Multiple feedbacks between chloroplast and whole plant in the context of plant adaptation and acclimation to the environment

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This review focuses on feedback pathways that serve to match plant energy acquisition with plant energy utilization, and thereby aid in the optimization of chloroplast and whole-plant function in a given environment. First, the role of source–sink signalling in adjusting photosynthetic capacity (light harvesting, photochemistry and carbon fixation) to meet whole-plant carbohydrate demand is briefly reviewed. Contrasting overall outcomes, i.e. increased plant growth versus plant growth arrest, are described and related to respective contrasting environments that either do or do not present opportunities for plant growth. Next, new insights into chloroplast-generated oxidative signals, and their modulation by specific components of the chloroplast’s photoprotective network, are reviewed with respect to their ability to block foliar phloem-loading complexes, and, thereby, affect both plant growth and plant biotic defences. Lastly, carbon export capacity is described as a newly identified tuning point that has been subjected to the evolution of differential responses in plant varieties (ecotypes) and species from different geographical origins with contrasting environmental challenges.

1. Introduction

Multiple pathways of communication, over both short and long distances, between the various functional parts of the plant are being elucidated. It appears self-evident that such communication pathways should enable the plant to respond appropriately to opportunities as well as challenges in its physical and biological environment. We place the communication between chloroplast and the whole plant into a context of the adjustment in form and function via physiological acclimation of individual plants to their growth environment as well as genetic adaptation of species and varieties (ecotypes) to the climate and other features of the habitats in which the respective lines evolved.

2. Matching energy acquisition with energy utilization: established and emerging signalling pathways serving to optimize chloroplast and whole-plant function in a given environment

Here, we review the signalling pathways serving to match light availability, and energy acquisition in photosynthesis, with energy utilization for plant growth, development and defences—in the context of plant genetic make-up, developmental status and a wide range of environmental conditions. Figure 1 presents the principal functional sequence from solar energy acquisition and sugar production to sugar distribution throughout the plant (solid arrows), starting with light absorption in the light-harvesting complexes (light harvesting) and primary photochemistry (in photosystem II (PSII) and the oxygen-evolving complex (OEC)) supporting photosynthetic electron transport and CO₂ fixation.
(in the Calvin cycle (CC)), followed by the loading of sugars into the phloem conduits and the transport of sugars to the plant’s sinks (sugar-consuming and sugar-storing tissues). In addition, figure 1 features a host of feedback loops falling into two principal categories of either (i) (dashed arrows along the top) providing feedback from the plant’s sugar-transport system and its sinks, via modulation of foliar sugar/starch content, back to light harvesting, photochemistry and CO2 fixation or (ii) (dotted arrows along the bottom) providing both feed-forward and feedback loops (dotted arrows along the bottom) associated with chloroplast redox balance (determined by the ratio, and action of, oxidants and antioxidants) as influenced by the environment and by the chloroplast’s photoprotective network. In the following, we will examine examples of source- and sink-driven signalling networks, and will argue that both source- and sink-driven signals modulate phloem loading/transport, and thereby impact plant response to environmental stresses.

(a) Sink-to-source signalling in photosynthetic adjustment

It has long been recognized that the leaf’s capacity for photosynthesis is adjusted in response to the level of demand generated by the plant’s sinks for the products of photosynthesis [1–3]. A plant’s inherent growth rate, developmental status and the opportunity (or lack thereof) for plant growth presented in the environment all contribute to setting this ‘sink demand’ as the level of plant sink strength. For example, (i) short-lived annual species tend to have higher inherent growth rates than slowly growing evergreens, (ii) a plant in its reproductive state tends to have greater sink strength than a vegetative plant and (iii) soil conditions (e.g. water and nutrient availability, temperature and soil texture/structure), together with climatic features (e.g. light availability, temperature, humidity), cumulatively generate either plant growth-promoting or plant growth-impeding environmental conditions. It has been shown that regulation of foliar photosynthetic capacity occurs via modulation of the expression of photosynthetic genes involved in e.g. light harvesting, photochemistry and other thylakoid processes, and CO2 fixation (e.g. [4–6]). The negative feedback via limiting carbon export was established by studying photosynthetic repression in response to inhibition of carbon export from leaves via e.g. girdling of branches or trees (e.g. [7–9]) or cold-girdling of stems [1,5]; the negative feedback from limiting sink strength was demonstrated e.g. via photosynthetic repression in response to removal of fruit and other sinks (e.g. [7,10–12]). Foliar sugar and/or starch accumulation are typically observed under either limiting carbon export at the leaf level or limiting plant sink strength [13].

(b) Contrasting overall outcomes in environments with contrasting opportunities for plant growth: increased plant growth versus plant growth arrest

Figure 2 illustrates that two principal scenarios with opposite outcomes can be distinguished by examining the level of ‘opportunity’ for plant growth presented by the environment. We will illustrate these scenarios for the example of overall plant growth response to resource availability in the environment, while acknowledging that the same principles also apply to other more complex features, including temporal dynamics in the environment and in plant-organ-specific growth patterns. Plant response to opportunity presented by the environment presumably involves the feedback loops detailed in §2a and some of those discussed in §2c.
On the one hand (figure 2, left-hand side), there is the scenario of an increase in the opportunity for plant growth presented by the environment. Such a scenario can arise via increased availability of previously limiting resources such as light, water and/or nutrients, a shift to a more favourable temperature regime, etc., experienced by a species with the appropriate genetic adaptations and developmental state allowing it to upregulate photosynthetic capacity, carbon export capacity (see §3) and growth rate. As an additional response to increased light availability, any plant not increasing its growth and photosynthesis rates enough to use all of the additional available light will probably upregulate its capacity for thermal dissipation of any excess of absorbed light [14,15] and/or employ a variety of mechanisms to decrease light interception, e.g. via changes in leaf angle or leaf reflectance [16–19], or decrease light absorption, via decreases in chlorophyll content and light-harvesting capacity (e.g. [20,21]).

On the other hand (figure 2, right-hand side), limiting or no opportunity for growth is presented in environments with either limiting or excessive resources (like water, nutrients) or unfavourable temperatures. Such environmental conditions typically result in reduced growth or even growth arrest and a resulting decrease in plant sink strength [13]. Furthermore, in the latter environments, photosynthetic depression, with decreased photosynthetic capacity and light-harvesting capacity and/or inactivation of photochemistry (‘photoinhibition’), is seen [13,22,23].

While decreased photosynthetic capacity and light-harvesting capacity are features that have long been associated with photosynthetic depression under sink limitation (see above), the phenomenon of the ‘photoinhibition’ of photosynthesis— involving an inactivation of PSII and/or OEC—is commonly instead interpreted as a sign of ‘damage’ (e.g. [24–31]). We have recently summarized evidence showing that, in all cases in which photoinhibitory inactivation of photochemistry and foliar carbohydrate levels were both assessed, photoinhibition was found to be associated with greater accumulation of foliar sugar and/or starch [13]. Viewing such responses in the context of the whole plant in its environment thus offers opportunities for a more holistic interpretation of responses like the phenomenon of ‘photoinhibition’. The invariable association of photoinhibition with foliar carbohydrate accumulation in plants under field conditions suggests that the tacit assumption that photoinhibition limits plant productivity (e.g. [32–40]) should be re-assessed for the possibility that limiting plant productivity instead triggers photoinhibition [13]. Based on the above-described data, the possibility that inactivation of PSII and/or OEC is, in fact, a principal component (together with decreased light harvesting and CO₂ fixation) of photosynthetic repression under certain sink limiting conditions should be seriously considered.

There is a third scenario (figure 2, middle), where light availability is suddenly increased for a plant that is not genetically adapted for, and/or finds itself in an environment not suitable for, increasing carbon export and/or plant sink strength. Such a scenario is not common in natural settings, but is a frequently used experimental approach to study photoinhibition in response to sudden transfer of shade-grown plants to high light. We have shown that sudden transfer of evergreens grown under non-fluctuating low light levels to highly excessive light results in photoinhibition of PSII and of photosynthetic capacity accompanied by foliar carbohydrate accumulation [13,41]. Especially in evergreens, which, as their name implies, do not commonly lower their chlorophyll content (or their light-harvesting capacity), photoinhibition is associated with a conversion of the otherwise rapidly reversible, flexible thermal dissipation of excess light (assessed from non-photochemical quenching (NPQ) of chlorophyll fluorescence) to a less flexible form of thermal dissipation (sustained NPQ) remaining continuously engaged for prolonged time periods [14,15,22,41–43]. An analogous scenario for annuals, as species presumably possessing sufficient carbon
was not significant, double mutant npq1 lut2 exhibited significantly greater wall ingrowths than wild-type when transferred from low to high growth light. The PsbS-deficient npq4 mutant exhibited significantly greater oxylipin levels than wild-type under herbivore attack in the field but not in the absence of herbivores.

### Table 1. Summary of evidence for a role of the antioxidant tocopherol, the photosystem II protein PsbS and the xanthophyll zeaxanthin in modulating cell wall ingrowths in phloem-loading complexes, herbivore resistance and oxylipin levels. While the npq1 mutant exhibited a trend for increased cell wall ingrowths that was not significant, double mutant npq1 lut2 exhibited significantly greater wall ingrowths than wild-type when transferred from low to high growth light. The PsbS-deficient npq4 mutant exhibited significantly greater oxylipin levels than wild-type under herbivore attack in the field but not in the absence of herbivores.

<table>
<thead>
<tr>
<th>Mutant or Treatment</th>
<th>Resulting Effect</th>
<th>Sources</th>
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<tbody>
<tr>
<td>vte mutant (tocopherol-/vitamin E-deficient)</td>
<td>Calllose deposition resulting in enhanced wall ingrowths in phloem-loading complexes</td>
<td>[57]</td>
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<tr>
<td>Oxilipin (methyl jasmonate) treatment</td>
<td>Enhanced wall ingrowths in phloem-loading complexes</td>
<td>[58]</td>
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<tr>
<td>npq1 lut2 mutants (zeaxanthin- and lutein-deficient)</td>
<td>Enhanced wall ingrowths in phloem-loading complexes</td>
<td>[56]</td>
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<tr>
<td>npq4 mutant (PsbS-deficient)</td>
<td>Enhanced herbivore resistance</td>
<td>[59]</td>
</tr>
<tr>
<td>Vte mutant (tocopherol-/vitamin E-deficient)</td>
<td>Enhanced oxylipin levels and enhanced ROS levels</td>
<td>[60–63]</td>
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<td>npq1 mutant (zeaxanthin-deficient)</td>
<td>Enhanced oxylipin levels</td>
<td>[56]</td>
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<td>[59,64]</td>
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export capacity and sink strength to respond to increased light availability, is a sudden transfer from low to high growth light environment under limiting soil nitrogen supply, which results in photoinhibition as well [44].

### (c) Oxidative signals and phloem blocking/occlusion as modulated by chloroplast photoprotection

In the lower portion of figure 1 (dotted arrows), selected examples are featured for the generation of oxidative signals associated with light-harvesting and primary photochemistry in plants adapted and acclimated to environments where leaves absorb a greater level of light than they are able to use through electron transport, a large fraction of the resulting excess excitation energy is safely dissipated (as heat; figure 1) via thermal dissipation of excess excitation energy [23]. The low remaining levels of excitation energy in excess of what is used in electron transport lead to the formation of reactive oxygen species (ROS; figure 1). At low concentrations, ROS trigger formation of oxidative signals (figure 1), while massive ROS formation presumably leads to cellular damage [45,46]. In light-harvesting complexes, excess excitation energy promotes the formation of (electronically excited) singlet oxygen as a form of ROS, while charge separation in primary photochemistry can give rise to the formation of singly reduced superoxide as the second major form of ROS arising in the light reactions [47]. Chloroplast antioxidant defences (figure 1) de-excite, and thereby detoxify, both singlet oxygen and superoxide, but highly excessive light levels presumably allow remaining ROS to trigger multiple redox-associated signalling pathways. For selected reviews of various examples of the many different components of the redox-signalling network originating in the chloroplast, see the studies of Munne-Bosch et al. [48], Baginsky & Link [49], Dietz et al. [50], Foyer et al. [51] and Mullineaux et al. [52]. We will highlight a selected example of one such signalling pathway, leading to the lipid-peroxidation-derived oxylipin messengers (figure 1), that had previously been shown to be involved in the regulation of the biosynthesis of chloroplast antioxidants and facilitators of thermal dissipation [53–55] and are now emerging as themselves being subjected to modulation by some of these same chloroplast antioxidants and facilitators of thermal dissipation [56] (table 1). We are highlighting this specific example among the many redox-signalling networks because of the connections that have been made between this particular signalling pathway and phloem loading/transport.

Table 1 summarizes evidence for modulation of the formation of oxylipin messengers by the antioxidant vitamin E (tocopherols) and two factors directly associated with thermal energy dissipation in the light-harvesting complexes, the xanthophylls zeaxanthin and lutein and the PSII protein PsbS. Mutants deficient in the antioxidant vitamin E, in the xanthophylls zeaxanthin and lutein or in the PsbS protein generated greater levels of ROS [62,64] and greater levels of oxylipin messengers compared with wild-type (table 1). The key oxylipin studied in this work (figure 1 and table 1) was the stress hormone jasmonic acid, known to regulate plant growth and development as well as (biotic) defences against pathogens and pests (e.g. [65]). In addition, the latter studies also gave attention to actual evidence for altered biotic defences: it was found that the PsbS-deficient mutant (with lower levels of flexible NPQ and enhanced production of ROS and jasmonic acid) was more resistant to herbivore attack than the wild-type under field conditions (table 1). Similarly, evidence was also accumulated for an alteration of plant responses associated with defences against pathogens. An occlusion of the leaf’s phloem-loading complexes and its long-distance phloem conduits presumably blocks the internal spreading of pathogens (like fungi and viruses that use these transport channels) within the plant [66–68]. Mutants deficient in the antioxidant vitamin E, and thus forming increased levels of jasmonic acid (table 1), were shown to be impaired in phloem loading, carbon export, and plant growth and accumulated foliar carbohydrates (for a review, see [48] and also [57,63,69–72]). Similarly, mutants deficient in zeaxanthin exhibited not only enhanced oxylipin production but also increased cell wall deposition in phloem-loading cells (table 1).

It has been recognized that cell wall architecture is a component of plant biotic defence: ‘A rigid cell wall can fend off pathogen attack by forming an impenetrable, physical barrier’ [73, p. 549]. The specific occlusion of the gateways involved in phloem loading and carbon export from leaves is a defence mechanism that presumably comes at a cost to plant growth and thus has far-reaching implications. It is increasingly recognized that combinations of abiotic and biotic stresses in a plant’s environment can either exacerbate or ameliorate each other [74].
Likewise, multiple plant-hormone-based signalling pathways exhibit both antagonistic and cooperative interactions [75–77]. It is feasible that trade-offs may exist not only between plant growth versus defence and thus plant abiotic and biotic stress resistance, but also between plant resistance to pests versus pathogens, and/or between additional specific features. For example, greater vitamin E content of certain pepper varieties was associated with inhibition of the development of an insect pest of pepper [78]—whereas decreased vitamin E content of Arabidopsis mutants was associated with enhanced putative pathogen defence via phloem occlusion (table 1). Future research should examine combinations of individual plant species and specific pests or pathogens in specific abiotic environments.

3. Adaptation to contrasting environmental challenges with respect to carbon export-related features

In addition to being the site of the above-described occlusion (table 1 and figure 1) in the possible service of defence against pathogens that spread through the phloem, phloem-loading complexes were recently proposed to be the target point for overall adjustment of foliar carbon export capacity in response to environmental change [79,80]. Differences in the adjustability of phloem loading and carbon export were, furthermore, shown to be excellent predictors for differences in photosynthetic acclimation to the environment between plant varieties (ecotypes) and species from different geographical regions [81]. The latter work indicates that control points and within-plant signalling networks are subjected to genetic adaptation of plants to their environment over evolutionary time. These responses are featured in figures 1 and 2 as (increased, decreased or genetically limited) carbon export capacity (phloem loading/sugar transport) and photosynthetic capacity.

Firstly, it was shown that number and size of the cells loading sugars into the phloem’s sugar-transporting sieve tubes as well as number and size of the sieve tubes are adjusted to growth temperature in the winter annual Arabidopsis thaliana and the summer annual squash, Cucurbita pepo L. cv. Italian Zucchini Romanesco, both grown at 25°C under a 9-h photoperiod of 1000 μmol photon m$^{-2}$ s$^{-1}$. ***p < 0.001. For further details on methods and growing conditions, see Cohu et al. [80,81].

Figure 3. Differences in total cross-sectional sieve tube area per foliar sugar-loading vein induced by growth temperature (temp.) and growth light environment in Arabidopsis ecotypes from Sweden (filled squares) and Italy (filled circles) (as well as in the model line Col-0 (open triangles)). Sieve tubes are also referred to as sieve elements in the literature on plant vasculature. *p < 0.05; ***p < 0.001; light intensity is given in μmol photon m$^{-2}$ s$^{-1}$.

Figure 4. (a) Light- and CO$_2$-saturated photosynthetic rate (measured at 25°C), (b) sieve element number per foliar minor vein and (c) foliar vein density, in the winter annual Arabidopsis thaliana and the summer annual squash, Cucurbita pepo L. cv. Italian Zucchini Romanesco, both grown at 25°C under a 9-h photoperiod of 1000 μmol photon m$^{-2}$ s$^{-1}$. ***p < 0.001. For further details on methods and growing conditions, see Cohu et al. [80,81].
export—and a high photosynthetic capacity—at cool growth temperatures despite an increased viscosity of the phloem sap (and other limitations to sugar transport) under these cool temperatures. The latter concomitant upregulation of photosynthetic capacity and phloem-loading capacity also co-occurs with upregulation of other leaf anatomical features, e.g. increased leaf thickness and, especially, an increase in the number of the chloroplast-packed palisade layers of the leaf [82]. Future research should elucidate which signalling networks are involved in this concerted upregulation. Transcription factors, such as CBF/DREB (C-repeat binding factor/dehydration-responsive-element-binding protein), involved in plant adaptation/acclimation to cold stress [83,84], are attractive candidates as key components of such networks.

Furthermore, local varieties (ecotypes) of Arabidopsis adapted to the latitudinal extremes (Sweden versus Italy) of this species’ geographical range exhibited different sensitivities to environmental cues. Only the Swedish ecotype responded to a decrease in growth temperature (without an increase in growth light intensity) with an increase in the size of the sieve tubes of the leaves’ minor veins (figure 3; see also [80,81]). A combination of both low temperature and high light during plant growth was required to elicit a similar response in the Italian ecotype (figure 3; see also [80,81]). These findings suggest that the two ecotypes of this winter annual species do not differ in the principal ability to adjust leaf anatomy to cool growth temperature, but do differ in the intensity of cues required to elicit such a response. The greater responsiveness of the Swedish ecotype to cool temperature makes intuitive sense. These responses are consistent with putative genetic differences in the operation of the signalling networks involved.

In addition to differential adaptations to the environment between members (ecotypes) of a given winter annual species, different strategies are also seen between winter annual and summer annual species. In contrast to winter annuals, summer annuals typically germinate in the spring, grow over the summer and complete their life cycle in the autumn. Figure 4 shows similar photosynthetic capacities in both the winter annual Arabidopsis and the summer annual squash grown under warm temperatures and high light. Anatomical adjustments to phloem-loading cells are made by plants using either of the two major mechanisms for active sugar loading into the sugar-transporting phloem (sieve) tubes (see [85]), i.e. apoplastic loading via membrane transporters (as the phloem-loading mode used by Arabidopsis) and symplastic loading through plasmodesmatal openings in the cell wall (as the phloem-loading mode used by squash). However, while carbon export in Arabidopsis apparently relies on a few large veins per area of leaf (large number of sieve tubes per given sugar-loading vein and a low vein density per leaf area), carbon export in squash instead apparently relies on many small veins per area of leaf (small number of sieve tubes per given sugar-loading vein and a high vein density per leaf area; figure 4). Future research should address the implications of this difference in number (density) and size of sugar-loading and –exporting leaf veins between winter and summer annuals (for data on additional species, see [79]) for sugar export and phloem loading in source leaves (as well as, possibly, for sugar import and phloem unloading in sink tissues), and e.g. tolerance of cold versus hot temperatures as well as for abiotic versus biotic stress tolerance.

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