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Products of leaf primary carbon metabolism modulate the developmental programme determining plant morphology

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Abstract

Considerable effort has been expended on understanding the genetic networks that regulate leaf development and morphology, however, less attention has been given to the role of leaf carbon status in modulating the plant developmental programme. Unexpected changes in plant development have been observed in response to changes in leaf metabolism. The focus of this review will be to discuss how manipulation of leaf carbon metabolic pathways, such as the photosynthetic carbon reduction cycle and trehalose biosynthesis, has provided insights into links between metabolism and development.

Key words: Carbon metabolism, leaf development, leaf morphology, phenotypic plasticity, regulation.

Introduction

The primary role of the plant shoot is to produce leaves to capture light energy and to convert this to sugars in the process of photosynthesis. During seedling development leaves are produced from primordia generated by the shoot apical meristem. As the young seedling progresses from the juvenile vegetative phase, through the mature vegetative, to the reproductive stage, changes in leaf shape and size occur. These processes are under the control of an endogenous developmental programme and a number of the genes involved have now been identified (Poethig, 1990, 2003; Piazza *et al*., 2005). In addition to endogenous genetic mechanisms, shoot and leaf morphology is also modulated by environmental factors such as daylength and light availability (Björkman, 1981; Evans, 1996; Evans and Poorter, 2001; Adams and Langton, 2005; Kozuka *et al*., 2005). Changes in leaf and shoot morphology have been observed in transgenic plants with altered leaf metabolism suggesting that the plant developmental programme can be modulated by the metabolic status of the leaf. As yet the mechanism(s) that are involved in mediating these changes in growth and development in response to changes in metabolism have not been identified. However, some insight into the way in which leaf metabolism affects the plant developmental programme is now emerging. The main focus of this review will be to summarize the impact of transgenic changes in photosynthetic carbon fixation and trehalose metabolism, on leaf and shoot development and to discuss the mechanisms that may be responsible for this. Dramatic and unexpected leaf phenotypes have occurred in response to relatively minor changes in leaf metabolism other than photosynthesis and selected examples are presented to demonstrate the link between metabolic process in plants and development.

Effect of manipulation of photosynthetic carbon fixation

Optimizing photosynthetic carbon assimilation with the environment plays a crucial role in determining fitness and survival. Studies of biodiversity have shown that the relationship between photosynthetic capacity of leaves and investment of biomass in leaf area is conserved across species (Reich *et al*., 1997). This would suggest that plants have evolved mechanisms to ensure co-ordination of leaf development with metabolism. Given that photosynthesis in mature source leaves determines the availability of carbohydrate essential for plant growth, it is likely that products of primary metabolism may also play a role in mediating development.

The first enzyme in the Calvin cycle to be studied using antisense technology was Rubisco. Antisense Rubisco

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plants with reductions in photosynthesis accumulated less carbohydrate, resulting in changes in the root-to-shoot ratios and specific leaf area (specific leaf area (*SLA*) defines leaf area per unit of leaf carbon mass (m^2 kg^{-1}) (Quick *et al*., 1991; Fichtner *et al*., 1993; Stitt and Schulze, 1994). Indeed, a near linear relationship between photosynthetic rate, starch levels, and *SLA* over a wide range of reductions in Rubisco activity was observed (Fichtner *et al*., 1993). Interestingly, the decrease in carbohydrate availability seen in the Rubsico antisense plants also caused a delay in the normal timing of developmental events. The leaves produced on the shoots of plants in the juvenile phase of development are smaller and simpler than those of the adult plant and the Rubisco antisense plants continued to produce this type of leaves until node 11, in contrast with the wild-type plants which had adult leaves by node 8 (Tsai *et al*., 1997). This suggested that these plants had an extended period in the juvenile vegetative phase. Increased leaf longevity was also observed, suggesting that senescence was delayed in the antisense Rubisco plants (Tsai *et al*., 1997; Miller *et al*., 2000). These results provided the first evidence that reductions in photosynthetic carbon assimilation, resulting in reduced availability of carbohydrate (source strength), not only had an impact on plant yield but also played a role in modulating the developmental programme of the shoot. The only confounding factor in these studies is that the antisense plants used only had 20% of the wild-type Rubisco activity and, as a consequence, large changes in nitrate and the nitrogen status of the leaves as Rubisco constitutes approximately 25% of leaf protein. This raises the possibility that the changes in development observed in the anitsense Rubisco plants were due not only to an altered carbon status but that changes in N status may also be having an impact (Masle *et al*., 1993; Fichtener *et al*., 1993).

By contrast to Rubisco the enzyme sedoheptulose-1,7-*bis*phosphatase (SBPase: EC 3.1.3.37) accounts for less than 1% of leaf protein, therefore antisense down-regulation of this enzyme would not lead to large perturbations in nitrogen balance and hence direct effects of photosynthesis on leaf development could be examined. SBPase functions in the regenerative phase of the Calvin cycle where it catalyses the dephosphorylation of sedoheptulose-1,7-*bis*phosphate. Transgenic tobacco plants with small reductions in SBPase activity were found to have decreased rates of photosynthetic carbon fixation (Harrison *et al*., 1998, 2001; Olcer *et al*., 2001). Analyses of the SBPase antisense plants revealed that small changes in photosynthesis resulted in a reduction in starch accumulation in the source leaves and changes in leaf and shoot development (Lawson *et al*., 2006). As with the Rubisco antisense plants, a change in *SLA* was evident in the SBPase antisense plants, but no delay in the development of the juvenile phase of vegetative growth was seen. What was interesting about the impact of reduced SBPase activity was that growth showed a bimodal response to reductions in SBPase activity (Lawson *et al*., 2006). Antisense plants with small reductions in SBPase activity and, concomitantly, small decreases in the end of day levels of starch, were shorter and produced smaller, thicker leaves (reduced specific leaf area) when compared with wild-type plants. By contrast, plants with large reductions in SBPase activity had low levels of both starch and sucrose, and in these plants the specific leaf area was increased and the height of the plants was either similar to, or taller, with thinner stems when compared with wild-type plants. Similar changes in shoot morphology have been observed in some species in response to light levels in the growth environment (Björkman, 1981; Evans, 1996). In growth conditions where light is limiting, and the carbohydrate status of the leaf reduced, plants produce thinner, larger leaves and longer, thinner stems to maximize light capture. Recently, evidence has been provided indicating a role for photoassimilated sucrose in the regulation of leaf growth patterns in response to shade (Kozuka *et al*., 2005). It is possible that the reductions in source capacity in the SBPase antisense plants alter the metabolic signals that mimic the acclimatory responses to different light environments, but as yet no candidate molecules mediating this response have been identified. At the whole plant level increased *SLA* would attenuate the effects of decreased photosynthesis. This is likely to be beneficial to the plant as resources are invested in leaf area to increase light capture, rather than photosynthetic machinery. A correlation between an increase in specific leaf area and reduced photosynthetic rates was observed in the Calvin cycle antisense plants (Stitt and Schulze, 1994; Price *et al*., 1995; Banks *et al*., 1999; Raines 2003). The conservation of response in this diverse set of transgenic plants suggests a common signal coming either directly or indirectly from photosynthesis that can influence shoot development.

Impact of transgenic manipulation of trehalose metabolism

A striking impact on specific leaf area and its relationship to photosynthesis and carbohydrate metabolism has been observed in plants expressing *E. coli* genes encoding enzymes in the trehalose pathway. The trehalose pathway, once believed to be of curiosity interest in plants, is now known to be indispensable and probably universal playing a central role in regulating carbon use for growth (Eastmond *et al*., 2002; Schluepmann *et al*., 2003). It is thought that a key role is played by the signal metabolite trehalose 6-phosphate (T-6-P) (Schluepmann *et al*., 2003, 2004; Pellny *et al*., 2004; Lunn *et al*., 2006). Remarkably, elevated T-6-P in transgenic tobacco leads to a decrease in *SLA* and up to a 40% increase in photosynthetic carbon assimilation per unit leaf area, due mainly to increased

Rubisco activity (Pellny *et al*., 2004). However, because *SLA* and leaf area per plant is lower, photosynthesis at the whole plant level does not differ from the wild type. In plants expressing transgenes encoding trehalose phosphate phosphatase or trehalose phosphate hydrolase to reduce T-6-P content, *SLA* was increased and, in this case, photosynthetic capacity per unit leaf area was lower but, at the whole plant level, greater leaf area compensated for the decrease in photosynthetic capacity. The changes in leaf development observed in plants with altered T-6-P levels may be a consequence of the altered allocation of carbon between starch and sucrose. The recent finding that high levels of T-6-P stimulate the post-translational activation of ADP glucose pyrophosphorylase, thereby increasing starch biosynthesis, provides a link between cytosolic metabolism and the chloroplast (Kolbe *et al*., 2005; Lunn *et al*., 2006). This work supports the central role of T-6-P in regulating carbon metabolism in plants and the possibility that T-6-P effects on leaf development can be explained through this route. T-6-P may link cytosolic metabolism with that of the chloroplast, but the full mechanistic basis for this has yet to be established. The mode of action of T-6-P and regulation of T-6-P metabolism are two important questions to be addressed to further our understanding of how this signalling network functions.

A role for starch/sucrose ratios and T-6-P in regulating leaf development?

The similarities in leaf phenotypes (altered *SLA*), together with changes in starch-to-sucrose ratios, found in the plants with reduced Calvin cycle activity and plants with altered T-6-P levels, raises the interesting possibility that a common mechanism was responsible for these changes. These results suggest that a balance between the utilization and storage of fixed carbon plays a role in the modulation of leaf development. The importance of sucrose-to-starch ratios for growth was demonstrated by analysis of tomato plants overexpressing sucrose phosphate synthase (SPS). This manipulation has the effect of directing the newly fixed carbon into sucrose, at the expense of starch accumulation. A small change in this balance in favour of sucrose accelerated floral development and increased fruit yield in tomato but, if an excess of newly fixed carbon was used to synthesize sucrose, then no increase in growth was evident (Sharkey *et al*., 2004). The mechanism underlying the increase in plant yield in the overexpressing SPS lines is not clear but it was not due to a stimulation in photosynthesis. The major effect was to partition a higher proportion of newly fixed carbon directly into sucrose, resulting in an increase in the sucrose-to-starch ratio.

Three further studies have highlighted the potential importance of the regulation of carbon allocation to starch and sucrose biosynthesis. Changes in the starch-to-sucrose ratio were evident in the SBPase antisense plants and, in source leaves, starch levels were decreased in response to small reductions in SBPase activity. Interestingly, in these plants with small reductions in SBPase activity, sucrose accumulated in the young leaves, despite having lower levels of starch in the source leaves. To explain this result it was suggested that the plant was able to 'sense' the source limitation and, to compensate, the growth of the shoot was reduced, imposing a temporary sink limitation in the young leaves (Olcer *et al*., 2001; Lawson *et al*., 2006). A more recent study where daily starch accumulation was limited by altering the length of the photoperiod provides support for this hypothesis. Reductions in the levels of starch accumulation during the photoperiod resulted in a depletion of sugar levels at the end of the night but, surprisingly, when sugar became available at the start of the next photoperiod, it was not used and starch accumulated (Gibon *et al*., 2004). It is likely that this accumulation of carbohydrate occurs in response to reduced growth caused by a shortage of sugars for growth in the dark phase. The importance of carbon allocation over the diurnal cycle, was also shown using a range of transgenic plants with altered metabolism (Kehr *et al*., 1998). Taken together the studies described in this section highlight the potential importance of the availability of carbohydrate, for utilization in the dark, in the regulation of growth and development. The mechanism by which plants sense changes in the availability of carbohydrate and how this feeds through to effects on growth are not known. A number of recent studies have provided evidence showing that the majority of starch degradation at night occurs through the hydrolytic pathway, resulting in the formation of maltose and glucose in the cytosol (Fig. 1). This opens up the possibility that starch to sucrose conversion and the resulting altered starch: sucrose ratios can be sensed by tracking flux through the cytosolic hexokinase (Sharkey *et al*., 2004). This mechanism is likely to form part of a complex regulatory network, involving T-6-P, that maintains a balance between carbon storage and utilization. How such changes in carbon balance are then linked to changes in development is not known, but hexokinase may play a central role in this process by integrating the effects of light, hormones, and nutrient status (Rollond and Sheen, 2005) (Fig. 1).

Changes in non-photosynthetic metabolism impact on leaf development

In addition to the impact of changes in photosynthetic carbon fixation on development, evidence is accumulating to suggest that development in plants is also sensitive to changes in the flux of carbon in the chloroplast, between the chloroplast and the cytosol and also in the cytosol. A number of mutants have been produced in which the flux of carbon has been altered and this has led to dramatic and

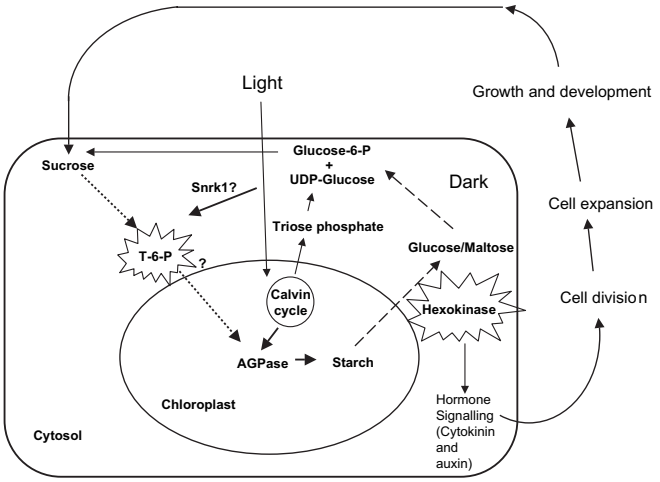

Fig. 1. The central role of metabolism in mediating the regulation of growth and development. During the day light absorbed is used to fix carbon in the Calvin cycle producing intermediates for both starch and sucrose biosynthesis (solid arrows). Starch produced during the photoperiod is broken down at night into glucose and maltose via the hydrolytic pathway to provide sucrose for growth at night (broken lines). Levels of sucrose in the cytosol are communicated to the chloroplast by T-6-P by an, as yet, unknown process and increased T-6-P activates AGPase increasing starch accumulation (dashed line). The mechanism by which T-6-P modulates AGPase activity is also not known but may be mediated by thioredoxin. It has been proposed that the glucose sensor, hexokinase, integrates light, hormone, and nutrient signalling to control plant development. Under conditions where starch levels are reduced, sucrose supply is restricted and growth is slowed to compensate. Conversely, when growth is restricted, sucrose accumulates and starch levels increase.

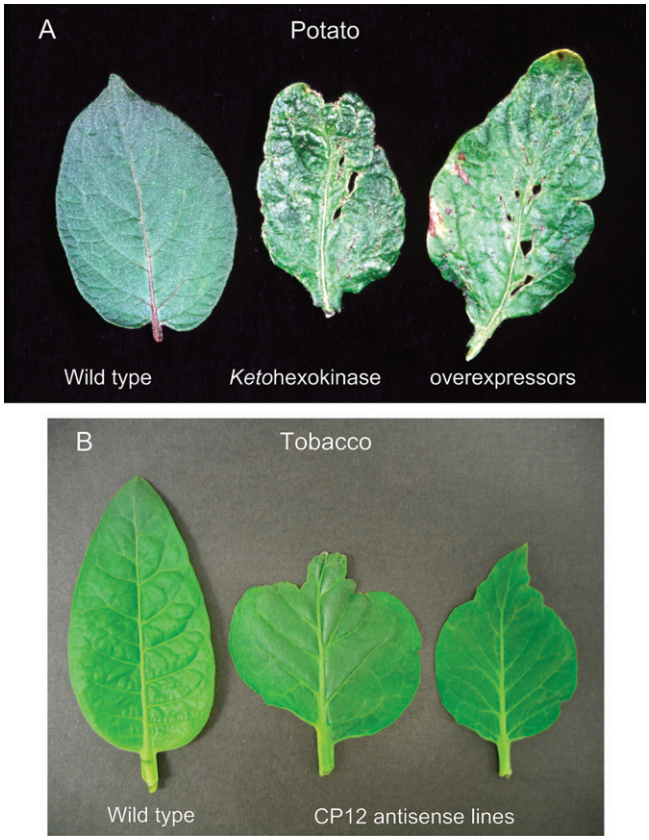

Fig. 2. Leaf phenotypes. (A) Potato plants over-expressing *E. coli keto*hexokinase. (B) Antisense tobacco plants with reduced levels of the chloroplast protein CP12. The shape of the leaf is dramatically modified in both of these plants with increased leaf thickness and prominent veins. Figure 2A was provided by Dr P Geigenberger and Dr A Fernie, MPI, Potsdam (see Geigenberger *et al*., 2004 for the full reference) and is reproduced by kind permission of Springer Science and Business Media.

unexpected changes in leaf shoot morphology. Selected examples are discussed below to highlight some of the effects of metabolic perturbations on leaf development.

The enzyme *keto*hexokinase (KHK) is not found in plants, but in *E. coli* it catalyses the conversion of fructose to fructose-1-phosphate. Ectopic expression of KHK in transgenic potato plants creates an alternative pathway of fructose metabolism and leads to severe abnormalities in leaf area and shape (Fig. 2A). The main metabolic effect of this manipulation is to increase the levels of triose phosphates and glyceraldehyde. KHK expression also resulted in a reduction in photosynthetic carbon fixation by as much as 50%, and flux of carbon to both starch and sucrose was also decreased. An increase in respiratory activity was evident and the activity of glucose-6-phosphate dehydrogenase, the first enzyme in the oxidative pentose phosphate pathway, was increased by 50%, suggesting an increased demand for NAD(P)H in these plants. (Geigenberger *et al*., 2004). A severe leaf phenotype, with characteristics similar to that observed in the KHK overexpressing potato plants, was observed in antisense tobacco plants with reduced levles of a small chloroplast protein, CP12 (Fig. 2B). This CP12 antisense phenotype was not expected as the proposed role for CP12 is in the regulation of two enzymes in the Calvin cycle phosphoribulokinase (PRKase) and NADP-glyceraldehyde-2-phosphate dehydrogenase (GAPDH). Given that neither the PRKase (Paul *et al*., 1995) or the GAPDH (Paul *et al*., 1995; Price *et al*., 1995) antisense plants exhibited such a phenotype, it seems likely that CP12 plays a wider role in chloroplast metabolism over an above regulating the activity of PRK and GAPDH (C Raines *et al*., unpublished observations). Interestingly, analysis of the CP12 antisense plants revealed an increase in the activity of glucose-6-phosphate dehydrogenase and a decrease in plastid malate dehydrogenase activity in the light. These data indicate that, as with the KHK overexpressing potato, the NADPH status of the CP12 plants was altered, suggesting that a change in redox state may be part of the underlying cause for the abnormal leaf phenotypes. An alternative explanation may be that changes in carbon fluxes are occurring, altering the availability of compounds such as homones essential for the control of leaf development.

Large changes in carbon fluxes with a reallocation of photosynthate from sucrose biosynthesis into organic acids also occurred in plants with reductions in the levels of the plastidic 2-oxoglutarate/malate translocator DiT1. These plants had altered leaf morphology, irregular leaf margins,

and reduced apical dominance (Schneidereit *et al*., 2006). Changes in leaf phenotype have also been observed in *Arabidopsis* plants with a mutation in the crumpled leaf gene, *CRL*, encoding a protein of the plastid outer envelope. No function has been found for the product of this gene but it is possible that it has a role in metabolism at the interface between the chloroplast and the cytosol (Asano *et al*., 2004). These two examples provide further evidence of the importance of regulating carbon fluxes in determining plant development. Systematic analysis of plants with leaf phenotypes such as those described here will provide invaluable information on the regulatory networks involved in co-ordinating metabolism and growth.

Conclusions

This review discusses evidence from the current literature that highlights the links between leaf metabolism and development. In a diverse set of Calvin cycle antisense plants similar changes in leaf development were noted, suggesting that there may be a common signal from photosynthesis that regulates this aspect of development. Part of this mechanism may involve T-6-P as a signal molecule to maintain a balance between the utilization and storage of carbohydrates. How these metabolites are sensed by the plant and how cell division and expansion are influenced by changes in metabolism are future challenges that need to be resolved to enable the maximum potential to be gained from transgenic manipulation of photosynthetic metabolism to increase yields.

Manipulation of pathways of chloroplast metabolism, other than photosynthesis has yielded a number of mutants with altered leaf phenotypes, but the signalling pathways involved are at present unknown. Products from phenolic acid metabolism, sterol biosynthesis, and isoprenoid biosynthesis have been shown to have an impact on the regulation of leaf development (Streatfield *et al*., 1998; Tamagnone *et al*., 1998; Estevez *et al*., 2000; Carland *et al*., 2002). Understanding the factors regulating the flux of carbon between these pathways will be important if plants are to be fully exploited as biofactories for the production of commercially important compounds.

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