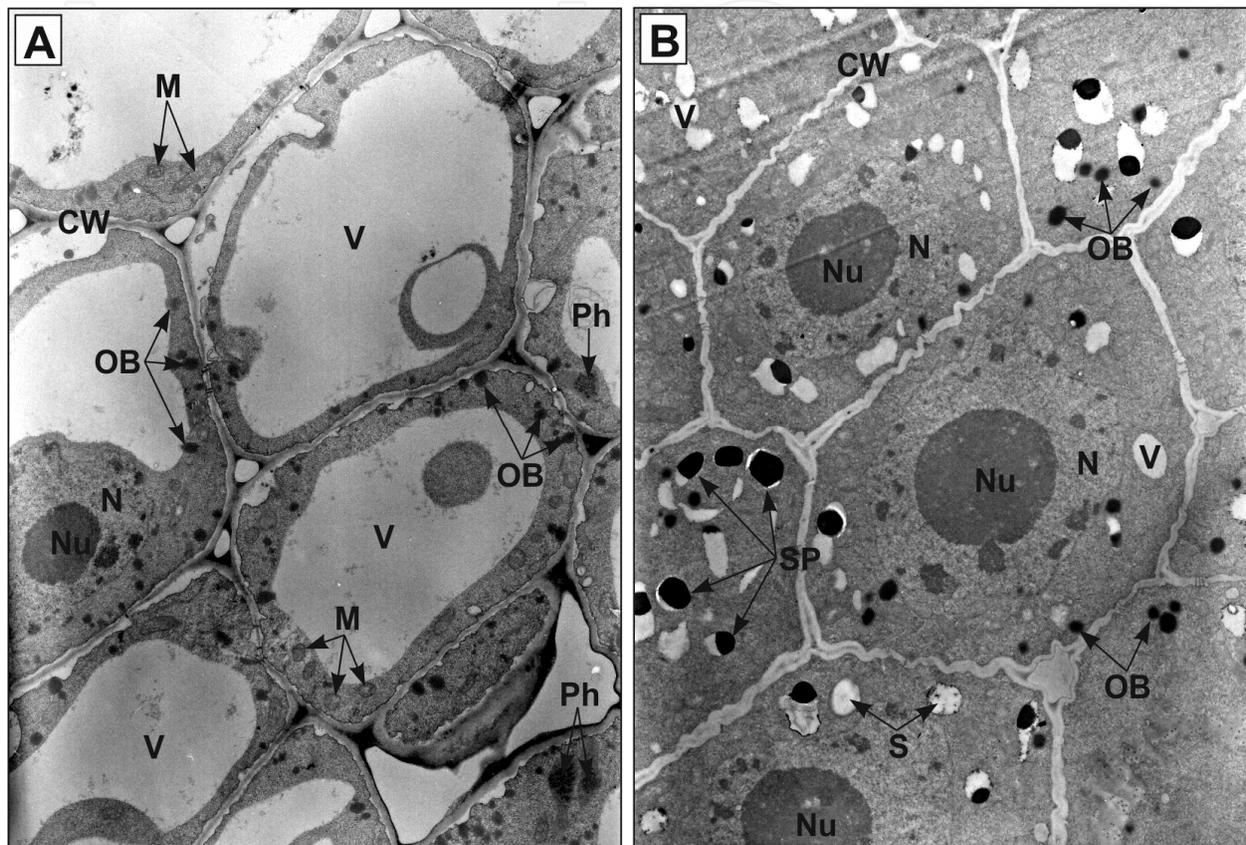
Plant reactions to sugar deficits have been studied with the use of a wide range of research methods: electron microscopy (Borek and Ratajczak 2002, Borek et al. 2006, 2011, 2012a), confocal microscopy (Morkunas and Bednarski 2008, Morkunas et al. 2011, 2012), enzymatic activity assays and metabolite assays, e.g. by using HPLC (Morkunas et al. 2010), nuclear 1HNMR spectroscopy (Brouquisse et al. 2007, Kim et al. 2007), and <sup>13</sup>C and <sup>31</sup>P-NMR spectroscopy (Vauclare et al. 2010, Gout et al. 2011). Electron paramagnetic resonance (EPR) spectroscopy is applied to measure free radicals (Morkunas et al. 2004, Morkunas and Bednarski 2008, Morkunas et al. 2008, 2012). Novelty includes various methods of molecular biology: assays of gene expression (Buchanan-Wollaston et al. 2005) and application of reporter genes (Lee et al. 2007). Moreover, sugar-starvation-induced promoters have been used to obtain useful recombinant proteins (Xu et al. 2011a, 2011b).

### 3. Morphological, anatomical, and ultrastructural changes under sugar starvation

Sugar starvation causes changes in growth, as shown in organ cultures *in vitro* and in cell suspensions. For example, significant differences are observed in morphological structure of embryo axes of narrowleaf lupine (*Lupinus angustifolius* L.), white lupine (*Lupinus albus* L.), yellow lupine (*Lupinus luteus* L.), Andean lupine (*Lupinus mutabilis* Sweet) and garden pea (*Pisum sativum* L.) cultured *in vitro* under sugar starvation and with 60 mM sucrose. Radicles of *Pisum* and *Lupinus* spp. cultured under sugar starvation were shorter and thicker than sugar-fed radicles. The length and fresh weight of starved isolated embryo axes was about 2-fold lower than those of sugar-fed axes (Morkunas 1997, Borek et al. 2012a). In contrast to lupine isolated embryo axes, lupine seedlings were much bigger when they were grown on medium without sucrose (Borek et al. 2012a).

Light micrographs of radicle cross sections revealed that starved radicles were thicker than sugar-fed radicles. Cortical cells of sugar-fed embryo axes were rounded, with large

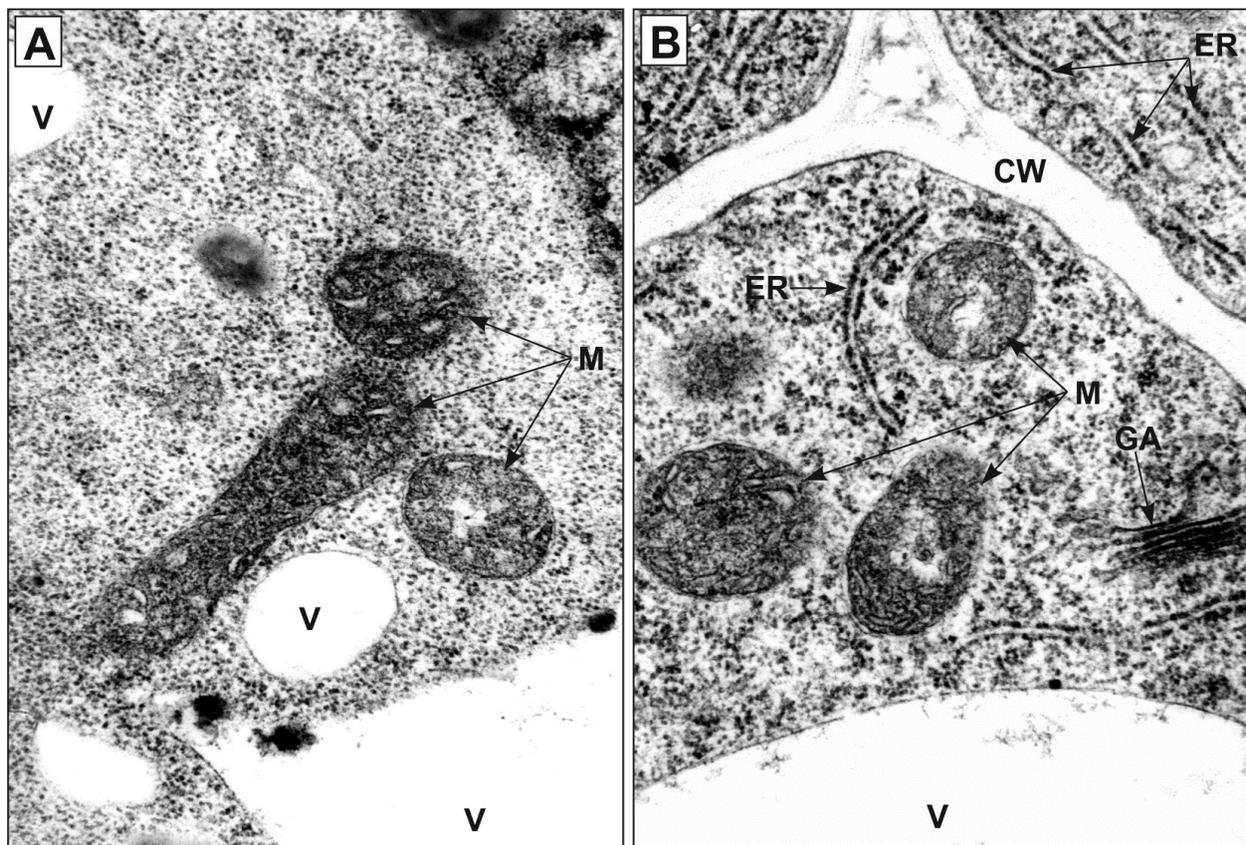
intercellular spaces. Many of the observed cells were dividing or have just divided. The cells lacked a central vacuole; instead, they contained very numerous small vacuoles. The major feature distinguishing cortical cells of starved lupine (as compared to sugar-fed lupine) was the presence of a large central vacuole with marginal cytoplasm.



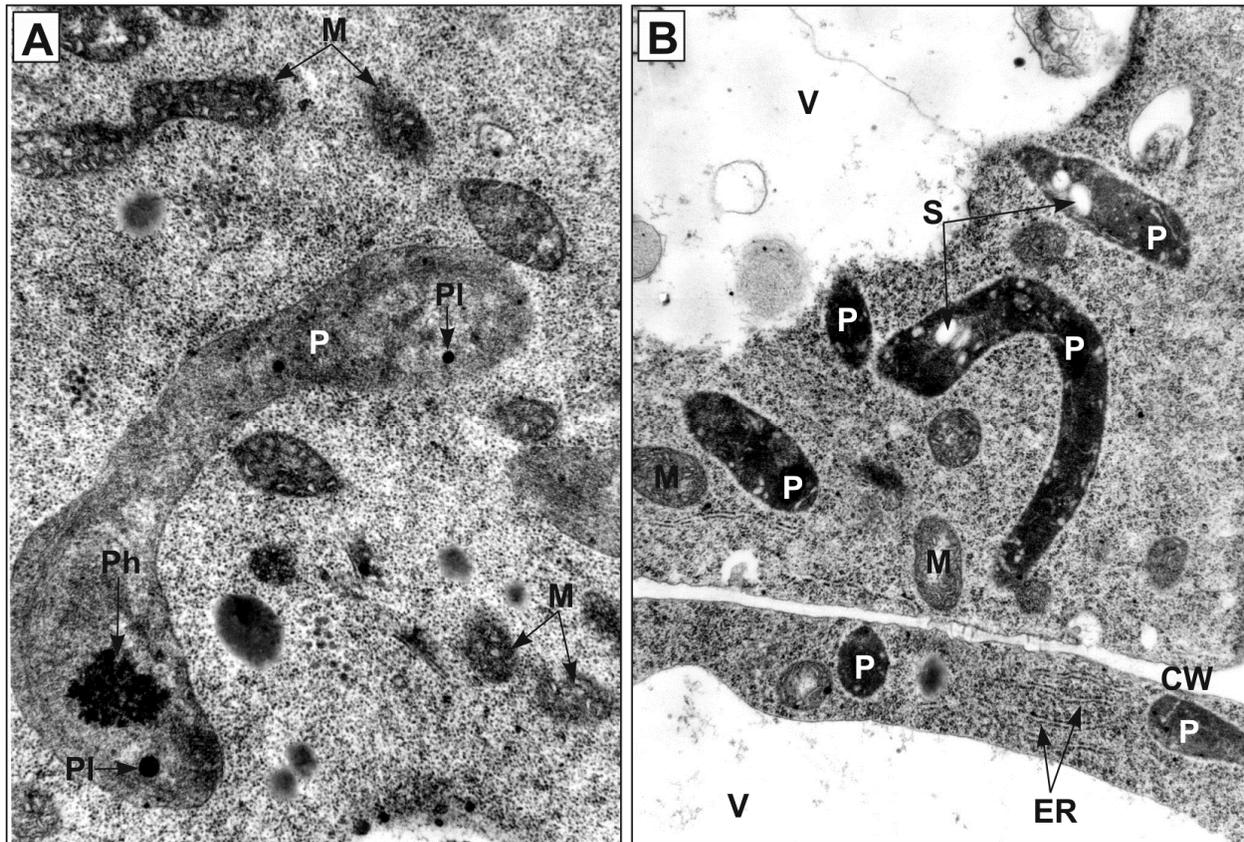
**Figure 1.** Ultrastructure of root meristematic zone cells of yellow lupine embryo axes grown for 96 h *in vitro* on medium without sucrose (A; sugar starvation) and on medium with 60 mM sucrose (B). CW cell wall, M mitochondrion, N nucleus, Nu nucleolus, OB oil body, Ph phytoferritin, S starch, SP storage protein, V vacuole.

Ultrastructural studies of the root meristematic zone of sucrose-starved lupine embryo axes showed that (in contrast to cells of embryo axes fed with sucrose) their cytoplasm, along with endoplasmic reticulum, mitochondria, plastids, ribosomes, and nuclei, were forced to the periphery by growing vacuoles (Fig. 1A, B). In spite of gradual autolysis of cytoplasmic proteins, in starved cells mitochondria were protected as organelles maintaining cellular respiration. Electron micrographs reveal that the inner mitochondrial membrane in starved pea cells is well developed, forming numerous cristae (Fig. 2A), in contrast to sucrose-fed cells, with less developed cristae (Fig. 2B). Oxygraphic studies of mitochondria isolated from starved embryos of *Pisum sativum* and *Lupinus angustifolius* have shown that they are active and exercise respiratory control (Morkunas et al. 2000, 2003). The phenomenon of mitochondria protection against autolysis under sugar starvation was observed also in other

species and plant tissues (Baysdorfer et al. 1988, Brouquisse et al. 1991). However, Couée et al. (1992) reported that under conditions of carbohydrate starvation, mitochondria could be degraded. Those authors revealed heterogeneity of mitochondria isolated from root tips of maize (*Zea mays*) and fractionated on Percoll density gradients, i.e. higher- and lower-density mitochondria. The higher-density mitochondria from glucose-starved maize root tips retained the ultrastructure and most of the respiratory properties of non-starved mitochondria. By contrast, lower- and intermediate-density mitochondria were absent in the mitochondrial fractions from glucose-starved maize root tips and were not detected *in situ*. Interestingly, in plastids of starved pea cells, large amounts of phytoferritin were accumulated (Fig. 3A), while only starch grains and tubular structures were found in plastids of cells with a high level of sucrose (Fig. 3B) (Morkunas 1997). Lowering carbohydrate level in storage tissues of germinating seeds enhances the breakdown of reserves. In cotyledons of 4-day-old lupine seedlings and in excised lupine cotyledons grown on medium without sucrose, the deposits of storage protein were clearly smaller than in organs fed with sucrose. Starch granules and oil bodies were also smaller and less numerous (Fig. 4A, B). Similarly, the cell walls, containing hemicelluloses (important storage compounds in lupine seeds) were thinner (Borek et al. 2006, 2011, 2012a).



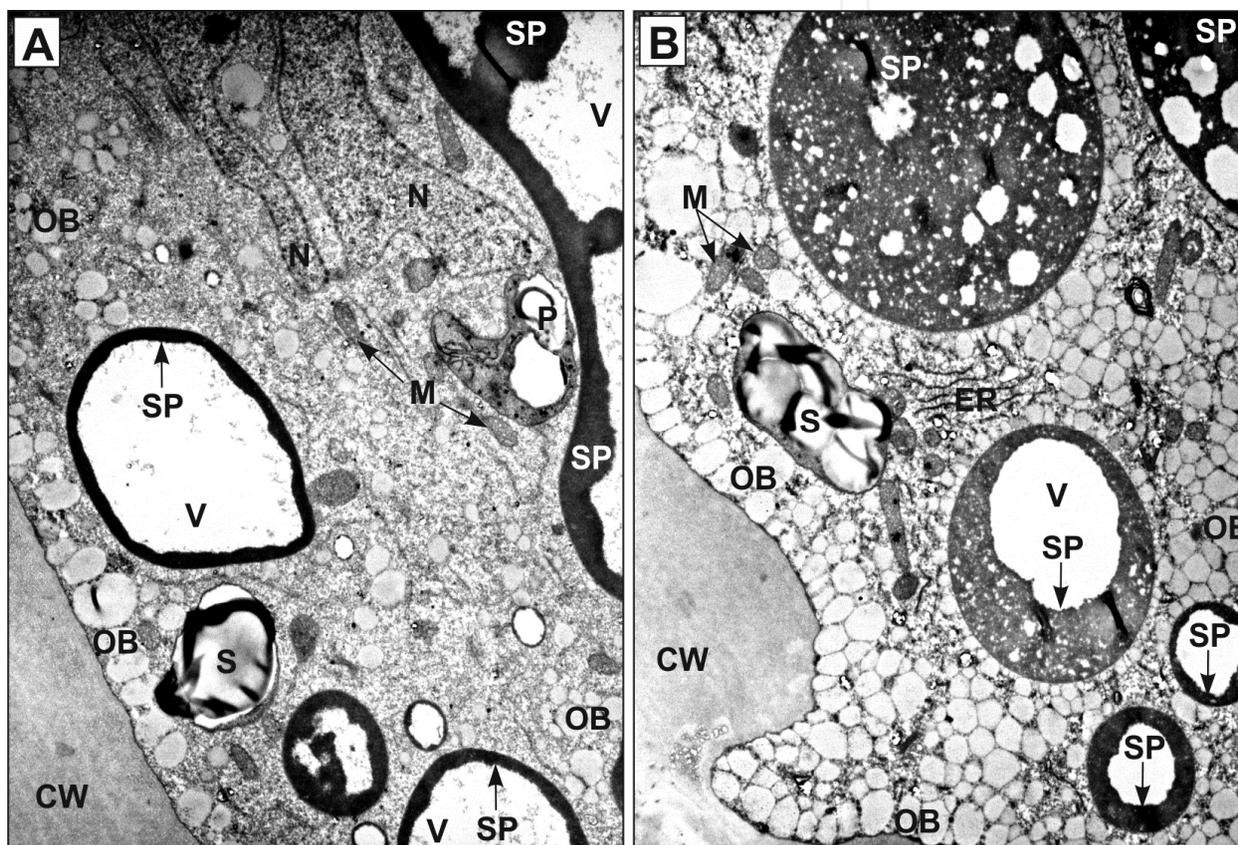
**Figure 2.** Mitochondria of pea embryo axes grown for 72 h *in vitro* on medium without sucrose (A; sugar starvation) and on medium with 60 mM sucrose (B). CW cell wall, ER endoplasmic reticulum, GA Golgi apparatus, M mitochondrion, V vacuole.



**Figure 3.** Plastids of pea embryo axes grown for 72 h *in vitro* on medium without sucrose (A; sugar starvation) and on medium with 60 mM sucrose (B). CW cell wall, ER endoplasmic reticulum, M mitochondrion, P plastid, Ph phytoferritin, PI plastoglobule, S starch, V vacuole.

Research conducted on *Arabidopsis* cell suspension revealed that sugar starvation caused an immediate arrest of cell growth, together with a rapid degradation of cellular proteins. Cell divisions, as indicated by cell number and accumulation of biomass, were stopped immediately after the initiation of sucrose starvation. Observations of the morphology of starved cells using Nomarski interference microscopy and confocal laser-scanning microscopy revealed that in sucrose-free medium, the width of the cytoplasm was markedly decreased, while the vacuole was enlarged (Rose et al. 2006). Carbon starvation induced autophagy in plant cells, as confirmed by experiments on model systems, e.g. on cell suspensions of sycamore (Journet et al. 1986), rice (Chen et al. 1994), *Arabidopsis* (Rose et al. 2006) and in pea and lupine embryo axes grown *in vitro* (Morkunas 1997, Morkunas et al. 2003, Borek et al. 2011, 2012a, 2012b). Vacuolar autophagy starts as early as several hours after sugar starvation. Sequestration of portions of the cytoplasm with organelles (except for the nucleus) by endomembranes results in formation of autophagic vacuoles, followed by their fusion with other vacuoles. During periods of nutrient starvation, the autophagic process can be reinitiated in plant cells that are already vacuolated (Chen et al. 1994, Aubert et al. 1996, Moriyasu and Ohsumi 1996, Moriyasu and Klionsky 2003, Moriyasu et al. 2003). Carbohydrate starvation-induced autophagy is associated with an increase in intracellular proteolysis (James et al. 1996, Moriyasu and Ohsumi 1996, Thompson and Vierstra 2005) and a marked degradation of membrane polar lipids (Aubert et al. 1996, Inoue and

Moriyasu 2006). In addition, Rose et al. (2006) revealed that concomitantly, the number of transvacuolar strands is decreased dramatically. Starvation-induced acidic compartments were most frequent in the dense perinuclear cytoplasm, close to the large central vacuole. In vacuoles of starved cells, quinacrine was strongly accumulated. The role of autophagy in adaptation to sugar starvation has been investigated primarily because of its impact on basal metabolism and biomass production. The intracellular degradation of cytoplasmic components by means of autophagy produces amino acids, phospholipids, and other elements that are necessary for basic metabolism and essential biosynthetic pathways. In starved cells the initiation of autophagy is needed to obtain respiratory substrates (Journet et al. 1986, Chen et al. 1994, Aubert et al. 1996).



**Figure 4.** Ultrastructure of parenchyma cells of white lupine excised cotyledons grown for 96 h *in vitro* on medium without sucrose (A) and on medium with 60 mM sucrose (B). CW cell wall, ER endoplasmic reticulum, M mitochondrion, N nucleus, Nu nucleolus, OB oil body, Ph phytoferritin, S starch, SP storage protein, V vacuole.

#### 4. Plant metabolism under sugar starvation

The studies have shown that in most cases, sugar starvation triggers a specific sequence of events in plant cells. The cells subjected to sugar starvation at first adapt to the lack of carbohydrates through gradual replacement of carbohydrate metabolism by protein and lipid metabolism. Such metabolic reorganization may result in autophagy (Saglio and Pradet 1980, Journet et al. 1986, Brouquisse et al. 1991, Rose et al. 2006). Brouquisse et al.

(1992) report that during sugar starvation of maize root meristems, 3 phases can be distinguished:

1. acclimation (from 0 to 30-35 h), when cellular carbohydrate levels and respiration rate decrease, while nitrogen is released from storage proteins through their degradation;
2. survival phase (from 30-35 to 90-100 h), involving intensive breakdown of proteins and lipids, release of  $P_i$  phosphorylcholine, and free amino acids; this phase can be reversed by sugar feeding;
3. cell disorganization (more than 100 h), when the level of all metabolites and enzymatic activity are significantly decreased and the changes are irreversible, leading to death.

During sugar starvation at the acclimation and survival phases, the total protein content decreases. This is associated with a temporary increase in free amino acids and an increase in proteolytic activity (Tassi et al. 1992, James et al. 1993, Moriyasu and Ohsumi 1996, Borek and Ratajczak 2002). *In vitro* studies of embryo axes of lupines and garden pea have revealed a dramatic decrease in protein concentration (especially the soluble fraction) in starved cells (Morkunas 1997, Borek et al. 2012a). The decrease in cytosolic proteins in starved cells was correlated with an increase in the activity of proteolytic enzymes, i.e. endo- and exopeptidases (Morkunas et al. 1999, Borek and Ratajczak 2002). The increased activity of proteases could result from activation of enzymatic proteins that were already present in cells but in a bound or inactive form. Their increased activity could also result from *de novo* synthesis of the enzymes, caused by release of their genes from catabolite repression. The increased activity of proteases does not confirm unambiguously that the lack of sugar causes an enhanced expression of genes of these enzymes, because enzymatic activity can be controlled in other ways, e.g. by allosteric regulation or by phosphorylation and dephosphorylation, which is the most common type of reversible covalent modification (Berg et al. 2002). However, a study of starved maize root meristems shows that the increased activity of proteolytic enzymes under sugar starvation is an effect of enhanced transcription and translation (James et al. 1993). It also has been evidenced that proteases are closely associated with some cell compartments. Optimal acidic pH for endo- and carboxypeptidase, which increased under starvation, suggested that proteases were located in vacuoles or in lysosomes (Feller 1986, Mikola and Mikola 1986, Huffaker 1990). Accumulation of autolysosomes was observed in tobacco suspension cells cultured under sucrose starvation conditions in the presence of a cysteine protease inhibitor E-64. Experiments with fluorescent dyes, green fluorescent protein (GFP), and endocytosis markers (FM4-64 and Lucifer Yellow CH) suggested that there is a membrane flow from the plasma membrane to autolysosomes. Using fluorescent dyes and markers of the central vacuole (GFP-AtVam3p, sporamin-GFP and gamma-VM23-GFP), the transport of components of the central vacuole to autolysosomes was displayed (Yano et al. 2004). When cells of tobacco were treated with E-64c cysteine protease inhibitor, then both protein degradation and protease activation were inhibited. Simultaneously, many spherical bodies accumulated in the cytosol, beside the nucleus. The bodies, with acidic pH inside, contained acid phosphatase, which is a marker enzyme of autolysosomes. The bodies accumulated because E-64c inhibitor blocked the degradation of the proteins contained in them.

Consequently, the normally short life span of these bodies was significantly prolonged, and their observation was possible. In all probability, the observed spherical bodies were autolysosomes participating in protein degradation during autophagy (Moriyasu and Ohsumi 1996). During advanced sugar starvation, autophagy involves cytosolic proteins and even whole organelles can be degraded. Plastids, ribosomes, and endoplasmic reticulum are consumed relatively early, whereas the plasma membrane, mitochondria, and peroxisomes persist longer (Baker and Graham 2002). The increased activity of proteolytic enzymes and autophagic processes observed under sugar starvation are certainly adaptive processes enabling the cells to maintain continuous energy supply under sugar starvation. Amino acids released from degraded cytosolic proteins, storage proteins, and organelles are used as respiratory substrates (Brouquisse et al. 1991, Ratajczak et al. 1996, Yu 1999, Borek et al. 2001, Gonzali et al. 2006).

A perfect example of enzymes induced under sugar starvation is glutamate dehydrogenase (GDH). This is a mitochondrial enzyme that catalyzes the synthesis and degradation of glutamate, which is one of the central amino acids of nitrogen metabolism in plants (Lehmann and Ratajczak 2008, Borek et al. 2011). The reversibility of the reaction catalyzed by GDH *in vivo* is considered as alternative and depends on carbon and ammonium status (Lehmann et al. 2011). In sucrose-starved pea and lupine embryo axes, a remarkable increase in GDH activity was observed (Morkunas et al. 2000, Lehmann et al. 2003, 2010, Borek and Nuc 2011, Borek et al. 2012a). Sugar starvation caused also a significant increase in a number of GDH isoenzymes (Morkunas et al. 2000, Lehmann et al. 2003, 2010). The increased GDH activity caused by sugar deficit was also recorded in callus of *Nicotiana plumbaginifolia* (Maestri et al. 1991) and in carrot cell suspension (Robinson et al. 1992). Under sugar starvation, plant mitochondria may adapt to the stress conditions by increasing their ability to use amino acids as respiratory substrates. Thus the increase in GDH activity under sugar starvation may be the reason for the higher oxidation of glutamate by mitochondria. GDH catalyzing the oxidative deamination of glutamate, in cooperation with the corresponding aminotransferases, is the main pathway of amino acid catabolism in plants (Lea 1993, Lehmann and Ratajczak 2008). During this process, a large quantity of ammonia is produced. Nitrogen in this form is toxic, so its utilization is necessary. One of the possibilities of ammonia detoxification is the synthesis of asparagine, whose content increases during sugar starvation. In sugar-starved suspension cells of sycamore, asparagine content increased steadily, whereas the cell protein content declined progressively (Genix et al. 1990).

The higher activity of the enzymes of protein and amino acid catabolism under sugar starvation probably results from the release of the genes encoding them from catabolite repression (caused by sugar). Catabolite repression is of fundamental importance since it allows cells to adapt their metabolism to carbon sources other than sugar when they are in a habitat where sugar is unavailable. If sugar is present in the medium, then the synthesis of enzymes of the catabolism of carbon sources other than carbohydrates is inhibited. This applies to proteolytic enzymes, amino acid dehydrogenases (mostly GDH), alcohol dehydrogenase, and isocitrate dehydrogenase. Other studies of the influence of sugar deficit on plant cell metabolism show that already in the initial phase of starvation (after 1.5 h),

specific proteins appear, known as carbohydrate-responsive proteins (Baysdorfer et al. 1988). They may be the proteins associated with the phase of tissue acclimation to sugar starvation. Prolonged starvation results in synthesis of many specific proteins known as starvation-related proteins (STP) (Tassi et al. 1992). The genes encoding them (at least some of them) are normally under catabolite repression caused by carbohydrates. Their expression is initiated when the level of sugar in the cell falls below a critical level.

During sugar starvation, respiration rate declines rapidly in embryos of garden pea (Morkunas et al. 2000) and yellow lupine (Borek et al. 2011). The lower respiration rate is caused by the deficit of respiratory substrates rather than by mitochondrial degradation. In mitochondria isolated from starved pea embryos, respiratory activity (with glutamate as a substrate) is 60% higher than in those isolated from embryos fed with 60 mM sucrose (Morkunas et al. 2000). A similar relationship was observed in mitochondrial fractions from starved and control (sugar-fed) embryos of narrowleaf lupine (Morkunas et al. 2003). The concentration of mitochondrial proteins in starved cells was slightly lower than in control cells, and the activity of a mitochondrial marker enzyme, NAD<sup>+</sup>-dependent isocitrate dehydrogenase, was reduced in starved embryos only slightly. Besides, mitochondria isolated from lupine embryo axes cultivated without sucrose, exhibited respiration coupled with oxidative phosphorylation. They exhibited a respiration control ratio of 2 for succinate as a respiratory substrate. Mitochondria isolated from starved embryo axes oxidized glutamate and malate more intensively than mitochondria from embryo axes fed with sucrose. Ultrastructural analysis of cells of starved pea embryo axes shows that the inner mitochondrial membrane is highly convoluted, forming many folds (cristae, Fig. 2A). A similar finding was reported by Journet et al. (1986) who studied sycamore cell suspension. Mitochondria were protected in sycamore cells even as late as after 60 h of starvation, and then an addition of sugar to the medium resulted in intensive respiration. In starved cultures, sugar reserves were used up very quickly. Respiration rate was maintained at a high level for up to 30 h of starvation. In that period, respiratory substrates were provided by degradation of starch, phosphate esters, and proteins. Morkunas et al. (2000) also showed that the transfer of pea embryo axes from sugar starvation conditions onto the medium with sucrose after 48 h recovered their respiration activity. Metabolic adaptations of starved embryos of lupine (Morkunas et al. 2003) and pea (Morkunas et al. 2000) were quite effective physiologically, enabling their survival, and their transfer to a medium with sucrose allowed restoration of normal growth. Similarly, Couée et al. (1992), who studied maize root tips cultured *in vitro*, found that after 48 h of sugar starvation they still had a significant pool of functional mitochondria. Carbohydrate stress caused degeneration of lower-density mitochondria, but not of higher-density mitochondria, which was proved by measurements of respiratory activity of mitochondria and of fumarase activity (mitochondrial marker enzyme).

Under sugar starvation, the increased utilization of cytosolic proteins and storage proteins, is accompanied by a more intensive catabolism of lipids, aimed to supply respiratory substrates. In turn, sugar feeding significantly retards the breakdown of storage lipid. In the research on the regulatory function of sugars in lipid metabolism, sucrose and glucose are predominantly taken under consideration. Sucrose is particularly important in seed tissues

because storage lipid is synthesized from this sugar in developing seeds and sucrose is one of the main end products of storage lipid breakdown during seed germination. In seedlings of *Arabidopsis* (a model oil-seed plant), 1% sucrose significantly retards the breakdown of storage lipid (Eastmond et al. 2000), while 0.3 M glucose nearly eliminates the mobilization of this storage compound (To et al. 2002). Seedlings grown for 22 days on glucose solution contained about 80% of their seed storage lipid. In contrast, 22-day-old seedlings grown in equi-molar sorbitol retained only 4-5% of their seed storage lipid. This result additionally proves that the effect of glucose is not due to the osmotic potential of the media (To et al. 2002).

Transcriptome analysis of sucrose-starved rice suspension cells has revealed a decrease in the expression of transcripts involved in fatty acid synthesis but induced those involved in fatty acid degradation, such as 3-ketoacyl-CoA thiolase, fatty acid multifunctional proteins, and acyl-CoA oxidase (Wang et al. 2007). An increased expression of genes encoding lipase and enzymes linked to fatty acid  $\beta$ -oxidation was noted in sucrose-starved *Arabidopsis* suspension cells (Contento et al. 2004). Similarly, in dark-induced senescent *Arabidopsis* leaves an enhanced gene expression of lipase and enzymes involved in fatty acid  $\beta$ -oxidation was observed (Buchanan-Wollaston et al. 2005). Glucose starvation remarkably enhances the intensity of lipid degradation in isolated maize root tips. After 24 h of glucose starvation, the rate of oxidation of palmitic acid to CO<sub>2</sub> was increased 2.5-fold. The overall  $\beta$ -oxidation of fatty acids (measured as acetyl-CoA formation) was increased up to 5-fold in glucose-starved roots. An increase in catalase activity was observed as well (Dieuaide et al. 1992). Catalase is not directly involved in fatty acid  $\beta$ -oxidation but is involved in detoxification of H<sub>2</sub>O<sub>2</sub> produced in glyoxysomes by the activity of acyl-CoA oxidase when acyl-CoA is converted into trans-2-enoyl-CoA. Sucrose starvation in *Arabidopsis* suspension cells caused a 5.7-fold increase in the expression of acyl-CoA oxidase-4, which is the first committed step of short-chain fatty acid  $\beta$ -oxidation. A significant increase in acyl-CoA oxidase-4 activity in suspension cells was observed after 12 h of sucrose starvation, whereas a similar effect in one-week-old seedlings was noted after 2 days of sucrose starvation. The significant increase in gene expression of catalase-3 (glyoxysomal isoform) was observed already after 6 h of the removal of sucrose from the medium of *Arabidopsis* suspension cells. An increase in catalase activity was observed 6 h later, i.e. after 12 h of sucrose starvation. In seedlings the increase in catalase activity was observed after 2 days of sucrose starvation. About half of the catalase activity observed during sucrose starvation was due to glyoxysomal isoform 3 (Contento and Bassham 2010). In cucumber suspension cells, the expression of genes encoding 2 marker enzymes of the glyoxylate cycle (isocitrate lyase and malate synthase) was induced during deficits of sucrose, mannose and fructose in the medium (Graham et al. 1994). The above-mentioned results suggest that the enhanced gene expression patterns and enzymatic activities involved in lipolysis, fatty acid  $\beta$ -oxidation, and glyoxylate cycle, facilitate the production of acetyl-CoA from the lipid, which may be directly utilized by respiration to sustain energy production and growth when carbohydrates are exhausted.

Another set of data concerns the regulation of lipid metabolism by sucrose in developing and germinating protein lupine seeds (storage protein content up to 45% of seed dry

matter). Restriction in sucrose feeding of developing embryos of yellow lupine (lipid content about 6% of seed dry matter), white lupine (lipid content 7-14%), and Andean lupine (lipid content about 20%) caused a decrease in storage lipid accumulation during seed development (Borek et al. 2009). Ultrastructural investigations of cotyledons of 4-day-old yellow, white, and Andean lupine seedlings showed that oil bodies were smaller and less numerous when sugar level was decreased in tissues (Borek et al. 2006, 2011, 2012a). A peculiar and puzzling feature was observed in 4-day-old sucrose-starved isolated embryo axes of yellow, white, and Andean lupine grown *in vitro*. Their lipid level was by 43, 44, and 70% higher, respectively, than in sucrose-fed organs (Borek et al. 2012b). Lipase and catalase activity increased when sugar reserves were depleted in yellow lupine germinating seed organs (Borek et al. 2006). However, the enzymes involved in further steps of lipid breakdown (cytosolic aconitase, isocitrate lyase, NADP<sup>+</sup>-dependent cytosolic isocitrate dehydrogenase) were more active in sucrose-fed tissues (Borek and Nuc 2011). Changes in enzymatic activity caused by sucrose starvation were accompanied by modification of gene expression. Lipase mRNA level was higher, while mRNA levels for cytosolic aconitase and NADP<sup>+</sup>-dependent isocitrate dehydrogenase were lower in yellow lupine cotyledons and embryo axes whose sugar content was decreased (Borek and Nuc 2011). During yellow lupine seed germination, lipid-derived carbon skeletons are used for sugar synthesis but some of them are also used for amino acid formation (mainly asparagine, glutamine, and glutamate). In sugar-deficient conditions in tissues, this carbon flow from storage lipid to amino acids is significantly inhibited (Borek et al. 2003, Borek and Ratajczak 2010).

## 5. The role of sugar starvation in plant growth and development

As mentioned above, sugar in plants is not only an energetic and structural substrate but it also regulates the expression of many genes. Sugar may play a role of hormonal-like signal in a variety of eukaryotic cell types. The best known are the signaling pathways in yeasts (*Saccharomyces cerevisiae*), but many similar pathways have also been discovered in plants (Rolland et al. 2006). The time-course monitoring of gene expression profiles in suspension cells was investigated by Wang et al. (2007). After 12, 24, and 48 h of sucrose-starvation in rice cells, the expression of 867 genes was increased, while the expression of 855 genes was inhibited. Most of these genes encoded enzymes associated with metabolism. It is characteristic that genes associated with catabolism were up-regulated, while genes involved in biosynthetic pathways were down-regulated. Importantly, Wang et al. (2007) succeeded to identify the genes responsible for transcription and translation, which were the second and third major categories of genes in each group significantly affected by sucrose starvation. Response-to-stress genes were the fourth largest group of regulated genes, which suggests that most of the stress-associated genes were also significantly up-regulated by sucrose starvation. Admittedly, the model of cell suspension used by Wang and his research team is a rather “artificial” system, but identification of the genes affected by sugar starvation proved to be very useful for interpretation of results of research on the role of sugar starvation in the regulation of various processes of plant growth and development and in plant responses to biotic and abiotic stresses.

Most of the data on the regulatory role of sugar starvation in plant development was obtained as a result of research on senescence of plant organs, particularly of leaves and flowers. Controversies between groups of researchers on the role of sugar in leaf senescence may partly result from selection of various experimental models. Buchanan-Wollaston et al. (2005) identified over 800 genes of *Arabidopsis*, whose expression was up- or down-regulated during senescence. Those authors came to a conclusion that gene expression patterns during natural leaf senescence, compared with those identified when senescence is artificially induced in leaves by darkness or sucrose starvation in cell suspension cultures, showed not only similarities but also considerable differences. In this chapter, the role of sugar starvation in senescence is discussed in section 6.

There is also a rich literature on participation of the sugar signal in mobilization of storage materials during seed germination. Interaction between sugar and gibberellin in germinating barley grain is well documented (Thomas and Rodriquez 1994). Depending on the seedling's demand for carbohydrates, the rate of starch mobilization in barley endosperm is regulated by a combination of the sugar signal with gibberellin. This regulation is a textbook example of the role of feedback in metabolic adaptation to developmental needs of the plant (Bewley and Black 1994). Results of research on the role of sugar starvation in the expression of amylase genes have some practical applications. Promoter of the gene of rice  $\alpha$ -amylase 3D (RAmy3D), which is induced by sugar starvation, has proved to be very useful in transgenic plant cell cultures producing recombinant proteins, including plant-made pharmaceutical and plant-made industrial proteins (Park et al. 2010, Huang and McDonald 2012). For example, this promoter has been used for achieving high-level expression in rice cell suspension cultures of many therapeutic proteins, including human growth hormone, human  $\alpha$ 1-antitrypsin, human granulocyte-macrophage colony stimulating factor, bryodin-1, lysozyme and human serum albumin (Xu et al. 2011a, 2011b). Virtually every recombinant protein expressed in this rice suspension cell system has produced significantly higher secreted protein levels than attained with any other plant cell expression system tested (Xu et al. 2011a).

The role of sugar starvation in the control of storage lipid mobilization in germinating seeds was mentioned above. Storage protein mobilization in germinating seeds is also affected by sugar starvation. A good model for research on this phenomenon is yellow lupine seed. Its main storage compounds are proteins, which account for up to 45% of seed dry weight (Duranti et al. 2008, Borek et al. 2012a, 2012b). In ripe lupine seeds, starch is absent (Duranti et al. 2008, Borek et al. 2006, 2011). Sugar starvation releases from catabolite repression not only the genes of proteolytic enzymes but also the genes of amino acid catabolism. This allows the use of amino acid carbon skeletons both as sources of energy and as structural components. Interestingly, transitional starch appears during germination of yellow lupine seeds both in cotyledons and in seedling embryo axes (Borek et al. 2006, 2011, 2012a). This phenomenon can be explained by analogy with the appearance of transitional starch in leaves during photosynthesis. Sugar signal inhibits the expression of genes of some photosynthetic proteins (Sheen 1990, Krapp et al. 1993), so carbohydrates must be quickly converted into the neutral starch. In cotyledons of germinating lupine, carbohydrates are

synthesized through gluconeogenesis (Borek et al. 2003, Borek and Ratajczak 2010, Borek et al. 2011), so transitional starch synthesis prevents the inhibition by sugar of the continued mobilization of storage lipids and proteins. Transitional starch synthesis in seedling embryo axes may help to maintain the gradient necessary for sugar transport between the source (cotyledons) and the sink (embryo axes). Hypocotyl elongation of etiolated seedlings was suppressed by sugar. However, it is possible that maintenance of an appropriate level of carbohydrates in the embryo axis prevents the inhibition of hypocotyl elongation in etiolated seedlings (Jang et al. 1997, Rolland et al. 2002, Yang et al. 2004, Borek et al. 2012a). The major task of the hypocotyl during epigeal germination (e.g. in lupine) is to bring the cotyledons above the ground. Thus excessively deep sowing may lead to a loss of the ability to transform cotyledons from storage materials into photosynthetic organs (Elamrani et al. 1994).

Sugar signal in cooperation with hormone signaling pathways, may control a wide range of processes related to plant growth and development (Rolland et al. 2006). Sugar and nitrogen are the main factors that modify plant morphology, as they determine the growth rate and transition to the next developmental stages. Transcription analyses show that sugar and inorganic nitrogen act as both metabolites and signaling molecules (Price et al. 2004). There are many recent reports about the role of sugar starvation in processes of shoot growth reduction and root proliferation enhancement during phosphorus deficits (Ciereszko et al. 2005, Karthikeyan et al. 2007, Polit and Ciereszko 2009). The importance of sugar in plant responses to biotic and abiotic stresses is discussed in section 7.

## 6. Sugar starvation versus senescence

Shaded leaves age more quickly. Their sugar content declines as a result of limited photosynthesis, so their senescence may be induced by sugar starvation. Chung et al. (1997) discovered in *Arabidopsis* plants a gene called *sen 1*, which was expressed during leaf senescence. A particularly strong expression of the gene was observed in excised leaves kept in darkness, which simultaneously showed symptoms of accelerated senescence. When the excised leaves kept in darkness were treated with sugar, then the *sen 1* gene promoter was repressed. On the basis of the results, the cited authors postulated that sugar starvation induces leaf senescence. Further research on plant tissue senescence, e.g. with the use of a hexokinase mutant (as mentioned above, hexokinase is a sugar sensor), confirmed that a low level of sugar may induce leaf senescence (Moore et al. 2003). However, some other authors reported that leaf senescence is induced by a high level of sugar (Parrott et al. 2005, Pourtau et al. 2006). The dispute on which of these contradictory hypotheses is true was one of the most interesting scientific debates in the field of plant biology in the last decade. A detailed outline of the debate was presented by van Doorn (2008). He critically analyzed numerous works whose results support one or the other hypothesis. He did not question the results, but warned against their simplistic interpretation and unjustified generalization. Van Doorn did not support unambiguously any of the hypotheses, but concluded that "it is quite possible that neither of the two hypotheses is correct". On the basis of gene expression

analyses during natural and induced leaf senescence, Buchanan-Wollaston et al. (2005) proposed a model of cooperation of the sugar signaling pathway with abscisic acid, jasmonate, ethylene, salicylic acid, and cytokinin signaling pathways.

As in the case of leaf senescence, there is some controversy about the role of sugar starvation in flower senescence. Sugar starvation may be involved in the process of flower senescence because application of sugars to cut flowers generally delays its visible symptoms (van Doorn 2004). However, petals showing symptoms of senescence have a relatively high sugar content. Arron and Munné-Bosch (2012) assayed a large number of sugars and hormones in various organs of uncut and cut lily flowers. Addition of sucrose to the vase solution elevated the level of sugar in various organs of cut flowers, but primarily it altered the hormonal balance of floral tissues. Those results confirm the earlier findings that sucrose added to the vase solution accelerates the senescence of cut flowers with the participation of hormones. Hoeberichts et al. (2007) used cDNA microarrays to characterize senescence-associated gene expression in petals of cut carnation (*Dianthus caryophyllus*) flowers. The plants were treated with ethylene, silver thiosulphate (which blocks the ethylene receptor), or sucrose. Ethylene accelerated flower senescence and enhanced senescence-associated gene expression. Silver thiosulphate and sucrose had an opposite effect. The cited authors conclude that sucrose may slow down flower senescence by a negative regulation of the ethylene signal pathway. Cut flowers proved to be a good model for research on senescence regulation in plants, participation of hormones in this process (Tripathi and Tuteja 2007, and references cited there) and the role of sugar as a regulator of gene expression (van Doorn and Woltering 2008). Those studies enriched our knowledge but also provided some useful tips for horticultural practice.

At the cellular level, sugar starvation in senescent organs and in organs that have not initiated the senescence program causes similar or identical symptoms: degradation of cellular structures and degradation of many cellular components, such as proteins and lipids. Nevertheless, the goals of these transformations differ. In the case of senescence, valuable metabolites are removed from the cells destined to die, to feed other cells in other plant organs, e.g. in developing leaves or maturing seeds. During sugar starvation of non-senescent organs, protein and lipid degradation is aimed to supply substrates for respiratory processes, which provide the energy necessary for survival. Senescence is a stage of programmed death, during which a low level of sugar may be necessary for activation of some senescence-associated genes, whose expression is repressed by sugar. However, sugar starvation can be used in plant development in many other processes, not only in programmed cell death. As mentioned above, sugar signaling may, in cooperation with plant hormones, play a major role in plant response to environmental factors and in shaping of plant morphology. However, even at the level of cells, sugar starvation, if not associated with programmed cell death, may show other symptoms than during senescence. For example, generation of free radicals (which play an important role in programmed cell death) may be neutralized by initiation of scavenging free radicals whenever cells need to be protected against the negative effects of sugar starvation (Morkunas et al. 1999, 2003). The role of sugars in plant defense response against free radicals is discussed in section 7.

## 7. Effect of sugar starvation on plant response to biotic and abiotic stress factors

Depending on the duration of sugar starvation, considerable ultrastructural and metabolic changes were observed in plant cells (see above). Moreover, it was interesting whether sugar starvation (as a nutritional stress) is accompanied by changes in the redox status of the cell, like in the case of plant response to other stress factors (biotic and abiotic). In plant response to abiotic stress factors, the increased generation of free radicals, including reactive oxygen species (ROS), attests to a greater influence of the stress factor, whereas during the plant-pathogen interaction, particularly at an early stage of the infection, an increased generation of free radicals in plant cells is favorable (Morkunas and Bednarski 2008). Free radicals may be toxic to the pathogenic microorganism, hamper the penetration of host tissues (through initiation of lignification of host cell walls), activate the expression of host defense genes, and participate in signal transduction over short and long distances.

Under sugar starvation, synthesis of free radicals is intensified, particularly after 72 and 96 h of starvation of lupine embryo axes *in vitro* (Morkunas et al. 2003). Electron Paramagnetic Resonance (EPR) analyses of free radicals detected the presence of free radicals with a signal at  $g = 2.0060$ . Sugar starvation caused a 2-fold increase in the concentration of free radicals in embryo axes cultured *in vitro* for 72 h. Spectroscopic splitting factors ( $g$ -values) indicated that these radicals might be quinones involved in electron transport pathways. Thus, intensified synthesis of free radicals during nutritional stress and other stresses on plant cells may result in a transient oxidative stress due to enhanced ROS generation (Polle et al. 1990, Schraudner 1992, del Rio 1996, Polle 1996, Purvis 1997, Becana et al. 1998, Chamnongpol 1998, Prasad et al. 1999). Concentrations of free radicals increased also in aging plants (Leshem 1981, Thompson et al. 1987, Pastori and del Rio 1997). However, generation of free radicals during sugar starvation may be neutralized by a number of adaptive mechanisms protecting the cells from oxidative damage (Morkunas et al. 1999, 2003). An example of such a mechanism is the accumulation of large amounts of phytoferritin in plastids of starved lupine and pea cells. This fact was interpreted as a defense mechanism aimed at inactivation of iron ions ( $Fe^{2+}$ ), as it prevents ROS generation. Results of malondialdehyde (MDA) assays supported this interpretation (Morkunas et al. 1999). Ferritin, which binds iron ions ( $Fe^{2+}$ ) prevented ROS generation in Fenton reaction (Becana et al. 1998). Besides, in starved cells an antioxidant system was activated, i.e. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activity was higher than in sugar-fed tissues (Borek et al. 2006, Morkunas and Gmerek 2007, Morkunas and Bednarski 2008). Additionally, an increase was observed in the ability of starved embryo axes to take up  $Mn^{2+}$  from the growth medium, which seems to be an interesting adaptation, probably associated with increased SOD activity (Morkunas et al. 2003). All stresses generate ROS, potentially leading to oxidative damage. However, it must be emphasized that during nutritional stress, such as sugar starvation, specific defense mechanisms are induced, alleviating the results of oxidative stress.

Apart from the well-known classical antioxidant mechanisms, sugars and sugar-metabolizing enzymes are important players in the defense against oxidative stress (Bolouri-Moghaddam et al. 2010). Those authors hypothesized that the synergistic interaction of sugars (or sugar-like compounds) and phenolic compounds is a part of an integrated redox system. It quenches ROS and contributes to stress tolerance, especially in tissues or organelles with high soluble sugar concentrations.

Moreover, sugar-signaling pathways interact with stress pathways in a complex network, which modulates metabolic plant responses (Ho et al. 2001, Tran et al. 2007). Soluble sugars may either act directly as negative signals or as modulators of plant sensitivity, so they can also play important roles in cell responses to stress-induced remote signals (Rosa et al. 2009). Descriptive ecological and agronomic studies have revealed a strong correlation between soluble sugar concentrations and stress tolerance. However, since energy and resources are required for plants to cope with abiotic and biotic stress conditions, the source-sink partitioning between different organs is a crucial component of the mechanisms of stress tolerance (Ho 1988, Krapp and Stitt 1995). Recent studies for increasing tolerance to environmental stress, through metabolic engineering of compatible solutes have shown that increased concentrations of soluble sugars and/or other osmolytes increase plant tolerance to abiotic stresses, such as drought, salinity, and cold (Rathinasabapathi 2000).

Energy and resources are required for plants to cope with abiotic and biotic stress conditions, so in sugar starvation conditions, when metabolic processes are slowed down (e.g. respiration), the plants are more sensitive to stress factors. For example, the link between low sugar content and plant sensitivity to fungal diseases known as “low-sugar diseases”, was first reported over 50 years ago by Horsfall and Dimond (1957). It must be emphasized that many environmental factors, such as insufficient light, high humidity, excessive nitrogen fertilization, and excessively deep sowing, may cause a decrease in the level of carbohydrates in host plant tissues, contributing to an increase in plant sensitivity to fungal infection (Vidhyasekaran 1974a, 1974b, Morkunas et al. 2004, Huber and Thompson 2007, Yoshida et al. 2008, Morkunas et al. 2010, Morkunas et al. 2012). By contrast, low temperature can induce plant resistance to specific pathogens, because it results in accumulation of soluble carbohydrates, affecting the cell water potential and other osmotically-active molecules, as well as accumulation of pathogenesis-related (PR) proteins (Tronsmo 1993, Thomashow 1998, Hiilovaara-Teijo et al. 1999, Płazek and Żur 2003, Yuanyuan et al. 2009). Sugar starvation of host plant cells facilitates the development of fungal pathogens because water potential is much higher there than when the concentration of soluble carbohydrates in cells is high. A lack of carbohydrates in the growth medium caused a decrease in endogenous levels of soluble carbohydrates in lupine embryo axes (Morkunas et al. 2005, Borek et al. 2006), and facilitated the development of *Fusarium* infection. This was reflected in an increased area of necrotic spots, more intensive post-infection changes in cell ultrastructure, and in limited elongation and fresh weight increments of embryo axes, as compared to inoculated axes with a high endogenous level of carbohydrates (Morkunas et al. 2005, Morkunas and Bednarski 2008). Besides, Morkunas and Bednarski (2008) observed on transmission electron micrographs the occurrence of

hyphae of the pathogenic fungus *Fusarium oxysporum* in intercellular spaces of inoculated embryo axes cultured without sucrose as early as 72 h after inoculation, while no fungi were found in intercellular spaces of inoculated embryo axes cultured with sucrose. Cook and Papendick (1978) report that a high level of carbohydrates is a strong osmoticum in host plant cells, decreasing the water potential and inhibiting fungal growth. Moreover, in the absence of sugar, genes encoding cell wall-degrading extracellular enzymes secreted by fungal pathogens are released from catabolite repression (Akimitsu et al. 2004). These enzymes may contribute to pathogenesis by degrading wax, cuticle, and cell walls, which aids in tissue invasion and pathogen dissemination (Carapito et al. 2009, Kikot et al. 2009). Moreover, the induction of defense in host plant cells is cost-intensive (Swarbrick et al. 2006). Thus, under sugar starvation, where respiration rate is lowered, acquisition of the energy necessary to initiate defense responses, such as synthesis of PR proteins, phenylpropanoids, and papilla formation, is limited (Morkunas et al. 2005, Morkunas and Gmerek 2007, Morkunas et al. 2007, Morkunas and Bednarski 2008, Morkunas et al. 2011). It needs to be emphasized that a very important defense strategy against *Fusarium oxysporum* in inoculated lupine embryo axes cultured with sucrose involved a high accumulation of flavonoids, including isoflavonoids (Morkunas et al. 2005, 2007), fast stimulation of tissue lignification (Morkunas and Gmerek 2007), and intensive generation of superoxide anion radical (Morkunas and Bednarski 2008). This mechanism was not observed in inoculated embryo axes cultured without sucrose.

## 8. Conclusions

At some stages of plant development, sugar content may decrease considerably (so-called sugar starvation), usually under the influence of adverse environmental factors (biotic or abiotic). In this chapter, we have shown that sugar starvation in plant cells, depending on its duration, causes both ultrastructural and physiological-biochemical changes of varied severity. In non-senescent cells, the changes are aimed to maintain respiration and other basic metabolic processes at a specified level. Thus under conditions of sugar starvation, a priority is the acquisition of energy necessary for cell survival, even at the expense of organelles except the nucleus and the system supplying energy, i.e. mitochondria. The changes taking place during sugar starvation in the acclimation phase or even in the survival phase can be reversed, as long as the respiratory system functions properly. Carbon, nitrogen, and phosphorus are the major elements whose availability affects plant growth and development, so the sugar deficit signal in cooperation with nitrogen and phosphorus signaling pathways may influence plant morphology. Moreover, it is interesting that during sugar starvation, the observed processes resemble senescence. Many symptoms are identical, e.g. autophagy, decrease in protein content, and strong activation of proteolytic enzymes. However, senescence is controlled by programmed cell death. By contrast, if sugar starvation concerns non-senescent cells, then the initiated reactions cause cell acclimation to such conditions. At first these are reactions that protect cells against irreversible degradation of cell structures, especially those responsible for conservation and realization of genetic information as well as energy supply. At the second phase of sugar

starvation, defense strategies are initiated, e.g. activation of the enzymatic antioxidant system, manganese ion uptake, phytoferritin accumulation, protecting cells against the destructive influence of an excess of free radicals (e.g. membrane damage). However, the mechanisms underlying the processes used by plant cells to survive sugar starvation require further research. Currently we should focus on explanation of the controlling mechanisms, which may involve signal molecules. Their synthesis may, to some extent, alleviate the influence of stress factors, and thus play an important role in plant acclimation to stress conditions. In plant cells, in response to environmental factors (either abiotic or biotic), many signaling pathways are initiated. Knowledge of the signaling pathways initiated under conditions of sugar starvation, and their cross-talk in the network, would certainly greatly help to explain the molecular mechanisms underlying plant reactions to sugar starvation.

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## 9. References

- Agulló-Antón MÁ, Sánchez-Bravo J, Acosta M, Druege U (2011) Auxins or sugars: what makes the difference in the adventitious rooting of stored carnation cuttings? *J Plant Growth Reg* 30: 100-113.
- Akimitsu K, Isshikia A, Ohtanib K, Yamamotoa H, Eshelc D, Prusky D (2004) Sugars and pH: A clue to the regulation of fungal cell wall-degrading enzymes in plants. *Physiol Mol Plant Pathol* 65: 271-275.
- Arrom L, Munné-Bosch S (2012) Sucrose accelerates flower opening and delays senescence through a hormonal effect in cut lily flowers. *Plant Sci* 188-189: 41-47.
- Aubert S, Gout E, Bligny R, Marty-Mazars D, Barrieu F, Alabouvette J, Marty F, Douce R (1996) Ultrastructural and biochemical characterization of autophagy in higher plant cells subjected to carbon deprivation: control by the supply of mitochondria with respiratory substrates. *J Cell Biol* 133: 1251-1263.

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- Azad AK, Ishikawa T, Ishikawa T, Sawa Y, Shibata H (2008) Intracellular energy depletion triggers programmed cell death during petal senescence in tulip. *J Exp Bot* 59: 2085-2095.
- Baker A, Graham IA (2002) Plant peroxisomes: Biochemistry, cell biology and biotechnological applications. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Baysdorfer C, Warmbrodt RD, Van Der Woude WJ (1988) Mechanisms of starvation tolerance in pearl millet. *Plant Physiol* 88: 1381-1387.
- Becana M, Moran JF, Iturbe-Ormaetxe I (1998) Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. *Plant Soil* 201: 137-147.
- Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry. Fifth edition, WH Freeman, New York.
- Bewley JD, Black M (1994) Seeds. Physiology of development and germination. Second edition, Plenum Press, New York, London.
- Bolouri-Moghaddam MR, Le Roy K, Xiang L, Rolland F, Van den Ende W (2010) Sugar signalling and antioxidant network connections in plant cells. *FEBS J* 277: 2022-2037.
- Borek S, Kubala S, Kubala S (2012a) Regulation by sucrose of storage compounds breakdown in germinating seeds of yellow lupine (*Lupinus luteus* L.), white lupine (*Lupinus albus* L.) and Andean lupine (*Lupinus mutabilis* Sweet). I. Mobilization of storage protein. *Acta Physiol Plant* 34: 701-711.
- Borek S, Kubala S, Kubala S, Ratajczak L (2011) Comparative study of storage compound breakdown in germinating seeds of three lupine species. *Acta Physiol Plant* 33: 1953-1968.
- Borek S, Morkunas I, Ratajczak W, Ratajczak L (2001) Metabolism of amino acids in germinating yellow lupin seeds. III Breakdown of arginine in sugar-starved organs cultivated in vitro. *Acta Physiol Plant* 23: 141-148.
- Borek S, Nuc K (2011) Sucrose controls storage lipid breakdown on gene expression level in germinating yellow lupine (*Lupinus luteus* L.) seeds. *J Plant Physiol* 168: 1795-1803.
- Borek S, Pukacka S, Michalski K (2012b) Regulation by sucrose of storage compounds breakdown in germinating seeds of yellow lupine (*Lupinus luteus* L.), white lupine (*Lupinus albus* L.) and Andean lupine (*Lupinus mutabilis* Sweet). II. Mobilization of storage lipid. *Acta Physiol Plant* 34: 1199-1206.
- Borek S, Pukacka S, Michalski K, Ratajczak L (2009) Lipid and protein accumulation in developing seeds of three lupine species: *Lupinus luteus* L., *Lupinus albus* L., and *Lupinus mutabilis* Sweet. *J Exp Bot* 60: 3453-3466.
- Borek S, Ratajczak L (2010) Storage lipids as a source of carbon skeletons for asparagine synthesis in germinating seeds of yellow lupine (*Lupinus luteus* L.). *J Plant Physiol* 167: 717-724.
- Borek S, Ratajczak W (2002) Sugars as a metabolic regulator of storage protein mobilization in germinating seeds of yellow lupine (*Lupinus luteus* L.). *Acta Physiol Plant* 24: 425-434.
- Borek S, Ratajczak W, Ratajczak L (2003) A transfer of carbon atoms from fatty acids to sugars and amino acids in yellow lupine (*Lupinus luteus* L.) seedlings. *J Plant Physiol* 160: 539-545.
- Borek S, Ratajczak W, Ratajczak L (2006) Ultrastructural and enzymatic research on the role of sucrose in mobilization of storage lipids in germinating yellow lupine seeds. *Plant Sci* 170: 441-452.

- Brouquisse R, Gaudillère JP, Raymond P (1998) Induction of a carbon-starvation-related proteolysis in whole maize plants submitted to light/dark cycles and to extended darkness. *Plant Physiol* 117: 1281-1291.
- Brouquisse R, James F, Pradet A, Raymond P (1992) Asparagine metabolism and nitrogen distribution during protein degradation in sugar-starved maize root tips. *Planta* 188: 384-395.
- Brouquisse R, Rolin D, Cortès S, Gaudillère M, Evrard A, Roby C (2007) A metabolic study of the regulation of proteolysis by sugars in maize root tips: effects of glycerol and dihydroxyacetone. *Planta* 225: 693-709.
- Brouquisse R, James F, Raymond P, Pradet A (1991) Study of glucose starvation in excised maize root tips. *Plant Physiol* 96: 619-626.
- Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, Lin JF, Wu SH, Swidzinski J, Ishizaki K, Leaveret CJ (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. *Plant J* 42: 567-585.
- Carapito RI, Imberty A, Jeltsch JM, Byrns SC, Tam PH, Lowary TL, Varrot A, Phalip V (2009) Molecular basis of arabinobio-hydrolase activity in phytopathogenic fungi. Crystal structure and catalytic mechanism of *Fusarium graminearum* GH93 exo- $\alpha$ -L-arabinanase. *J Biol Chem* 284: 12285-12296.
- Chamnonpol S (1998) Defense activation and enhanced pathogen tolerance induced by H<sub>2</sub>O<sub>2</sub> in transgenic tobacco. *Proc Natl Acad Sci USA* 95: 5818-5823.
- Chen MH, Liu LF, Chen YR, Wu HK, Yu SM (1994) Expression of  $\alpha$ -amylases, carbohydrate metabolism, and autophagy in cultured rice cells is coordinately regulated by sugar nutrient. *Plant J* 6: 625-636.
- Cho JI, Ryoo N, Eom JS, Lee DW, Kim HB, Jeong SW, Lee YH, Kwon YK, Cho MH, Bhoo SH, Hahn TR, Park YI, Hwang I, Sheen J, Jeon JS (2009) Role of the rice hexokinases OsHXK5 and OsHXK6 as glucose sensors. *Plant Physiol* 149: 745-759.
- Cho YH, Yoo SD (2011) Signaling role of fructose mediated by FINS1/FBP in *Arabidopsis thaliana*. *PLoS Genet* 7: e1001263. doi:10.1371/journal.pgen.1001263.
- Chung BC, Lee SY, Oh SA, Rhew TH, Nam HG, Lee CH (1997) The promoter activity of *sen1*, a senescence-associated gene of *Arabidopsis*, is repressed by sugars. *J Plant Physiol* 151: 339-345.
- Ciereszko I, Johansson H, Kleczkowski LA (2005) Interactive effects of phosphate deficiency, sugar and light/dark conditions on gene expression of UDP-glucose pyrophosphorylase in *Arabidopsis*. *J Plant Physiol* 162: 343-353.
- Contento AL, Bassham DC (2010) Increase in catalase-3 activity as a response to use of alternative catabolic substrates during sucrose starvation. *Plant Physiol Biochem* 48: 232-238.
- Contento AL, Kim SJ, Bassham DC (2004) Transcriptome profiling of the response of *Arabidopsis* suspension culture cells to Suc starvation. *Plant Physiol* 135: 2330-2347.
- Cook RJ, Papendick RI (1978) Role of water potential in microbial growth and development of plant disease with special reference to postharvest pathology. *Hortsciences* 13: 559-564.
- Couée I, Murielle J, Carde JP, Brouquisse R, Raymond P, Pradet A (1992) Effects of glucose starvation on mitochondrial subpopulations in the meristematic and submeristematic regions of maize root. *Plant Physiol* 100: 1891-1900.

- del Rio LA (1996) Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. *Biochem Soc Trans* 24: 434-438.
- Dieuaide M, Brouquisse R, Pradet A, Raymond P (1992) Increased fatty acid  $\beta$ -oxidation after glucose starvation in maize root tips. *Plant Physiol* 99: 595-600.
- Dorne AJ, Bligny R, Rebeille F, Roby C, Douce R (1987) Fatty acid disappearance and phosphorylcholine accumulation in higher plant cells after a long period of sucrose deprivation. *Plant Physiol Biochem* 25: 589-595.
- Duranti M, Consonni A, Magni C, Sessa F, Scarafoni A (2008) The major proteins of lupin seed: characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends Food Sci Tech* 19: 624-633.
- Eastmond PJ, Germain V, Lange PR, Bryce JH, Smith SM, Graham IA (2000) Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. *Proc Natl Acad Sci USA* 97: 5669-5674.
- Elamrani A, Gaudillère JP, Raymond P (1994) Carbohydrate starvation is a major determinant of the loss of greening capacity in cotyledons of dark-grown sugar beet seedlings. *Physiol Plant* 91:56-64.
- Farrar JF (1981) Respiration rate of barley roots: its relation to growth, substrate supply and the illumination of the shoot. *Ann Bot* 48: 53-63.
- Feller U (1986) Proteolytic enzymes in relation to leaf senescence. In: Dalling MJ (ed.) *Plant proteolytic enzymes*. 2. CRC Press, Boca Raton, pp. 49-68.
- Genix P, Bligny R, Martin JB, Douce R (1990) Transient accumulation of asparagine in sycamore cells after a long period of sucrose starvation. *Plant Physiol* 94: 717-722.
- Gibson SI (2005) Control of plant development and gene expression by sugar signaling. *Curr Opin Plant Biol* 8: 93-102.
- Gonzali S, Loreti E, Solfanelli C, Novi G, Alpi A, Perata P (2006) Identification of sugar-modulated genes and evidence for *in vivo* sugar sensing in *Arabidopsis*. *J Plant Res* 119: 115-123.
- Gout E, Bligny R, Douce R, Boisson AM, Rivasseau C (2011) Early response of plant cell to carbon deprivation: *in vivo*  $^{31}\text{P}$ -NMR spectroscopy shows a quasi-instantaneous disruption on cytosolic sugars, phosphorylated intermediates of energy metabolism, phosphate partitioning, and intracellular pHs. *New Phytologist* 189: 135-147.
- Graham IA, Derby KJ, Leaver CJ (1994) Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. *Plant Cell* 6: 761-772.
- Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *J Exp Bot* 59: 93-109.
- Hanson J, Smeekens S (2009) Sugar perception and signaling - an update. *Curr Opin Plant Biol* 12: 562-567.
- Hara M, Oki K, Hoshino K, Kuboi T (2003) Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyls. *Plant Sci* 164: 259-265.
- Harrington GN, Bush DR (2003) The bifunctional role of hexokinase in metabolism and glucose signaling. *Plant Cell* 15: 2493-2496.
- Hiilovaara-Teijo M, Hannukkala A, Griffith M, Yu XM, Pihakaski-Maunsbach K (1999) Snow-mold-induced apoplastic proteins in winter rye leaves lack antifreeze activity. *Plant Physiol* 121: 665-673.

- Ho LC (1988) Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annu Rev Plant Physiol Plant Mol Biol* 39: 355-378.
- Ho SL, Chao YC, Tong WF, Yu SM (2001) Sugar coordinately and differentially regulates growth- and stress-regulated gene expression via a complex signal transduction network and multiple control mechanisms. *Plant Physiol* 125: 877-890.
- Hoerberichts FA, van Doorn WG, Vorst O, Hall RD, van Wordragen MF (2007) Sucrose prevents up-regulation of senescence-associated genes in carnation petals. *J Exp Bot* 58: 2873-2885.
- Horsfall JG, Dimond AE (1957) Interactions of tissue sugar, growth substances, and disease susceptibility. *Z Pflanzenkr Pflanzenschutz* 64: 415-421.
- Huang TK, McDonald KA (2012) Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. *Biotechnol Adv* 30: 398-409.
- Huber DM, Thompson IA (2007) Nitrogen and plant disease. In: Datnoff LE, Elmer WH, Huber DM (eds.) *Mineral nutrition and plant disease*. APS, St Paul, MN, pp. 31-44.
- Huffaker RC (1990) Proteolytic activity during senescence of plants. *New Phytol* 116: 199-231.
- Inoue Y, Moriyasu Y (2006) Autophagy is not a main contributor to the degradation of phospholipids in tobacco cells cultured under sucrose starvation conditions. *Plant Cell Physiol* 47: 471-480.
- James F, Brouquisse R, Pradet A, Raymond P (1993) Changes in proteolytic activities in glucose-starved maize root tips. Regulation by sugars. *Plant Physiol Biochem* 31: 845-856.
- James F, Brouquisse R, Suire C, Pradet A, Raymond P (1996) Purification and biochemical characterization of a vacuolar serine endopeptidase induced by glucose starvation in maize roots. *Biochem J* 320: 283-292.
- Jang JC, León P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. *Plant Cell* 9: 5-19.
- Jang JC, Sheen J (1994) Sugar sensing in higher plants. *Plant Cell* 6: 1665-1679.
- Johnson R, Ryan CA (1990) Wound-inducible potato inhibitor II genes: enhancement of expression by sucrose. *Plant Mol Biol* 14: 527-536.
- Journet EP, Blingy R, Douce R (1986) Biochemical changes during sucrose deprivation in higher plant cells. *J Biol Chem* 261: 3193-3199.
- Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG (2007) Phosphate starvation responses are mediated by sugar signaling in *Arabidopsis*. *Planta* 225: 907-918.
- Kerr PS, Rufty TW Jr, Huber SC (1985) Changes in nonstructural carbohydrates in different parts of soybean (*Glycine max* [L.] Merr.) plants during a light/dark cycle and in extended darkness. *Plant Physiol* 78: 576-581.
- Kikot GE, Hours RA, Alconada TM (2009) Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. *J Basic Microbiol* 49: 231-241.
- Kim SW, Koo BC, Kim J, Liu JR (2007) Metabolic discrimination of sucrose starvation from *Arabidopsis* cell suspension by <sup>1</sup>H NMR based metabolomics. *Biotech Bioprocess Eng* 12: 653-661.
- Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr Opin Plant Biol* 7: 235-246.

- Koch KE (1996) Carbohydrate-modulated gene expression in plants. *Annu Rev Plant Physiol Plant Mol Biol* 47: 509-540.
- Krapp A, Hofmann B, Schäfer C, Stitt M (1993) Regulation of the expression of *rbcS* and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? *Plant J* 3: 817-828.
- Krapp A, Stitt M (1995) An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady state transcript levels after cold-girdling source leaves. *Planta* 195: 313-323.
- Lea PJ (1993) Nitrogen metabolism. In: *Plant Biochemistry and Molecular Biology* (ed.) Lea PJ, Leegood RC, John Wiley and Sons, Chester, 5: 155-180.
- Lee EJ, Matsumura Y, Soga K, Hoson T, Koizumi N (2007) Glycosyl hydrolases of cell wall are induced by sugar starvation in *Arabidopsis*. *Plant Cell Physiol* 48: 405-413.
- Lehmann T, Dabert M, Nowak W (2011) Organ-specific expression of glutamate dehydrogenase (GDH) subunits in yellow lupine. *J Plant Physiol* 168: 1060-1066.
- Lehmann T, Ratajczak L (2008) The pivotal role of glutamate dehydrogenase (GDH) in the mobilization of N and C from storage material to asparagine in germinating seeds of yellow lupine. *J Plant Physiol* 165: 149-158.
- Lehmann T, Ratajczak L, Deckert J, Przybylska M (2003) The modifying effect of sucrose on glutamate dehydrogenase (GDH) activity in lupine embryos treated with inhibitors of RNA and protein synthesis. *Acta Physiol Plant* 25: 325-335.
- Lehmann T, Skrok A, Dabert M (2010) Stress-induced changes in glutamate dehydrogenase activity imply its role in adaptation to C and N metabolism in lupine embryos. *Physiol Plant* 138: 35-47.
- Leshem YY (1981) Oxy free radicals and plant senescence. In: *What's new in Plant Physiol* 12: 1-4.
- Li Y, Lee KK, Walsh S, Smith C, Handingham S, Sorefan K, Cawley G, Bevan MW (2006) Establishing glucose- and ABA-regulated transcription networks in *Arabidopsis* by microarray analysis and promoter classification using a Relevance Vector Machine. *Genome Res* 16: 414-427.
- Loreti E, De Bellis L, Alpi A, Perata P (2001) Why and how do plant cells sense sugars? *Annals Bot* 88: 803-812.
- Loreti E, Povero G, Novi G, Solfanelli C, Alpi A, Perata P (2008) Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in *Arabidopsis*. *New Phytologist* 179: 1004-1016.
- Lothier J, Lasseur B, Prudhomme MP, Morvan-Bertrand A (2010) Hexokinase-dependent sugar signaling represses fructan exohydrolase activity in *Lolium perenne*. *Func Plant Biol* 37: 1151-1160.
- Maestri E, Restivo FM, Gulli M, Tassi I (1991) Glutamate dehydrogenase regulation in callus cultures of *Nicotiana plumbaginifolia*: effect of glucose feeding and carbon source starvation of the isoenzymatic pattern. *Plant Cell Environm* 14: 613-618.
- Mikola L, Mikola J (1986) Occurrence and properties of different types of peptidases in higher plants. In: *Dalling MJ (ed.) Plant proteolytic enzymes*. 1. Boca Raton, CRC Press, pp. 97-117.

- Mohapatra PK, Patro L, Raval MK, Ramaswamy NK, Biswal UC, Biswal B (2010) Senescence-induced loss in photosynthesis enhances cell wall  $\beta$ -glucosidase activity. *Physiol Plant* 138: 346-355.
- Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* 300: 332-336.
- Moriyasu Y, Hattori M, Jauh GY, Rogers JC (2003) Alpha tonoplast intrinsic protein is specifically associated with vacuole membrane involved in an autophagic process. *Plant Cell Physiol* 44: 795-802.
- Moriyasu Y, Klionsky DJ (2003) Autophagy in plants. In: Klionsky DJ (ed.) *Autophagy*. Landes Bioscience, Georgetown, TX, pp: 208-215.
- Moriyasu Y, Ohsumi Y (1996) Autophagy in tobacco suspension-cultured cells in response to sucrose starvation. *Plant Physiol* 111: 1233-1241.
- Morkunas I (1997) Regulation by carbohydrates of germinating legume seeds metabolism. PhD thesis, Department of Plant Physiology, Adam Mickiewicz University, Poznań, Poland.
- Morkunas I, Bednarski W (2008) *Fusarium oxysporum* induced oxidative stress and antioxidative defenses of yellow lupine embryo axes with different level of sugars. *J Plant Physiol* 165: 262-277.
- Morkunas I, Bednarski W, Kopyra M (2008) Defense strategies of pea embryo axes with different levels of sucrose to *Fusarium oxysporum* and *Ascochyta pisi*. *Physiol Mol Plant Pathol* 72: 167-178.
- Morkunas I, Bednarski W, Kozłowska M (2004) Response of embryo axes of germinating seeds of yellow lupine to *Fusarium oxysporum*. *Plant Physiol Biochem* 42: 493-499.
- Morkunas I, Borek S, Ratajczak W, Ratajczak L, Antonowicz J (1999) Accumulation of phytoferritin caused by carbohydrate stress in plastids of embryos in lupin and pea cultivated *in vitro*. In: *Zesz Probl Post Nauk Rol*, Wyd. ZFR PAN, Warszawa, 469: 247-256.
- Morkunas I, Formela M, Marczak Ł, Stobiecki M, Bednarski W (2012) The mobilization of defense mechanisms in the early stages of pea seed germination against *Ascochyta pisi*. *Protoplasma* doi: 10.1007/s00709-012-0374-x.
- Morkunas I, Garnczarska M, Bednarski W, Ratajczak W, Waplak S (2003) Metabolic and ultrastructural responses of lupine embryo axes to sugar starvation. *J Plant Physiol* 160: 311-319.
- Morkunas I, Gmerek J (2007) The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. *J Plant Physiol* 164: 185-194.
- Morkunas I, Kozłowska M, Ratajczak L, Marczak Ł (2007) Role of sucrose in the development of *Fusarium* wilt in lupine embryo axes. *Physiol Mol Plant Pathol* 70: 25-37.
- Morkunas I, Lehmann T, Ratajczak W, Ratajczak L, Tomaszewska B (2000) The involvement of glutamate dehydrogenase in the adaptation of mitochondria to oxidize glutamate in sucrose starved pea embryos. *Acta Physiol Plant* 22: 389-394.
- Morkunas I, Marczak J, Stachowiak M, Stobiecki (2005) Sucrose-induced lupine defense against *Fusarium oxysporum*: Sucrose-stimulated accumulation of isoflavonoids as a defense response of lupine to *Fusarium oxysporum*. *Plant Physiol Biochem* 43: 363-373.

- Morkunas I, Narożna D, Nowak W, Samardakiewicz S, Remlein-Starosta D (2011) Cross-talk interactions of sucrose and *Fusarium oxysporum* in the phenylpropanoid pathway and the accumulation and localization of flavonoids in embryo axes of yellow lupine. *J Plant Physiol* 168: 424-433.
- Morkunas I, Stobiecki M, Marczak Ł, Stachowiak J, Narożna D, Remlein-Starosta D (2010) Changes in carbohydrate and isoflavonoid metabolism in yellow lupine in response to infection by *Fusarium oxysporum* during the stages of seed germination and early seedling growth. *Physiol Mol Plant Pathol* 75: 46-55.
- Müller J, Wiemken A, Aeschbacher RA (1999) Trehalose metabolism in sugar sensing and plant development. *Plant Sci* 147: 37-47.
- Nicolai M, Roncato MA, Canoy AS, Rouquié D, Sarda X, Freyssinet G, Robaglia C (2006) Large-scale analysis of mRNA translation states during sucrose starvation in *Arabidopsis* cells identifies cell proliferation and chromatin structure as targets of translational control. *Plant Physiol* 141: 663-673.
- Pace GGM, Volk RJ, Jackson WA (1990) Nitrate reduction in response to CO<sub>2</sub>-limited photosynthesis. *Plant Physiol* 92: 286-292.
- Park CI, Lee SJ, Kang SH, Jung HS, Kim DI, Lim SM (2010) Fed-batch cultivation of transgenic rice cells for the production of hCTLA4Ig using concentrated amino acids. *Process Biochem* 45: 67-74.
- Parrott D, Yang L, Shama L, Fischer AM (2005) Senescence is accelerated, and several proteases are induced by carbon "feast" conditions in barley (*Hordeum vulgare* L.) leaves. *Planta* 222: 989-1000.
- Pastori GM, del Rio LA (1997) Natural senescence of pea leaves. *Plant Physiol* 113: 411-418.
- Płazek A, Żur I (2003) Cold-induced plant resistance to necrotrophic pathogens and antioxidant enzyme activities and cell membrane permeability. *Plant Sci* 164: 1019-1028.
- Polit JT, Ciereszko I (2009) In situ activities of hexokinase and fructokinase in relation to phosphorylation status of root meristem cells of *Vicia faba* during reactivation from sugar starvation. *Physiol Plant* 135: 342-350.
- Polle A (1996) Developmental changes of antioxidative systems in tobacco leaves as the affected by limited sucrose export in transgenic plants expressing yeast invertase in apoplastic space. *Planta* 198: 253-262.
- Polle A, Chakrabarti K, Schürmann W, Rennenberg H (1990) Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway spruce (*Picea abies* L., Karst). *Plant Physiol* 94: 312-319.
- Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A (2006) Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in *Arabidopsis*. *Planta* 224: 556-568.
- Prasad KVSK, Saradhi PP, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. *Environ Exp Bot* 42: 1-10.
- Price J, Laxmi A, Martin SKST, Jang JC (2004) Global transcription profiling reveals multiple sugar signal transduction mechanisms in *Arabidopsis*. *Plant Cell* 2004 16: 2128-2150.
- Purvis AC (1997) Role of the alternative oxidase in limiting superoxide production by plant mitochondria. *Physiol Plant* 100: 165-170.

- Ramon M, Rolland F, Sheen J (2008) Sugar sensing and signaling. In: The Arabidopsis book. Rockville, MD: American Society of Plant Biologists; doi:10.1199/tab.0117.
- Ratajczak W, Lehmann T, Polcyn W, Ratajczak L (1996) Metabolism of amino acids in germinating yellow lupin seeds. I. The decomposition of  $^{14}\text{C}$ -aspartate and  $^{14}\text{C}$ -glutamate during the imbibition. *Acta Physiol Plant* 18: 13-18.
- Rathinasabapathi B (2000) Metabolic engineering for stress tolerance: Installing osmoprotectant synthesis pathways. *Annals Bot* 86: 709-716.
- Robinson SA, Stewart GR, Phillips R (1992) Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension cultures. *Plant Physiol* 98: 1190-1195.
- Roby C, Martin JB, Bligny R, Douce R (1987) Biochemical changes during sucrose deprivation in higher plant cells. *J Biol Chem* 262: 5000-5007.
- Roitsch T, Bittner M, Godt DE (1995) Induction of apoplastic invertase of *Chenopodium rubrum* by D-glucose and a glucose analog and tissue specific expression suggest a role in sink source regulation. *Plant Physiol* 108: 285-294.
- Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu Rev Plant Biol* 57: 675-709.
- Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. *Plant Cell* 14 (suppl.): S185-S205.
- Rolland F, Sheen J (2005) Sugar sensing and signalling networks in plants. *Biochem Soc Trans* 33: 269-270.
- Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M, Prado FE (2009) Soluble sugars - metabolism, sensing and abiotic stress. *Plant Signaling Behavior* 4: 388-393.
- Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F (2006) Starvation-induced expression of autophagy-related genes in Arabidopsis. *Biol Cell* 98: 53-67.
- Saglio PH, Pradet A (1980) Soluble sugars, respiration, and energy charge during aging of excised maize root tips. *Plant Physiol* 66: 516-519.
- Sahulka J, Lisa L (1978) The influence of exogenously supplied sucrose on glutamine synthetase and glutamate dehydrogenase levels in excised *Pisum sativum* roots. *Biol Plant* 20: 446-452.
- Salanoubat M, Belliard G (1989) The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis and sucrose concentration. *Gene* 84: 181-185.
- Schraudner M (1992) Biochemical plant responses to ozone. III. Activation of the defense related proteins  $\beta$ -1,3-glucanase and chitinase in tobacco leaves. *Plant Physiol* 99: 1321-1328.
- Sheen J (1990) Metabolic repression of transcription in higher plants. *Plant Cell* 2: 1027-1038.
- Sheen J, Zhou L, Jang JC (1999) Sugars as signaling molecules. *Curr Opin Plant Biol* 2: 410-418.
- Sherson SM, Alford HL, Forbes SM, Wallace G, Smith SM (2003) Roles of cell-wall invertases and monosaccharide transporters in the growth and development of *Arabidopsis*. *J Exp Bot* 54: 525-531.
- Sinha AK, Romer U, Kockenberger W, Hofmann M, Elling L, Roitsch T (2002) Metabolism and non-metabolizable sugars activate different signal transduction pathways in tomato. *Plant Physiol* 128: 1480-1489.

- Smeekens S (2000) Sugar induced signal transduction in plants. *Annu Rev Plant Physiol Plant Mol Biol* 51: 49-81.
- Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. *Curr Opin Plant Biol* 13: 274-279.
- Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. *Plant Physiol* 140: 637-246.
- Swarbrick PJ, Schulze-Lefert P, Scholes JD (2006) Metabolic consequences of susceptibility and resistance in barley leaves challenged with powdery mildew. *Plant Cell Environ* 29: 1061-1076.
- Tassi F, Maestri E, Restivo FM, Marminoli N (1992) The effects of carbon starvation on cellular metabolism and protein and RNA synthesis in *Gerbera* callus cultures. *Plant Sci* 83: 127-136.
- Thomas BR, Rodriquez R (1994) Metabolite signal regulate gene expression and source/sink relations in cereal seedlings. *Plant Physiol* 106: 1235-1239.
- Thomashow MF (1998) Role of cold-responsive genes in plant freezing tolerance. *Plant Physiol* 118: 1-7.
- Thompson AR, Vierstra RD (2005) Autophagic recycling: lessons from yeast help define the process in plants. *Curr Opin Plant Biol* 8: 165-173.
- Thompson JE, Legge RL, Barber RF (1987). The role of free radicals in senescence and wounding. *New Phytol* 105: 317-344.
- To JPC, Reiter WD, Gibson SI (2002) Mobilization of seed storage lipid by *Arabidopsis* seedlings is retarded in the presence of exogenous sugars. *BMC Plant Biol* 2: 4.
- Tran LS, Nakashima K, Shinozaki K, Yamaguchi Shinozaki K (2007) Plant gene networks in osmotic stress response: from genes to regulatory networks. *Methods Enzymol* 428: 109-128.
- Tripathi SK, Tuteja N (2007) Integrated signaling in flower senescence. *Plant Signal Beh* 2: 437-445.
- Tronsmo AM (1993) Resistance to winter stress factors in half-sib families of *Dactylis glomerata*, tested in a controlled environment. *Acta Agric Scand* 43: 89-96.
- Tsukaya H, Ohshima T, Naito S, Chino M, Komeda Y (1991) Sugar dependent expression of the CHS-A gene for chalcone synthase from petunia in transgenic *Arabidopsis*. *Plant Physiol* 97: 1414-1421.
- Usadel B, Blasing OE, Gibon Y, Retzlaff K, Hohne M, Gunther M, Stitt M (2008) Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in *Arabidopsis* rosettes. *Plant Physiol* 146: 1834-1861.
- van Doorn WG (2004) Is petal senescence due to sugar starvation? *Plant Physiol* 134: 35-42.
- van Doorn WG (2008) Is the onset of senescence in leaf cells of intact plants due to low or high sugar levels? *J Exp Bot* 59: 1963-1972.
- van Doorn WG, Woltering EJ (2008) Physiology and molecular biology of petal senescence. *J Exp Bot* 59: 453-480.
- Vauclare P, Bligny R, Gout E, De Meuron V, Widmer F (2010) Metabolic and structural rearrangement during dark-induced autophagy in soybean (*Glycine max* L.) nodules: an electron microscopy and  $^{31}\text{P}$  and  $^{13}\text{C}$  nuclear magnetic resonance study. *Planta* 231: 1495-1504.

- Vidhyasekaran P (1974a) Possible role of sugars in restriction of lesion development in finger millet leaves infected with *Helminthosporium tetramera*. *Physiol Plant Pathol* 4: 457-467.
- Vidhyasekaran P (1974b) Finger millet helminthosporiose, a low sugar disease. *Z Pflanzenkr Pflanzenschutz* 81: 28-38.
- Visser RG, Stolte A, Jacobsen E (1991) Expression of a chimaeric granule-bound starch synthase-GUS gene in transgenic potato plants. *Plant Mol Biol* 4: 691-699.
- Walsh KB, Vessey JK, Layzell DB (1987) Carbohydrate supply and N<sub>2</sub> fixation in soybean. *Plant Physiol* 85: 137-144.
- Wang HJ, Wan AR, Hsu CM, Lee KW, Yu SM, Jauh GY (2007) Transcriptomic adaptations in rice suspension cells under sucrose starvation. *Plant Mol Biol* 63: 441-463.
- Webster PL, Henry M (1987) Sucrose regulation of protein synthesis in pea root meristem cells. *Environ Exp Bot* 27: 253-262.
- Webster PL, van't Hof J (1973) Polyribosomes in proliferating and non-proliferating root meristem cells. *Am J Bot* 60: 117-121.
- Wittenbach VA (1977) Induced senescence of intact wheat seedlings and its reversibility. *Plant Physiol* 59: 1039-1042.
- Wittenbach VA, Lin W, Hebert RR (1982) Vacuolar localization of proteases and degradation of chloroplasts in mesophyll protoplasts from senescing primary wheat leaves. *Plant Physiol* 69: 98-102.
- Xu J, Dolan MC, Medrano G, Cramer CL, Weathers PJ (2011a) Green factory: Plants as bioproduction platforms for recombinant proteins. *Biotechnol Adv* doi:10.1016/j.biotechadv.2011.08.020 (in press).
- Xu J, Ge X, Dolan MC (2011b) Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. *Biotechnol Adv* 29: 278-299.
- Yang Z, Zhang L, Diao F, Huang M, Wu N (2004) Sucrose regulates elongation of carrot somatic embryo radicles as a signal molecule. *Plant Mol Biol* 54: 441-459.
- Yano K, Matsui S, Tsuchiya T, Maeshima M, Kutsuna N, Hasezawa S, Moriyasu Y (2004) Contribution of the plasma membrane and central vacuole in the formation of autolysosomes in cultured tobacco cells. *Plant Cell Physiol* 45: 951-957.
- Yoshida M, Nakajima T, Tonooka T (2008) Effect of nitrogen application at anthesis on *Fusarium* head blight and mycotoxin accumulation in breadmaking wheat in the western part of Japan. *J Gen Plant Pathol* 74: 355-363.
- Yu SM (1999) Cellular and genetic responses of plants to sugar starvation. *Plant Physiol* 121: 687-693.
- Yuanyuan M, Yali Z, Jiang L, Hongbo S (2009) Roles of plant soluble sugars and their responses to plant cold stress. *African J Biotech* 8: 2004-2010.