

Transcriptional-Metabolic Networks in β -Carotene-Enriched Potato Tubers: The Long and Winding Road to the Golden Phenotype^{1[C][W][OA]}

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Vitamin A deficiency is a public health problem in a large number of countries. Biofortification of major staple crops (wheat [*Triticum aestivum*], rice [*Oryza sativa*], maize [*Zea mays*], and potato [*Solanum tuberosum*]) with β -carotene has the potential to alleviate this nutritional problem. Previously, we engineered transgenic “Golden” potato tubers overexpressing three bacterial genes for β -carotene synthesis (*CrtB*, *CrtI*, and *CrtY*, encoding phytoene synthase, phytoene desaturase, and lycopene β -cyclase, respectively) and accumulating the highest amount of β -carotene in the four aforementioned crops. Here, we report the systematic quantitation of carotenoid metabolites and transcripts in 24 lines carrying six different transgene combinations under the control of the *35S* and *Patatin* (*Pat*) promoters. Low levels of *B-I* expression are sufficient for interfering with leaf carotenogenesis, but not for β -carotene accumulation in tubers and calli, which requires high expression levels of all three genes under the control of the *Pat* promoter. Tubers expressing the *B-I* transgenes show large perturbations in the transcription of endogenous carotenoid genes, with only minor changes in carotenoid content, while the opposite phenotype (low levels of transcriptional perturbation and high carotenoid levels) is observed in Golden (*Y-B-I*) tubers. We used hierarchical clustering and pairwise correlation analysis, together with a new method for network correlation analysis, developed for this purpose, to assess the perturbations in transcript and metabolite levels in transgenic leaves and tubers. Through a “guilt-by-profiling” approach, we identified several endogenous genes for carotenoid biosynthesis likely to play a key regulatory role in Golden tubers, which are candidates for manipulations aimed at the further optimization of tuber carotenoid content.

Carotenoids are a group of widely distributed isoprenoid pigments exerting vital roles in photoprotection and as free radical scavengers. In addition, they represent essential components of light-harvesting and reaction center complexes of photosynthetic organisms (for review, see Hirschberg, 2001). When accumulated in chromoplasts, carotenoids confer their colors to many flowers and fruits, contributing substantially to plant-animal interactions (Cunningham and Gantt, 1998; Hirschberg, 2001; Fraser and Bramley, 2004). In addition, carotenoids are the precursors of

several physiologically important apocarotenoids (Bouvier et al., 2005; Auldrige et al., 2006), like the phytohormone abscisic acid, originating from 9-cis-violaxanthin and -neoxanthin (Schwartz et al., 1997). A further example of plant apocarotenoids is represented by the strigolactones, which serve both as interspecific signaling molecules (Bouwmeester et al., 2007) and as regulators of shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008). In animals, the best-known apocarotenoids are vitamin A (retinol) and its derivatives, which are involved in different processes, including vision, immune response, development, and reproduction (Ross et al., 2000). This essential compound is either taken up directly from animal foodstuff or synthesized from dietary carotenoids with provitamin A activity, such as β -carotene, α -carotene, and cryptoxanthin. Provitamin A carotenoids are converted into retinal via symmetrical cleavage reactions catalyzed by the β -carotene cleavage oxygenase I, which has been characterized from several species, including humans (for review, see Moise et al., 2005). The coverage of dietary vitamin A demand through plant carotenoids is particularly important in developing countries, where the consumption of animal products is low.

Vitamin A deficiency is the major cause of child blindness and increases the probability of disease and

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death from severe infections (<http://www.who.int/nutrition/topics/vad/en/>). Vitamin A deficiency is a widespread public health problem in developing countries, but it is also encountered among the elderly, heavy drinkers, and smokers in poor urban populations of developed countries (<http://www.who.int/nutrition/topics/vad/en/>). β -Carotene exhibits the highest provitamin A activity and accumulates in several plants such as carrot (*Daucus carota*), apricot (*Prunus armeniaca*), and peach (*Prunus persica*) but not in major crops such as wheat (*Triticum aestivum*), rice (*Oryza sativa*), and potato (*Solanum tuberosum*). In the Copenhagen Consensus 2008 list of priorities, vitamin A supplements for children and food biofortification rank first and third, respectively (<http://www.copenhagenconsensus.com/Default.aspx>). Therefore, the improvement of provitamin A content has been an important goal of plant biotechnology, and several successful efforts have been undertaken to improve the provitamin A content in major crops: "Golden" rice (Ye et al., 2000; Paine et al., 2005), tomato (*Solanum lycopersicum*; Romer et al., 2000; Rosati et al., 2000; Fraser et al., 2002; Dharmapuri et al., 2002), canola (*Brassica napus*; Shewmaker et al., 1999; Ravanello et al., 2003), maize (*Zea mays*; Aluru et al., 2008), and flaxseed (*Linum usitatissimum*; Fujisawa et al., 2008).

In plants, the synthesis of carotenoids (Hirschberg, 2001; Fraser and Bramley, 2004; Dellapenna and Pogson, 2006) is initiated by the enzyme phytoene synthase (PSY), which mediates the condensation of two molecules of geranylgeranyl diphosphate, leading to the colorless carotene phytoene. The conjugated double bond system of phytoene is then extended by phytoene desaturase (PDS) and ζ -carotene desaturase (ZDS) to yield lycopene. The pathway leading from phytoene to all-trans-lycopene involves at least one further enzyme, the carotene isomerase CrtISO. Lycopene is the substrate for the lycopene- β - and - ϵ -cyclases (LCY-b and LCY-e), which catalyze the formation of β - and α -carotene. β -Carotene and α -carotene are hydroxylated into zeaxanthin and lutein, respectively, by the action of heme (LUT1, LUT5) and nonheme (CHY1, CHY2) carotene hydroxylases. Zeaxanthin is a constituent of the xanthophyll cycle and can be reversibly converted into violaxanthin via antheraxanthin. These reactions are mediated by the enzymes zeaxanthin epoxidase (ZEP) and violaxanthin deepoxidase. In contrast to plants, some nonphotosynthetic bacteria, such as *Erwinia*, utilize only three enzymes (i.e. phytoene synthase CrtB, phytoene desaturase/isomerase CrtI, and lycopene cyclase CrtY) to perform the synthesis of β -carotene from geranylgeranyl diphosphate (Fig. 1).

In recent years, three strategies have been followed to enhance the carotenoid content of potato tubers. The first one was a "blocking" strategy and relied on the posttranscriptional gene silencing of key biosynthetic steps: the CHY hydroxylase, acting on β -carotene (Diretto et al., 2007b; van Eck et al., 2007), the ZEP epoxidase, acting on zeaxanthin (Romer et al., 2002),

and the LCY-e cyclase, acting on lycopene and starting the α -branch (Diretto et al., 2006; Fig. 1). The second one was a "push" strategy and relied on the overexpression of biosynthetic genes in a constitutive or tuber-specific manner. This included the overexpression of single genes such as *CrtB* (Ducreux et al., 2005), of 1-deoxy-D-xylulose 5-phosphate synthase (involved in the synthesis of the isoprenoid precursor IPP; Morris et al., 2006b) or of β -carotene ketolase (Morris et al., 2006a; resulting in the production of ketocarotenoids), or of combinations of two or three genes (minipathways; Gerjets and Sandmann, 2006; Diretto et al., 2007a). The third strategy, termed "sink engineering," relied on the overexpression of regulatory genes causing differentiation of chromoplasts, which in turn act as storage structures for carotenoids (Lopez et al., 2008).

Quantitatively, the most important results have been obtained using the second strategy: through the tissue-specific overexpression of a minipathway containing the *CrtB*, *CrtI*, and *CrtY* bacterial genes, we have produced Golden potato tubers containing up to 47 $\mu\text{g g}^{-1}$ dry weight of β -carotene (Diretto et al., 2007a). This value represents the highest β -carotene level ever reported in the four major staple crops: maize, rice, wheat, and potato. Golden tubers not only display a massive accumulation of carotenoids but also an increase of endogenous carotenoid biosynthesis transcripts.

In order to define the specific role of the various components of the minipathway (transgenes as well as promoters controlling them) in modulating carotenoid biosynthesis in the tuber, and in order to clarify the conditions leading to the Golden phenotype, we report here on the systematic molecular and biochemical characterization of transgenic plants harboring six different combinations of the *CrtB*, *CrtI*, and *CrtY* genes under the control of constitutive (35S) and tuber-specific (*Patatin* [*Pat*]) promoters.

RESULTS AND DISCUSSION

Golden tubers were obtained using the plasmid *pP-YBI*, encoding the bacterial minipathway enzymes *CrtB*, *CrtI*, and *CrtY*, leading to β -carotene synthesis under the control of the tuber-specific *Pat* promoter (Diretto et al., 2007a; Table I). Additional transgenic plants were generated using five different plasmids, harboring *CrtI* alone (*I*) or in combination with *CrtB* (*BI*) and *CrtY* (*YBI*; Diretto et al., 2007a). In all cases, *CrtB* was put under the control of the tuber-specific *Pat1* promoter, a strategy previously shown to result in an increase of β -carotene content (Ducreux et al., 2005). Genes mediating the next steps in the pathway (*CrtI* and/or *CrtY*) were put under the control of the constitutive 35S promoter (*pK* series) or of the tuber-specific *Pat2* promoter (*pP* series; Diretto et al., 2007a; Table I). For each construct, 10 transgenic lines were selected and grown in the greenhouse under controlled

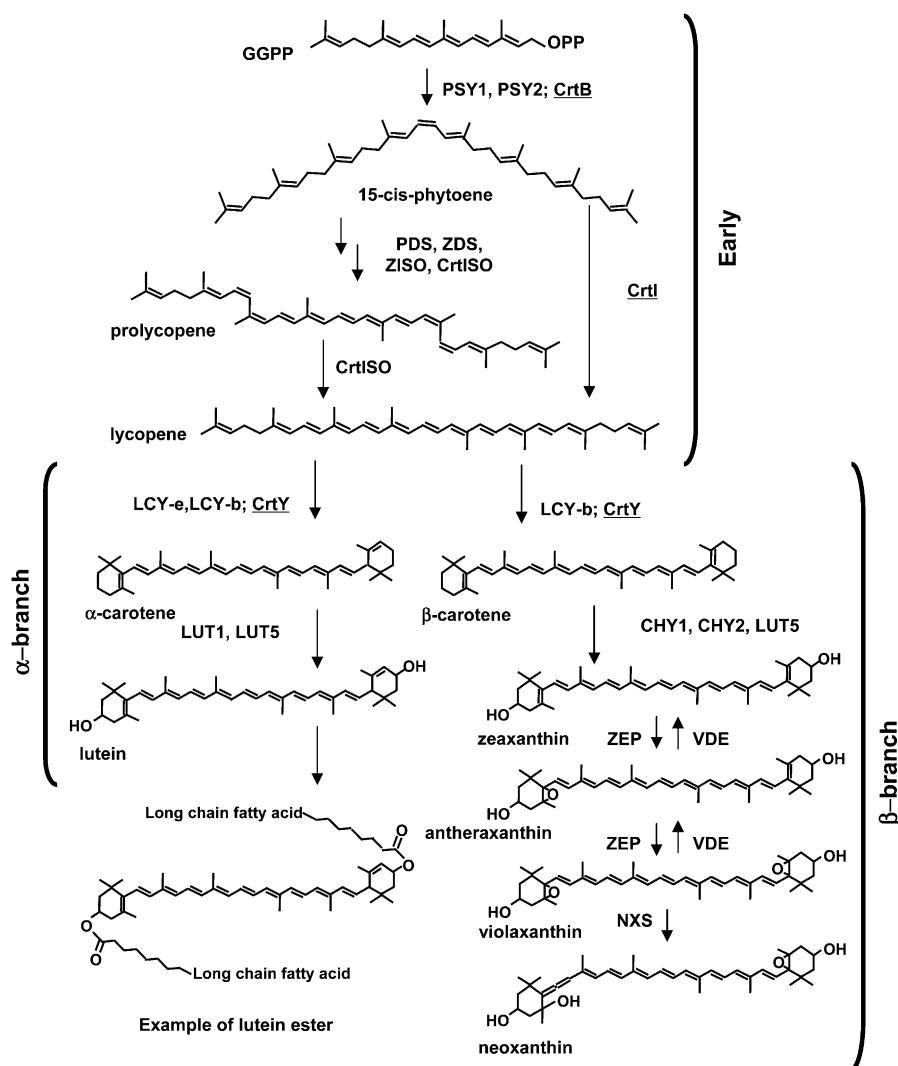


Figure 1. Carotenoid biosynthesis in potato leaves/tubers. Bacterial enzymes are underlined.

conditions. Based on their tuber carotenoid content (Diretto et al., 2007a), the lines obtained were designated as “expressor” or “nonexpressor,” exhibiting modified or unchanged carotenoid contents, respectively. The *pK-YBI* and *pP-YBI* constructs produced only two expressor lines, each with significantly increased carotenoid content. Therefore, two nonexpressor and two expressor lines for each construct were selected for further characterization in this work.

***CrtY-B-I* Expression Causes Unscheduled Carotenoid Accumulation in Vitro**

During in vitro regeneration, the *YBI* expressor lines exhibited a phenotype that was not observed with any of the other transformants. Explants from *pP-YBI* expressor lines formed orange calli (Fig. 2A), while in vitro plantlets from *pK-YBI* expressor lines had a yellow-orange appearance (Fig. 2B). The carotenoid content of calli and plantlets was determined via diode array HPLC (Table II). Wild-type plantlets exhibited low carotenoid levels (approximately $75 \mu\text{g g}^{-1}$ dry

weight), of which about 13% was β-carotene. The carotenoid levels of *pP-YBI* plantlets were similar to those of the wild type, while the *pK-YBI* plantlets contained around 1.5-fold higher amounts of total carotenoids, of which approximately 16% was β-carotene, and 1.4-fold lower amounts of total chlorophylls, which explained their yellow-orange appearance. In contrast to the plantlets, the carotenoid content of *pK-YBI* calli was almost identical to that of wild-type calli, while the orange calli derived from *pP-YBI* expressor lines contained massive (up to 1 mg g^{-1} dry weight) amounts of carotenoids, with β-carotene being the major carotenoid (about 75% of total carotenoids). The phenotype of the *pK-YBI* plantlets disappeared in greenhouse-grown plants.

Expression of *CrtB-I* Genes Induces Striking Alterations in Leaf Pigment Composition and Gene Expression

We reported previously that the *pK-YBI* expressor lines exhibit a pale leaf color (Diretto et al., 2007a). As shown in Figure 2C, this phenotype was already

Table 1. Gene constructs analyzed

Plasmid	Promoter-Gene Combinations
<i>pK-I</i>	<i>35S:CrtI</i>
<i>pK-BI</i>	<i>Pat1:CrtB</i> , <i>35S:CrtI</i>
<i>pK-YBI</i>	<i>Pat1:CrtB</i> , <i>35S:CrtI</i> , <i>35S:CrtY</i>
<i>pP-I</i>	<i>Pat2:CrtI</i>
<i>pP-BI</i>	<i>Pat1:CrtB</i> , <i>Pat2:CrtI</i>
<i>pP-YBI</i>	<i>Pat1:CrtB</i> , <i>Pat2:CrtI</i> , <i>Pat2:CrtY</i>

observed in *pK-I* expressor leaves and became more pronounced in *pK-BI* and *pK-YBI* expressor leaves. Thus, the phenotype can be attributed to the expression of the *CrtI* transgene, which is expressed in all three types of leaves, and is intensified by the additional expression of *CrtB* and *CrtY*. In agreement with the visual phenotype, HPLC analysis showed that carotenoid content decreased significantly in leaves of *pK* expressor lines (Fig. 3A), while no significant variations were observed in leaves of expressor *pP* lines and of nonexpressor lines (Supplemental Table S1). Moreover, the reduction of pigment content was progressive, from *pK-I* to *pK-YBI* (Fig. 3A). It has been reported previously that overexpression of *CrtI* in tobacco (*Nicotiana tabacum*) and tomato leaves leads to alterations in pigment content and composition, including a decrease in total carotenoids and α -xanthophylls, an increase of β -carotene and β -xanthophylls, and a reduction of the chlorophyll-carotenoid ratio (Misawa et al., 1994; Romer et al., 2000). The changes in pigment composition reported here were quantitatively and qualitatively more severe: as shown in Supplemental Table S1, the amounts of chlorophylls and carotenoids were reduced approximately 25% and 34%, respectively. Moreover, the reduction affected all three carotenoid classes, α - and β -xanthophylls and β -carotene (Fig. 3A). The more pronounced phenotype reported here might be attributed to species-specific differences between tomato/tobacco and potato, or it may be the result of the codon-optimized *CrtI* gene used in this study (Al-Babili et al., 2006), probably leading to higher *CrtI* protein amounts. However, western-blot experiments were unable to reveal any *CrtI* protein in leaves of the expressor lines (data not shown).

Compared with *pK-I* leaves, the *pK-BI* leaves showed lower pigment content and paler color. This suggested that *CrtB* was contributing to the phenotype and thus that it was expressed in leaves, in spite of its control by the *Pat1* tuber-specific promoter. Indeed, real-time reverse transcription (RT)-PCR analyses revealed low, but detectable, *CrtB* transcript levels in leaves of the *pK-BI* and *pK-YBI* lines (Supplemental Table S2). Comparably low, but detectable, levels of expression in leaves were measured for *CrtI* and *CrtY* under the control of the *Pat2* promoter (Supplemental Table S2). These observations suggest that both *Pat* promoters used in this work were “leaky” and allowed low levels of expression in leaves. In the case of the *CrtB* gene, these low levels of expression resulted in an enhancement of the leaf phenotype caused by *CrtI*

expression. It is also notable that coexpression of *CrtI* and *CrtB* led to lower amounts of *CrtI* transcript, compared with the expression of *CrtI* as a single transgene (Supplemental Table S2).

To evaluate possible transcriptional perturbations of endogenous carotenoid genes, and to determine their contribution to leaf pigment composition, their transcript levels were monitored by real-time RT-PCR. All gene expression data were normalized to the housekeeping tubulin gene. Normalization to another housekeeping gene, ubiquitin, resulted in similar expression trends (data not shown). The results are analytically shown in Supplemental Table S2 and summarized in Figure 3B. Overexpression of *CrtI* led to a repression of “early” transcripts mediating lycopene synthesis and to an induction of transcripts in the α - and β -branches. These transcriptional alterations are consistent with the general reduction of carotenoid content (Fig. 3A). Combined expression of *CrtI* and *CrtB* further repressed early carotenoid transcripts and *LCY-e* (Fig. 3B) and, at the pigment level, further reduced β -carotene and β -xanthophyll contents, while α -xanthophyll content was increased (Fig. 3A). Finally, the additional expression of the *CrtY* β -cyclase partially restored the levels of β -xanthophylls (Fig. 3A) but further repressed the transcripts of xanthophyll biosynthesis enzymes (Fig. 3B).

These data indicate that leaf carotenoid levels are directly related to the expression levels of early genes, particularly *PSY1*, *PSY2*, and *PDS* (Fig. 3), suggesting that the early biosynthetic flux leading to lycopene (Fig. 1) in transgenic leaves is mainly controlled by the endogenous enzymes. In contrast, the levels of α - or

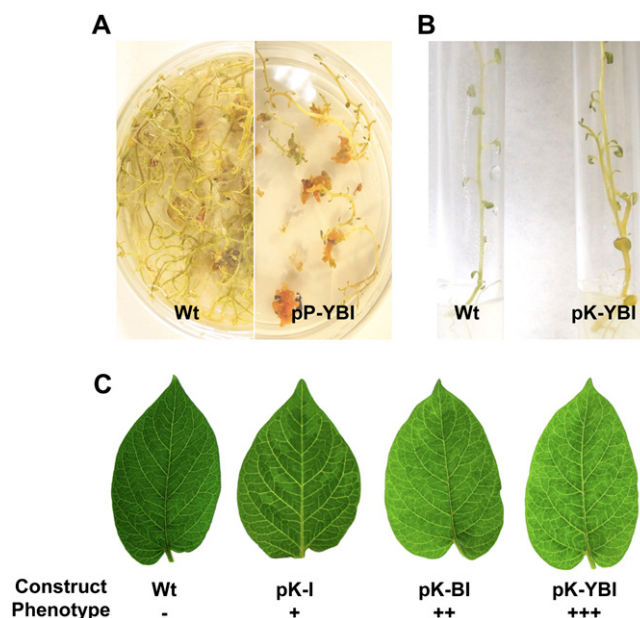


Figure 2. Phenotypes of transgenic plants. A, In vitro phenotypes of explants. B, In vitro phenotypes of regenerated plantlets. C, Leaf phenotypes of expressor plants grown in the greenhouse. Wt, Wild type.

Table II. HPLC analysis of chlorophyll and carotenoid contents in wild-type and Golden calli and plantlets

Values shown are $\mu\text{g g}^{-1}$ dry weight. Carotenoid composition was measured via diode array HPLC on a minimum of four different calli or stems. Fold change with respect to the wild type is reported for each pigment and for each line. Fold change with respect to the wild type was not calculated in cases in which a metabolite was not detected (indicated by dash).

Sample	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Violaxanthin + Neoxanthin + Other Xanthophylls	Lutein	β -Carotene	α/β -Xanthophyll	Total Carotene
Calli								
Wild type	8.8 $\pm$ 1.7	1.7 $\pm$ 0.5	10.9 $\pm$ 2.0	10.2 $\pm$ 1.9	10.5 $\pm$ 1.7	6.1 $\pm$ 1.3	1.0	48.1 $\pm$ 2.5
pK-YBI 41 (E)	9.1 $\pm$ 1.6	1.2 $\pm$ 0.4	10.7 $\pm$ 1.7	10.6 $\pm$ 1.7	10.3 $\pm$ 3.0	8.9 $\pm$ 1.1	1.0	50.8 $\pm$ 4.8
Fold change	1.0	0.7	0.9	1.0	1.0	1.5	1.0	1.1
pK-YBI 42 (E)	8.0 $\pm$ 1.4	1.1 $\pm$ 0.4	9.4 $\pm$ 1.7	10.9 $\pm$ 1.9	11.0 $\pm$ 2.7	8.8 $\pm$ 1.8	1.0	49.2 $\pm$ 3.3
Fold change	0.9	0.7	0.9	1.1	1.1	1.4	1.0	1.0
pP-YBI 17 (E)	0.00	0.0	0.00	0.00	206.9 $\pm$ 38.8	605.0 $\pm$ 90.22	–	811.8 $\pm$ 84.8
Fold change	–	–	–	–	19.8	99.0	–	16.9
pP-YBI 30 (E)	0.00	0.0	0.0	0.0	302.8 $\pm$ 49.8	787.3 $\pm$ 76.3	–	1,090.1 $\pm$ 128.4
Fold change	–	–	–	–	29.0	128.9	–	22.6
Plantlets								
Wild type	250.1 $\pm$ 29.6	102.7 $\pm$ 18.1	361.3 $\pm$ 40.8	21.3 $\pm$ 4.9	42.7 $\pm$ 6.5	10.3 $\pm$ 1.5	0.5	74.3 $\pm$ 8.5
pK-YBI 41 (E)	173.1 $\pm$ 18.2	66.7 $\pm$ 10.3	241.3 $\pm$ 37.4	24.7 $\pm$ 4.0	71.2 $\pm$ 7.8	18.1 $\pm$ 1.8	0.4	113.9 $\pm$ 25.8
Fold change	0.7	0.7	0.7	1.2	1.7	1.8	0.7	1.5
pK-YBI 42 (E)	162.4 $\pm$ 28.0	53.5 $\pm$ 6.4	13.7 $\pm$ 3.0	22.9 $\pm$ 5.2	74.5 $\pm$ 10.3	18.4 $\pm$ 4.3	0.3	115.8 $\pm$ 30.0
Fold change	0.7	0.5	0.7	1.1	1.7	1.8	0.6	1.6
pP-YBI 17 (E)	237.3 $\pm$ 41.4	95.3 $\pm$ 16.8	334.1 $\pm$ 45.5	20.0 $\pm$ 2.0	42.1 $\pm$ 6.4	11.4 $\pm$ 2.1	0.5	73.5 $\pm$ 5.4
Fold change	1.0	0.9	0.9	0.9	1.0	1.1	1.0	1.0
pP-YBI 30 (E)	255.3 $\pm$ 32.8	114.1 $\pm$ 27.4	370.2 $\pm$ 52.7	22.2 $\pm$ 4.0	44.7 $\pm$ 6.6	12.1 $\pm$ 2.1	0.5	79.0 $\pm$ 6.8
Fold change	1.0	1.1	1.0	1.0	1.1	1.2	1.0	1.1

β -branch xanthophylls tend to be inversely related to the levels of transcripts in the corresponding branches (Fig. 3). This might be the result of branch-specific negative feedback mechanisms, in which the compounds of one branch repress the transcripts in the other branch. The existence of such a mechanism would explain how leaves manage to maintain a constant α -xanthophyll- β -xanthophyll ratio.

In summary, overexpression of *CrtI* represses leaf carotenoid levels, and this phenotype is potentiated by coexpression of *CrtB*. Our interpretation is that the all-trans desaturation/isomerization pathway catalyzed by the *CrtI* enzyme competes with the endogenous poly-cis pathway catalyzed by the plant enzymes (Fig. 1; Bartley et al., 1999; Giuliano et al., 2002). Also, through an unknown mechanism, *CrtI* overexpression results in the repression of endogenous early genes (Fig. 3B). One possible hypothesis is that this repression is mediated by the accumulation of trace amounts of all-trans-lycopene, or of one of its degradation products, in leaves, which are undetected by the chromatographic system used here. Both the reduced synthetic flux, resulting from repression of early endogenous genes, and the competition at the enzyme level for the precursor pool result in inefficient synthesis of the final products β -carotene, β -xanthophylls, and α -xanthophylls (Fig. 3A).

Why does *CrtB* potentiate this effect? Our hypothesis is that, even at the low expression levels observed in *pK-BI* leaves, the enzyme forms a complex with *CrtI* and diverts, through metabolic channeling, a larger

part of the geranylgeranyl diphosphate pool into the all-trans pathway, resulting in greater competition with the endogenous, poly-cis desaturases (Bartley et al., 1999). Since *CrtI* is an enzyme of bacterial origin, it is unlikely that it forms an efficient complex with the endogenous cyclases; thus, the lycopene it forms would be less readily available to the latter enzymes, resulting in lower levels of final products in the pathway (Fig. 3A). Finally, it is possible that the carotenoids produced by the bacterial enzymes are incorrectly compartmentalized within the plastid membranes and are thus less readily assembled into pigment-protein complexes, resulting in lower overall carotenoid accumulation.

It is noteworthy that these metabolic effects are observed in spite of the fact that the levels of expression of the transgenes are low, compared with those of endogenous genes (Fig. 3B), and that the levels of the *CrtI* protein are undetectable by western blotting (data not shown). Possibly, higher expression levels would result in lethal bleaching of leaves and are counter-selected immediately after transformation. Since *CrtB* potentiates the leaf-bleaching phenotype induced by *CrtI*, only events with low expression of both transgenes would survive, explaining why coexpression of *CrtB* reduces *CrtI* expression, as noted above.

The Golden Phenotype Requires Coordinated Expression of the *Y-B-I* Genes in Tubers

Carotenoid composition and gene expression in tubers were analyzed, respectively, by HPLC and

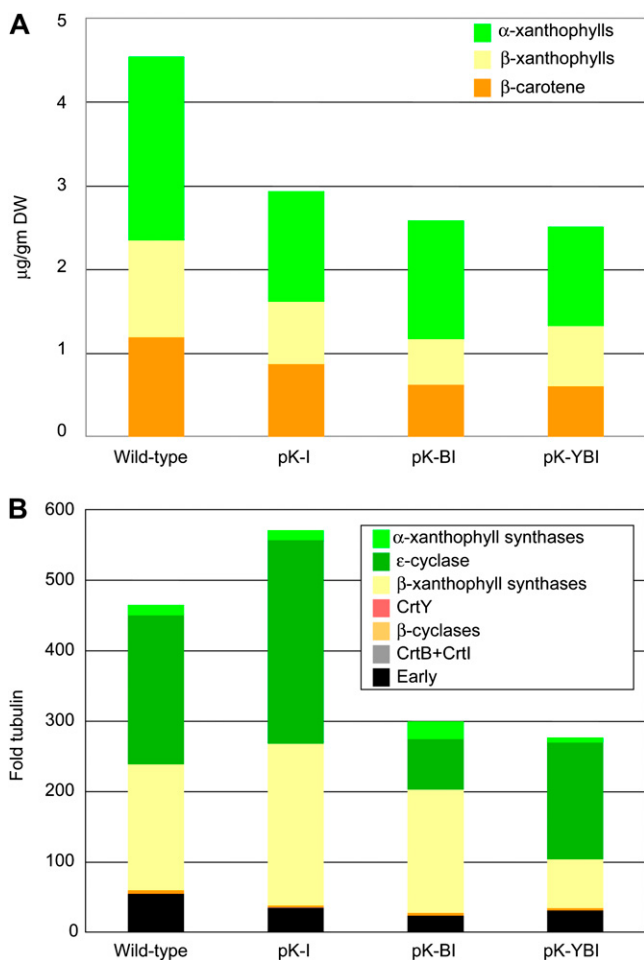


Figure 3. Expression of endogenous carotenoid metabolites and transcripts in the different segments of the leaf carotenoid pathway. A, Metabolite data (from Supplemental Table S1). B, Transcript data (from Supplemental Table S2). Transcript data are normalized to the house-keeping tubulin transcript. Early transcripts include *PSY1*, *PSY2*, *PDS*, *ZDS*, and *CrtISO*. α -Xanthophyll and β -xanthophyll synthases include *LCY-e*, *LCY-b*, *LUT1*, and *LUT5* and *LCY-b*, *CHY1*, *CHY2*, *LUT5*, *ZEP*, and *NXS*, respectively. See also Figure 1. DW, Dry weight. [See online article for color version of this figure.]

real-time RT-PCR. The detailed data are shown in Supplemental Tables S3 and S4 and summarized in Figure 4. An increase in total carotenoid content was observed in all expressor lines, with a maximum level (approximately 20-fold) in Golden (*pP-YBI*) tubers. In most cases, the differences in carotenoid composition of tubers overexpressing the same transgene combination under the control of the *35S* or the *Pat* promoter (*pK* versus *pP* series) were quantitative rather than qualitative: the *pK* series showed more modest modifications of carotenoid content (Fig. 4A), corresponding to lower transgene expression levels in tubers (Fig. 4B).

Overexpression of *CrtI* alone led to a strong increase of β -branch carotenoids (Fig. 4A). This increase is reminiscent of that described in tomato fruits over-

expressing *CrtI* (Romer et al., 2000) but, in contrast to tomato fruits, results in the accumulation of β -xanthophylls rather than of β -carotene (Fig. 4A). The absence of β -carotene accumulation in *CrtI* tubers is probably due to the high expression of endogenous transcripts encoding β -xanthophyll synthases in this tissue (Fig. 4B), which is sufficient to convert β -carotene into downstream β -xanthophylls.

Simultaneous overexpression of *CrtB* and *CrtI* resulted in the accumulation of β -xanthophylls (Fig. 4A), but to lower levels than overexpression of *CrtI* alone. These lower levels are likely the result of the lower transgene expression in *B-I* versus *I* tubers (Fig. 4B; Supplemental Table S4). As stated above, we believe that this lower expression results from a counterselection of high-expressing lines during “in vitro” culture,

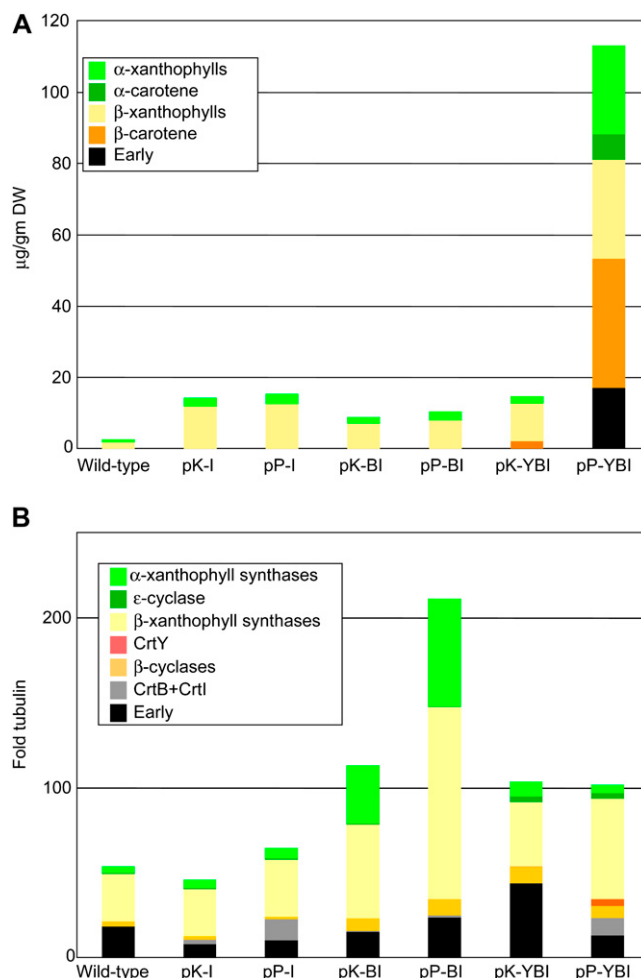


Figure 4. Expression of endogenous carotenoid metabolites and transcripts in the different segments of the tuber carotenoid pathway. A, Metabolite data (from Supplemental Table S3). B, Transcript data (from Supplemental Table S4). Transcript data are normalized to the house-keeping tubulin transcript. Early transcripts, α -xanthophyll, and β -xanthophyll synthases are as in Figure 3. DW, Dry weight. [See online article for color version of this figure.]

due to the leaky expression of the *Pat* promoter in leaves. At the transcriptional level, *CrtB-I* overexpression resulted in a large up-regulation of α - and β -xanthophyll synthases and of *NXS* (Fig. 4B; Supplemental Table S4), which, however does not result in a corresponding increase of α - and β -xanthophylls (Fig. 4A). *NXS* has been previously shown to catalyze the synthesis of neoxanthin in vitro (Al-Babili et al., 2000), but it is also almost identical, in its primary structure, to tomato *CYC-b* and to pepper (*Capsicum annuum*) capsanthin-capsorubin synthase, which both exhibit lycopene β -cyclase activity (Hugueney et al., 1995; Ronen et al., 2000). Therefore, it is likely that *NXS* is a lycopene β -cyclase and that its increased expression in tubers overexpressing *CrtB-I* is responsible for the lack of accumulation of lycopene (Fig. 4A; Supplemental Table S3), the product of the *CrtB-I* minipathway. The accumulation of β -carotene and β -xanthophylls instead of lycopene was also observed in Golden rice overexpressing *PSY* and *CrtI* in the endosperm (Ye et al., 2000) and was suggested to be the result of the activities of the downstream genes *LCY-b* and *CHY* (Schaub et al., 2005). Additionally, it is possible that potato tubers and/or rice endosperm are unable to accumulate lycopene because of the lack of specialized “sink” structures. In fact, lycopene-accumulating plastids are characterized by a different ultrastructure compared with other carotenoid-accumulating chromoplasts (Deruere et al., 1994).

The simultaneous overexpression of *CrtB*, *CrtI*, and *CrtY* led to the accumulation of β -carotene. However, this accumulation was relatively modest (on the order of $2 \mu\text{g g}^{-1}$ dry weight) when *CrtI* and *CrtY* expression was driven by the 35S promoter, leading to low expression levels in tubers (Fig. 4). In contrast, expression of all three transgenes under the control of the *Pat* promoter control resulted in a much higher (10- to 100-fold) expression of the transgenes (Fig. 4B), in a massive (20-fold) increase of total carotenoids (Fig. 4A), and in a dramatic shift in the xanthophyll-carotene ratio, with phytoene, α -carotene, and β -carotene accounting for 50% of total carotenoids (Fig. 4A).

Antibodies raised to the *CrtI* proteins were used for western-blot analysis: extracts of Golden tubers show a signal with an apparent molecular mass of about 55 kD, corresponding to that of the mature *CrtI* protein (Supplemental Fig. S1). This implies that in Golden tubers, the *CrtI* protein is correctly imported into plastids. In contrast, *pK-YBI* tubers showed very low, or undetectable, signal. A similar lack of signal was observed in tubers and leaves of all the other lines (data not shown).

The levels of transcripts encoding carotenoid biosynthesis enzymes were modified in all transgenic tubers. (1) The most highly expressed phytoene synthase, *PSY1*, was down-regulated in all transgenic tubers, with the exception of *pK-YBI* (Supplemental Table S4). The second dedicated gene in the pathway, *PDS*, was up-regulated in most transgenic tubers, with the exception of Golden (*pP-YBI*) tubers. This regula-

tion of endogenous early genes can be attributed to the *CrtI* transgene, which is expressed in all transgenic tubers. A similar repression of *PSY1* and induction of *PDS* have been observed in tomato fruits overexpressing *CrtI* (Romer et al., 2000). (2) *LCY-b* was up-regulated in the *B-I* and *Y-B-I* series, especially in the Golden *pP-YBI* tubers, while the β -cyclase paralog *NXS* was down-regulated in the latter but induced in all other *BI* and *YBI* expressor lines (Fig. 4B; Supplemental Table S4). *LCY-e* was repressed in the *B-I* series but up-regulated in the *Y-B-I* series, particularly in Golden tubers (Fig. 4B). The high levels of expression of *LCY-b* and *LCY-e* in Golden tubers are consistent with the accumulation of α - and β -carotene (Fig. 4A). (3) Apart from the nonuniform response of *CHY2*, other transcripts encoding α - and β -xanthophyll synthases were strongly up-regulated in *B-I* tubers (Fig. 4B). Particularly *CHY1*, encoding a β -branch nonheme hydroxylase, was induced in all *B-I* and *Y-B-I* lines (Supplemental Table S4); P450 carotenoid hydroxylases (*LUT5*, *LUT1*) were strongly up-regulated in *B-I* and to a lower extent in *pK-YBI* tubers, while *LUT1* was strongly repressed in Golden tubers (Supplemental Table S4). The induction of hydroxylases in *B-I* tubers and the repression of *LUT1* in Golden tubers provide a rationale for the lack of β -carotene accumulation in the former and for α -carotene accumulation in the latter.

In summary, most transgene combinations induced the levels of endogenous carotenoid transcripts, with the exception of *PSY1* in most tubers and *LUT1* and *NXS* in Golden tubers. The transcriptional alterations observed (Fig. 4B) were generally consistent with the HPLC data (Fig. 4A). However, they do not explain some biochemical phenotypes, such as phytoene accumulation in Golden tubers. The simultaneous overexpression of *CrtB* and *CrtI* genes resulted in the highest deregulation/induction of endogenous transcripts, which however did not result in a comparable increase in carotenoid content. In contrast, Golden tubers show a relatively modest induction of endogenous transcripts, affecting mainly the β -branch, and a high expression level of all three transgenes (Fig. 4B). This high expression of all three transgenes, combined with a minor perturbation of endogenous gene expression, can be considered as a “golden ratio,” allowing the accumulation of high levels of β -carotene in tubers.

The data suggest that both negative and positive feedback mechanisms may be acting to influence the endogenous transcript levels in Golden tubers. The high levels of carotenoids and β -carotene contained in these tubers seem to trigger a repression of *PSY1*, which is likely a key gene in tuber carotenogenesis, and an induction of β -xanthophyll synthases and lycopene ε -cyclase. The latter regulatory events are predicted to counteract the accumulation of high levels of carotenoids and β -carotene (Fig. 1). Thus, it is possible that, by silencing of the *CHY1*, *CHY2*, *LUT5*, and *LCY-e* genes, the levels of β -carotene in golden

tubers may increase even further. This has been experimentally confirmed for the *LCY-e* and *CHY* genes (Diretto et al., 2006, 2007b; van Eck et al., 2007).

Correlation and Network Analysis of Transcripts and Metabolites in Leaves and Tubers

To further explore relationships between gene expression and the accumulation of selected metabolites in the different transgenic lines, we used three different approaches: hierarchical clustering and correlation matrices and networks of the transcript and metabolite levels in leaves and tubers.

Hierarchical clustering (Claverie, 1999) generates a heat map, in which the genes or metabolites (rows) and transgenic lines (columns) are clustered together according to their fold changes with respect to the wild type. The clustering algorithm treats each row or column as numerical vectors and rearranges their position in the data table according to a predefined measure of similarity (Pearson distance). Different shades of red and green indicate different levels of increase or decrease, respectively (Fig. 5). In the leaf and tuber heat maps, all expressor lines containing the same transgene combination clustered together on the left side of the heat map, forming a distinct group from the cluster of nonexpressor lines and of the wild type. Golden (*pP-YBI*) tubers showed the largest overall perturbations and were found on the extreme left, followed by *BI*, *pK-YBI*, and *pK-I* tubers. Leaves of the *pK* lines, expressing one or more transgenes under the control of the constitutive 35S promoter, show the largest transcriptional/metabolic perturbations and cluster preferentially on the left side, away from the wild type (Fig. 5A). Leaves of the two Golden lines cluster instead very close to wild-type leaves, indicating very low levels of metabolic-transcriptional perturbation.

The *Crt* transgenes are coregulated in both leaf and tuber heat maps, clustering at the top in the dendrograms. In leaves (Fig. 5A), *CrtI* and *CrtY* are most tightly coregulated, in agreement with the fact that their expression is controlled by identical promoters; however, the transgene showing the closer coregulation with endogenous genes is *CrtB*. In tubers (Fig. 5B), all three transgenes are coregulated with each other and with three carotenes (phytoene, α -carotene, and β -carotene), which appear *de novo* in Golden tubers. While the clustering of *CrtB* with phytoene and of *CrtY* with β -carotene are expected on the basis of the biochemical reactions they catalyze (Fig. 1), their clustering with α -carotene is less expected, since synthesis of the latter compound requires the combined action of β - and ϵ -cyclase.

All leaf carotenoids and tuber xanthophylls cluster separately from the transgenes as well as from the endogenous genes, albeit some closer correlations are observed with the latter: in *pK* leaves, a cluster comprising early transcripts (*PSY1*, *PSY2*, *CrtISO*) as well as β -branch transcripts (*LCY-b*, *NXS*, *CHY2*) is corepressed with carotenoid metabolites (Fig. 5A). In

Golden tubers, transcripts from all three biosynthetic branches (*ZDS*, *CrtISO*, *LCY-b*, *CHY1*, *ZEP*, *LCY-e*) are coinduced with xanthophylls (Fig. 5B). Some transcripts also show coregulation with each other: leaf clusters include *PDS/LUT5/CHY1/LUT1*, *ZDS/LCY-e/ZEP*, and *PSY1/PSY2/NXS* (Fig. 5A), while tuber clusters include *PDS/CrtISO*, *LCY-b/CHY1*, *ZEP/ZDS*, and *LUT1/LUT5* (Fig. 5B).

We then applied pairwise correlation analysis (Steuer, 2006), a method that calculates the Pearson correlation coefficient for the fold change of all transcript-metabolite pairs in the expressor lines with respect to their nonexpressor counterparts (Fig. 6). Positive correlations are shown in different shades of red, while negative correlations are shown in different shades of blue. Besides the correlations described above, novel and significant correlations (greater than +0.60 and less than -0.60) emerge from this analysis. Leaf carotenoids positively correlate with most endogenous carotenoid transcripts (Fig. 6A), while negative correlations are observed with transgene transcripts, particularly *CrtI*; among endogenous transcripts, *PSY1*, *PSY2*, *CrtISO*, *LCY-b*, and *CHY2* show the highest number of positive correlations to metabolites and other transcripts, thus indicating a "master switch" role of these genes. In tubers (Fig. 6B), *PSY1* shows significant negative correlations to the end products of the β -branch, violaxanthin and neoxanthin, and of the α -branch, lutein. This is an indication that this transcript may be under negative feedback control by carotenoid end products. *NXS* shows a significant negative correlation with violaxanthin, which is the neoxanthin precursor; this is consistent with the function proposed for this gene as a neoxanthin synthase (Al-Babili et al., 2000; Bouvier et al., 2000). Besides the above correlations, *Crt* transgenes display the largest number of negative and positive correlations to endogenous transcripts/metabolites in both tissues: *CrtI* shows a remarkably high number of negative correlations in leaves (Fig. 6A), while *CrtB* and *CrtY* display a comparable number of positive correlations in tubers. As noted previously, these two transgenes show very high correlations with phytoene, α -carotene, and β -carotene, the three compounds that appear *de novo* in Golden tubers.

Each matrix was transformed into a correlation network to highlight potential differences in the topology and strength of the overall transcript-metabolite correlations in different tissues and transgene combinations. A correlation network is a graph depicting all-versus-all correlations in a group of variables showing quantitative variation (for review, see Albert, 2007; Krishnan et al., 2008). In this case, these variables are the fold variations in transcript-metabolite levels in transgenic expressor lines with respect to the wild type. The correlation network (Fig. 7) is composed of a number of nodes: metabolites (diamonds), endogenous genes (circles), transgenes (triangles). The color of a line, or edge, joining two nodes corresponds to a positive (red) or negative (blue) correlation, while its

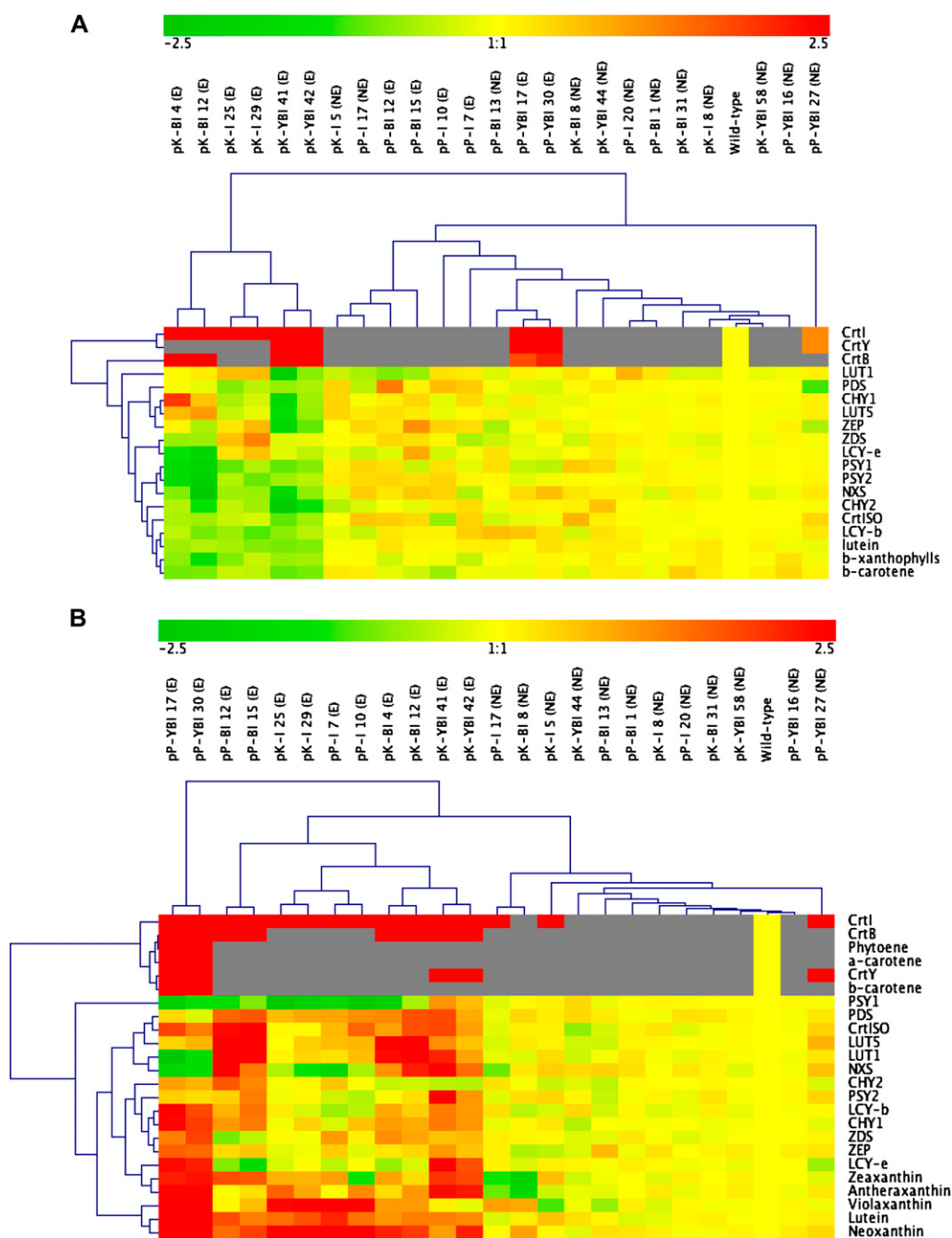


Figure 5. Hierarchical clustering of transcript/metabolite fold changes in transgenic leaves and tubers. A, Leaf data. B, Tuber data. Colored squares represent the values of $\log_2$ -transformed fold changes of a transcript or metabolite with respect to the wild type, according to the color scale shown. Gray squares indicate no detectable expression of the corresponding transcript/metabolite. Hierarchical clustering was calculated both on columns and rows, applying the Pearson correlation coefficient with the average linkage algorithm. For details, see “Materials and Methods.”

thickness represents the strength of the correlation (i.e. the absolute value of the Pearson correlation coefficient $[|\rho|]$ of that particular pair). The present network is defined by the following parameters (G. Giuliano, F. Scossa, and G. Diretto, unpublished data): “node strength” (ns) is the average of the

absolute values of all correlation coefficients of a given node with all other nodes:

$$ns = \text{Avg}|\rho|$$

and “network strength” (NS) is the average of all node strengths:

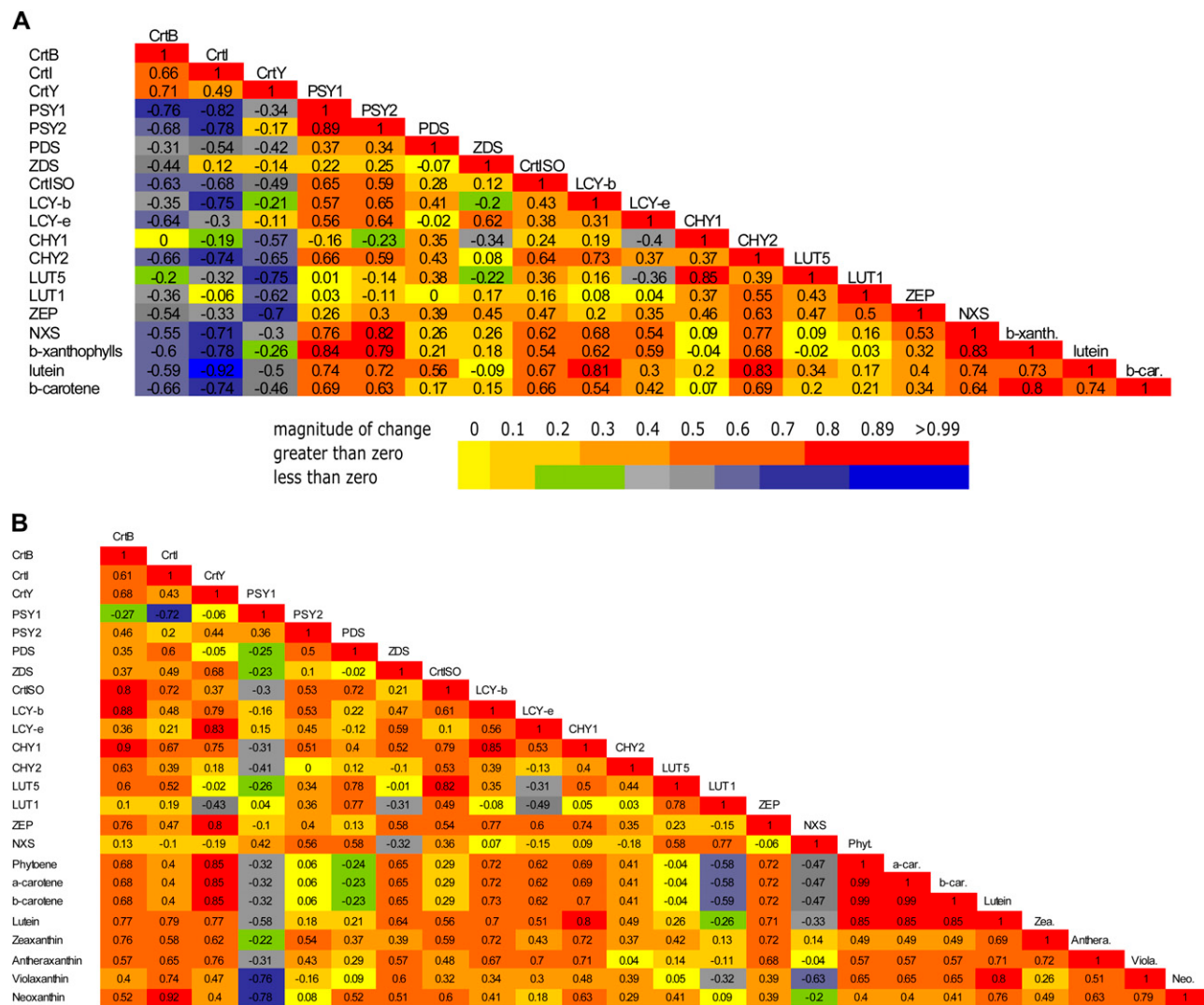


Figure 6. Matrix correlation analysis of transcript and metabolite levels in transgenic leaves and tubers. Visualization of the correlations is shown between carotenoid metabolites and transcript levels in wild-type and transgenic leaves (A) and tubers (B). Each square represents the correlation between the gene/metabolite heading the column and the gene/metabolite heading the row. Correlation coefficients and significances were calculated by applying the Pearson formula using Excel and visualized using the Heatmapper software. Pearson p values are shown and highlighted with different shades of red (positive correlations) or blue (negative correlations). For details, see “Materials and Methods.”

$$NS = \text{Avg}(ns)$$

The sizes of the symbols in Figure 7 are proportional to the node strengths, while the absolute values of such strengths are shown in Supplemental Table S5.

Network strength is high (greater than 0.70) in leaves and tubers for most transgene combinations (with the exception of the *pP-I* tuber network, which has a network strength of 0.59). This is perhaps not surprising, since we are dealing with a group of compounds and transcripts involved in the same metabolic pathway and thus expected to be coregulated. Additionally, network strength increases from

single to triple constructs, indicating that the latter increase the overall level of coregulation between the nodes of the network (transgenes, endogenous genes, and carotenoid metabolites). However, this increase occurs with different kinetics in *pK* leaves and *pP* tubers. The major increase in network strength in leaves (from 0.72 to 0.92) is observed between *pK-BI* and *pK-YBI* plants (Fig. 7). In other terms, the addition of the *CrtY* transgene to the *CrtB* and *CrtI* ones brings a sizable increase of the total coregulation in the network. This increase is observed in the absence of significant changes in leaf metabolite levels (Fig. 3A); that is, it is mainly due to transcriptional perturba-

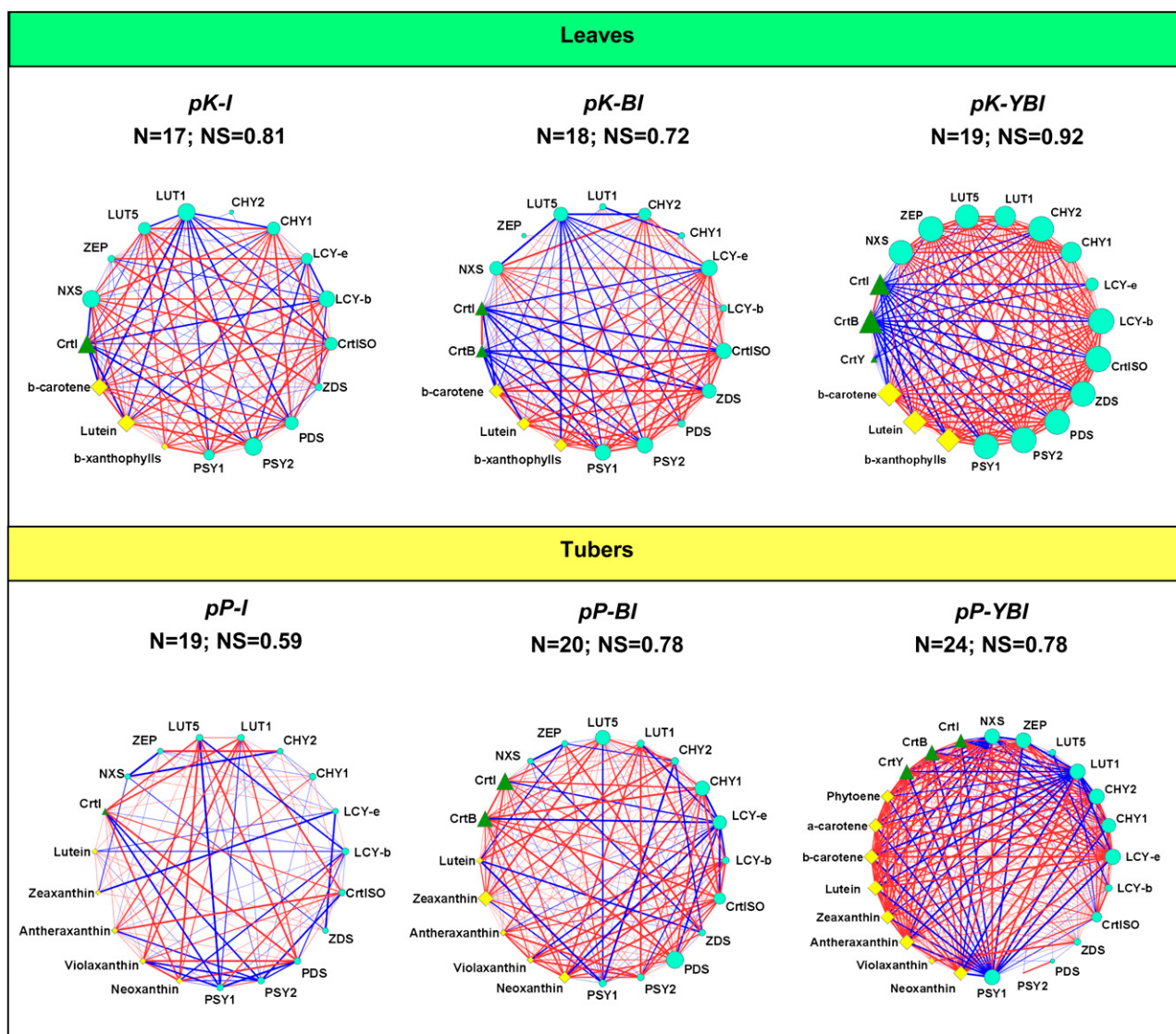


Figure 7. Correlation networks of carotenoid transcripts and metabolites in transgenic leaves and tubers. Networks are visualized as circles, with each node representing an endogenous transcript (turquoise circles), a transgene (green triangles), or a metabolite (yellow diamonds). Lines joining the nodes represent correlations; direct correlations are shown in red, while inverse correlations are in blue. Node sizes are proportional to the respective node strengths, which are shown in Supplemental Table S5. Number of nodes (N) and network strength (NS) are shown on top of each network.

tions. This increase is the result of a generalized increase in the strength of all nodes (Fig. 7; Supplemental Table S5) rather than of specific ones. The colors of the edges indicate that transgenes show negative correlations, and endogenous genes show positive correlations, with most other nodes.

In contrast, the major increase in tuber network strength is observed between *pP-I* and *pP-BI* plants, while the major increase in carotenoid content is observed between *pP-BI* and *pP-YBI* tubers, in the absence of an increase in network strength. Again, this means that the addition of the *CrtB* transgene to the *CrtI* one causes a major increase of the coregulation within the tuber network (Fig. 7), and again, this is

mainly due to increases in coregulation of transcript rather than metabolite levels (Fig. 4). Unlike in leaves, transgenes show mostly positive correlations with carotenoid levels, while endogenous genes show more variable trends: the *PSY1* and *LUT1* nodes are weak in *pP-BI* tubers and become very strong, with mostly negative correlations to the rest of the network, in Golden tubers. We define such strong nodes, showing a large number of either positive or negative correlations, as “hubs” (G. Giuliano, F. Scossa, and G. Diretto, unpublished data). Thus, in the Golden tuber network, the *CrtB*, *CrtI*, and *CrtY* transgenes are positive hubs and the *PSY1*, *LUT1*, and *NXS* endogenous genes are negative ones (Fig. 7).

In addition to the real-time analysis of carotenoid genes, a global transcriptomic analysis of Golden tubers is under way using the Potato Oligo Chip Initiative microarray (Kloosterman et al., 2008). The preliminary results indicate transcriptional perturbations in a range of metabolic pathways (such as glycolysis, starch synthesis and mobilization, lipid biosynthesis and degradation, biosynthesis of secondary metabolites other than carotenoids), albeit the frequency of transcriptional alterations in those pathways is substantially lower than in the carotenoid pathway. As an example, we show in Supplemental Table S6 the regulation of genes in those pathways that are metabolically adjacent to the dedicated carotenoid pathway: 1-deoxy-D-xylulose 5-phosphate and mevalonate pathways, chlorophyll and gibberellin biosynthesis, biosynthesis of quinones, tocochromanols, and apocarotenoids. The metabolic relations between these pathways and the carotenoid pathway are shown in Supplemental Figure S2. As can be seen, several differentially regulated genes are found in these pathways, especially in the mevalonate, tocochromanol, and gibberellin pathways.

CONCLUSION

We used a combined approach based on the quantitative biochemical and molecular characterization of carotenoid metabolites and transcripts in 24 transgenic potato lines containing the *CrtB*, *CrtI*, and *CrtY* genes in different combinations. Our data allow a general and extensive assessment of carotenoid regulation in potato leaves and tubers in six different genetic manipulation trials. *CrtI* expression, alone or in combination with *CrtB* and *CrtY*, strongly interferes with leaf carotenogenesis, both at the metabolite and transcript levels. Phytoene, α -carotene, and β -carotene accumulate only in Golden tubers: at the transcript level, this phenotype can be explained by several processes: *CrtY* transgenic expression, which, contrary to what was observed in rice and canola, is necessary for β -carotene accumulation; down-regulation of *LUT1*, which possibly represses the transformation of α -carotene into lutein; and a golden ratio value in the transcription of endogenous genes. The accumulation of phytoene is common in many transgenic lines and therefore appears to be a bottleneck for further metabolism. It could be due to compartmentation of this carotenoid within the plastid, making it metabolically inert.

This study also provides additional potential targets for the rational design of further metabolic engineering experiments. Network correlation analysis showed an unexpectedly high degree of overall correlation in transcript-metabolite levels, which is increased by the sequential addition of transgenes. This increase in correlation does not often correspond to major changes in metabolite levels (i.e. it is predominantly observed at the transcript levels). Transcript levels seem to “react” to the perturbation introduced by the genetic

manipulations. This is, to some extent, expected: since regulatory relations between transcripts and metabolites are aimed at maintaining metabolite homeostasis, a “reaction” of the endogenous transcript network is likely to counteract an induced perturbation in metabolite abundance. Through a “guilt-by-profiling” approach (Tian et al., 2008), we have identified a series of endogenous genes showing such a regulatory behavior in Golden tubers. Besides *PSY1*, *LCY-e*, *CHYs*, and *ZEP*, which have been previously shown by us and others to be effective in modulating β -carotene and total carotenoid contents, the cytochrome P450 genes (*LUT1* and *LUT5*) were identified as potential modulators of potato carotenogenesis. Such genes are obvious candidates for manipulations aimed at the further optimization of the carotenoid content of Golden tubers. Differential regulation of genes for general isoprenoid metabolism is also found in Golden tubers. Although this is unlikely to play a major role in carotenoid accumulation, it probably reflects a regulatory cross talk between carotenoid biosynthesis and wider isoprenoid metabolism that deserves further investigation.

MATERIALS AND METHODS

Unless indicated differently, molecular biology methods were as described (Sambrook et al., 1989). Construct design, plant transformation, and selection were described previously (Diretto et al., 2007a). Wild-type (cv Desirée) and *Crt* transgenic rooted potato (*Solanum tuberosum*) plantlets were adapted in a transgenic greenhouse in pots (diameter, 25 cm) in a soil mixture composed of one-third sand and two-thirds sterile soil (Terraplast 2; BASF). Photoperiod was set at 14 h of light and 10 h of dark, with temperature set at 24°C during the light period and at 16°C during the dark period; irrigation was performed two times per week and always at the same time of the day. Three plants for each construct were grown, and a random block design strategy was used. Pooled leaf samples from three plants for each line were collected 45 d after transplantation and frozen at –80°C. During the late phase of growth, the day temperature was kept around 20°C in order to promote tuberization. During tuberization, irrigation was reduced in order to prevent tuber decay. After flowers fell and vines turned yellow (growth stage V), plants were cut and tubers were kept in the soil for 10 d in order to promote tuber hardening. Tubers from the lower two-thirds of the pot (“deep” tubers) were collected separately from superficial ones, washed in water, dried briefly at room temperature, cut in pieces, pooled, and frozen at –80°C. All carotenoid and RT-PCR measurements were conducted on at least four different deep tubers from three plants for each line (12 tubers in total) in order to avoid single tuber-specific variations.

HPLC assays were performed according to established protocols (Diretto et al., 2007a) using acetone-petroleum ether:diethyl ether (2:1, v/v) extraction; for leaf samples, Tris-HCl was added to the acetone phase in order to stabilize chlorophylls. All total carotenoid measurements in different lines were normalized using spectrophotometry.

RNA isolation, real-time RT-PCR conditions, and primers were described previously (Diretto et al., 2007b) and were performed according to established practices (Giuliano et al., 1993; Udvardi et al., 2008). Briefly, total RNA isolation was performed using a Tris-borate/phenol-chloroform-isoamyl alcohol (25:24:1)-based extraction followed by potassium acetate/lithium chloride/sodium chloride precipitation steps (Lopez-Gomez and Gomez-Lim, 1992) and DNase I purification at 37°C for 20 min. RNA purity was checked using a Nanodrop ND-1000 (NanoDrop Technologies), and only RNAs showing A_{260}/A_{280} and A_{260}/A_{230} ratios between 1.8:2.0 and 2.0:2.2, respectively, were selected. RNA integrity was assayed by using an Agilent 2100 Bioanalyzer, and only RNAs with a 28S/18S ratio between 1.5 and 2.0 were selected. A total of 500 ng of total RNA was retrotranscribed to cDNA using a murine leukemia virus reverse transcriptase/RNase inhibitor/oligo(dT)-based

mix (Applied Biosystems). cDNA quality was assessed using two pairs of primers for a reference gene (tubulin) in order to obtain a 1.0-kb amplicon in both standard and real-time PCR conditions. Three independent biological replicates for each independent line were used, and the “threshold cycle” values were determined for each gene using a standard curve. Primer pairs for each gene were designed using the same parameters (e.g. primer temperature of $60.0^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$, length of 18–25 bases, GC content between 40% and 60%) in order to generate a unique 120- to 150-bp PCR product. Normalization to a housekeeping gene (tubulin, ubiquitin, or actin) and to wild-type values was applied to raw data to obtain relative expression levels.

For western-blot analysis, 120 mg (fresh weight) of ground tuber tissue was incubated at 98°C in 150 μL of sample buffer (65 mM Tris-HCl, pH 6.75, 4% [w/v] SDS, 10% [v/v] β -mercaptoethanol, and 20% [v/v] glycerol) for 15 min. Samples were then centrifuged for 20 min at 18,000g, and 50 μL of the supernatant was applied to SDS-PAGE (acrylamide concentration of 12%; Laemmli, 1970). Applied protein amounts were verified by Coomassie Brilliant Blue staining of a gel run in parallel. For immunodetection, anti-CrtI antibodies, raised in rabbit against CrtI expressed in *Escherichia coli* (kindly provided by Syngenta), were used. For detection, horseradish peroxidase-coupled secondary anti-rabbit antibodies and the ECL system (Amersham Biosciences Europe) were employed according to the manufacturer's protocol.

Statistical analysis (one-way ANOVA plus Tukey's pairwise comparison) was performed using the PAST software (Hammer et al., 2001). Hierarchical clustering was performed on data tables organized as follows: each row representing a gene or a metabolite, columns for the different transgenic lines, and data points showing the associated $\log_2$ fold changes with respect to the wild type. Clustering was performed using Genesis version 1.7.2 (<http://genome.tugraz.at/genescient/>; Sturn et al., 2002) and calculated on both rows and columns using Pearson correlation and average linkage as agglomeration rule. Correlation matrices were built in Microsoft Excel based on the data tables as described above and were then visualized with Heatmapper plus (Bio-array resource for Arabidopsis Functional Genomics; <http://www.bar.utoronto.ca/>; Verhaak et al., 2006). Correlation networks were assembled manually from the corresponding matrices. Each data pair in the matrix was converted to a single line (edge) connecting two nodes. In all network diagrams, edge thickness is proportional to the absolute value of the Pearson correlation coefficient ($|r|$), while node sizes are proportional to their node strengths. Direct ($r > 0$) and inverse ($r < 0$) correlations are shown in red and blue, respectively. Different node shapes were used to distinguish endogenous transcripts from transgenes and metabolites. Networks were visualized as circle layouts with Cytoscape version 2.6.2 (www.cytoscape.org; Cline et al., 2007).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Expression of the CrtI protein in tubers.

Supplemental Figure S2. Isoprenoid metabolism in Golden tubers.

Supplemental Table S1. HPLC analysis of leaf chlorophyll and carotenoid content.

Supplemental Table S2. Real-time RT-PCR analysis of endogenous carotenoid gene expression in leaves.

Supplemental Table S3. HPLC analysis of tuber carotenoid content.

Supplemental Table S4. Real-time RT-PCR analysis of endogenous carotenoid gene expression in tubers.

Supplemental Table S5. Node strengths of the network shown in Figure 7.

Supplemental Table S6. Expression in Golden tubers of isoprenoid metabolism genes.

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Supplemental Table I – HPLC analysis of leaf chlorophyll and carotenoid content (mg/g dry weight)

Carotenoid composition was measured via diode array HPLC on a minimum of 4 different leaves from 2 different plants (8 leaves in total). Fold change with respect to the wild-type is reported for each pigment and for each line. Asterisks indicate significance of the fold change in an ANOVA test (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$). For details, see Methods.

	Chl <i>a</i>	Chl <i>b</i>	Total Chl	β-xanth.	Lutein	β-car.	β-xanth/ Lutein	Total Car	Chl/ Car
Wild-type	14.5±1.67	4.67±0.75	19.21±2.97	1.16±0.25	2.20±0.52	1.19±0.31	0.53	4.55±1.08	4.22
pK-I 25 (E)	10.1±1.25*	3.58±0.39	13.67±2.73	0.64±0.04**	1.40±0.08*	0.88±0.22*	0.46	2.93±0.33**	4.67
Fold change	0.69	0.77	0.71	0.55	0.64	0.74	0.87	0.64	1.11
pK-I 29 (E)	10.25±1.39*	3.47±0.61*	13.72±3.04	0.86±0.12*	1.23±0.33**	0.86±0.23*	0.70	2.96±0.69*	4.64
Fold change	0.70	0.74	0.71	0.74	0.56	0.72	1.33	0.65	1.10
pK-I 5 (NE)	13.87±2.97	5.03±1.35	18.90±3.38	1.14±0.10	2.10±0.08	1.40±0.39	0.54	4.64±0.57	4.07
Fold change	0.95	1.08	0.98	0.98	0.95	1.18	1.03	1.02	1.04
pK-I 8 (NE)	16.2±2.88	5.08±1.23	21.56±4.84	1.36±0.11	2.56±0.36	1.37±0.23	0.53	5.29±0.77	4.07
Fold change	1.12	1.09	1.12	1.17	1.16	1.15	1.00	1.16	0.97
pP-I 7 (E)	15.54±2.88	4.85±1.32	20.39±2.28	0.86±0.33	2.44±0.93	1.12±0.51	0.35	4.42±1.57	4.61
Fold change	1.07	1.04	1.06	0.74	1.11	0.94	0.67	0.97	1.09
pP-I 10 (E)	14.77±3.73	4.99±1.83	19.76±3.49	1.08±0.29	2.27±0.46	0.74±0.17	0.48	4.09±0.93	4.83
Fold change	1.02	1.07	1.03	0.93	1.03	0.62	0.90	0.90	1.15
pP-I 17 (NE)	15.28±3.89	5.27±1.74	20.55±3.85	1.20±0.15	2.14±0.31	1.36±0.33	0.56	4.69±0.80	4.38
Fold change	1.05	1.13	1.07	1.03	0.97	1.14	1.06	1.03	1.04
pP-I 20 (NE)	13.9±4.22	4.81±0.56	19.22±4.86	1.11±0.22	2.48±0.33	1.35±0.28	0.45	4.94±0.33	3.89
Fold change	0.96	1.03	1.00	0.96	1.13	1.13	0.84	1.09	0.92
pK-BI 4 (E)	9.75±2.52	3.10±0.89*	12.85±2.99*	0.67±0.19*	1.36±0.37*	0.62±0.18*	0.49	2.64±0.75*	4.87
Fold change	0.67	0.66	0.67	0.58	0.62	0.52	0.93	0.58	1.15
pK-BI 12 (E)	9.59±2.10*	3.04±1.01*	12.73±2.49*	0.41±0.01***	1.48±0.07***	0.63±0.04***	0.28	2.53±0.12**	5.03
Fold change	0.66	0.65	0.66	0.36	0.67	0.53	0.53	0.56	1.19
pK-BI 8 (NE)	14.37±3.49	5.03±1.75	19.40±3.77	1.14±0.26	2.47±0.44	1.01±0.16	0.46	4.62±0.86	4.20
Fold change	0.99	1.08	1.01	0.98	1.12	0.85	0.88	1.01	0.99
pK-BI 31 (NE)	15.25±4.86	5.44±0.66	20.73±5.73	1.22±0.35	2.39±0.28	1.65±0.33	0.51	5.26±0.81	3.94
Fold change	1.05	1.16	1.08	1.05	1.09	1.39	0.96	1.16	0.93
pP-BI 12 (E)	15.84±4.18	4.52±1.30	20.36±4.06	0.92±0.10	2.67±0.21	1.06±0.11	0.34	4.65±0.42	4.38
Fold change	1.09	0.97	1.06	0.79	1.21	0.89	0.65	1.02	1.04
pP-BI 15 (E)	14.38±3.69	4.87±1.59	19.25±47	1.13±0.33	2.30±0.49	0.92±0.25	0.49	4.35±1.07	4.42
Fold change	0.99	1.04	1.00	0.97	1.05	0.78	0.93	0.96	1.05
pP-BI 1 (NE)	14.44±2.59	4.71±1.48	19.11±5.10	1.08±0.29	2.18±0.10	1.09±0.24	0.49	4.35±0.77	4.39
Fold change	1.00	1.00	1.00	0.93	0.99	0.92	0.93	0.96	1.04
pP-BI 13 (NE)	13.74±3.50	4.58±1.49	18.32±3.92	1.18±0.32	2.49±0.67	1.07±0.28	0.47	4.74±1.28	3.86
Fold change	0.94	0.98	0.95	1.01	1.13	0.90	0.90	1.04	0.91
pK-YBI 41 (E)	9.02±2.21*	2.85±0.39**	12.31±3.33*	0.71±0.10**	1.16±0.28***	0.58±0.17**	0.61	2.45±0.66**	5.02
Fold change	0.62	0.61	0.64	0.61	0.53	0.49	1.15	0.54	1.19
pK-YBI 42 (E)	8.69±0.77*	2.70±0.70*	11.83±2.18**	0.73±0.14**	1.22±0.28***	0.63±0.15***	0.60	2.58±0.80**	4.58
Fold change	0.60	0.58	0.62	0.63	0.55	0.53	1.13	0.57	1.08
pK-YBI 44 (NE)	15.05±2.40	5.20±1.17	20.59±4.68	1.34±0.49	2.51±0.39	1.33±0.22	0.53	5.18±0.55	3.97
Fold change	1.03	1.11	1.07	1.16	1.14	1.12	1.00	1.14	0.94
pK-YBI 58 (NE)	13.77±3.55	4.44±0.67	18.43±5.03	1.13±0.23	2.41±0.34	1.30±0.10	0.47	4.84±0.98	3.81
Fold change	0.95	0.95	0.95	0.97	1.09	1.09	0.89	1.06	0.90
pP-YBI 17 (E)	14.32±2.37	5.05±1.09	19.44±3.68	1.08±0.12	2.11±0.33	1.07±0.16	0.51	4.26±0.39	4.56
Fold change	0.98	1.08	1.07	0.93	0.96	0.90	0.96	0.94	1.08
pP-YBI 30 (E)	15.11±2.85	4.99±0.38	20.34±4.06	1.22±0.44	2.68±0.51	1.28±0.40	0.46	5.18±1.48	3.93
Fold change	1.04	1.07	1.06	1.05	1.22	1.08	0.87	1.14	0.93
pP-YBI 16 (NE)	15.76±4.09	5.06±1.75	21.01±6.86	1.42±0.45	2.06±0.27	1.53±0.19	0.69	5.01±1.50	4.19
Fold change	1.09	1.08	1.09	1.22	0.94	1.29	1.30	1.10	1.00
pP-YBI 27 (NE)	14.77±2.38	4.54±1.06	18.71±3.69	1.09±0.18	2.00±0.16	1.27±0.22	0.55	4.36±0.29	4.29
Fold change	1.02	0.97	0.97	0.94	0.91	1.07	1.04	0.96	1.02

Supplemental Table II – Real Time RT-PCR analysis of endogenous carotenoid gene expression in leaves

Gene expression was measured via Real Time RT-PCR analysis on a minimum of 4 different leaves from 2 different plants (8 leaves in total). Data are normalized for the expression of the tubulin gene. Fold change with respect to the wild-type is reported for each gene and for each line. Asterisks indicate significance of the fold change in an ANOVA test (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$). For details, see Methods.

	<i>CrtB</i>	<i>CrtI</i>	<i>CrtY</i>	<i>PSY1</i>	<i>PSY2</i>	<i>PDS</i>	<i>ZDS</i>	<i>CrtISO</i>	<i>LCY-b</i>	<i>CHY1</i>	<i>CHY2</i>	<i>LUT5</i>	<i>ZEP</i>	<i>NXS</i>	<i>LCY-e</i>	<i>LUT1</i>
Wild-type	nd	nd	nd	30.88±8.34	7.38±0.66	9.08±0.36	0.79±0.22	7.00±1.26	5.09±0.20	6.75±1.48	10.06±0.50	25.78±7.22	148.81±31.25	0.18±0.01	211.70±27.52	2.30±0.55
pK-I 25 (E)	-	0.5923±0.0449	-	15.13±3.09**	5.02±0.66*	4.99±1.23**	1.21±0.13*	5.18±0.56	3.10±0.20*	4.39±0.67*	7.24±1.31*	19.08±0.52	177.09±35.71	0.11±0.02*	256.16±31.76	3.61±0.35*
Fold change	-	-	-	0.49	0.68	0.55	1.52	0.74	0.61	0.65	0.72	0.74	1.19	0.62	1.21	1.57
pK-I 29 (E)	-	0.7155±0.0366	-	20.69±2.47*	3.69±0.89**	6.35±1.18*	1.84±0.29*	5.60±0.98	2.39±0.66**	5.33±1.35	6.24±1.21*	22.69±3.87	238.10±41.67	0.11±0.03*	321.79±76.21	3.63±0.64
Fold change	-	-	-	0.67	0.50	0.70	2.32	0.80	0.47	0.79	0.62	0.88	1.60	0.61	1.52	1.58
pK-I 5 (NE)	-	nd	-	33.96±7.41	8.57±1.33	12.17±0.82	0.81±0.08	7.07±2.09	4.48±1.07	8.98±0.40	6.84±1.31	34.55±2.32	145.84±14.88	0.15±0.01	186.30±25.40	1.70±0.23
Fold change	-	-	-	1.10	1.16	1.34	1.02	1.01	0.88	1.33	0.68	1.34	0.98	0.85	0.88	0.74
pK-I 8 (NE)	-	nd	-	32.16±6.77	7.09±1.09	9.23±2.07	0.66±0.14	7.03±1.76	4.79±1.27	6.17±1.69	11.00±2.75	25.15±4.45	152.54±22.22	0.15±0.03	222.78±30.84	2.08±0.66
Fold change	-	-	-	1.04	0.96	1.02	0.84	1.00	0.94	0.91	1.09	0.98	1.03	0.83	1.05	0.90
pP-I 7 (E)	-	nd	-	26.86±6.18	7.90±0.66	12.89±2.00	0.52±0.02	9.52±1.12	7.02±0.20*	8.64±0.40	12.68±1.91	26.56±1.03	186.02±4.46	0.12±0.01*	201.12±10.59	2.65±0.02
Fold change	-	-	-	0.87	1.07	1.42	0.66	1.36	1.38	1.28	1.26	1.03	1.25	0.68	0.95	1.15
pP-I 10 (E)	-	nd	-	37.36±3.09	9.89±0.07*	14.07±1.36*	0.84±0.02	4.13±1.26*	5.49±0.25	5.94±0.34	11.67±2.01	23.46±1.29	200.90±5.95	0.24±0.01*	167.25±29.64	2.78±0.23
Fold change	-	-	-	1.21	1.34	1.55	1.06	0.59	1.08	0.88	1.16	0.91	1.35	1.35	0.79	1.21
pP-I 17 (NE)	-	nd	-	41.07±5.87	9.38±0.66	6.35±0.82	0.85±0.01	10.22±2.10	3.76±0.20	6.48±0.13	8.15±1.21	29.65±0.77	196.43±34.23	0.25±0.01*	156.66±21.17	1.50±0.07*
Fold change	-	-	-	1.33	1.27	0.70	1.07	1.46	0.74	0.96	0.81	1.15	1.32	1.40	0.74	0.65
pP-I 20 (NE)	-	nd	-	27.32±3.65	6.99±2.06	9.55±2.34	0.88±0.13	7.11±2.06	5.53±1.88	6.22±1.76	9.81±2.55	26.06±4.87	140.23±28.87	0.21±0.08	208.45±30.45	2.50±0.31
Fold change	-	-	-	0.88	0.95	1.05	1.11	1.02	1.09	0.92	0.97	1.01	0.94	1.67	0.98	1.09
pK-BI 4 (E)	0.0006±0.0002	0.1029±0.0117	-	9.88±2.40***	2.29±0.22***	8.81±0.73	0.48±0.02*	4.62±1.27*	3.61±0.10*	25.85±2.70***	6.84±0.10*	41.77±8.77	159.23±14.88	0.10±0.01**	84.68±19.05**	2.37±0.32
Fold change	-	-	-	0.32	0.31	0.97	0.61	0.66	0.71	3.83	0.68	1.62	1.07	0.55	0.40	1.03
pK-BI 12 (E)	0.0005±0.0001	0.0928±0.0285	-	8.03±1.85***	1.62±0.52***	8.08±1.36	0.49±0.06*	4.34±0.91*	2.70±0.25**	10.06±1.96*	4.02±0.10**	50.02±6.45**	98.22±11.90*	0.03±0.01***	59.28±10.41**	2.62±0.78
Fold change	-	-	-	0.26	0.22	0.89	0.62	0.62	0.53	1.49	0.40	1.94	0.66	0.19	0.28	1.14
pK-BI 8 (NE)	nd	nd	-	44.15±12.97	7.53±1.99	9.35±2.27	0.62±0.04	11.55±1.89	4.02±0.51	6.07±0.20	11.07±2.92	25.78±4.13	132.44±7.44	0.21±0.02	213.82±25.40	2.78±0.58
Fold change	-	-	-	1.43	1.02	1.03	0.78	1.65	0.79	0.90	1.10	1.00	0.89	1.20	1.01	1.21
pK-BI 31 (NE)	nd	nd	-	29.76±2.77	7.65±2.07	9.09±2.44	0.71±0.16	6.59±1.77	5.01±1.87	7.11±1.66	9.77±2.88	27.01±5.78	145.22±38.76	0.15±0.05	201.87±36.23	2.70±0.43
Fold change	-	-	-	0.96	1.04	1.00	0.90	0.94	0.98	1.05	0.97	1.05	0.98	0.83	0.95	1.17
pP-BI 12 (E)	nd	nd	-	37.67±8.95	9.75±1.26	22.24±4.27*	1.05±0.03	9.87±2.10	5.95±0.46	7.63±0.13	9.76±0.80	31.97±5.67	175.60±5.95	0.21±0.01	179.95±8.47	1.24±0.02**
Fold change	-	-	-	1.22	1.32	2.45	1.32	1.41	1.17	1.13	0.97	1.24	1.18	1.20	0.85	0.54
pP-BI 15 (E)	nd	nd	-	23.16±6.18	9.16±2.22	9.80±2.27	0.99±0.12	9.10±2.03	5.65±0.31	8.84±1.08	8.75±0.40	29.65±4.13	313.99±99.70*	0.25±0.04	381.07±48.69*	1.40±0.02*
Fold change	-	-	-	0.75	1.24	1.08	1.24	1.30	1.11	1.31	0.87	1.15	2.11	1.42	1.80	0.61
pP-BI 1 (NE)	nd	nd	-	33.65±4.77	8.01±2.22	9.27±2.74	0.83±0.23	6.73±1.81	4.82±1.25	6.88±2.04	9.49±2.37	24.99±6.82	157.87±39.54	0.21±0.05	222.97±47.33	1.87±0.37
Fold change	-	-	-	1.09	1.08	1.02	1.05	0.96	0.95	1.02	0.94	0.97	1.06	1.20	1.05	0.81
pP-BI 13 (NE)	nd	nd	-	38.29±7.41	7.61±2.44	8.72±2.63	0.60±0.02	5.46±1.26	7.94±1.63	5.74±0.20	9.16±2.62	28.88±6.19	96.73±2.98*	0.18±0.01	256.16±52.93	1.91±0.18
Fold change	-	-	-	1.24	1.03	0.96	0.75	0.78	1.56	0.85	0.91	1.12	0.65	1.01	1.21	0.83
pK-YBI 41 (E)	0.0006±0.0002	0.2466±0.0281	0.0841±0.0102	15.13±1.24**	4.58±0.30*	5.54±0.54**	0.68±0.12	3.43±0.70**	2.90±0.31***	2.02±0.57**	2.11±0.11***	8.77±1.03***	41.67±10.42***	0.05±0.01***	179.95±12.70	0.51±0.15***
Fold change	-	-	-	0.49	0.62	0.61	0.86	0.49	0.57	0.30	0.21	0.34	0.28	0.30	0.85	0.22
pK-YBI 42 (E)	0.0006±0.0002	0.2755±0.0269	0.0219±0.0019	17.29±0.93**	5.09±0.52*	5.99±0.91**	0.71±0.16	4.34±0.21**	3.51±0.15**	4.18±0.20*	3.82±0.60***	15.47±0.26*	72.92±17.86**	0.10±0.02**	152.43±27.52	1.31±0.18***
Fold change	-	-	-	0.56	0.69	0.66	0.89	0.62	0.69	0.62	0.38	0.60	0.49	0.58	0.72	0.57
pK-YBI 44(NE)	nd	0.0002±0.0001	nd	40.76±5.25	7.31±0.89	10.53±2.81	0.95±0.21	7.91±1.82	6.15±1.58	6.61±0.34	15.29±4.23	27.07±5.41	148.81±23.81	0.19±0.05	245.58±57.16	2.28±0.51
Fold change	-	-	-	1.32	0.99	1.16	1.19	1.13	1.21	0.98	1.52	1.05	1.00	1.09	1.16	0.99
pK-YBI 58(NE)	nd	nd	nd	31.87±4.77	7.01±1.65	8.99±2.03	0.72±0.05	6.88±1.44	5.25±1.64	6.57±1.77	10.42±3.32	25.44±4.56	140.58±16.65	0.16±0.03	200.36±28.57	1.92±0.45
Fold change	-	-	-	1.03	0.95	0.99	0.91	0.98	1.03	0.97	1.04	0.99	0.95	0.89	0.95	0.84
pP-YBI 17 (E)	0.0003±0.0001	0.0011±0.0002	0.0011±0.0002	22.85±3.40	6.28±1.48	7.63±2.45	0.68±0.09	6.16±1.26	7.48±1.98	7.76±0.13	11.17±2.11	22.17±7.99	141.37±40.18	0.23±0.07	190.53±23.29	2.51±0.44
Fold change	-	-	-	0.74	0.85	0.84	0.86	0.88	1.47	1.15	1.11	0.86	0.95	1.28	0.90	1.09
pP-YBI 30 (E)	0.0004±0.0001	0.0012±0.0003	0.0012±0.0004	21.92±2.78	6.94±2.29	7.35±1.09	0.78±0.05	6.44±0.63	6.15±0.71	6.01±1.21	10.66±0.70	23.46±1.55	174.11±20.83	0.27±0.07	184.18±55.04	2.14±0.61
Fold change	-	-	-	0.71	0.94	0.81	0.98	0.92	1.21	0.89	1.06	0.91	1.17	1.55	0.87	0.93
pP-YBI 16 (NE)	nd	nd	nd	30.76±5.73	7.17±2.15	8.88±2.47	0.82±0.20	6.91±1.58	4.91±1.33	6.60±2.56	9.69±2.88	25.29±4.44	139.87±22.76	0.17±0.03	229.06±30.32	2.11±0.21
Fold change	-	-	-	1.00	0.98	0.98	1.04	0.99	0.96	0.98	0.96	0.98	0.94	0.94	1.08	0.92
pP-YBI 27 (NE)	nd	0.0002±0.0001	0.0002±0.0001	31.80±6.48	7.90±1.18	4.09±1.14*	0.77±0.15	9.24±1.19	6.00±0.25	7.36±1.42	9.76±1.71	28.88±4.38	98.22±20.18	0.20±0.05	205.35±46.57	2.48±0.39
Fold change	-	-	-	1.03	1.07	0.45	0.97	1.32	1.18	1.09	0.97	1.12	0.66	1.16	0.97	1.08

Supplemental Table III – HPLC analysis of tuber carotenoid content (µg/g dry weight).

Carotenoid composition was measured via diode array HPLC on a minimum of 4 different tubers from 3 different plants (12 tubers in total). Fold variation with respect to the wild-type is reported for each carotenoid specie and for each line. Asterisks indicate significance of the fold variation in an ANOVA test (*:p ≤ 0.05; **:p ≤ 0.01; ***: p ≤ 0.001). For details, see Methods.

	Phytoene	α-carot.	β-carot.	Lutein	Zea	Anthera	Viola	Neo	Other Xanth.	β- / α-	Esters	Total
Wild-type	0.00	0.00	0.00	0.85±0.22	1.40±0.36	0.61±0.15	0.73±0.17	0.61±0.04	0.55±0.16	3.94	1.25±0.47	6.01±1.58
pK-I 25 (E)	0.00	0.00	0.00	2.28±0.40**	2.40±0.54*	1.79±0.48*	3.81±0.78***	3.89±0.56***	2.38±0.88	5.21	2.82±1.02	19.38±4.66**
Fold change	-	-	-	2.69	1.72	2.94	5.21	6.38	4.25	-	2.25	3.22
pK-I 29 (E)	0.00	0.00	0.00	2.74±0.79*	2.33±0.44	1.11±0.28*	5.02±0.84***	3.49±0.61***	3.98±0.94***	4.36	1.71±0.16	20.48±4.17***
Fold change	-	-	-	3.23	1.66	1.82	6.88	5.72	7.10	-	1.37	3.41
pK-I 5 (NE)	0.00	0.00	0.00	1.28±0.05	2.16±0.10	0.47±0.14*	0.34±0.08	0.63±0.08	0.53±0.09	3.46	1.60±0.40	7.01±0.95
Fold change	-	-	-	1.51	1.55	0.77	0.46	1.03	0.95	-	1.28	1.17
pK-I 8 (NE)	0.00	0.00	0.00	0.75±0.14	1.20±0.24	0.70±0.17	0.60±0.11	0.67±0.19	0.71±0.21	4.23	1.40±0.37	6.03±1.98
Fold change	-	-	-	0.88	0.86	1.15	0.82	1.10	1.30	-	1.12	1.00
pP-I 7 (E)	0.00	0.00	0.00	3.62±0.34***	2.70±1.02	0.60±0.15	5.41±0.03***	3.27±0.14***	1.07±0.16	3.31	2.89±0.50**	19.57±2.35***
Fold change	-	-	-	4.26	1.92	0.98	7.41	5.36	1.92	-	2.32	3.26
pP-I 10 (E)	0.00	0.00	0.00	2.24±0.46**	0.57±0.12**	1.36±0.35*	7.42±1.16***	3.77±0.79**	2.92±0.47**	5.86	2.60±0.66*	20.87±4.01***
Fold change	-	-	-	2.64	0.41	2.23	10.16	6.18	5.21	-	2.08	3.47
pP-I 17 (NE)	0.00	0.00	0.00	0.70±0.08	0.59±0.07***	0.31±0.09**	1.39±0.35	0.62±0.39	0.63±0.07	4.16	1.23±0.20	5.46±1.25
Fold change	-	-	-	0.82	0.42	0.51	1.90	1.01	1.12	-	0.98	0.91
pP-I 20 (NE)	0.00	0.00	0.00	0.89±0.28	1.56±0.20	0.55±0.08	0.76±0.21	0.59±0.15	0.48±0.08	3.89	1.23±0.26	6.06±1.77
Fold change	-	-	-	1.05	1.11	0.90	1.04	0.97	0.87	-	0.98	1.01
pK-BI 4 (E)	0.00	0.00	0.00	1.74±0.78	2.59±0.81	0.76±0.17	1.46±0.45*	2.69±0.82*	1.33±0.52	4.31	2.69±0.86*	13.27±4.40*
Fold change	-	-	-	2.05	1.85	1.25	1.99	4.41	2.37	-	2.16	2.21
pK-BI 12 (E)	0.00	0.00	0.00	2.05±0.50*	1.77±0.48	1.23±0.52	1.45±0.28*	2.29±0.66**	1.59±0.14***	3.29	2.84±0.22***	13.22±2.81***
Fold change	-	-	-	2.41	1.26	2.01	1.99	3.76	2.84	-	2.27	2.20
pK-BI 8 (NE)	0.00	0.00	0.00	0.95±0.23	0.36±0.10*	0.18±0.04*	1.34±0.19	1.15±0.08	0.64±0.13	3.19	0.96±0.55	5.58±1.31
Fold change	-	-	-	1.12	0.26	0.29	1.83	1.88	1.14	-	0.77	0.93
pK-BI 31 (NE)	0.00	0.00	0.00	0.94±0.22	1.39±0.34	0.63±0.15	0.69±0.03	0.58±0.11	0.56±0.03	3.50	1.49±0.26	6.28±1.48
Fold change	-	-	-	1.11	1.00	1.03	0.95	0.95	1.02	-	1.19	1.04
pP-BI 12 (E)	0.00	0.00	0.00	2.86±0.30***	4.66±0.63***	0.60±0.16	0.77±0.19	1.64±0.25**	1.70±0.24***	2.68	3.24±0.49**	15.47±2.25***
Fold change	-	-	-	3.37	3.33	0.98	1.05	2.69	3.04	-	2.59	2.57
pP-BI 15 (E)	0.00	0.00	0.00	2.06±0.58*	4.32±0.59**	0.75±0.23	1.14±0.11	2.23±0.49**	1.35±0.17***	4.10	4.07±1.77*	16.02±4.12***
Fold change	-	-	-	2.43	3.08	1.24	1.56	3.65	2.41	-	3.26	2.66
pP-BI 1 (NE)	0.00	0.00	0.00	0.71±0.16	1.40±0.31	0.61±0.13	0.81±0.27	0.72±0.10	0.73±0.09	4.99	1.28±0.30	6.26±1.66
Fold change	-	-	-	0.83	1.00	1.00	1.11	1.18	1.33	-	1.02	1.04
pP-BI 13 (NE)	0.00	0.00	0.00	0.90±0.20	1.31±0.32	0.53±0.14	0.44±0.10	0.74±0.14	0.73±0.12	3.36	1.15±0.22	5.81±1.23
Fold change	-	-	-	1.06	0.94	0.87	0.60	1.22	1.30	-	0.92	0.97
pK-YBI 41 (E)	0.00	0.00	2.08±0.39	2.04±0.44***	5.46±1.71*	3.06±0.94*	0.81±0.22	1.19±0.28	0.92±0.24	6.18	3.56±1.10*	19.12±4.66**
Fold change	-	-	-	2.40	3.90	5.01	1.11	1.95	1.84	-	2.85	3.18
pK-YBI 42 (E)	0.00	0.00	2.37±0.71	1.87±0.53*	4.79±1.28**	2.85±0.79*	0.64±0.27	2.70±0.52***	0.67±0.22	7.14	4.62±1.39*	20.51±3.38***
Fold change	-	-	-	2.20	3.42	4.67	0.88	4.43	1.34	-	3.70	3.41
pK-YBI 44 (NE)	0.00	0.00	0.00	1.05±0.41	1.09±0.38	0.55±0.22	0.67±0.28	0.53±0.18	0.48±0.11	2.70	2.01±0.46*	6.38±2.38
Fold change	-	-	-	1.23	0.78	0.90	0.92	0.87	0.96	-	1.61	1.06
pK-YBI 58 (NE)	0.00	0.00	0.00	0.83±0.18	1.51±0.34	0.69±0.16	0.69±0.20	0.65±0.10	0.68±0.19	4.26	1.07±0.21	6.12±1.50
Fold change	-	-	-	0.98	1.08	1.13	0.95	1.07	1.24	-	0.86	1.02
pP-YBI 17 (E)	15.77±5.11	6.85±1.39	41.21±4.66	26.16±4.29***	6.28±2.59*	3.73±1.28*	9.17±2.59**	4.31±1.03**	0.77±0.18	9.32	2.55±0.79	116.8±37.72**
Fold change	-	-	-	30.78	4.49	6.11	12.56	7.06	1.54	-	2.04	19.43
pP-YBI 30 (E)	18.45±4.07	7.39±1.76	31.27±6.39	23.77±5.39***	6.77±1.69**	3.27±1.07**	18.63±4.86***	3.29±0.72**	0.86±0.23*	10.05	2.89±0.66*	116.59±27.69***
Fold change	-	-	-	27.97	4.83	5.36	25.52	5.39	1.72	-	2.31	19.40
pP-YBI 16 (NE)	0.00	0.00	0.00	0.88±0.11	1.39±0.20	0.57±0.16	0.77±0.20	0.69±0.10	0.59±0.04	3.89	1.17±0.14	6.06±1.66
Fold change	-	-	-	1.04	1.00	0.93	1.05	1.13	1.07	-	0.94	1.01
pP-YBI 27 (NE)	0.00	0.00	0.00	0.95±0.38	1.21±0.40	0.71±0.24	0.69±0.09	0.79±0.31	0.59±0.13	3.58	0.99±0.37	5.93±1.85
Fold change	-	-	-	1.12	0.86	1.16	0.94	1.29	1.18	-	0.79	0.99

Supplemental Table IV – Real Time RT-PCR analysis of endogenous carotenoid gene expression in tubers

Gene expression was measured via Real Time RT-PCR analysis on a minimum of 4 different tubers from 3 different plants (12 tubers in total). Data are normalized for the expression of the tubulin gene. Fold change with respect to the wild-type is reported for each gene and for each line. Asterisks indicate significance of the fold change in an ANOVA test (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$). For details, see Methods.

	<i>CrtB</i>	<i>CrtI</i>	<i>CrtY</i>	<i>PSY1</i>	<i>PSY2</i>	<i>PDS</i>	<i>ZDS</i>	<i>CrtISO</i>	<i>LCY-b</i>	<i>CHY1</i>	<i>CHY2</i>	<i>LUT5</i>	<i>ZEP</i>	<i>NXS</i>	<i>LCY-e</i>	<i>LUT1</i>
Wild-type	nd	nd	nd	13.09± 1.31	3.12± 0.75	0.82± 0.17	0.051± 0.004	1.49± 0.33	1.22± 0.26	3.21± 0.71	15.87± 5.08	6.14± 1.60	4.02± 0.76	3.25± 1.01	0.72± 0.20	1.03± 0.24
pK-I 25 (E)	-	2.2149± 0.1733	-	1.31± 0.13***	2.90± 0.50	1.28± 0.25	0.053± 0.007	1.55± 0.30	1.39± 0.55	3.02± 0.83	15.08± 1.11	6.14± 1.47	3.46± 0.93	2.18± 0.58*	0.59± 0.14	1.11± 0.18
Fold change	-	-	-	0.10	0.93	1.57	1.03	1.04	1.14	0.94	0.95	1.00	0.86	0.67	0.81	1.07
pK-I 29 (E)	-	2.8019± 0.32029	-	3.01± 0.26***	3.24± 0.31	1.51± 0.02**	0.048± 0.006	1.53± 0.55	1.00± 0.06	4.36± 0.03*	16.66± 2.70	8.60± 1.11	3.74± 1.41	1.33± 0.25***	0.74± 0.19	1.38± 0.03*
Fold change	-	-	-	0.23	1.04	1.84	0.95	1.03	0.82	1.36	1.05	1.40	0.93	0.41	1.02	1.33
pK-I 5 (NE)	-	0.0012± 0.0001	-	14.26± 2.75	4.08± 1.18	0.92± 0.07	0.053± 0.012	1.62± 0.55	0.99± 0.15	3.75± 0.93	11.90± 3.33	6.57± 1.54	2.90± 0.28	4.45± 1.23	0.80± 0.16	1.30± 0.22
Fold change	-	-	-	1.09	1.31	1.13	1.03	1.09	0.81	1.17	0.75	1.07	0.72	1.37	1.10	1.26
pK-I 8 (NE)	-	nd	-	14.05± 3.88	3.33± 1.20	0.77± 0.10	0.050± 0.021	1.77± 0.44	1.02± 0.30	3.33± 1.07	15.34± 4.50	6.11± 1.66	4.22± 1.66	3.07± 1.12	0.87± 0.27	1.01± 0.18
Fold change	-	-	-	1.07	1.07	0.94	0.98	1.19	0.84	1.04	0.97	0.99	1.05	0.95	1.21	0.98
pP-I 7 (E)	-	10.0064± 0.0265	-	3.80± 0.13***	2.40± 0.28	1.51± 0.16**	0.100± 0.022*	2.38± 0.30**	0.76± 0.17*	2.63± 0.03	23.64± 3.17	9.52± 2.03	5.68± 0.52	0.91± 0.13***	0.51± 0.09	1.26± 0.14
Fold change	-	-	-	0.29	0.77	1.85	1.97	1.60	0.62	0.82	1.49	1.55	1.41	0.28	0.71	1.22
pP-I 10 (E)	-	14.9054± 3.0849	-	2.36± 0.65***	2.12± 0.06*	1.75± 0.03***	0.056± 0.008	4.26± 0.70**	0.86± 0.15*	4.84± 0.93*	15.39± 1.11	10.08± 3.07	3.46± 0.08	2.11± 0.52	0.74± 0.18	1.59± 0.39
Fold change	-	-	-	0.18	0.68	2.14	1.10	2.86	0.70	1.51	0.97	1.64	0.86	0.65	1.02	1.54
pP-I 17 (NE)	-	0.0021± 0.0005	-	11.12± 2.49	2.68± 0.84	0.74± 0.19	0.053± 0.014	1.28± 0.36	1.50± 0.10	2.85± 0.73	19.20± 0.32	4.98± 1.38	4.43± 0.72	1.66± 0.55*	0.78± 0.01	0.92± 0.32
Fold change	-	-	-	0.85	0.86	0.90	1.10	0.86	1.23	0.89	1.21	0.81	1.10	0.51	1.08	0.89
pP-I 20 (NE)	-	nd	-	13.45± 4.55	2.78± 0.05	0.99± 0.11	0.043± 0.012	1.26± 0.31	1.11± 0.28	2.99± 0.44	16.04± 5.00	6.02± 1.99	4.06± 0.33	2.88± 0.33	0.72± 0.15	1.22± 0.11
Fold change	-	-	-	1.03	0.89	1.21	0.84	0.85	0.91	0.93	1.01	0.98	1.01	0.89	1.00	1.18
pK-BI 4 (E)	0.0021± 0.0002	0.3619± 0.0222	-	3.01± 0.65***	3.24± 0.16	1.82± 0.36**	0.110± 0.015***	2.81± 0.43	2.19± 0.16**	6.25± 0.64*	11.74± 1.59	35.14± 6.08***	4.75± 1.21	6.79± 1.37*	0.44± 0.03**	5.19± 1.07***
Fold change	-	-	-	0.23	1.04	2.23	2.16	1.89	1.79	1.95	0.74	5.72	1.18	2.09	0.61	5.02
pK-BI 12 (E)	0.0018± 0.0003	0.4519± 0.0217	-	8.51± 1.44*	3.86± 0.41	2.70± 0.73***	0.093± 0.002***	4.88± 0.65**	1.70± 0.17	5.65± 0.67*	12.06± 0.16	73.05± 10.63***	5.27± 1.05	14.56± 3.70**	0.59± 0.12	9.48± 1.04***
Fold change	-	-	-	0.65	1.24	3.30	1.83	3.28	1.39	1.76	0.76	11.89	1.31	4.48	0.81	9.17
pK-BI 8 (NE)	nd	nd	-	13.61± 2.36	3.86± 0.81	1.06± 0.16	0.054± 0.008	1.65± 0.33	0.87± 0.11	3.40± 0.74	15.23± 3.65	7.19± 0.31	2.70± 0.64*	3.77± 0.49	0.59± 0.17	0.86± 0.23
Fold change	-	-	-	1.04	1.24	1.30	1.05	1.11	0.71	1.06	0.96	1.17	0.67	1.16	0.82	0.83
pK-BI 31 (NE)	nd	nd	-	12.77± 2.80	3.74± 0.55	1.04± 0.05	0.047± 0.013	1.45± 0.44	1.34± 0.14	3.09± 0.55	14.66± 3.04	6.44± 1.06	3.86± 1.22	3.54± 0.33	0.77± 0.14	1.09± 0.28
Fold change	-	-	-	0.98	1.20	1.20	0.92	0.97	1.10	0.96	0.92	1.05	0.96	1.08	1.07	1.06
pP-BI 12 (E)	0.2610± 0.0441	0.8461± 0.2865	-	4.32± 0.92***	4.24± 1.12	2.32± 0.57*	0.027± 0.003**	7.56± 0.71***	2.03± 0.35*	6.42± 0.42***	49.51± 6.19**	109.18± 7.99***	5.15± 0.85	19.14± 4.13***	0.40± 0.11**	8.67± 1.90***
Fold change	-	-	-	0.33	1.36	2.84	0.53	5.08	1.66	2.00	3.12	17.77	1.28	5.89	0.55	8.38
pP-BI 15 (E)	0.3484± 0.0482	0.9397± 0.2409	-	7.07± 2.05**	7.57± 0.59**	2.61± 0.67**	0.037± 0.002*	11.96± 1.62***	3.17± 0.06***	7.92± 1.51*	36.02± 3.17**	93.70± 20.95***	5.96± 0.12**	8.19± 2.47**	0.24± 0.05***	17.16± 2.77***
Fold change	-	-	-	0.54	2.43	3.19	0.72	8.04	2.59	2.47	2.27	15.25	1.48	2.52	0.33	16.59
pP-BI 1 (NE)	nd	nd	-	13.41± 3.41	2.99± 0.44	0.78± 0.22	0.061± 0.021	1.62± 0.55	1.28± 0.22	3.55± 0.22	15.22± 3.77	6.31± 1.99	4.29± 1.33	3.87± 1.01	0.66± 0.10	1.27± 0.27
Fold change	-	-	-	1.02	0.96	0.95	1.17	1.09	1.05	1.11	0.96	1.03	1.07	1.19	0.92	1.23
pP-BI 13 (NE)	nd	nd	-	11.78± 3.08	2.31± 0.09	0.94± 0.30	0.044± 0.005	1.19± 0.35	0.97± 0.22	3.14± 0.03	11.43± 3.33	4.73± 0.55	6.40± 1.09	4.39± 0.23*	0.69± 0.09	0.98± 0.30
Fold change	-	-	-	0.90	0.74	1.15	0.87	0.80	0.79	0.98	0.72	0.77	1.59	1.35	0.96	0.95
pK-YBI 41 (E)	0.0038± 0.0008	0.2211± 0.0365	0.0338± 0.0106	27.09± 3.27**	21.44± 4.52***	2.83± 0.86**	0.077± 0.011*	5.15± 1.71**	2.97± 0.53*	7.92± 1.86*	11.11± 3.49	13.09± 2.76**	8.09± 2.33	19.56± 6.11**	4.47± 1.30**	4.47± 0.56***
Fold change	-	-	-	2.07	6.88	3.46	1.50	3.46	2.43	2.47	0.70	2.13	2.01	6.02	6.18	4.32
pK-YBI 42 (E)	0.0056± 0.0013	0.1398± 0.0275	0.0268± 0.0033	20.41± 2.22*	6.29± 1.34**	1.44± 0.39*	0.081± 0.018*	2.86± 0.61**	2.43± 0.35**	6.48± 1.22*	10.79± 1.11	9.28± 0.43*	6.00± 1.37	8.71± 1.56***	2.34± 0.40***	1.93± 0.40*
Fold change	-	-	-	1.56	2.02	1.76	1.59	1.92	1.99	2.02	0.68	1.51	1.49	2.68	3.24	1.87
pK-YBI 44 (NE)	nd	0.0027± 0.0007	0.0007± 0.0001	17.14± 3.53	3.27± 0.69	1.06± 0.28	0.043± 0.007	0.88± 0.25*	1.58± 0.38	2.18± 0.55	13.33± 4.28	4.79± 1.41*	3.34± 1.05	4.13± 0.94	0.77± 0.20	0.80± 0.22
Fold change	-	-	-	1.31	1.05	1.29	0.84	0.59	1.29	0.68	0.84	0.78	0.83	1.27	1.07	0.77
pK-YBI 58 (NE)	nd	nd	nd	12.88± 3.05	3.01± 0.66	0.86± 0.20	0.050± 0.016	1.55± 0.30	1.09± 0.22	2.89± 0.43	15.09± 4.50	6.09± 1.91	4.11± 1.09	3.11± 0.80	0.81± 0.22	0.87± 0.10
Fold change	-	-	-	0.98	0.96	1.05	0.98	1.04	0.89	0.90	0.95	0.99	1.02	0.96	1.13	0.85
pP-YBI 17 (E)	1.5310± 0.1201	10.8411± 1.9231	3.8371± 0.3561	2.75± 0.31***	4.33± 0.53	1.06± 0.15	0.121± 0.020***	5.33± 1.42*	9.14± 2.31***	16.52± 4.62**	30.31± 4.92*	8.29± 1.72	11.87± 2.62**	0.91± 0.26***	3.65± 1.00***	0.09± 0.05***
Fold change	-	-	-	0.21	1.39	1.30	2.37	3.58	7.47	5.15	1.91	1.35	2.95	0.28	5.05	0.09
pP-YBI 30 (E)	1.3791± 0.4709	6.5733± 1.0643	3.8963± 0.2863	4.58± 1.20***	3.93± 0.84	0.68± 0.18	0.182± 0.057**	3.60± 0.42***	4.17± 1.23***	11.84± 3.35**	26.50± 6.19	10.14± 2.76	11.15± 2.05**	1.04± 0.22***	3.19± 0.77***	0.33± 0.11***
Fold change	-	-	-	0.35	1.26	0.83	3.56	2.42	3.41	3.69	1.67	1.65	2.77	0.32	4.41	0.32
pP-YBI 16 (NE)	nd	nd	nd	13.62± 3.66	3.21± 1.22	0.79± 0.15	0.049± 0.017	1.44± 0.28	1.18± 0.32	3.22± 1.05	15.77± 4.50	6.33± 1.86	4.05± 1.05	3.04± 0.60	0.80± 0.20	0.98± 0.11
Fold change	-	-	-	1.04	1.03	0.96	0.98	0.97	0.97	1.00	1.00	1.03	1.00	0.94	1.11	0.95
pP-YBI 27 (NE)	nd	0.0025± 0.0004	0.0025± 0.0008	12.69± 3.20	4.14± 0.50	0.97± 0.23	0.055± 0.014	1.99± 0.28	1.27± 0.36	2.79± 0.90	13.01± 4.13	10.38± 3.19	4.75± 1.37	4.91± 1.27	0.45± 0.14	1.23± 0.28
Fold change	-	-	-	0.97	1.33	1.18	1.07	1.34	1.04	0.87	0.82	1.69	1.18	1.51	0.62	1.19

Supplemental Table V – Node strengths of the network shown in Figure 7

Node strengths (ns) are calculated as the mean of the absolute values of Pearson correlation coefficients radiating from a single node. SD=standard deviation.

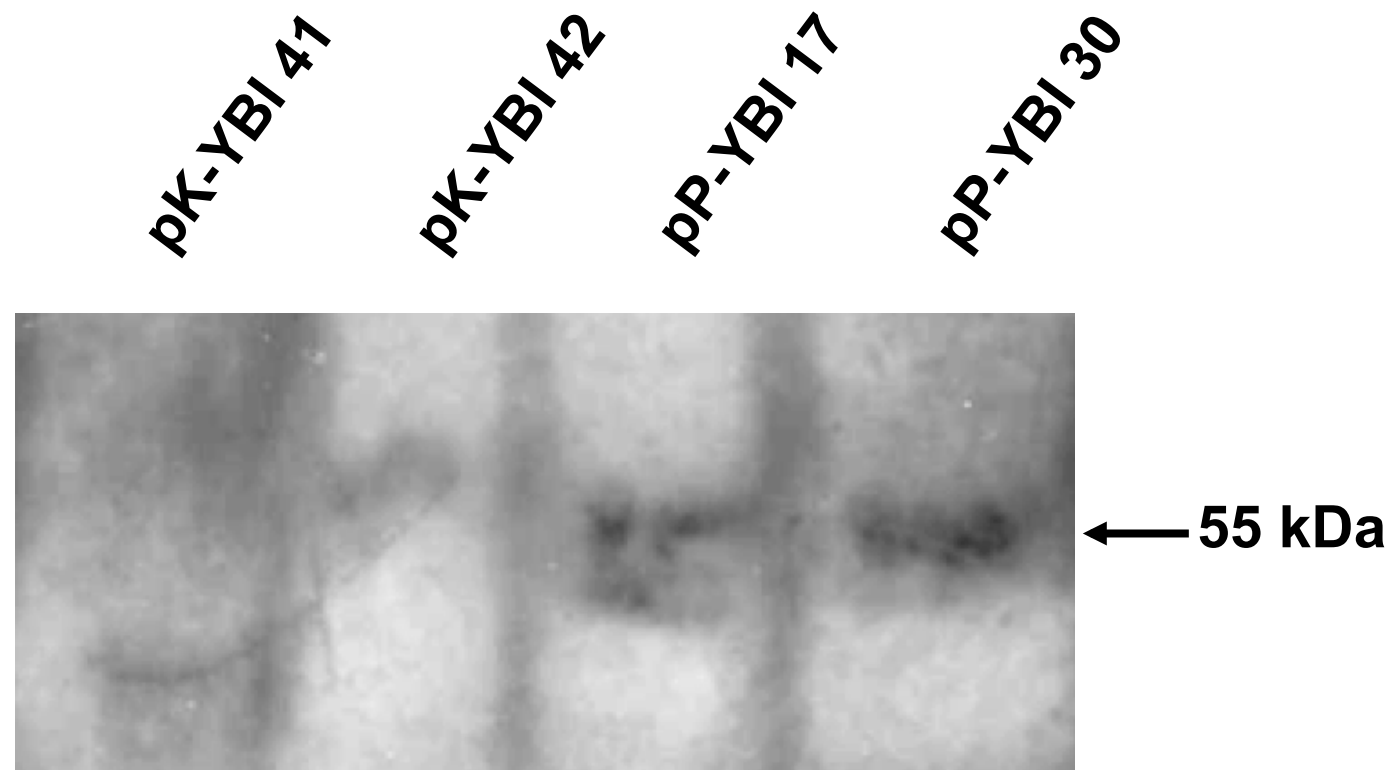
	Leaves						Tubers					
	pK-I		pK-BI		pK-YBI		pP-I		pP-BI		pP-YBI	
Node	ns	SD	ns	SD	ns	SD	ns	SD	ns	SD	ns	SD
<i>PSY1</i>	0.852	0.124	0.762	0.254	0.945	0.039	0.707	0.272	0.806	0.195	0.880	0.224
<i>PSY2</i>	0.858	0.115	0.840	0.206	0.944	0.070	0.616	0.289	0.770	0.207	0.533	0.119
<i>PDS</i>	0.796	0.179	0.736	0.216	0.956	0.040	0.691	0.309	0.881	0.141	0.186	0.164
<i>ZDS</i>	0.812	0.127	0.735	0.176	0.749	0.089	0.575	0.235	0.719	0.213	0.781	0.178
<i>CrtISO</i>	0.973	0.099	0.616	0.218	0.960	0.049	0.639	0.331	0.864	0.153	0.841	0.192
<i>LCY-b</i>	0.858	0.102	0.758	0.165	0.952	0.041	0.644	0.220	0.820	0.185	0.789	0.214
<i>LCY-e</i>	0.839	0.111	0.844	0.199	0.843	0.072	0.473	0.263	0.868	0.131	0.871	0.254
<i>CHY1</i>	0.789	0.185	0.556	0.200	0.899	0.102	0.532	0.215	0.878	0.144	0.864	0.232
<i>CHY2</i>	0.552	0.214	0.821	0.198	0.938	0.038	0.440	0.287	0.786	0.203	0.852	0.267
<i>LUT5</i>	0.696	0.221	0.840	0.187	0.940	0.072	0.706	0.275	0.854	0.170	0.234	0.151
<i>LUT1</i>	0.889	0.107	0.328	0.178	0.915	0.082	0.648	0.343	0.841	0.161	0.845	0.274
<i>ZEP</i>	0.764	0.137	0.381	0.168	0.945	0.073	0.408	0.322	0.371	0.137	0.878	0.238
<i>NXS</i>	0.826	0.095	0.824	0.197	0.931	0.052	0.487	0.218	0.621	0.260	0.796	0.256
Phytoene	-	-	-	-	-	-	-	-	-	-	0.861	0.227
Lutein	0.852	0.096	0.806	0.238	0.944	0.064	0.695	0.208	0.791	0.205	0.876	0.244
α-carotene	-	-	-	-	-	-	-	-	-	-	0.868	0.237
β-carotene	0.876	0.108	0.719	0.176	0.944	0.068	-	-	-	-	0.876	0.234
Zeaxanthin	-	-	-	-	-	-	0.368	0.283	0.861	0.166	0.866	0.237
Antheraxanthin	-	-	-	-	-	-	0.579	0.300	0.603	0.271	0.879	0.233
Violaxanthin	-	-	-	-	-	-	0.690	0.320	0.612	0.242	0.754	0.176
Neoxanthin	-	-	-	-	-	-	0.718	0.280	0.868	0.141	0.874	0.226
β-xanthophylls	0.796	0.109	0.841	0.179	0.952	0.039	-	-	-	-	-	-
<i>CrtI</i>	0.898	0.081	0.819	0.226	0.920	0.076	0.690	0.328	0.880	0.149	0.859	0.224
<i>CrtB</i>	-	-	0.802	0.239	0.934	0.074	-	-	0.881	0.144	0.876	0.244
<i>CrtY</i>	-	-	-	-	0.935	0.085	-	-	-	-	0.871	0.245

Supplemental Table VI – Microarray analysis of isoprenoid metabolism genes
Fold-change (FC) with respect to Wt is shown as the result of 6 replicate experiments (3 biological and 2 technical replicas).

Oligo ID	Gene	Functional Annotation	pP-YBI 17		pP-YBI 30	
Mevalonate pathway			FC	p-value	FC	p-value
MICRO.352.C3_1694	HMGS	hydroxymethylglutaryl coenzyme A synthase	2.46±0.31	7.13E-04	1.95±0.78	0.041
MICRO.352.C1_977	HMGS	hydroxymethylglutaryl coenzyme A synthase	1.76±0.26	0.005	1.88±1.03	0.090
MICRO.3134.C7_526	HMGR	hydroxymethylglutaryl-coenzyme A reductase	0.99±0.08	0.726	0.94±0.15	0.478
MICRO.2814.C1_2293	HMGR	hydroxymethylglutaryl-coenzyme A reductase	1.43±0.13	0.005	1.17±0.32	0.327
MICRO.1162.C2_416	HMGR	hydroxymethylglutaryl-coenzyme A reductase	1.49±0.19	0.008	1.29±0.49	0.253
TBSK03372FA12.t3m.scf_343	HMGR	hydroxymethylglutaryl-coenzyme A reductase	1.32±0.11	0.007	1.04±0.29	0.793
MICRO.3134.C1_1922	HMGR	hydroxymethylglutaryl-coenzyme A reductase	1.46±0.17	0.007	1.34±0.17	0.018
MICRO.6420.C1_1036	HMGR1	hydroxymethylglutaryl-coenzyme A reductase	3.47±0.67	9.90E-04	1.31±0.38	0.149
MICRO.3134.C4_1187	HMGR2	hydroxymethylglutaryl-coenzyme A reductase	2.37±0.19	2.34E-04	2.00±0.28	0.002
MICRO.3134.C9_596	HMGR2	hydroxymethylglutaryl-coenzyme A reductase	1.60±0.30	0.015	1.56±0.18	0.005
MICRO.3134.C3_1103	HMGR2	hydroxymethylglutaryl-coenzyme A reductase	1.66±0.20	0.004	1.55±0.29	0.018
MICRO.3134.C6_1093	HMGR3	hydroxymethylglutaryl-coenzyme A reductase	1.04±0.11	0.577	1.30±0.21	0.048
cSTA3E23TH_447	HMGR3	hydroxymethylglutaryl-coenzyme A reductase	1.86±0.27	0.003	1.61±0.75	0.119
MICRO.5270.C2_1470	MVK	mevalonate kinase	0.91±0.03	0.015	0.74±0.10	0.020
MICRO.1866.C2_636	PMVK	phosphomevalonate kinase	0.75±0.05	0.004	0.95±0.07	0.261
bf_arrayxxx_0033h07.t7m.scf_405	PMVK	phosphomevalonate kinase	0.72±0.09	0.014	0.81±0.08	0.026
MICRO.1866.C1_2219	PMVK	phosphomevalonate kinase	1.73±0.08	1.66E-04	1.68±0.21	0.004
MICRO.2965.C1_1572	mvaD	mevalonate disphosphate decarboxylase	1.35±0.24	0.043	1.32±0.26	0.062
MICRO.2151.C2_323	FPPS	farnesyl diphosphate synthase	0.85±0.21	0.276	0.78±0.19	0.126
MICRO.2151.C3_724	FPPS	farnesyl diphosphate synthase	0.72±0.10	0.017	0.64±0.08	0.006
BF_LBCHXXXX_0042C09T3M.SCF_130	GPS	geranyl pyrophosphate synthase	1.26±0.11	0.012	1.32±0.62	0.303
bf_ivrootxx_0042c08.t3m.scf_593	GPS	geranyl pyrophosphate synthase	1.44±0.16	0.007	1.56±0.82	0.170
MICRO.16217.C1_273	GGPS	geranylgeranyl pyrophosphate synthase	1.31±0.08	0.003	1.45±0.44	0.088
BPLI13C5TH_322	GGPS1	geranylgeranyl pyrophosphate synthase	7.80±21.31	0.067	5.48±7.56	0.046
MICRO.4165.C14_923	GGPS2	geranylgeranyl pyrophosphate synthase	1.01±0.21	0.955	1.10±0.08	0.070
DOXP pathway						
MICRO.2309.C1_1656	DXS	1-D-deoxyxylulose-5-phosphate synthase	1.47±0.16	0.006	0.93±0.16	0.491
MICRO.2309.C2_824	DXS	1-D-deoxyxylulose-5-phosphate synthase	1.41±0.15	0.008	0.98±0.28	0.879
MICRO.5323.C3_934	DXR	1-D-deoxyxylulose-5-phosphate reductoisomerase	0.71±0.12	0.027	0.89±0.05	0.023
MICRO.5323.C4_1692	DXR	1-D-deoxyxylulose-5-phosphate reductoisomerase	0.65±0.08	0.005	0.64±0.05	0.001
MICRO.15807.C2_414	ispE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	0.87±0.18	0.263	0.96±0.05	0.201
MICRO.13619.C1_964	ispE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	1.98±0.64	0.023	1.76±0.58	0.040
MICRO.6382.C1_842	ispF	2-C-methyl-D-erythritol 2.4-cyclodiphosphate synthase	1.10±0.05	0.020	1.53±0.13	0.002
MICRO.890.C3_471	IspG	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	0.52±0.06	0.001	0.61±0.08	0.005
POAC055TP_870	Ipl	isopentenyl diphosphate isomerase	0.79±0.14	0.072	0.81±0.12	0.067
MICRO.1368.C12_575	Ipl2	isopentenyl diphosphate isomerase	1.50±0.27	0.019	1.66±0.22	0.005
BF_LBCHXXXX_0042C09T3M.SCF_130	GPS	geranyl pyrophosphate synthase	1.26±0.11	0.012	1.32±0.62	0.303
bf_ivrootxx_0042c08.t3m.scf_593	GPS	geranyl pyrophosphate synthase	1.44±0.16	0.007	1.56±0.82	0.170
MICRO.16217.C1_273	GGPS	geranylgeranyl pyrophosphate synthase	1.31±0.08	0.003	1.45±0.44	0.088
BPLI13C5TH_322	GGPS1	geranylgeranyl pyrophosphate synthase	7.80±21.31	0.067	5.48±7.56	0.046
MICRO.4165.C14_923	GGPS2	geranylgeranyl pyrophosphate synthase	1.01±0.21	0.955	1.10±0.08	0.070
Apocarotenoid and ABA biosynthesis						
MICRO.4825.C1_462	G10H	geraniol 10-hydroxylase	0.85±0.14	0.152	0.96±0.30	0.794
MICRO.9915.C1_814	G10H	geraniol 10-hydroxylase	0.51±0.18	0.029	0.83±0.35	0.417
MICRO.14898.C1_1060	HGO	10-hydroxygeraniol oxidoreductase	0.72±0.14	0.041	0.53±0.27	0.078
MICRO.8543.C1_1242	HGO	10-hydroxygeraniol oxidoreductase	0.92±0.09	0.191	0.71±0.15	0.048
MICRO.10327.C1_642	CCD1	carotenoid cleavage dioxygenase 1	0.55±0.08	0.003	0.70±0.08	0.007
MICRO.4340.C1_1	CCD1A	carotenoid cleavage dioxygenase 1A	0.67±0.07	0.004	0.79±0.14	0.072
MICRO.4340.C2_560	CCD1A	carotenoid cleavage dioxygenase 1A	0.53±0.06	0.002	0.68±0.14	0.034
bf_mxflxxxx_0012d12.t3m.scf_300	NCED	9-cis epoxycarotenoid dioxygenase	3.14±0.31	0.000	0.91±0.45	0.702
MICRO.11321.C1_756	NCED2	9-cis-epoxy-carotenoid dioxygenase	0.97±0.14	0.698	0.99±0.07	0.763
Chlorophyll biosynthesis						
MICRO.1051.C1_1434	PORA	NADPH:protochlorophyllide oxidoreductase	1.79±0.15	0.001	1.99±0.16	0.000
MICRO.1948.C1_1391	ChlP	geranylgeranyl reductase	0.97±0.06	0.332	2.03±0.54	0.012
MICRO.13625.C1_712	CAO	chloroplast chlorophyllide a oxygenase	1.25±0.50	0.327	1.36±0.57	0.223
MICRO.2538.C1_1161	ChlG	chlorophyll synthetase	0.99±0.07	0.812	1.17±0.15	0.087
MICRO.7616.C1_122	CH1	chlorophyllide a oxygenase	1.37±0.42	0.124	1.39±0.31	0.060
MICRO.6304.C1_759	CLH	chlorophyllase	0.77±0.20	0.129	0.84±0.27	0.340

Supplemental Table VI (continued)

Gibberellin biosynthesis						
MICRO.2534.C1_1263	CPS	ent-copalyl diphosphate synthase	2.02±0.22	0.001	2.08±0.15	2.68E-04
MICRO.10720.C2_566	KO	ent-kaurene oxidase	0.68±0.07	0.004	0.66±0.11	0.015
MICRO.2633.C1_702	KO	ent-kaurene oxidase	0.67±0.14	0.030	0.76±0.25	0.177
cSTA6G24TH_420	KA01	ent-kaurenoic acid oxidase	6.00±1.64	0.001	3.94±4.88	0.066
MICRO.1345.C1_1453	KA02	ent-kaurenoic acid hydroxylase	0.76±0.12	0.039	0.24±0.08	0.003
MICRO.7271.C1_651	KA13H	ent-kaurenoic acid 13-hydroxylase	3.29±1.86	0.020	8.14±2.35	0.001
Quinone biosynthesis						
MICRO.1948.C1_1391	GGR	geranylgeranyl reductase	0.97±0.06	0.332	2.03±0.54	0.012
MICRO.2151.C3_724	FPPS	farnesyl diphosphate synthase	0.72±0.10	0.017	0.64±0.08	0.006
MICRO.2151.C2_323	FPPS	farnesyl diphosphate synthase	0.85±0.21	0.276	0.78±0.19	0.126
MICRO.5886.C1_674	SPP	solanesyl diphosphate synthase	1.73±0.28	0.007	2.98±0.65	0.002
MICRO.5051.C1_795	SPP	solanesyl diphosphate synthase	1.96±0.37	0.006	3.59±1.05	0.003
MICRO.4207.C1_1359	HST	homogentisate geranylgeranyl transferase	0.90±0.13	0.245	0.92±0.07	0.134
MICRO.1059.C1_1306	VTE3	MPBQ/MSBQ methyltransferase 1	1.07±0.10	0.247	1.32±0.17	0.023
MICRO.572.C1_548	MenG/UbiE	S-adenosylmethionine:2-demethylmenaquinone methyltransferase-like	0.87±0.05	0.017	0.98±0.22	0.842
MICRO.14879.C1_1295	PPT1	para-hydroxy benzoate polyprenyl diphosphate transferase	0.74±0.25	0.155	0.94±0.32	0.720
cSTA41A18TH_74	COQ3/UbiG	hexaprenyldihydroxybenzoate methyltransferase/3-demethylubiquinone-9 3-methyltransferase	0.95±0.17	0.581	1.45±0.14	0.004
MICRO.10619.C2	COQ6/UbiH	ubiquinone biosynthesis monooxygenase/2-octaprenyl-6-methoxyphenol hydroxylase	0.87±0.08	0.058	0.96±0.15	0.643
Tocochromanol biosynthesis						
MICRO.2820.C1_1005	GGH	geranylgeranyl hydrogenase	1.14±0.38	0.462	1.42±0.17	0.009
MICRO.4207.C1_1359	HGGT	homogentisate geranylgeranyl transferase	0.90±0.13	0.245	0.92±0.07	0.134
bf_arrayxxx_0044c12.t3m.scf_615	VTE2-2	homogentisate phytyltransferase	0.86±0.17	0.239	1.03±0.09	0.571
MICRO.11205.C1_619	VTE2-2	homogentisate phytyltransferase	0.63±0.48	0.267	0.88±0.39	0.596
MICRO.1059.C1_1306	VTE3	MPBQ/MSBQ methyltransferase 1	1.07±0.10	0.247	1.32±0.17	0.023
MICRO.113.C1_291	VTE4	gamma-tocopherol methyltransferase	1.50±0.21	0.011	2.50±0.57	0.004
MICRO.113.C4_415	VTE4	gamma-tocopherol methyltransferase	2.02±0.28	0.002	3.12±0.20	0.000
MICRO.113.C2_41	VTE4	gamma-tocopherol methyltransferase	1.86±0.17	0.001	2.71±0.71	0.005



Supplemental Figure 1. Expression of the CrtI protein in tubers.

Total proteins from approx. 40 mg (fresh weight) of tuber tissue were loaded in each lane and analyzed using an anti-CrtI antibody.

