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REVIEW PAPER

Increasing tomato fruit quality by enhancing fruit chloroplast function. A double-edged sword?

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Abstract

Fruits are generally regarded as photosynthate sinks as they rely on energy provided by sugars transported from leaves to carry out the highly demanding processes of development and ripening; eventually these imported photosynthates also contribute to the fruit organoleptic properties. Three recent reports have revealed, however, that transcriptional factors enhancing chloroplast development in fruit may result in higher contents not only of tomato fruit-specialized metabolites but also of sugars. In addition to suggesting new ways to improve fruit quality by fortifying fruit chloroplasts and plastids, these results prompted us to re-evaluate the importance of the contribution of chloroplasts/photosynthesis to fruit development and ripening.

Key words: Chloroplasts, fruit, oxidative stress, plastid, *Solanum*, sugars, tomato.

Introduction

The tomato fruit is a model system for fleshy fruit ripening and at the same time is one of the most widely consumed vegetables worldwide, making it an ideal system to assess genes, processes, and environmental conditions affecting fruit ripening and quality (Klee and Giovannoni, 2011). The conspicuous presence of chloroplasts in the highly vacuolated cells of tomato fruit pericarp is responsible for the green colour which is characteristic of the tomato fruit before ripening, but the contribution of fruit chloroplast photosynthesis to fruit metabolite composition is not well understood (Lytovchenko *et al.*, 2011). Fruit ripening involves the acquisition of a series of fruit traits that make the fruit attractive and palatable; however, consumers often complain about the low organoleptic quality of the fruit in modern varieties (Causse *et al.*, 2010). The conversion from chloroplast to chromoplast is an important part of the ripening process and is normally associated with the dismantling of the photosynthetic machinery and the accumulation of carotenoids in chromoplasts and of sugars, organic acids, and volatile aroma compounds in the fruit cells (Gillaspy *et al.*, 1993; Klee and Giovannoni, 2011). Loss of organoleptic quality in modern varieties is due to a combination of reasons, from difficulty in selecting this complex quantitative trait, to conflicts in harmonizing organoleptic fruit quality with the interests of the different stakeholders in the tomato business/industry. Recent publications (Powell *et al.*, 2012; Pan *et al.*, 2013; Sagar *et al.*, 2013) reveal important links between photosynthesis in the developing fruit and quality of the ripe fruit that provides some insights into this question.

Fruit chloroplast development and fruit photosynthesis

It is generally assumed that leaves are the powerhouses of the plant as they provide photo-assimilates, while fruits and roots are basically heterotrophic as they rely on transport from leaves to grow and develop to their final size and

composition. If this is the case, what is the need for fruits of tomato to be green before ripening? Do fruit chloroplasts have any role? Do they have any effect on final fruit quality? To begin with, what do we know about chloroplast formation and fruit photosynthesis?

Our understanding of fruit chloroplast formation and of the role of fruit photosynthesis at the different stages of fruit organ development is somewhat incomplete when compared with that of the leaf. Similarly to those in the leaf, it is known that fruit chloroplast formation, chlorophyll synthesis, and assembly of the photosynthetic apparatus require exposure to light and the activation of a series of developmental cues. Chloroplast proteins involved in light-harvesting complexes, electron transfer, and CO_2 fixation are all expressed in the fruit cells and they are regulated by transcription factors, in a manner similar to that in leaves (Hetherington *et al.*, 1998; Carrara *et al.*, 2001). Recent proteomic analyses corroborate this at the protein level for all the components of photosynthesis, the Calvin cycle, and photorespiration reactions (Barsan *et al.*, 2010, 2012). There is, however, some fruit-specific regulation of nuclear-encoded photosynthetic genes (Sugita and Gruissem, 1987; Piechulla and Gruissem, 1987; Piechulla *et al.*, 1987; Wanner and Gruissem, 1991; Manzara *et al.*, 1993), the purpose of which is not clear, but is probably to optimize function in the context of the fruit.

Are these fruit chloroplasts capable of photosynthesis? Are fruit chloroplast net contributors or is the bulk of fruit development and carbon accumulation simply reliant on photoassimilates imported from leaves? What is the contribution of fruit chloroplasts and fruit photosynthesis to fruit metabolism before ripening? What role do chloroplast and fruit photosynthesis play in chloroplast to chromoplast conversion and in fruit quality at the red ripe stage?

The role of fruit photosynthesis in fruit metabolism and development has been extensively discussed (Piechulla *et al.*, 1987; Wanner and Gruissem, 1991; Schaffer and Petreikov, 1997; Carrari *et al.*, 2006; Steinhauser *et al.*, 2010), but, even now, information about its importance is controversial. One of the issues is whether the fruits are or are not net fixed-carbon producers. There are reports indicating that tomato fruits are unlikely to be net assimilators of CO_2 despite the high level of expression of photosynthetic genes in this organ (Blanke and Lenz, 1989; Carrara *et al.*, 2001). Exceedingly high expression of genes associated with photosynthesis occurs in specific fruit tissues with difficult access to light, such as the locules (Lemaire-Chamley *et al.*, 2005), which, although capable of photosynthesis (Laval-Martin *et al.*, 1977), are also likely to display higher rates of respiration. Moreover, the triose-phosphate and glucose-phosphate transporters are both active in tomato chloroplasts, indicating that they could, in principle, both import and export phosphoesters.

A number of studies in tomato support that the vast majority of photo-assimilates in the fruit are supplied by the leaves rather than produced *de novo* in the fruit (Hackel *et al.*, 2006; Schauer *et al.*, 2006; Zanor *et al.*, 2009; Do *et al.*, 2010). Consistent with this major import contribution to fruit, the correct development and sugar composition of fruit largely depend on the size of the photosynthate pool available in leaves and also on the sink strength of the fruit (Baldet *et al.*, 2006; Burstin *et al.*, 2007). Genetic analyses of fruit growth and composition have also confirmed the importance of both the size of the pool available (Schauer *et al.*, 2006) and the sink strength (Fridman *et al.*, 2000, 2004). These results and others coming from a series of studies involving quantitative trait locus (QTL) analysis, network analysis, and molecular biology analysis revealed for instance that the major QTLs for fruit size and fruit sugars have to do with genes affecting cell division/number of cells (Frary *et al.*, 2000) and auxin signalling (Cong *et al.*, 2008) in the initial stages of fruit development, as well as with the ability to convert imported sucrose into glucose and fructose by an invertase later in fruit development (Fridman *et al.*, 2004), all consistent with the importance of developing a strong sink organ and with no indications for a major contribution of fruit photosynthesis. Further support for the importance of leaf versus fruit photosynthesis comes from a number of studies where elevation of tomato leaf photosynthesis results in a proportional increase in fruit yield (Araujo *et al.*, 2011; Nunes-Nesi *et al.*, 2011).

The carbohydrate pool size in leaves and its partitioning between leaves and fruit are affected by a variety of environmental conditions including those cultural practices which are known to determine fruit growth and quality (Heuvelink, 1997; Gautier *et al.*, 2001; Bertin *et al.*, 2003). Furthermore, an increase in soluble sugars in ripe tomato fruit that have been exposed to salinity, as a cultural practice to obtain better quality tomatoes, appears to be a consequence of up-regulation of sucrose transport from leaves and increased activity of ADP-glucose pyrophosporylase in fruits during early development. This increase in sugar mobilization results in accumulation of starch in the immature fruits, and this affects later fruit quality as a source for sugars in red fruit (Yin *et al.*, 2010). Fruit size and fruit quality-related metabolite levels are often inversely correlated, further supporting that competition for imported resources is a critical determinant.

Seemingly to close the issue for good, Lytovchenko *et al.* (2011) indicated that fruit photosynthesis is not required for correct fruit development, or for the photosynthate accumulation in the fruit, including those metabolites impacting taste. In that study, transgenic Money Maker tomato plants, exhibiting decreased expression of the chlorophyll biosynthesis gene glutamate 1-semialdehyde aminotransferase (GSA) under the control of the pre-ripening fruit-specific TFM5 promoter showed a reduced photosynthetic rate, as determined by both CO_2 exchange and by the levels of intermediates of the Calvin–Benson cycle. Fruits of those plants were affected neither in size nor in any of the main primary or intermediary metabolites, thus suggesting that transport from leaves can compensate for loss of fruit photosynthesis. Only a delay in seed development was observed in those fruits, suggesting that fruit photosynthesis may be important for timely seed development. These results support the contention that the contribution of fruit photosynthesis to fruit formation and fruit energy metabolism is dispensable, although it may be relevant under specific environmental conditions.

Despite all the above, it remains the case that as much as 20% of the total carbon of the fruit has been estimated to result from photosynthetic activity in the fruit itself (Hetherington *et al.*, 1998). All three stages in which fruit development is traditionally described—cell division, cell enlargement, and ripening—contribute to final sugar accumulation in the fruit. In particular, the second stage is accompanied by the degradation of starch into soluble sugars (Davies and Cocking, 1965; Schaffer and Petreikov, 1997), with early studies already indicating that the level of soluble solids in ripe tomato fruit is related to the level of the starch in immature and mature green fruit (Davies and Cocking, 1965). The contribution of fruit photosynthesis to fruit growth and net sugar accumulation has been supported by early fruit shading experiments which analysed the rate of fruit growth and concluded that the fruit contributes by its own fixed carbon between 10% and 15% of the total (Tanaka *et al.*, 1974). There have been criticisms about these experiments as the bagging procedure may impact light receptors which are required for normal fruit development (Giliberto *et al.*, 2005; Azari *et al.*, 2010). Additional support for the contribution and importance of fruit photosynthesis comes from experiments showing a 15–20% negative effect on fruit development by depleting photosynthesis in the fruit following the antisense inhibition of the fruit chloroplastic fructose-1,6-bisphosphatase (FBPase; Obiadalla-Ali *et al.*, 2004). The importance of fruit photosynthesis in early fruit development is also suggested by the results of combined metabolomic and transcriptomic analyses (Wang *et al.*, 2009).

In addition to photosynthesis, fruit are able to fix carbon (refixation), via malate, with the CO_2 supply for this derived from mitochondrial respiration of imported carbon (Blanke and Lenz, 1989) rather than from intercellular/stomatal/diffusible CO_2, whose accessibility could be in part compromised in bulky organs such as the tomato fruit with a thick cuticle and near absence of stomata. The possibility also exists that CO_2 generated by the oxidative pentose pathway could be re-assimilated by Rubisco in green fruits, as shown in green seeds (Schwender *et al.*, 2004), thus providing further efficiency to the system.

Taking all these reports together, fruit photosynthesis contributes to fruit development and carbon economy. This contribution can be dispensable under normal growth but may become important under certain, limiting environmental conditions. The use of different genetic backgrounds and different environmental growth conditions (light) in the studies may also have contributed to the sometimes contradictory results. Interestingly, we now know that the genetic background is a determinant of the degree of contribution of fruit photosynthesis to fruit growth and fruit carbon economy.

Indeed, this conundrum about the importance of fruit chloroplasts and fruit photosynthesis has received an unexpected twist with the identification of the genetic nature of the 'uniform ripening' *u* mutation (Powell *et al.*, 2012). Tomato *U* gene mutants exhibit defective fruits in that chloroplast number and thylakoid grana are dramatically reduced as compared with the wild type, and yet fruit develops to normal size and ripens at the same time as the wild type, although the accumulation of sugars in red fruit is repressed by 10–15%. Consistent with this, the so-called uniform ripening tomato mutants show a pale green fruit phenotype at the mature green stage, which contrasts with the darker green-shouldered phenotype of the wild-type fruit (Yeager, 1935; Bohn and Scott, 1945; Kemp and Nonnecke, 1960). The introgression of the wild-type *U* locus into fruits of (*u*/*u*) converts tomato pale immature fruit into dark-green-shouldered fruits with higher starch accumulation. The *U* gene has been recently identified by positional cloning, and the *u* mutants were revealed to carry an additional A in a small A-repeat region in exon I of the Golden 2-like *GLK2* gene that introduces a premature termination codon in the encoded protein. Since *GLK2* is the predominant GLK form expressed in the fruit, and since GLKs are members of the GARP family of transcription factors that regulate the expression of chloroplast genes (Waters *et al.*, 2008, 2009; Waters and Langdale 2009), *u* mutants qualify as fruit chloroplast mutants, the rest of the plant's needs being satisfied by the *GLK1* gene, with redundant GLK roles in vegetative tissues.

Adding wild-type alleles of *GLK*, either by crossing or by genetic transformation under different promoter sequences, produces fruit with more chloroplasts that have more grana/thylakoids, and higher accumulation of chlorophyll and starch at the mature green stage (Powell *et al.*, 2012). Fruits with activated GLKs express higher levels of transcripts for photosystem II (PSII) and PSI components, as well as of other genes involved in sugar (*GLK2*) or other aspects of metabolism (*GLK1*) (Powell *et al.*, 2012; AG, unpublished results). Furthermore, *GLK*-overexpressing lines accumulate more sugars and lycopene in the red ripe stage, opening up a way to increase fruit quality by acting at the GLK level.

Most of the cultivated tomato varieties that fill the aisles of large supermarkets, including the variety used for the GSA experiment (Lytovchenko *et al.*, 2011), carry the *u* mutation and therefore have defective fruit chloroplasts with the associated effects of lower sugars and lycopene levels (see below) that they could potentially have.

No matter what the contribution of fruit chloroplasts is to net photosynthesis in green fruits, tomato fruits clearly undergo a physiological transition associated with the differentiation of chromoplasts from photosynthetically active chloroplasts occurring during fruit ripening (Buker *et al.*, 1998; Kahlau and Bock, 2008). This transition appears to be coupled with a decline in both nuclear- and plastid-encoded gene expression (Piechulla *et al.*, 1987; Wanner and Gruissem, 1991; Carrari *et al.*, 2006; Kahlau and Bock, 2008) and in enzymatic activities (Schaffer and Petreikov, 1997; Steinhauser *et al.*, 2010) that are associated with carbon assimilation and chloroplast components. Furthermore, most nuclear gene expression for chloroplast proteins, and all plastid-encoded photosynthesis gene expression, is developmentally regulated, already decreasing during the late stages of green fruit development prior to ripening (Kahlau and Bock, 2008) in anticipation of the partially phototrophic to completely heterotrophic shift occurring in the fruit at ripening. Only in some mutants is this process partially blocked, as is the case in the stay-green mutants (Barry *et al.*, 2008). It is

noteworthy that most of the proteins of the Calvin–Benson cycle, including Rubisco, and of the oxidative pentose pathway were identified in the proteome of the tomato chromoplast (Barsan *et al.*, 2010). If all these pathways were active, the CO_2 generated by the oxidative pentose pathway could be re-assimilated by Rubisco (Schwender *et al.*, 2004) to satisfy the specific metabolic needs of the ripe fruit.

Therefore, as fruits ripen, their chloroplasts are remodelled into chromoplasts that no longer contain chlorophyll but synthesize and accumulate lycopene, β-carotene, and other metabolites important for ripe fruit sensory and nutritional attributes (Hetherington *et al.*, 1998; Egea *et al.*, 2010; Klee and Giovannoni, 2011). How then can fruit chloroplasts and photosynthesis in green fruit affect the metabolite constitution of ripen fruit? Early studies have related the content of soluble solids in ripe tomato fruit to the starch level in the immature and mature green fruit stages (Dinar and Stevens, 1981; Schaffer and Petreikov, 1997), but this is probably only part of the story.

Genes encoding components of fruit chloroplast development and/or light signalling as genetic tools for fruit improvement

There is evidence that tomato fruits either selected from the available gene pool or engineered to have an increased number of highly active chloroplasts at the green stage will develop ripe tomatoes with more active chromoplasts, producing higher amounts of metabolites associated with organoleptic and nutritional quality (Isaacson *et al.*, 2002; Galpaz *et al.*, 2008; Nashilevitz *et al.*, 2010).

For example, the *high pigment 1*, *2*, and *3* (*hp1*, *hp2*, and *hp3*) tomato mutants possess altered chloroplast number and ultrastructure, resulting in fruits with elevated levels of chlorophyll, carotenoids, and flavonoids, and altered patterns of production of volatile aroma compounds (Yen *et al.*, 1997; Bino *et al.*, 2005; Kolotilin *et al.*, 2007; Galpaz *et al.*, 2008). These mutants have been of limited use in tomato breeding programmes due to negative pleiotropic effects of the light signal perception phenotype that affect other aspects of plant growth, making it also difficult to infer the contribution of fruit photosynthesis to the fruit quality phenotype (Yen *et al.*, 1997). Fortunately a number of studies using molecular genetic approaches on these mutants resulted in the identification of the corresponding mutations as components of the light signal transduction pathway and opened the way to a more targeted approach with fewer pleiotropic effects. A series of *hp2* alleles in tomato were revealed (Mustilli *et al.*, 1999) as mutations in DE-ETIOLATED1 (DET1), a negative regulator of light signal transduction; interestingly, *hp1* has also been identified as a mutation in a tomato UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1) homologue, whose *Arabidopsis* counterpart interacts with DET1, and therefore both genes are part of the light signalling pathway. The identification of the genes underlying the *hp* mutations allowed fruit targeted engineering (Davuluri *et al.*, 2004) using RNA interference (RNAi) driven by fruit-specific promoters, resulting in plants that do not present pleiotropic effects. The engineered fruit showed a higher content of quality metabolites (Davuluri *et al.*, 2005). Metabolomic and transcriptomic analysis of fruit with reduced DET1 expression failed to pinpoint the mechanism underlying the phenotype of enhanced plastids in the fruit. Although the authors identified an increased plastid biogenesis *per se*, they could not conclude that this was the primary effect (Enfissi *et al.*, 2010). Consistent with this, they failed to detect in the DET-modified plants any effect on the expression of either transcription factors regulating plastid biogenesis (e.g. GLK), structural proteins involved in plastid biogenesis (e.g. ribosomal proteins), or other genes involved in plastid division (FtsZ and MinD). Collectively, these data prompted the authors to suggest that, in the case of *hp* mutants, it is the initiation of core metabolic processes which drives subsequent plastid biogenesis to provide a defined cell compartment for these processes (Kolotilin *et al.*, 2007). Recent results involving epistasis studies and transcript profiling analysis also support that GLK2 acts independently of HP1/DDB1, suggesting that different routes regulate plastid/chlorophyll and carotenoid accumulation in tomato fruit and that GLK and DDB1 effects on fruit quality could be additive (Nguyen at al., 2014).

In contrast to the effects observed with DET modifications, the reinforcement of GLK resulted in fruit containing more and bigger chloroplasts with more and denser grana, with activated expression of transcripts for PSI and PSII components of the photosynthetic machinery. Some differential specificities of expression, with consequences on fruit metabolism, can be anticipated from the expression pattern in GLK1 and GLK2 overexpression lines (Powell *et al.*, 2012). Interestingly, no changes in the expression levels for the main regulatory light signal transduction genes are detected in fruits overexpressing GLKs (Powell *et al.*, 2012), suggesting that at least at the transcriptional level no interaction between the core light signal transduction and GLK exists in the fruit. The GLK2 green shoulder phenotype in U/U tomatoes appears to result from a latitudinal gradient of expression through the green fruit independent of the ripening gradient. Interestingly, none of the usual suspects—RIN (RIPENING INHIBITOR), NOR (NON-RIPENING), CNR (COLORLESS NON-RIPENING), TAGL1 (TOMATO AGAMOUS LIKE 1), and AP2 (APETALA 2)—seem to be involved in the regulation of this gradient. Neither GLK1 nor GLK2 affect general ripening control systems (RIN, NOR, CNR, and TAGL1) or ethylene biosynthesis and signalling (Nguyen *et al.*, 2014).

The combination of HY5 and GLKs, functioning downstream of light and auxin/cytokinin signalling pathways, is responsible for coordinated expression of the key genes in chloroplast biogenesis during root greening (Kobayashi *et al.*, 2012) and it is possible that this can be extended to aerial organs including fruits. In this respect, it is possible that the activity of GLKs on fruit chloroplasts is affected not only by light but also by the changing hormonal status of the fruit (Galpaz *et al.*, 2008; Klee and Giovannoni, 2011). A large body of experimental data supports the participation of phytohormones in regulating fruit chloroplast formation/

differentiation and evolution to a chromoplast during ripening (Egea *et al.*, 2010). For instance, down-regulation of the tomato auxin response factor ARF4 resulted in a fruit-specific dark-green phenotype and blotchy ripening of tomato fruit (Jones *et al.*, 2002), with cells in the outer pericarp tissue displaying more chloroplasts with more grana per cell. More recent work by Sagar *et al.* (2013) revealed that fruits of those plants accumulated higher amounts of transient starch at the immature stages and higher amounts of sugar at the red ripe stage. The effect of ARF4 on fruit plastid accumulation seems to be mediated through the transcriptional up-regulaton of SlGLKs in the fruit as both the fruit SlGLK2 (inactive protein in the microtom background) and ectopic expression in the fruit or the leaf of SlGLK1 transcripts increased in SlARF4-AS fruit tissues. This hypothesis is reinforced by the presence of a perfectly conserved canonical ARF-binding motif (TGTCTC box) in the promoter region of the *SlGLK1* gene (Sagar *et al.*, 2013). Highly pigmented fruit phenotypes can also be produced by ectopic expression in tomato of the cytokinin biosynthesis gene *ipt* from *Agrobacterium tumefaciens* (Martineau *et al.*, 1994) and by exogenous treatment with cytokinin (Mustilli *et al.*, 1999), therefore suggesting the possibility that cytokinins positively influence fruit chloroplast accumulation. All these examples support a role for hormones in fruit chloroplast development and open up new avenues of research for fruit photosynthesis modulation.

Other putative light signal transduction genes have proven useful to modify fruit traits. RNAi-mediated gene repression of two light signal transduction genes, *LeHY5* and *LeCOP1LIKE*, showed that these genes regulated fruit pigmentation antagonistically in tomato (Liu *et al.*, 2004). Although the results obtained are consistent with their role as positive and negative elements of the light signalling pathway in *Arabidopsis*, the mechanism by which they alter fruit pigmentation and quality is unknown.

Other fruit chloroplast mutants have also proven useful to alter fruit quality. Thus, the *green-flesh* and *chlorophyll retainer* mutations of tomato and pepper showed an inhibition of chlorophyll degradation caused by dysfunction of the corresponding homologues of the chloroplast-targeted STAY-GREEN protein of rice. Again, these mutations influenced fruit chloroplast development by delaying the degradation of chloroplast components during ripening, and impacted fruit quality in ripe fruit very positively (Barry *et al.*, 2008; Borovsky and Paran, 2008). Recently, the modification of other chloroplast proteins was found to impact fruit ripening and quality. Thus, the *Orange ripening* (OrrDS) mutant that encodes the M subunit of the plastidial NADH dehydrogenase complex (Nashilevitz *et al.*, 2010) is affected in multiple aspects of the fruit ripening process, including the ethylene climacteric and carotenogenic phases. In contrast, fruit of *lutescent 1* (*l1*) and *lutescent 2* (*l2*) tomato mutants (Barry *et al.*, 2012) show an early and progressive loss of chlorophyll, producing whitish-yellow fruit prior to the onset of ripening together with a delay in the onset of fruit ripening itself. Both *l1* and *l2* have been identified as tomato homologues of *EGY1*, a chloroplast-targeted zinc metalloprotease (Barry *et al.*, 2012) that is required for chloroplast development in *Arabidopsis* (Chen *et al.*, 2005). This supports the importance of a chloroplast signal that stimulates ripening and that eventually affects fruit quality.

More recently, Pan *et al.* (2013) using a network analysis approach identified a transcription factor related to the ARABIDOPSIS PSEUDO RESPONSE REGULATOR2-LIKE gene (APRR2-like) that influences pigmentation and ripening in tomato fruit. Most interestingly, transgenic tomato lines with the APRR2-like gene overexpressed under the control of the 35S promoter produced fruits with larger and more numerous plastids and consequently with higher chlorophyll levels in immature unripe fruits and higher carotenoid amounts in red ripe fruits. In *Arabidopsis*, this gene seems to be a transcriptional activator with a GARP DNA-binding domain and a receiver-like domain similar to ARR-B genes (Fitter *et al.*, 2002). Interestingly, GLK (Powell *et al.*, 2012) is the closest global relative of APPR2-like in tomato, athough the latter lacks the AREAEAA motif conserved in the GLK genes (Fitter *et al.*, 2002), suggesting a different specificity. Although the function of APRR2 in *Arabidopsis* has yet to be determined, its central position in the regulatory hub in tomato, its regulation during ripening, and the effect on RIN, ACO (ACC OXIDASE), and PG (POLYGALCURONASE) expression suggest an important role in the ripening process. On the other hand, the similarity of the APPR2-like ectopic overexpression phenotype to that of GLK-like genes, together with the alteration of APRR expression in *hp* mutant plants (Rohrmann *et al.*, 2011) indicates a link between the functional deficiency of the DDB1-mediated light signal transduction in the *hp1* mutant and APRR2-like gene expression and suggests a possible explanation for its implication in plastid development and metabolism.

All of these results further consolidate the idea that genes encoding relevant chloroplast biogenesis genes, or components of light signalling, represent genetic tools for enhancing fruit composition and nutritional value (Fig. 1).

Walking on the wild side: oxidative stress during normal fruit ripening and under stressful environmental conditions

If reinforcement of chloroplasts in the fruit results in better quality fruit, why was the *u* mutation leading to weak fruit chloroplasts introduced in the 1940s–1950s in the first place? The reason appears to be that in addition to providing a fruit that ripens more uniformly, with no green shoulders, it facilitated the determination of maturity crucial for the processing industry to avoid losses. Moreover, tomato breeders also know well that *u* fruits are less prone to develop cracking and yellow shoulders at the stem end, two disorders often found under certain stressful environmental conditions (Hobson and Davies, 1976; Peet, 1992). Absorbed solar energy may be harmful when it exceeds the capacity of the photosynthetic apparatus, resulting in photooxidative stress (Jarvis and López-Juez, 2013). Photooxidative stress occurs when light exceeds the coping capacity of the plethora of mechanisms that plants have developed to manage excess absorbed energy

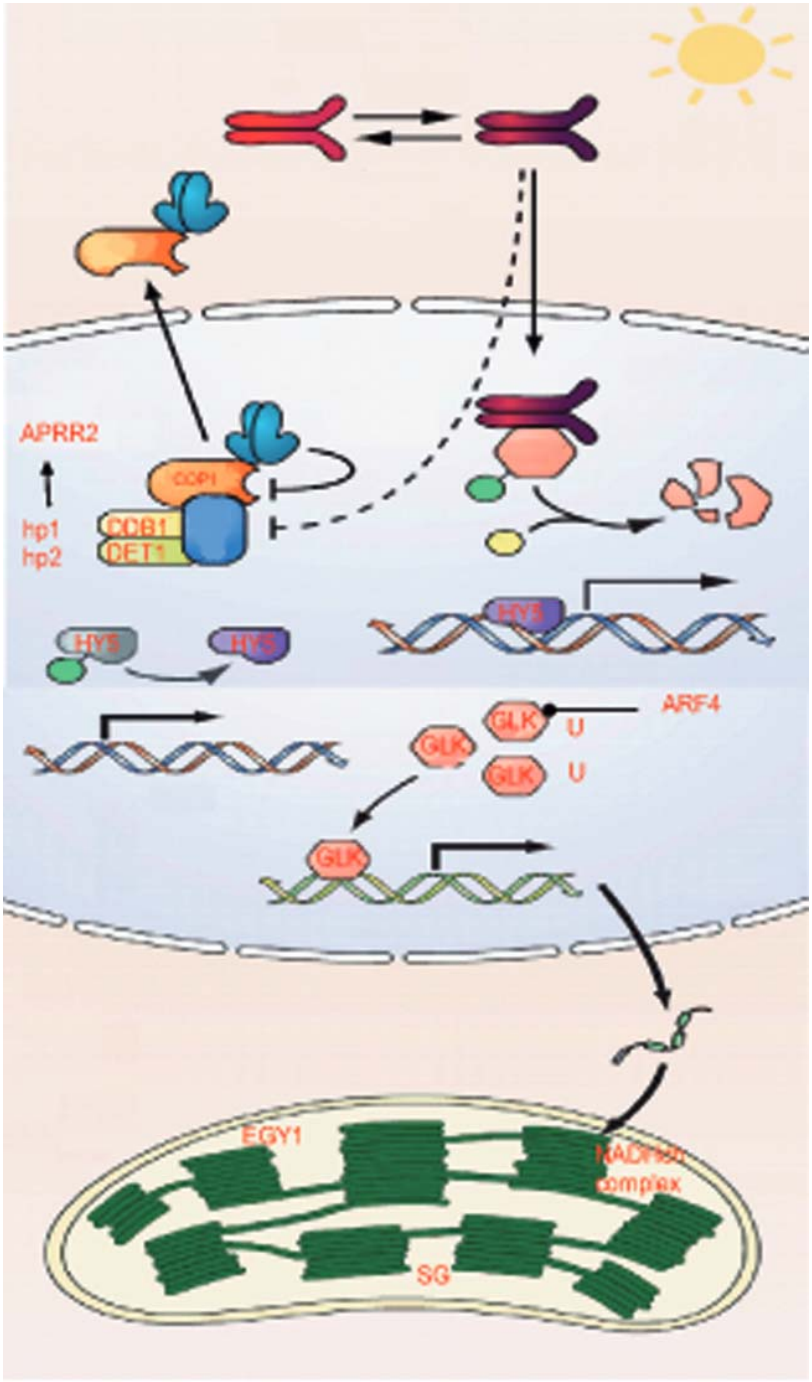

Fig. 1. Light signalling and chloroplast development factors affecting fruit quality. In red are those components that in tomato have been reported to affect fruit chloroplast development and fruit quality. COP1 and DE-ETIOLATED 1 (DET1/hp2) and UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1/hp1) cooperate in the dark to promote the ubiquitination and degradation of photomorphogenesis-promoting transcription factors such as the transcription factor HY5, whereas other light regulators probably act by repressing transcription of photomorphogenesis and pigment genes. Down-regulation of LeCOP1-like, DET1/hp2, and DDB1/hp1 leads to higher chlorophyll/plastid and carotene content in green and mature fruit, respectively, while HY5 down-regulation causes the opposite effects, as observed in *hp1*, *hp2*, *HY5*, and *LeCOP1*-like mutants. Exposure to light causes COP1 to exit from the nucleus and leads to the repression of DET1/hp2 and DBB1/hp1 and to active HY5 levels increasing. This enables G-box binding and transcriptional activation of photomorphogenesis and pigment genes. In a DBB/hp1-independent regulatory pathway, Golden 2-like MYB transcription factors (GLK1 and GLK2) participate; these are also major drivers of photosynthetic genes, with GLK2 having a predominant role in fruits. Overexpression of GLKs increased chlorophyll content, chloroplast number, and thylakoid grana stacks in green fruit, which results later in higher levels of carotenoids and sugars in ripe fruit. [Adapted by permission from Macmillan Publishers Ltd: *The EMBO Journal*. Waters MT, Langdale JA. The making of a chloroplast. *EMBO J*. 28, 2861–2873, figure 1, copyright (2009).]

(Murchie and Niyogi, 2011). Thus, part of the *U* fruit problem could be due to oxidative stress running out of control in very active fruit chloroplasts as they become overheated, or unable to cope with high irradiation operating in many industrial facilities (which use completely different conditions from those at the centres of origin and diversification where wild relatives of tomato first evolved). Fruits of wild tomato species are inherently small as an adaptation to the limitations imposed by water and other environmental stresses in nature, and limited fruit growth is favoured by survival and reproductive strategies during evolution over other fruit traits associated with growth and carbon gain, which are the focus of modern agriculture based on domesticated species with larger fruits.

Lhc/*cab* are among the most sensitive genes to photooxidative stress, and their transcription is turned off soon after plants are subjected to photooxidative conditions (Burgess and Taylor, 1987; Kawata and Cheung, 1990). The decline of *cab* mRNA in maturing tomato fruit delayed chloroplast deterioration in a mutant tomato fruit (Piechulla *et al.*, 1986) and coincided with an increase in superoxide dismutase (Livne and Gepstein, 1988). This strongly suggests that the decline in *cab* mRNA is in response to the increasingly photooxidative environment in maturing fruit.

On the other hand, control of redox chemistry is central to photosynthesis in the chloroplast and to cell survival, and therefore it is not surprising that plants go a long way in ensuring that naturally (Jarvis and López-Juez, 2013). This control is exerted even when there is an imbalance between the two photosystems and is governed by the redox stage of the plastoquinone, providing the plant with a sharp, rapid, and direct control of photosynthesis which is encoded universally within the chloroplast (Pfannschmidt *et al.*, 2009).

Oxidative stress is induced in tomato fruit upon exposure to high irradiance and high temperature, and, presumably, upon an increase in photosynthetically produced O_2 within a tissue in which gas exchange may be reduced because of the near absence of stomata. This may have consequences of fruit plastid bleaching and later lead to impairment in ripening that affects mainly the shoulders of the fruit. Yellow shoulder is a colour and ripening disorder of mature fruits (Francis *et al.*, 2000) (although probably induced when they are immature; RF-M, unpublished results) injuring all types of tomatoes under high light/temperature conditions. It will, nevertheless, affect particularly severely the fruits from genotypes lacking the *U* gene (Picha, 1987). It has long been known that non-uniform green *U* tomatoes are far more sensitive to cuticle cracking (Young, 1946; Young and MacArthur, 1947; Hudson, 1956). Similarly to the yellow shoulder disorder, fruit cracking is also more frequent when developing fruits are directly exposed to high irradiance (Peet, 1992). It is therefore not surprising that in order to protect fruit from the deleterious, quality-reducing effect of oxygen radicals produced from electron transfer going out of control under high irradiance/temperature, breeders selected fruits with fewer and weaker chloroplasts.

During fruit ripening, chloroplasts develop into chromoplasts, accumulating high amounts of liposoluble antioxidant compounds, such as lycopene and β-carotene, which presumably will protect the chromoplast and fruit cells in addition to serving as a visual cue for frugivores to eat the fruit and disperse the seed. Despite this, oxidative stress in the fruit is known to increase markedly during the last stages of normal tomato fruit ripening; and this has been proposed to be part of the ripening programme aimed at facilitating metabolic changes associated with ripening and fruit softening required for seed release (Jiménez *et al.*, 2002). Consistent with this, short shelf life tomato cultivars exhibit increased oxidative stress and reduced radical scavenging activity (Mondal *et al.*, 2004). Accordingly, engineering tomato fruit for higher antioxidant levels resulted in firmer fruits with extended fruit shelf life (Mehta *et al.*, 2002; Powell *et al.*, 2002; Smith *et al.*, 2002; Zhang *et al.*, 2013).

Interestingly, enhancing photoprotection has been proposed and demonstrated as a useful strategy to improve photosynthesis (Murchie and Niyogi, 2011). These approaches should be finely tuned and their beneficial effects should be weighed against the evidence that such actions may have a cost that could limit carbon availability, namely yield (Murchie and Niyogi, 2011).

In terms of applying these findings, all the data described above indicate that it should be possible to increase the fruit's own supply of healthy nutrients by enhancing fruit plastids at the different stages of fruit development (fortification of both chloroplasts and chromoplasts) while keeping oxidative stress under control. Understanding the mechanisms for achieving a fine redox control and manipulation of photo/thermo protection mechanisms in the plastid under real growth conditions will require further research to take full advantage of chloroplast fortification.

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