Is the onset of senescence in leaf cells of intact plants due to low or high sugar levels?

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Abstract
This review examines the hypotheses that developmental programmed cell death in leaves is mediated (i) by sugar starvation in the leaf cells or (ii) by sugar accumulation in these cells. Experimental evidence for both hypotheses is critically discussed and found to be lacking. For example, some papers show that sugars prevent senescence of cut leaves placed in darkness, and prevent low sugar levels in the leaves. In these tests, the sugars seem to replace photosynthesis, hence the results have little relevance to leaf senescence in intact plants in the light. Low nitrogen nutrition and high light results in earlier senescence than the low nitrogen treatment alone. This is accompanied by high sugar levels in the leaves. The results have led to the idea that accumulation of sugars is the cause of the additional effect, or more generally, that sugar accumulation is always the direct cause of leaf senescence. Results from over-expressing, or knocking out, hexokinase genes tend to support the high sugar hypothesis, but pleiotropic effects confound this conclusion. In addition, several experiments show the effects of treatments on senescence without the increase in leaf sugar levels. Nonetheless, sugar levels are usually measured in whole leaves. Such an overall level does not reflect the differences in the onset of senescence between tissues and cells, and can therefore not be used as an argument for or against either of the two hypotheses. It is argued that future work should determine the time line of the concentrations of various sugars in various cells and cellular compartments, in relation to senescence processes in the same cells. Taken together, the data are not decisive. It is possible that neither of the two hypotheses is correct.

Key words: Chloroplast, cytokinin, fructose, glucose, hexokinase, nitrogen partitioning, photosynthesis, programmed cell death, senescence, sucrose, yellowing.

Introduction
Leaf senescence during normal development, i.e. in the absence of pathogens, is associated with extensive degradation of macromolecules. The breakdown results in molecules that can be transported, via the phloem, to other parts of the plant (Bleecker and Patterson, 1997; Buchanan-Wollaston et al., 2003). Relatively early on during leaf senescence the chloroplasts become dismantled. The process includes degradation of internal membranes, a major part of the chloroplast proteins, and chlorophyll (Sukamoto, 2006; Hörtensteiner, 2006).

Leaf senescence is regulated by various external and internal factors. Examples are shortening of the days during autumn, drought, lack of nitrogen, and shading. Several signals connect these external factors to the onset of senescence. Senescence can also be due to internal factors such as lack of nutrients, or a change in sink–source relations, for example, at the onset of flowering or seed set.

The role of intercellular sugar levels as part of the pathway that induces leaf senescence has long been debated. Several authors found that a decrease in the rate of photosynthesis preceded leaf yellowing, which to some was suggestive of the idea that developmental leaf senescence is induced by low sugar levels (Quirino et al., 2000). According to one model, low sugar levels would increase ethylene production or sensitivity, whereby ethylene acts as an accelerator of the onset of leaf senescence (Grbic and Bleecker, 1995). However, several other authors adduced data that were interpreted to support the opposite idea: leaf senescence is due to elevated sugar

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levels in the cell rather than to sugar starvation (Yoshida, 2003; Wingler et al., 2006).

The present review examines the hypotheses that developmental leaf senescence in intact plants is (i) induced by carbohydrate starvation in the leaf cells, or (ii) due to elevated sugar levels in the cells. These hypotheses pertain to leaf senescence at the end of their normal life span, i.e. in the absence of pathogens and severe stress.

**Leaf senescence is induced by various factors, some of which might act through sugar levels**

The debate on the role of sugars in the regulation of the onset of leaf senescence might be confusing, at least in part, because there are several factors, and more than one pathway, leading to senescence. For example, transcripts of SAG12, a senescence-specific protease, become abundant in leaves that age and yellow on intact plants grown in the light. However, this increase in transcript abundance is not found after detaching leaves and placing them in darkness, which also hastens leaf senescence (Weaver et al., 1998; Noh and Amasino, 1999). This indicates that the two treatments do not induce identical pathways leading to senescence. Buchanan-Wollaston et al. (2005) studied gene expression during leaf senescence in intact Arabidopsis plants. A difference was found between leaves on plants placed in darkness and plants placed in light (the term ‘light’ will be used here to indicate a normal diurnal light–dark cycle). In both treatments, leaf yellowing was accompanied by increased transcript abundance of a large number of genes. However, the sets of genes in the two treatments only partially overlapped.

These data indicate that results of one treatment can not be used simply as an argument for what happens in another treatment, as the processes are not the same. A number of treatments that induce leaf yellowing might therefore be distinguished. The first is detachment. Severing a leaf prevents the flow of root-borne hormones, such as cytokinins, into the leaf. As leaf yellowing is highly sensitive to this hormone, detachment alone might induce yellowing. This was indeed found in experiments by Thimann et al. (1977), who did nonetheless find an effect of darkness in addition to that of detachment. The second is dark treatment. At least three methods of dark treatment should be distinguished. One is placing a detached leaf or leaf part in darkness, and comparing this with a treatment in light. The second is placing a whole plant in darkness, compared with a light treatment. In some species, placing whole plants in darkness delays yellowing in the lowermost leaves, compared to keeping the plants in the light, an effect possibly due to slower growth at the shoot tip (Ono et al., 1996; Weaver and Amasino, 2001). The third is shading individual older leaves on intact plants or placing such individual leaves on intact plants in complete darkness. Shading induced early leaf yellowing, which was largely explained by the lower rates of transpiration and the resulting lower flow of cytokinins into the leaf. Placing leaves of intact plants in darkness might be explained similarly (Pons and Jordi, 1998).

The results suggest that the question of what determines leaf senescence in intact plants in the light can only be studied by using exactly that material. Admittedly, a whole plant is a highly complex system in which leaf senescence is regulated by several internal factors such as the rate of growth of the shoot and the root, hormone flow into the leaf, nutrient uptake by the roots and nutrient requirements in the growing parts (Ono et al., 1996).

**Chloroplast dismantling**

Large-scale chloroplast dismantling is visibly observed as leaf yellowing, which is often used as a simple parameter of the progress of leaf senescence. Chloroplast degradation has been studied, in considerable detail, both at the ultrastructural and biochemical levels. The chloroplasts become considerably smaller, the thylakoid membranes dilate and then mostly disappear. Large droplets (plastoglobuli) containing a lipid-like substance accumulate in the chloroplast (Thomson and Platt-Aloia, 1988; Inada et al., 1998). Vesicles, apparently containing lipids, have been observed to leave the chloroplast (Guiamet et al., 1999), but it has remained unclear what happens with the contents of these vesicles. Reviews on chloroplast protein degradation (Hörtensteiner and Feller, 2002; Sakamoto, 2006) and chlorophyll catabolism (Hörtensteiner, 2006; Hörtensteiner and Lee, 2007) show that the degradation of several proteins of the photosynthetic machinery precedes chlorophyll degradation.

The signals that regulate the onset of chloroplast dismantling are as yet unclear (Lim et al., 2007). Cytokinins have a large effect on the onset of visible leaf senescence. The mechanism of action of cytokinins with regard to this process is still unknown (van Doorn, 2005; Riefler et al., 2006). One possible model is that cytokinin concentration controls a switch in gene expression. High cytokinin levels apparently cause expression of genes involved in photosynthesis and high net protein synthesis, whilst low levels of the hormone induce the expression of genes related to catabolism.

Chloroplast dismantling represents only part of the remobilization processes in the senescent leaf cell. It is accompanied by degradation processes in, for example, mitochondria, nuclei, peroxisomes, and vacuoles (Hopkins et al., 2007; Fischer, 2007).

**Sugar sensing and signalling**

The main role of sugars in metabolism is, obviously, its function as a carrier of energy and carbon. However,
sugars have several additional roles, such as the maintenance of osmotic potential and signalling the energy status of plant parts. At least three glucose signalling pathways have been discovered in yeast, each of which result in co-ordinate regulation of gene expression. The degree of regulation by sugars is even more complex in multicellular organisms. In plants, for example, the diurnal changes in enzyme activity relating to photosynthesis, carbon fixation, glycolysis, and glucose-phosphate metabolism is partially regulated through sugar signalling (Bläsing et al., 2005). In addition, multicellular plants exhibit rigorous co-ordination, and thus communication, between source and sink organs. Source activities such as photosynthesis, but also nutrient mobilization, are generally up-regulated under low sugar conditions. By contrast, sink activities such as growth and storage are up-regulated when sugars are present at high concentrations. Glucose levels in cells are continuously assessed by hexose kinases (HXKs), whilst some glucose signalling also occurs through a hexose kinase-independent pathway. HXKs are found to be associated with mitochondria, chloroplasts, the cytoplasm, and the nucleus. A complex interaction has been found between glucose signalling and signal transduction through hormones such as ABA, auxin, cytokinin, and ethylene. For example, HXK1 is present in high-molecular-weight complexes in the nucleus, where it controls transcription and the proteasome-mediated degradation of the EIN3 transcription factor, thereby counteracting the effect of ethylene (Rolland et al., 2006). Furthermore, sugars can prevent the up-regulation of EIN3 transcription factors during senescence (Hoebenriets et al., 2007), which might also act through HXK. These data indicate that it is plausible to assume that sugars might have a role in signalling at least some of the changes in overall plant status that lead to leaf senescence. The filling of the grains and its high demand on source leaves, for example, or the lack of nutrients in growing tissues, might, at least in part, promote leaf senescence by altering sugar signalling. The most parsimonious hypothesis, in this context, seems that a high demand for nutrients and sugars in growing plant parts, probably reflected in low N and sugar levels in these growing parts, sends a hormonal senescence signal to the older leaves.

Evidence in favour of the hypothesis that natural leaf senescence is induced by low sugar levels

The arguments that have been used in favour of the idea that low sugar levels in the leaf cells are the cause of leaf senescence, during normal development of intact plants growing at a diurnal light/dark cycle, are grouped here into (i) effects of sugar treatments, (ii) whole leaf sugar levels, and (iii) hexokinase mutants.

Sugar treatments

Thimann et al. (1977) studied senescence in segments of oat leaves floating on a solution, either in light or darkness. Infiltrating the segments with a solution containing glucose or sucrose inhibited yellowing in darkness and had no effect in the light. Treatment with mannitol was without effect. These data might be interpreted to suggest that low sugar levels in the leaves, as a result of lack of photosynthesis in darkness, are the cause of leaf yellowing. Thimann et al. (1977) showed that half of the decrease of chlorophyll could be prevented by sugar treatment. This was about the same as the difference between placing the detached leaves in the light or in darkness, suggesting that the sugars might undo the effect of darkness.

In the third and fourth leaves of Arabidopsis plants grown in the light, the transcript abundance of a senescence-associated gene, called sen 1, showed an increase by day 25, when the first flower was opening but no visible senescence was yet found in the leaves. Transcript abundance was still high by day 45, when several leaves had turned yellow. If cut leaves were placed in darkness, transcript abundance increased more rapidly than in leaves on plants in the light (Oh et al., 1996). Sugar treatment prevented the increase in sen 1 transcript abundance in cut leaves placed in darkness. The effect was mediated through the promoter of the gene. The authors suggested that low sugar levels might therefore be natural inducers of senescence in intact plants growing in the light (Chung et al., 1997). However, the data only show that sugars prevented the large effect of darkness in cut leaves.

In other experiments with Arabidopsis plants growing in the light, five genes, including a β-glucosidase and an asparagine synthetase, increased in transcript abundance by the time the leaves showed a little yellowing (one gene) or showed yellowing in as much as 75% of the leaf area (the other four genes). Placing the plants in darkness, or placing detached leaves in darkness, rapidly increased the transcript abundance of these five genes. Sucrose treatment of detached leaves placed in darkness prevented the expression of at least three genes (results on the two other genes were not reported). In this experiment, control leaves placed in light showed no increase in transcript abundance of two of these three genes. The third gene showed a small increase in transcript abundance, less than the increase in darkness (Fujiki et al., 2001). In suspension-cultured cells of Arabidopsis, cessation of sugar feeding induced the same five genes (Fujiki et al., 2000a). Similar results had been found with two other genes, both subunits of the branched-chain α-keto acid dehydrogenase. The two genes showed increased transcript abundance during leaf senescence in plants growing in the light, and a rapid increase in plants placed in darkness. Sucrose treatment of leaves placed in darkness prevented
the increase in transcript abundance induced by darkness (Fujiki et al., 2000b). Sugar levels were found to regulate tightly the promoter activity of these two genes in suspension-cultured Arabidopsis and tobacco cells (Fujiki et al., 2002). Again, these data show an effect of sugar only in darkness. The data did not show that lack of sugars induces leaf senescence in plants growing in the light.

NADP-dependent malic enzyme (NADP-ME) converts malate to pyruvate and NADPH. Overexpression of an NADP-ME gene in maize resulted in a 6–33-fold increase of activity. The transgenic plants showed no phenotype except for an earlier dark-induced leaf senescence. The effect of darkness on leaf senescence in the transgenic plants was counteracted by supplying malate, glucose, or sucrose. The data indicated that lack of a readily mobilized carbon source caused premature leaf senescence (Fahnenstich et al., 2007), and are in line with the other experiments here discussed. The data do not show that natural leaf senescence is due to such a lack of a readily metabolized carbon source.

The up-regulation of the cysteine protease SAG12 is specific for leaf senescence on the plant, and is not found in dark-induced leaf senescence of cut leaves. Noh and Amasino (1999) found that sugars (sucrose, glucose, and fructose) rapidly decreased the transcript abundance of SAG12 in Arabidopsis. In these tests, half-senescent leaves were detached from plants growing in the light, and were placed in sugar solutions in the light. These data show that sugars can repress the increase in transcript abundance of at least one gene that is very specific for developmental leaf senescence. The data do allow the suggestion that the increased transcript abundance of SAG12, during normal leaf senescence, is induced by low sugar levels. The same gene was rapidly induced by sugar deprivation in suspension-cultured Arabidopsis cells (Quirino et al., 2000).

Sugar levels
Quirino et al. (2000) found that isolated Arabidopsis leaves, held in darkness, first contained low levels of hexoses and disaccharides and then rapidly yellowed. The result was interpreted to favour the idea that low sugar levels can induce leaf yellowing. The results on sugar levels, however, pertain to whole leaves, which is not enough proof that a low sugar level in a cell precedes the chloroplast degradation in that cell. Cell death in leaves occurs first in the mesophyll, later in the epidermis, and still later in the tissues surrounding the vascular bundles (Lim et al., 2007). This may confound the conclusions drawn. Tissues at different stages of senescence should be subjected to separate analysis. Chloroplasts are mainly present in the mesophyll; hence sugar levels in the mesophyll should be compared with yellowing in the mesophyll. But even in the mesophyll, cells can be at various stages of senescence. This is true for cells close to the vascular bundle, which tend to senescence later than those further away. It is also true in leaves where senescence starts at the top or at the margins. So even further focusing at the individual cell level is necessary.

Hexokinase mutants
High sugar levels tend to repress photosynthesis. Hexokinase activity is involved in this regulation. The enzyme not only catalyses hexose phosphorylation but also senses hexose concentrations. Hexokinase overexpression has been found to produce hypersensitivity to glucose (Dai et al., 1999; Xiao et al., 2000). A gin2 mutant in Arabidopsis has a lesion in hexokinase 1 (HXX1), is glucose-insensitive, and shows delayed leaf senescence (Moore et al., 2003). This could be interpreted as evidence in favour of the hypothesis that a ‘low sugar’ signal induced leaf senescence, as there was no HXX to produce the normal sugar signal. Moreover, the glucose+ fructose levels were much lower in the mutant than in the wild type. However, the gin2 plants showed several pleiotropic effects, such as a very small root system, very small leaves, hypersensitivity to cytokinin, and insensitivity to auxin (Moore et al., 2003), suggesting that results on hexokinase overexpression or knockout mutants should be interpreted with extreme caution. The delay of leaf senescence, compared to wild-type, might, for example, also be attributed to hypersensitivity to endogenous cytokinin.

Taken together, the available data suggest that sugars may substitute for the effect of darkness on leaf senescence. Very little evidence favours the hypothesis that low sugar levels are triggers of leaf senescence in plants growing in the light. Thus far, only the results of Noh and Amasino (1999) seem good evidence in favour of the hypothesis. Sugar levels have been measured in whole leaves or leaf segments. This seems inadequate. Sugar levels should be determined per tissue, per cell, or even in various cellular compartments, in relation to various senescence processes, including chloroplast dismantling, in the same cell.

High sugar levels as a cause of leaf senescence
The arguments used in favour of the idea that high sugar levels in leaf cells are the cause of leaf senescence, during normal development of intact plants grown at a diurnal light/dark cycle, can be grouped into data on (i) treatments that induce high sugar levels in leaves, and (ii) hexokinase mutants.

Treatments that produce high sugar levels in leaves
Detached leaves of tobacco (Krapp et al., 1991) or barley (Parrott et al., 2005), incubated under strong light, showed accelerated yellowing, associated with sugar accumulation.
These data might suggest that high sugar levels can induce early yellowing in these species.

Placing leaves in solutions containing sugar reportedly hastens leaf yellowing in most cultivars of *Alstroemeria pelegrina* and some cultivars of *Lilium multilorum*, and in leaves on cut flowering stems of the same cultivars of these two species, as well as some *Chrysanthemum* cultivars (EJ Woltering, personal communication, 2007; WG van Doorn, unpublished results). These experiments were carried out under low light levels (15 μmol m⁻² s⁻¹). However, the same sugar treatments, at the same low light level, did not hasten leaf yellowing in cut flowering stems from a range of other genera (*Anemone, Antirrhinum, Asclepias, Aster, Campanula, Carthamus, Crocosmia, Dianthus, Eupatorium, Eustoma, Gladiolus, Gypsophila, Iris, Lysimachia, Matthiola, Rosa, Solidago, Solidaster, Tanacetum, Trachelium, Tulipa, Veronica*, and *Viburnum* (EJ Woltering, personal communication, 2007; WG van Doorn, unpublished results). The induction of leaf yellowing after feeding isolated tobacco leaves with glucose (Pourtau *et al.*, 2006) is not enough proof that glucose accumulation is part of the causal chain leading to yellowing, during normal leaf development in whole plants. The local high sugar levels in the cell walls might have led to cellular dehydration and this might induce yellowing because of water stress. These tests, therefore, do not show that high sugar levels within the cells induce leaf senescence.

Preventing phloem export by girdling treatments at the base of leaves on intact plants (Feller and Fischer, 1994) or barley plants (Parrott *et al.*, 2005) led to sugar accumulation in the leaves, which was associated with earlier leaf yellowing and, in the case of barley, with other indicators of leaf senescence such as an increase in protease activity. The data of Parrott *et al.* (2005) even show an association between leaf yellowing and a threshold sugar concentration in the leaves. These results suggest that high sugar levels in the leaf cells can, in principle, induce leaf senescence.

It is well known that low N or P nutrition of plants can induce early leaf senescence during the vegetative growth phase of plants. In experiments with sunflower (*Helianthus annuus*) grown at two N levels (8.0 and 0.8 mM nitrate) and two light levels (150 μmol m⁻² s⁻¹ and 450 μmol m⁻² s⁻¹), leaf senescence started about 10 d earlier in the plants grown at low N and high light, compared with the other treatments. Thus the effect of low N on senescence was only found at high light. The earlier leaf senescence was associated with much higher levels of glucose+sucrose+starch in the old leaves, clearly prior to the onset of senescence (Ono *et al.*, 1996). Further experiments showed that glucose and also sucrose levels were considerably higher in these leaves (Ono and Watanabe, 1997). These data show a positive correlation between advanced leaf senescence, as a result of nutrient stress during vegetative growth at high light levels, and leaf glucose and sucrose levels.

*Arabidopsis* plants showed earlier leaf senescence when grown at long days (16 light) rather than short days (12 h light), both at relatively low light levels (100 μmol m⁻² s⁻¹). Leaf senescence was even more advanced when plants were held at long days at relatively high light levels (200 μmol m⁻² s⁻¹). No positive correlation was found between leaf senescence and the glucose and fructose concentrations in the leaves of plants grown at low light levels, but the concentrations of these hexoses were higher in leaves of plants grown at relatively high light levels (Wingler *et al.*, 2006). The data showed that the time to leaf senescence in *Arabidopsis* is sometimes, but not always, correlated with overall leaf glucose and fructose concentrations.

When *Arabidopsis* plants were grown on agar at high and low N levels, the presence of 1–2% glucose or sucrose in the agar induced earlier leaf yellowing in the plants grown at low N levels. Addition of sorbitol or mannitol had no effect, indicating that the response was not due to an osmotic effect in the root zone (Pourtau *et al.*, 2004, 2006). The sugar treatment resulted in an early decrease of a chlorophyll fluorescence parameter (Fₒ/Fᵣ), which also decreased prior to normal developmental leaf yellowing (Wingler *et al.*, 2004). These data were taken to suggest the hypothesis that high sugar levels in the leaf cells are the cause of early leaf senescence. However, in these experiments, the effect of glucose feeding on senescence might be due to glucose accumulation in the leaf apoplast and therefore to osmotic stress in the leaf cells. It was argued that this osmotic stress hypothesis would not be valid since sorbitol and mannitol had no effect. This argument is not convincing though, as little of these two sugars may have been taken up by the roots and thus the sugars did not accumulate in the leaf apoplast to levels that induced osmotic stress. The concentration of sorbitol and mannitol in the leaves was not reported. Although Noh and Amasino (1999) reported that SAG12 accumulated during developmental leaf senescence in *Arabidopsis* and not as a result of 3 h of desiccation, the increase of the transcript abundance SAG12 (Pourtau *et al.*, 2006) in the leaves of plants held at high sugar levels in agar can not be taken as convincing evidence for the idea that high sugar levels and not osmotic stress induce leaf senescence, as the low osmotic potential in the cell walls might trigger water-stress-induced senescence over a much longer period than 3 h. Moreover, it is not known where in the leaf the SAG12 transcript accumulated. Only if water stress could be shown, under these conditions, to be unable to result in high abundance of the SAG12 transcript, would the data support the idea that there is another effect than water stress.

As described, it is well known that high sugar levels in a leaf tend to be related to a lower rate of photosynthesis,
due to feedback control. Jongeblod et al. (2004) studied natural ageing in leaves on intact plants of castor bean (Ricinus communis). Sugars in the leaves were shown to accumulate, followed by a decrease in chlorophyll concentration. This might also be interpreted by assuming that leaf senescence was due to high sugar levels. The data on sugar levels thus far pertain to whole leaves, and therefore to cells that are at various stages of senescence. They do not show where the sugars are in each cell, nor do they show the relationship with chloroplast dismantling in that cell. It is also not clear how the accumulated sugar is distributed between cells, organelles, and the cell walls. The high sugar levels might therefore even be the product of senescence-associated remobilization rather than the cause of senescence. It is known that various components, in particular proteins, are degraded prior to chlorophyll. The accumulation of sugars prior to chlorophyll degradation therefore is not fully convincing evidence in favour of the high sugar hypothesis. The sugars that are released after mobilization might be stored mainly in the vacuole of the senescing cell, be exported to the cell walls of the senescing cell, and/or to cells that transfer them to the phloem, and/or be present in the phloem. If high sugar levels are mainly present in the compartments mentioned above rather than in the cytoplasm, it is difficult to see how they would induce chloroplast dismantling. Thus each of the correlations, if found, between senescence and elevated sugar levels in whole leaves cannot be used to support the idea that high sugar levels in the cell are the cause of senescence.

Source and sink relationships might explain at least some of the relationships between leaf senescence and high sugar levels in these leaves. Growth of roots, leaves, or seeds can induce leaf senescence. However, the dry weight of new leaves or other tissues produced, and the mineral and sugar content of these tissues, is often not reported in studies on leaf senescence in whole plants. An exception is the work of Noh and Amasino (1999) which remarks that, under high light conditions with ample sugar supplies, Arabidopsis plants grow fast, reproduce abundantly, and show early leaf senescence. The HK1 mutant (gin2) cannot use the increased light input, shows delayed reproduction, and has a miniature root system and miniature leaves which exhibit delayed senescence. Thus the effect of the mutation on leaf senescence might be attributed not to the absence of a sugar signal but (among other possible explanations) simply to the lower growth rate of the shoot and root. Another exception is a study on sunflower plants grown at two N and two light levels (Ono et al., 1996; Ono and Watanabe, 1997). As described above, old leaves on plants grown at low N and high light senesced earlier than the old leaves on plants grown at the other conditions, and this was associated with higher sucrose and glucose levels in these leaves. The higher sugar levels in the old leaves, prior to visible senescence, was thought to be explained, at least partially, by the lower shoot growth at low N, which would reduce the demand for photosynthates. Indeed, reversal to high N levels in plants growing at low N and high light resulted in an increase in shoot growth, and a decrease of glucose and sucrose concentrations in the old leaves (Ono et al., 1996; Ono and Watanabe, 1997). Still, even these data do not show that the high sugar levels in the old leaves were the cause of their senescence.

Hexokinase mutants

Dai et al. (1999) produced transgenic tomato plants overexpressing a hexokinase (AtHXK1). The plants had reduced photosynthesis rates. Hexokinase overexpression resulted in a ‘high sugar level’ signal even in the absence of high sugar levels. Actually, the sugar content in mature leaves of the transgenic plants was lower than that in wild-type plants. The transgenic plants exhibited rapid yellowing of cotyledons and mature leaves, indicating an effect of hexokinase, signalling ‘high sugar’. The interpretation that early leaf yellowing was associated with a ‘high sugar’ signal (Yoshida, 2003; Lim et al., 2007) thus seems plausible. However, HK1 overexpression showed several pleiotropic effects, such as a very small shoot and root (Dai et al., 1999). HK1 knock-out plants also showed many pleiotropic effects, including hypersensitivity to cytokinin (Moore et al., 2003). The overexpression of HK1 might have the opposite effect, thus resulting in insensitivity to cytokinin. Anyway, the side-effects on plant growth makes it hard to draw any conclusion about the role of sugars in leaf senescence.

Taken together, the data do not exclude the possibility that, in some species, and in some conditions, a high level of sugars, in some cellular compartment, is the cause of leaf senescence. However, the hypothesis that high sugar levels in the cell is generally part of the causal chain leading to leaf senescence is as yet inadequately supported by experimental data. As in the case of the sugar starvation hypothesis, several data lack precision. Again the sugar levels in a cell, or even better in various cellular compartments, have to be studied in relation to the onset of chloroplast dismantling and other senescence processes in the same cell.

Effects of high ambient carbon dioxide concentrations

Exposure to high CO2 levels induced early leaf senescence in tobacco plants. The effect was accompanied by down-regulation of genes encoding proteins involved in photosynthesis. Soluble sugar levels in the leaves showed no correlation with senescence and transcript levels of the genes mentioned. Both leaf senescence and the down-regulation of genes encoding photosynthetic proteins were
delayed by expression of the cytokinin producing gene isopentenyltransferase (ipt) but again the levels of soluble sugars were about the same in wild-type and ipt transgenic plants. The results were interpreted to indicate that sugar accumulation can be excluded as a cause of the down-regulation of genes that encode photosynthetic proteins, and as a cause of the associated leaf yellowing (Ludewig and Sonnewald, 2000). Indeed, these data show that leaf yellowing can become advanced without the concomitant increase in soluble sugar concentration at the whole leaf level. However, the results do not exclude, of course, that high sugar levels at the cellular level are the cause of yellowing. The same objection as raised above must be advanced to these data, as the sugar data pertain to whole leaves rather than cells and are therefore not precise enough.

Other data show that high ambient carbon dioxide levels have variable effects on leaf senescence, which can be hastened, not be affected, or delayed. In these tests the leaf sugar levels were usually not determined. Conversely, high ambient carbon dioxide reportedly results in the accumulation of sugars in leaves or has no effect, but the correlation with senescence has usually not been reported (Wingler et al., 2006).

Effect of cell wall invertase activity: an argument for the sugar starvation or the sugar accumulation hypothesis?

Three types of invertases are present in higher plants, one localized to the vacuole, one to the cytoplasm, and one, called extracellular invertase, to the cell wall (Roitsch et al., 2000; Roitsch and González, 2004). In tobacco, Balibrea-Lara et al. (2004) showed that low cell wall invertase activity is required and necessary for the induction of leaf yellowing. Leaves of intact plants showed a sharp decrease in extracellular invertase activity prior to yellowing. Overexpression of a gene encoding an extracellular invertase, under the influence of the promoter of SAG12, resulted in higher extracellular invertase activity in the leaves and in a delay of leaf yellowing. The localized induction of the expression of an extracellular invertase in tobacco leaves, under the control of a chemical-induced promoter even resulted in green areas amidst a yellow background. In addition, the application of cytokinins, during leaf growth, increased the transcript abundance of a gene encoding an extracellular invertase, and increased the activity of the enzyme. Transgenic plants, in which the transcript abundance of a gene encoding an invertase-inhibitor was increased, showed earlier leaf yellowing, compared with non-transgenic control plants. Leaf yellowing in the transgenic plants was insensitive to treatment with kinetin (a cytokinin). This was in contrast with controls, where local kinetin applications on the leaf surface resulted in green segments in an otherwise yellow leaf. High expression of a gene encoding an invertase inhibitor thus prevented cytokinin action, which is an argument for the idea that cytokinin acts through the maintenance of high extracellular invertase activity. Taken together, and assuming that there are no side-effects on intracellular invertases, the data suggest that maintenance of extracellular invertase activity prevents the normal developmental leaf yellowing in tobacco. However, the tests do not show how the invertase exerts this effect. The results might be due to sugar concentrations in the cell, but it is not clear how this should be interpreted.

If the data are interpreted as showing a need for the import of sugars into the cell, in order to prevent it from senescing by sugar starvation, the result seems rather counterintuitive. One would think that if photosynthesis is no longer adequate to provide the cell with sugars, the cell can degrade other compounds to generate energy, and does not need low sugar levels as a signal to do so. It is not at all obvious that a photosynthetic cell would (i) be importing sugars from the apoplast (rather than exporting sugars to the apoplast), and (ii) that as soon as this sugar import stops the cell would start degradation of the chloroplasts.

Alternatively, one might postulate that low cell wall invertase is not required for bringing sugars into the cell, but for proper export of sugars out of the cell, during senescence. If invertase activity remained high, the transfer of sucrose from the cell into the cell wall, and subsequent uptake by the phloem could be impaired as the sucrose becomes converted back to hexoses. So a decrease in cellular invertase activity can be part of a concerted effort at sugar export to the phloem, during senescence. However, if this is so, it is not obvious why preventing the decrease in invertase activity prevents leaf yellowing. Therefore, it is at present not clear which of the two possible functions, or something else, underlies the relationship between low extracellular invertase activity and leaf yellowing.

Conclusions

Some experiments suggest that low sugar levels can induce leaf senescence, but other experiments indicate that high sugar levels can do the same. The experiments showing that sugars prevent senescence pertain, thus far, only to cut leaves placed in darkness. Under these conditions, sugars might solely replace the absence of photosynthesis. These data, therefore, are not enough evidence in favour of the idea that low sugar levels are the cause of leaf senescence in intact plants in the light. The best evidence for the idea that low sugar concentrations in the cell are in the pathway leading to yellowing comes from tests of Noh and Amasino (1999), who severed leaves that were already slightly senescent, placed them in a sugar solution and immediately found a decrease in the transcript abundance of senescence-associated genes.
The data showing that sugar feeding can induce leaf senescence pertain only to a few species, whereas in numerous other species there is no effect. Even if there is an effect of sugar feeding, this is not adequate evidence for the hypothesis that high sugars are in the causal chain that induces leaf senescence in intact plants of the same species. Similarly, the results on leaf girdling indicate that in a few species the accumulation of sugars in the leaves might induce leaf senescence, but if this is true it remains to be shown that this is also true for normal leaf senescence. Some other experiments have been interpreted to show that elevated sugar levels in leaves trigger the process of chloroplast dismantling (and senescence in general), but it was here argued that most of these experiments can also be interpreted, with good reason, differently. The hypothesis that elevated sugar levels in the cytoplasm is generally the cause of leaf senescence in intact plants is therefore very weakly supported. Some assertions found in the literature, for example, ‘sugar accumulation can trigger leaf senescence’, or ‘sugars are an important factor in the regulation of leaf senescence’ (Wingler et al., 2006), whereby the sugars are meant to accumulate in the cell prior to its senescence, are actually far from being justified.

It is quite possible that neither of the two hypotheses is correct. This might follow, for example, from the time line of chloroplast dismantling and other degradation processes. In many annual plants a progressive loss of photosynthesis occurs from the time of full leaf expansion (Hensel et al., 1993). This decrease of photosynthesis is preceded by reduced expression of genes encoding subunits of Rubisco, chlorophyll binding proteins, and various other proteins of the photosynthetic machinery (Hensel et al., 1993; Buchanan-Wollaston, 1997). The decrease in photosynthesis occurs concomitantly with a decline in the levels of several proteins involved in photosynthesis, and a decrease in Rubisco activity. Only by the time that photosynthesis has considerably decreased, does the level of chlorophyll start to decrease (Hensel et al., 1993). The ongoing decrease in photosynthesis reaches the compensation point, whereby leaves can no longer contribute to the assimilatory needs of the rest of the plant. Only by then the sugar levels in the cell may become low. Low or high sugar levels, if the process so conceived is correct, cannot be the cause of chloroplast dismantling and leaf yellowing. Low sugar levels that are the result of impaired chloroplast function might, at best, induce degradation in other parts of the cell, for example, that of proteins in proteasomes (in the cytoplasm and nucleus) and vacuoles, that of nucleic acids (in nuclei), and that of fatty acids (in peroxisomes).

The reduced expression of genes that code for proteins of the photosynthetic machinery is followed by increased expression of genes encoding proteins involved in cellular degradation. Both processes seem regulated by a decrease in cytokinin activity: high cytokinin levels maintain high expression of the genes involved in the synthesis of chloroplast proteins, whereas low cytokinin levels induce the expression of enzymes that degrade the chloroplast and the rest of the cell (Jordi et al., 2000; Lim et al., 2007). Once degradation is underway, sugars may accumulate inside the cell (for example, in the vacuole) and in the cell walls. One of the purposes of cellular remobilization during senescence is the production of sugars that can be transported out of the leaf. If so conceived, high sugar levels do not induce leaf senescence, but are the result of leaf senescence.

In plants grown at low N, there is good evidence for an association between high leaf sugar levels and leaf senescence (Ono et al., 1996; Ono and Watanabe, 1997; Wingler et al., 2006). Under these conditions the high sugar levels in the old leaves grown at low N was suggested to be due to low shoot growth at low N, as N (and not sugar) was the limiting factor for that growth (Ono et al., 1996; Ono and Watanabe, 1997). The results do not yet show whether high sugar levels in the old leaves are the cause or the result of senescence, at the cellular level. Nonetheless, at present it can not be excluded that, under conditions where sugars are not limiting the growth of other plant parts (roots, leaves, flowers, seeds), sugar accumulation in the cells of old leaves is among the causes of the senescence of these cells. This might even be in contrast with situations where sugars are the factor that limits the growth of the distant parts, as might happen, for example, during seed filling. It might be speculated that under these conditions leaf senescence might be associated with low sugar levels in the senescing cells, or even be due to low sugar levels in these cells. If this were the case, for example, in the experiments of Noh and Amasino (1999), at least some apparently conflicting results would be reconciled.

Future areas of research on leaf senescence in intact plants grown in a normal diurnal light cycle, a system which is to be preferred to others such as isolated leaves, leaf shading, or whole plants placed in darkness, should emphasize both the larger picture of sink source relations and focus on the finer details at the cellular level. Part of the larger picture is the development, prior to and during leaf senescence, of other plant parts, and the amount of nutrients and carbon that is involved in this development. In addition, as argued above at some length, the measurement of both sugar levels and senescence should take place at the level of tissues and cells.

References

Role of sugars in induction of leaf senescence


Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A. 2006. Effect of sugar-induced senescence on gene expression and...


