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Relative importance of chlorophyll metabolic genes for light-induced greening of potato tubers

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Abstract

Potato tuber greening occurs due to the chlorophyll accumulation upon exposure to light, however, fundamental information on tuber chlorophyll metabolism is lacking. We measured the effect of varying light exposure (0, 48, 96, and 168 h) on chlorophyll concentration and gene expression of enzymes in the chlorophyll metabolic pathway in the potato varieties that differ in greening propensity. Greening was associated with the upregulation of genes involved in chlorophyll biosynthesis, particularly glutamyl-tRNA reductase 1, magnesium-chelatase subunit H, and magnesium-protoporphyrin IX monomethyl ester cyclase, and downregulation of genes involved in chlorophyll cycling and degradation, including chlorophyllide *a* oxygenase, and pheophorbide *a* oxygenase. Our findings suggest that relative resistance to tuber greening propensity may be due to a weaker upregulation of chlorophyll biosynthesis genes and weaker downregulation of chlorophyll degradation genes that occurs in susceptible varieties. The association of these biosynthesis and degradation genes with greening susceptibility may provide possible breeding targets for the future development of more greening-resistant varieties.

Keywords: chlorophyll biosynthesis; chlorophyll degradation; chlorophyll metabolic pathway; tuber greening.

Introduction

Potato (*Solanum tuberosum* L.) tubers are underground-modified stems with cells filled with amyloplasts, nonpigmented starch-storing plastids. Upon exposure to light, amyloplasts, located in the peripheral cell layers of the tubers, differentiate into chloroplasts, which accumulate the green photosynthetic pigment, chlorophyll (Chl) (Anstis and Northcote 1973). This tuber-greening process is influenced by genetic, cultural, physiological

Highlights

- Chlorophyll gene expression associated with potato tuber greening was determined
- Light exposure alters the expression of both biosynthesis and degradation genes
- Greening resistance was associated with relative changes in chlorophyll synthesis and degradation gene regulation

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Abbreviations: ALA – aminolevulinic acid; *ANOVA* – analysis of variance; AtPORA – *Arabidopsis thaliana* NADPH:protochlorophyllide oxidoreductase A; AtPORB – *Arabidopsis thaliana* NADPH:protochlorophyllide oxidoreductase B; AtPORC – *Arabidopsis thaliana* NADPH:protochlorophyllide oxidoreductase C; CAO – chlorophyllide *a* oxygenase; Chl – chlorophyll; CHLD – magnesium-chelatase subunit D; CHLG – chlorophyll synthase; CHLH – magnesium-chelatase subunit H; CHLI – magnesium-chelatase subunit I; CHLM – magnesium protoporphyrin IX methyltransferase; CLH – chlorophyllase; CRD – magnesium-protoporphyrin IX monomethyl ester cyclase; DVR – divinyl chlorophyllide *a* 8-vinyl-reductase; EF1α – elongation factor 1 alpha; GluTR – glutamyl-tRNA reductase; GSA – glutamate-1-semialdehyde 2,1-aminomutase; GUN4 – genomes uncoupled 4; HCAR – 7-hydroxymethyl chlorophyll *a* reductase; HEMA1 – glutamyl-tRNA reductase 1; HEMA2-like – glutamyl-tRNA reductase 2-like; HEMB – delta-aminolevulinic acid dehydratase; HEMC – porphobilinogen deaminase; HEMD – uroporphyrinogen-III synthase; HEME – uroporphyrinogen decarboxylase; HEMF – coproporphyrinogen-III oxidase; HEMG – protoporphyrinogen oxidase; NOL – chlorophyll(ide) *b* reductase NOL; NYC – chlorophyll(ide) *b* reductase NYC1; OsCHLH – rice magnesium-chelatase subunit H; OsCRD1 – rice magnesium-protoporphyrin IX monomethyl ester cyclase; PAO – pheophorbide *a* oxygenase; POR – NADPH:protochlorophyllide oxidoreductase; PPH – pheophytinase; qPCR – quantitative polymerase chain reaction; RCCR – red chlorophyll catabolite reductase; Rubisco – ribulose-1,5-bisphosphate carboxylase; SNPs – single nucleotide polymorphisms.

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(Tanios *et al.* 2020a,b,c), and environmental factors. Tuber greening may occur following light exposure at any stage along the potato supply chain and poses a major challenge to both growers and retailers, leading to loss of tuber marketability (Bamberg *et al.* 2015, Tanios *et al.* 2018).

Plastids are cellular organelles with high morphological and functional diversity found in plants and algae (Liebers *et al.* 2017). Starting from an undifferentiated small proplastid, these organelles can develop into four different forms, chloroplasts, chromoplasts, amyloplasts, and etioplasts, which can interconvert into each other upon environmentally induced alterations in plant development such as light exposure (Anstis and Northcote 1973, Liebers *et al.* 2017). Knowledge of the structural and molecular changes associated with plastid transition and Chl biosynthesis in potato tubers is limited. Differentiation events described include the elongation of vesicles into thylakoids, the appearance of ribosomes in the stroma, and the synthesis of ribulose-1,5-bisphosphate carboxylase (Rubisco) and other proteins (Zhu *et al.* 1984).

Chlorophyll is essential for light harvesting and energy transfer during photosynthesis (Chen 2014). The Chl metabolic pathway (Fig. 1) can be divided into three stages: (*1*) synthesis of Chl *a* from glutamate; (*2*) the interconversion between Chl *a* and Chl *b*; and (*3*) degradation of Chl *a* into a nonfluorescent Chl catabolite. Important progress has been made in the elucidation and characterization of genes involved in chlorophyll metabolism and the determination of their light regulation in leaves, particularly in the model plant, *Arabidopsis thaliana* (Tanaka and Tanaka 2007, Liu *et al.* 2017). In contrast, there are very few studies that have examined the expression of genes and regulatory pathways related to Chl metabolism during the greening process of potato tubers. Okamoto *et al.* (2020) showed four Chl biosynthesis genes (HEMA1, GSA, CHLH, and GUN4) were upregulated under white, blue, and red light but not under far-red light in tubers of a relatively susceptible potato variety. Wang *et al.* (2025) examined the expression of 17 chlorophyll-associated genes noting those involved in Chl biosynthesis were upregulated upon light exposure whilst those associated with degradation and Chl cycling were downregulated.

To confirm key genes that control Chl synthesis in potato tubers and to determine their importance for the observed variation in tuber greening propensity amongst cultivars, we first assessed the expression of chlorophyll-metabolic genes over time following exposure to light in cv. 'Maranca'. We then compared gene expression between two varieties ('Kennebec' and 'Nicola') that vary in their tuber greening response. We hypothesized that the chlorophyll biosynthesis genes most relevant for tuber greening would fulfil at least the following criteria: (*1*) be strongly upregulated by light treatment, and (*2*) be more strongly induced in a greening susceptible than resistant potato variety.

Materials and methods

Plant materials: Three potato varieties were used in this study, 'Nicola', 'Maranca', and 'Kennebec', known to have a low, mid, and high susceptibility to greening, respectively (Tanios *et al.* 2020c). Planting material was

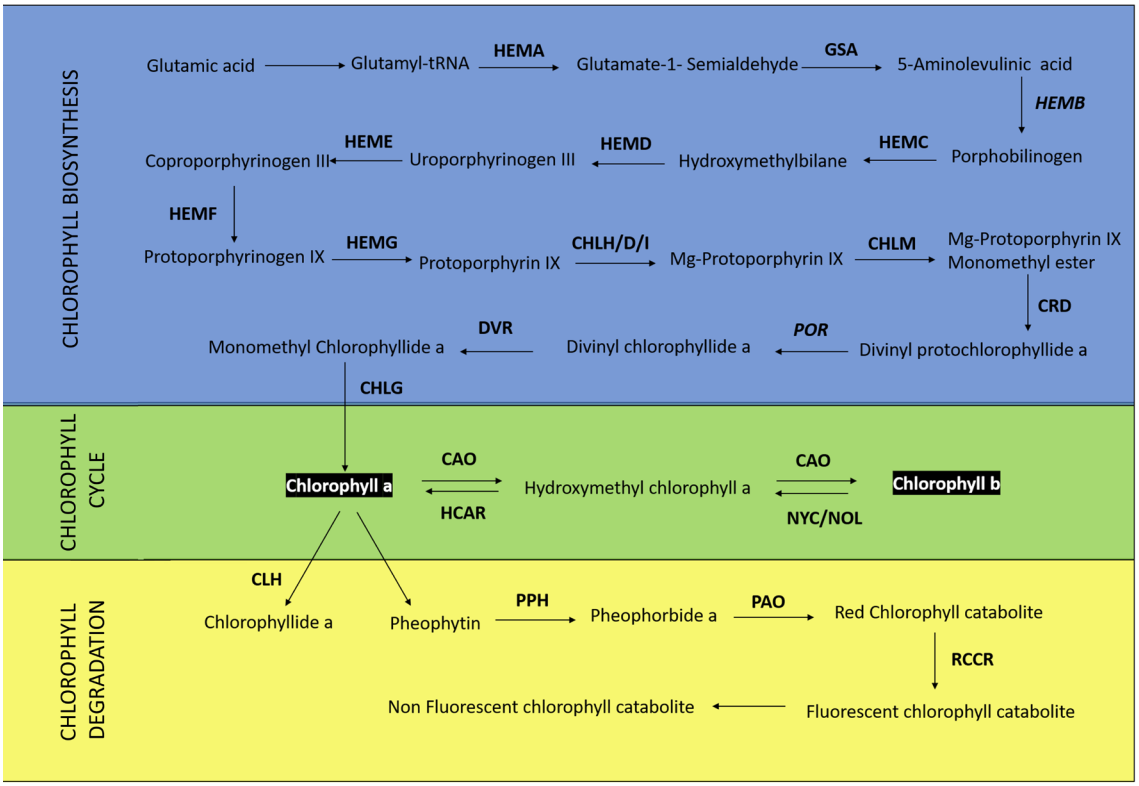

Fig. 1. A simplified scheme of chlorophyll metabolic pathway in angiosperms. Gene abbreviations are given in Table 1S.

either certified seed tubers ('Maranca') or four-week-old tissue culture plants ('Nicola' and 'Kennebec') cultured in Murashige and Skoog medium, pH 5.8, (Murashige and Skoog 1962) supplemented with sucrose (30 g L^{-1}), ascorbic acid (0.04 g L^{-1}), casein hydrolysate (0.5 g L^{-1}), and agar (8 g L^{-1}) at 22°C with a 16-h photoperiod under cool white fluorescent lamps [65 µmol(photon) m^{-2} s^{-1}]. Plants were grown from seed tubers or transplanted tissues cultured plantlets in a potting mix containing sand, peat, and composted pine bark (10:10:80; pH 6.0) premixed with *Osmocote* 16–3.5–10 NPK resin-coated fertilizer (*Scotts Australia Pty Ltd*., Baulkham Hills, Australia), under glasshouse conditions, with temperatures between 18 and 24°C. Fully expanded leaves were collected from 4-week-old 'Maranca' plants. Each variety was harvested following natural senescence. Any tubers that formed close to the soil surface were discarded while the rest were stored in the dark at room temperature for approximately 30 d to allow post-harvest maturation.

Expression analysis of chlorophyll-metabolic genes after light exposure ('Maranca'): Tubers of cv. 'Maranca' were exposed to light for 0, 48, 96, and 168 h with three tubers per light treatment. Treatments were referred to as T0, T48, T96, and T168, respectively. Light treatment involved uniformly sized tubers free of visible damage and were subject to continuous exposure to fluorescent light (Fig. 1S, *supplement*) with an intensity of 12 µmol(photon) m^{-2} s^{-1} at the tuber surface at room temperature. Tubers were arranged in rows and their places within the row were repositioned daily to avoid any possible bias of positioning from variation in light intensity, ensuring that the orientation of the tuber remained the same. Following light exposure, Chl concentration within tubers and the expression of genes encoding Chl biosynthesis and degradation enzymes from the different light-treated samples were assessed as described below.

Comparative expression analysis of chlorophyll-metabolic genes after light exposure ('Nicola' and 'Kennebec'): 'Nicola' (greening-resistant) and 'Kennebec' (greening-susceptible) tubers (Tanios *et al.* 2020c) were exposed to the same light treatment for 0, 96, and 168 h with three tubers per light treatment per cultivar. Treatments were referred to as T0, T96, and T168, respectively. Following light exposure, Chl concentration within tubers and the expression of genes encoding Chl biosynthesis and degradation enzymes from the different light-treated samples were compared, as described below.

Chlorophyll analysis: Three periderm disks (1.5 mm thick and 1 cm diameter) were cut using a cork borer from the stem, the middle, and the bud end of each tuber periderm. Leaf disc samples were obtained from the top, middle, and bottom of each leaf. The discs were snap-frozen in liquid nitrogen and ground to powder using a mortar and pestle. Samples were extracted with 5 mL of N,N-dimethylformamide for Chl analysis. All samples were stored at 4°C in the dark for 24 h. After centrifugation for 15 min at 2,500 × *g*, the absorbance was measured with a spectrophotometer (*Thermo Scientific Spectronic 200E*) at 647 and 664 nm. Chl concentrations were determined according to Porra *et al.* (1989), using the equation: Total Chl = 17.67 (A_{647}) + 7.12 (A_{664}) and expressed in mg kg^{-1}(FM).

Expression analysis of genes encoding enzymes in the chlorophyll metabolic pathway using quantitative real-time PCR: Three periderm disks (1.5 mm thick and 1 cm diameter) were cut using a cork borer from each tuber periderm and leaf tissue, frozen in liquid nitrogen and ground to powder using a mortar and pestle. Total RNA was isolated using the *PowerPlant® RNA* isolation kit (*QIAGEN*) according to the manufacturer's instructions. Following DNase treatment (*DNase Max TM kit*, *QIAGEN*), RNA yield was quantified using the *Qubit® 2.0* fluorometer (*Life Technologies*, Mulgrave, VIC, Australia). One microgram of RNA was reverse transcribed using the *iScript* reverse transcription supermix (*BIO-RAD*) following the manufacturer's protocol. Potato nucleotide sequences for each target gene were obtained from the *NCBI* database. Gene-specific primers were designed using *NCBI/Primer-Blast* (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 1S, *supplement*). There is the potential for SNPs within primer regions to affect PCR efficiency, we compared primer sequences against available data for the potato variety, 'Russet Burbank' (Table 1S) with the assumption that lack of any variation between diverse genotypes would suggest conserved sequences. For each gene, a standard curve was generated using a cDNA serial dilution. The qPCR reactions were carried out with one microliter cDNA using *iTaq Universal SYBR Green Supermix* (*BIO-RAD*) in a *Rotor Gene 6000* instrument (*QIAGEN*) with a thermocycle of 95°C for 15 min, followed by 40 cycles of 95°C for 30 s, 60°C for 60 s, and 72°C for 60 s. Two technical and three independent biological replicates were used for each gene. Samples for which the difference in Ct value between the technical replicates was larger than one, were excluded from the analysis. Expression of the target genes was normalized to the reference gene EF1α (Nicot *et al.* 2005), and relative transcript abundances were calculated as 2^{-dCt}.

Statistical analysis: The effect of time on the total Chl concentration of 'Maraca' was analysed by one-way analysis of variance (*ANOVA*). The effect of light treatment (T0, T48, T96, T168) and variety ('Maranca' and 'Kennebec') and their interaction on total Chl concentration and gene expression were analysed by two-way *ANOVA*. Values of the gene expression data required log transformation to meet assumptions of normality and homogeneity of variance in *ANOVA*. *Fisher*'s protected least significant difference post hoc tests were used to determine significant differences between treatment means. Relative expression mean values were log^2 transformed and hierarchical cluster analysis of differentially expressed genes was conducted based on Euclidean distance and Ward's minimum variance clustering method. Analysis was performed using *BioVinci Software* (*Bioturing*, San Diego, CA, USA). *Pearson*'s

correlation analysis between Chl concentration and relative expression for each gene was conducted using *SPSS v24* software (*SPSS™ Inc*., Armonk, NY, USA).

Results

Chlorophyll concentration and gene expression in 'Maranca' tubers and leaves: Artificial light exposure treatments for 0, 48, 96, and 168 h at room temperature revealed that no Chl was detected in the periderm of 'Maranca' tubers stored in darkness (T0), with Chl concentrations gradually increasing with the duration of light treatment (Fig. 2). In 'Maranca' leaves, Chl concentration was on average 7.2-fold higher compared to tubers after exposure to 168 h of light (41 mg kg^{-1} *vs* 5.7 mg kg^{-1}).

The expression of most Chl metabolic genes was noticeably higher in potato leaves when compared to tubers exposed to light for 168 h (Fig. 3). However, hierarchical clustering of Chl-biosynthesis genes in potato tuber revealed three major expression patterns (Fig. 3). Genes in group 1 were highly upregulated by light including glutamyl-tRNA reductase 1 (HEMA1), magnesium-protoporphyrin IX monomethyl ester cyclase (CRD), and magnesium-chelatase subunit H (CHLH) which showed strong and sustained RNA expression that increased with light exposure and was highly correlated with tuber Chl content (Table 1); and magnesium protoporphyrin IX methyltransferase (CHLM), NADPH:protochlorophyllide oxidoreductase (POR), and 7-hydroxymethyl chlorophyll *a* reductase (HCAR) which also showed strongly increasing RNA expression to T48 but then tended to decrease at T168.

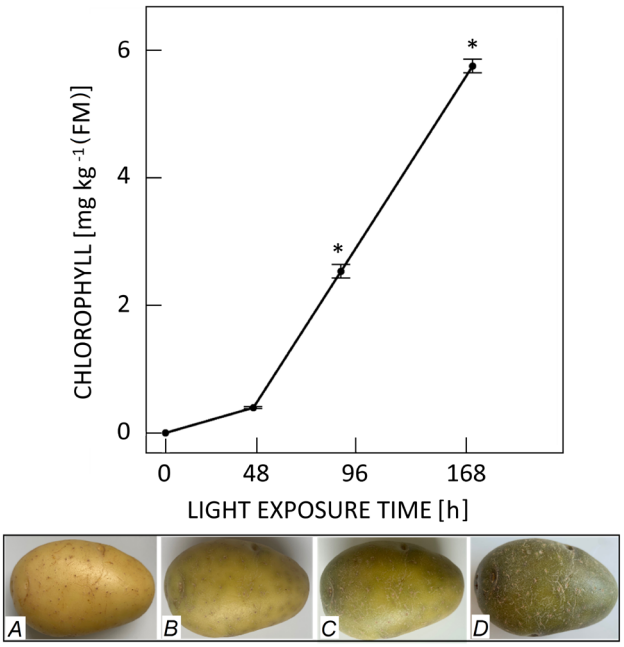

Fig. 2. Total chlorophyll concentration in potato tubers of the 'Maranca' variety following light exposure for 0 (*A*), 48 (*B*), 96 (*C*), and 168 h (*D*). Data was analysed by one-way *ANOVA* at $P<0.005$. * Indicates a significant increase compared with T0. $n = 3$.

Expression of genes in group 2 either gradually increased upon light exposure (or remained relatively constant across the light treatments (Fig. 3). Most of the genes in group 2, showed significant positive correlations with tuber Chl content (Table 1), however,

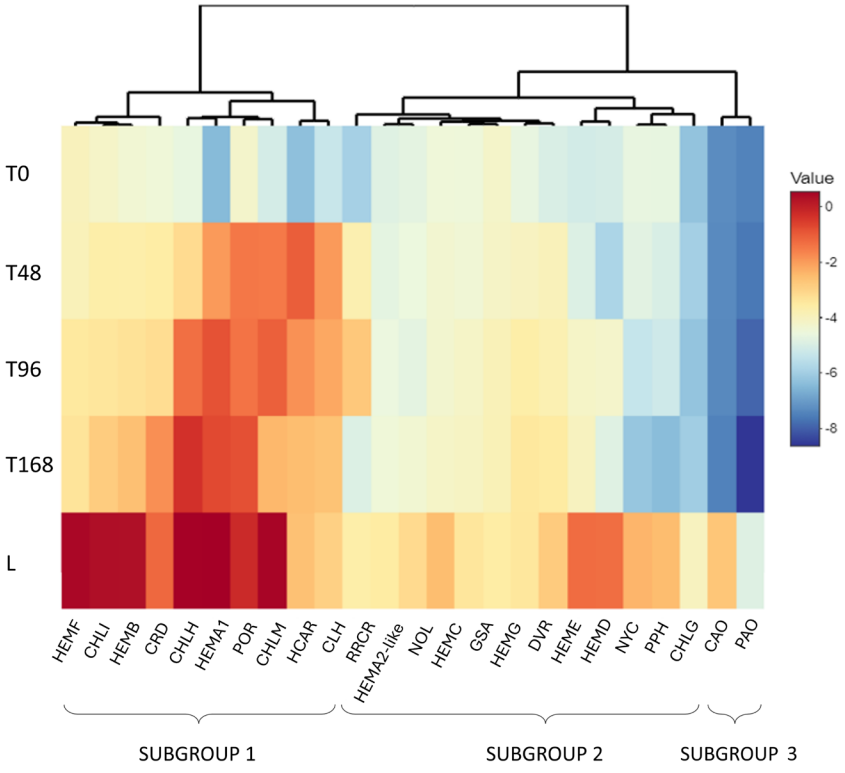

Fig. 3. Expression profiles of chlorophyll-metabolic genes in potato tubers of 'Maranca' following light exposure for 0, 48, 96, and 168 h (as denoted by T0, T48, T96, and T168, respectively) and 4-week-old plant leaves (as denoted by L). Gene expression was analysed by quantitative real-time PCR and normalised to the reference gene, EF1α. Data represent $\log^2$ average expression values from three biological and two technical replications. Data was clustered concerning the gene expression profile with dendrograms shown on the top of the heatmap using *BioVinci* software (*BioTuring*, San Diego, CA, USA). Gene abbreviations are given in Table 1S. $n = 3$.

Table 1. *Pearson*'s correlation coefficients between chlorophyll concentration and relative expression of the genes over 0, 96, and 160 h for the 'Maranca' variety. The symbols *, ** and *** represent statistical significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Gene	*R*
HEMA1	0.800**
HEMA2-like	0.618*
GSA	0.799**
HEMB	0.966**
HEMC	0.753**
HEMD	0.385
HEME	0.897**
HEMF	0.923**
HEMG	0.805**
CHLH	0.862**
CHLD	0.975**
CHLI	0.966**
CHLM	–0.108
CRD	0.997**
POR	0.092
DVR	0.818**
CHLG	0.132
CAO	–0.262
HCAR	0.160
NYC	–0.946**
NOL	0.703*
CLH	–0.024
PPH	–0.951**
PAO	–0.952**
RCCR	0.209

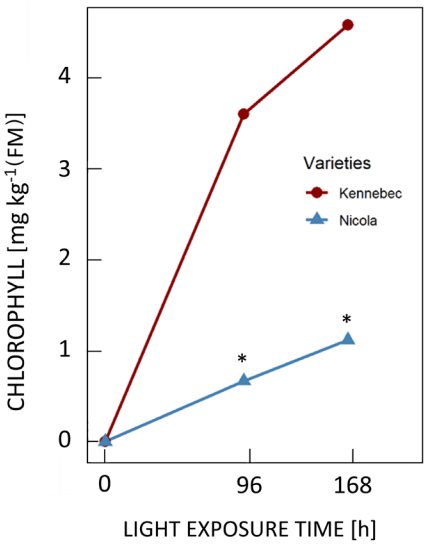

Fig. 4. Total chlorophyll concentration in potato tubers of 'Nicola' and 'Kennebec' following light exposure for 0, 96, and 168 h. Data was analysed by two-way *ANOVA* followed by *Fischer*'s protected LSD post-hoc test. * Indicates a significant treatment effect within a measurement date. $n = 3$. $P<0.05$.

expression of group 2 genes was less influenced by light than those in group 1. Genes in group 3 either gradually decreased in expression following light exposure [pheophorbide *a* oxygenase (PAO)], and were negatively correlating with tuber Chl content (Table 1), or showed low constitutive expression in the dark, that remained constant after light exposure [chlorophyllide *a* oxygenase (CAO); Fig. 3].

Chlorophyll concentration and gene expression in 'Nicola' compared to 'Kennebec' tubers: Two varieties, 'Nicola' and 'Kennebec', known to have a low and high susceptibility to greening were exposed to light for 0, 48, 96, and 169 h. At T0, no Chl was detected in 'Nicola' and 'Kennebec' tubers, with concentrations gradually increasing with the duration of light treatment (Fig. 4). At T96 and T168, Chl concentration was respectively 6-fold and 4-fold higher in 'Kennebec' compared to 'Nicola' (Fig. 5).

There were significant differences in gene expression between 'Kennebec' and 'Nicola' tubers (Fig. 5; Table 2S, *supplement*). Of the 17 Chl-biosynthesis genes, most of them increased after light exposure for both varieties. However, after 168 h of light exposure, 14 of them were significantly higher in 'Kennebec' compared to 'Nicola' except for HEMA2-like, HEMF, and CHLG (Fig. 5). Overall, most Chl-biosynthesis genes were highly positively correlated with tuber Chl concentration for both cultivars (Table 2). In contrast, there were negative correlations between NYC, CLH, PPH, and Chl concentration. Of the four Chl cycle genes, only HCAR was significantly higher in 'Kennebec' than 'Nicola' following light exposure for both 96 and 168 h. Regardless of variety, the expression of NYC, PPH, and PAO decreased after light exposure (Fig. 5), showing a significant negative correlation with tuber Chl concentration (Table 2).

Discussion

We found good support for our first hypothesis that Chl biosynthesis genes are upregulated in light-induced potato greening. In particular, there was strong upregulation of Chl-biosynthesis genes, HEMA1, CHLH, and CRD, with concurrent downregulation of key genes in the chlorophyll cycle and degradation, including CAO and PAO. This confirms and expands on the findings of Wang *et al*. (2025). Furthermore, as proposed by our second hypothesis, there was evidence of greater upregulation and downregulation of these Chl biosynthesis and degradation genes observed in the tuber greening-susceptible variety compared to the resistant variety. In potato leaves, in contrast, there was a much stronger upregulation of Chl-biosynthesis genes and only limited downregulation of the Chl-degradation genes. Based on these results, we propose that these genes are key transcriptional regulatory genes in potato tuber greening.

Our data revealed that potato greening is associated with the activation of a series of genes involved in Chl biosynthesis, which were also responsible for the differential greening response between greening-resistant

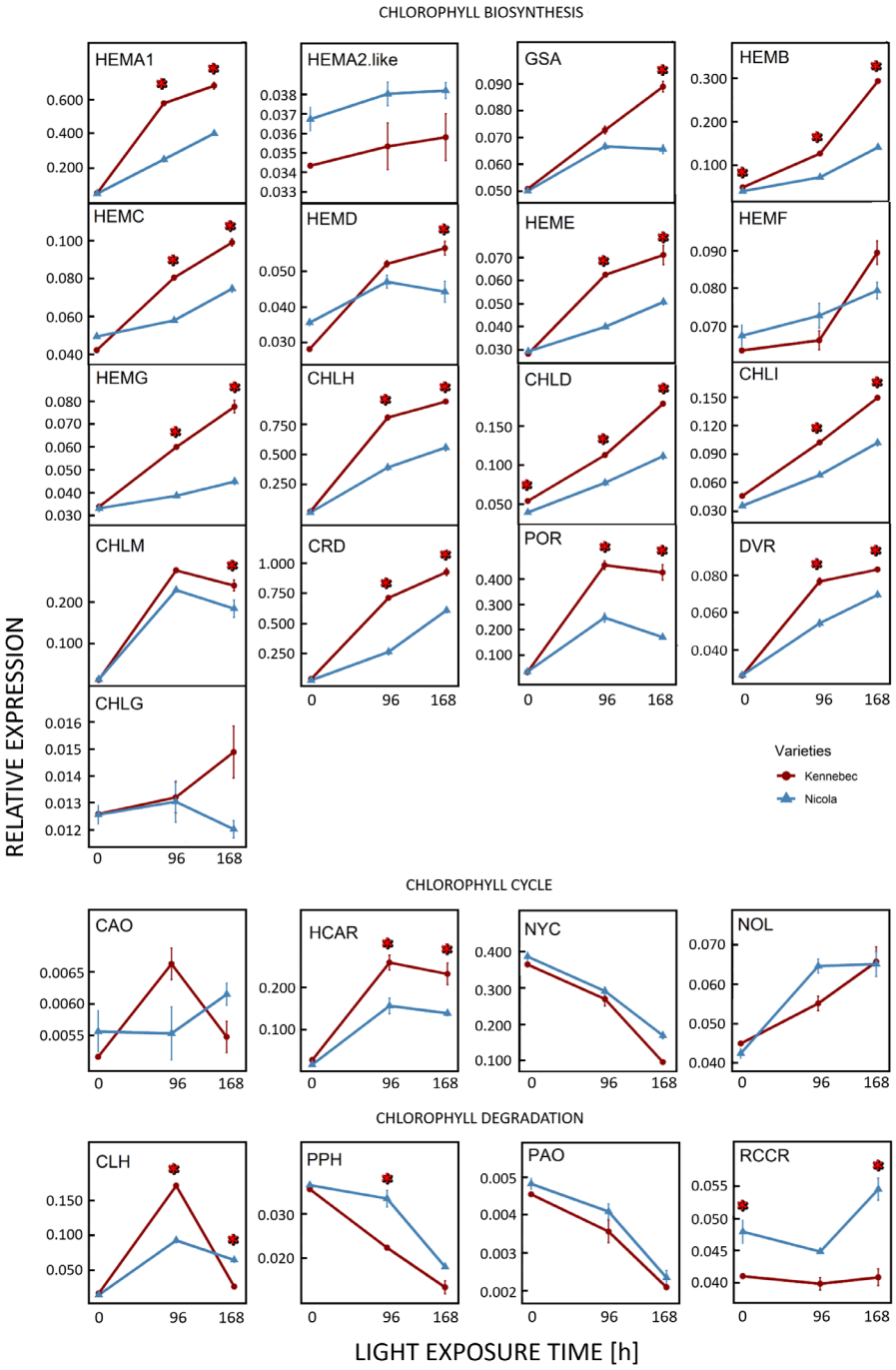

Fig. 5. Expression profiles of chlorophyll metabolic genes in potato tubers of 'Nicola' (*blue line*) and 'Kennebec' (*red line*), following light exposure for 0, 96, and 168 h. Gene expression was analysed by quantitative real-time PCR and normalised to the reference gene, EF1α. Data represent average expression values from three biological and two technical replications ± SE. Data was analysed by two-way *ANOVA* followed by *Fischer*'s protected LSD post-hoc test. * Indicates a significant treatment effect at the same time point of the two varieties. $n = 3$. Gene abbreviations are given in Table 1S. A summary of *ANOVA* output for each gene is provided in Table 2S.

and susceptible varieties. In potato, the two genes encoding HEMA1 and HEMA2-like, sharing 66% amino acid sequence identity, showed different light regulation. Only the expression of HEMA1 was found to be strongly upregulated by light, while that of HEMA2-like was independent of light. Similar results were reported in potato tubers (Okamoto *et al.* 2020) and in *Arabidopsis* leaves, with three isoforms of HEMA (HEMA1, HEMA2, HEMA3) (Ujwal *et al.* 2002, Yuan *et al.* 2017), where HEMA1 was highly expressed in photosynthetic tissues and responded to light (McCormac *et al.* 2001, McCormac and Terry 2002). In contrast, HEMA2 expression is found exclusively in nonphotosynthetic tissues and is not responsive to illumination (Kumar *et al.* 1996).

Table 2. *Pearson*'s correlation coefficients between chlorophyll concentration and relative gene expression during tuber greening of 'Nicola' (greening-resistant variety) and 'Kennebec' (greening-susceptible variety). The symbols *, ** and *** represent statistical significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Gene	'Nicola' *R*	'Kennebec' *R*
HEMA1	0.993**	0.995**
HEMA2-like	0.632	0.309
GSA	0.866**	0.965**
HEMB	0.949**	0.864**
HEMC	0.954**	0.990**
HEMD	0.665	0.986**
HEME	0.980**	0.978**
HEMF	0.748*	0.704*
HEMG	0.909**	0.967**
CHLH	0.980**	0.996**
CHLD	0.988**	0.938**
CHLI	0.982**	0.963**
CHLM	0.802**	0.941**
CRD	0.965**	0.997**
POR	0.708*	0.956**
DVR	0.992**	0.989**
CHLG	–0.211	0.579
CAO	0.450	0.458
HCAR	0.834**	0.925**
NYC	–0.972**	–0.871**
NOL	0.873**	0.877**
CLH	0.702*	0.365
PPH	–0.874**	–0.957**
PAO	–0.909**	–0.884**
RCCR	0.511	–0.142

Another key regulatory gene in the pathway is CHLH, encoding a subunit of the Mg-chelatase. Although our results showed that the expression of the three Mg-chelatase subunits (CHLH, CHLD, CHLI), which catalyse the insertion of Mg^{2+} into protoporphyrin IX, was induced by light and was higher in the susceptible compared to the resistant variety, CHLH was the most strongly upregulated and thus, is one of the major determinants of the flux of protoporphyrin IX into Chl biosynthesis in tubers. Consistent with our results, CHLH expression was rapidly induced by light in potato tubers (Okamoto *et al.* 2020, Wang *et al.* 2025) and in *Arabidopsis* foliar material (Matsumoto *et al.* 2004, Stephenson and Terry 2008) and T-DNA insertion mutation in OsCHLH caused a high reduction in Chl biosynthesis and subsequent seedling-lethal phenotype in rice (Jung *et al.* 2003).

A third key Chl-biosynthesis gene, which was shown in this study to be strongly induced upon light exposure is CRD1, which catalyses a cyclical reaction in the creation of the E ring of chlorophyll and form 3,8-divinyl-protochlorophyllide. Similar upregulation in light-exposed potato tubers was found by Wang *et al.* (2025). In rice, OsCRD1 was mainly expressed in green tissues and mutation in OsCRD1 led to a deficiency in Chl biosynthesis, chloroplast development, and decreased photosynthetic capacity (Wang *et al.* 2017).

The expression of CHLM, POR, and HCAR was also strongly light-induced, although it tended to reduce at T168. CHLM was shown to be constitutively expressed at the transcriptional and translational levels during greening in etiolated barley and tobacco (Yuan *et al.* 2017) and in *Chlamydomonas reinhardtii* mutants, in which defects in CHLM resulted in reductions of PSI and PSII core components as well as light-harvesting proteins (Meinecke *et al.* 2010). In contrast, mRNA levels of CHLM remained constant during greening in *Arabidopsis* (Matsumoto *et al.* 2004) and tobacco and barley seedlings (Alawady and Grimm 2005). Several POR gene families have been identified in angiosperms, which catalyse the photoreduction of protochlorophyllide (Pchlide) *a* to chlorophyllide *a*, that encode highly conserved POR polypeptides, with different light- and development-dependent regulatory mechanisms. For example, three POR genes (PORA, PORB, and PORC) were found in *A. thaliana*, where PORA expression is downregulated by light, while PORB is less light sensitive, and its mRNA and protein remain almost unchanged, and PORC is upregulated in response to light and is postulated to enable elevated rates of Chl synthesis under high light (Su *et al.* 2001). Database search of the *S. tuberosum* genome showed the existence of three POR homologs, sharing more than 80% amino acid sequence identity. In this study, only the expression of one POR homolog was studied, showing strong upregulation by light, sharing 75.80, 75.87, and 76.24% identity with AtPORA, AtPORB and AtPORC, respectively. Wang *et al.* (2025) also showed upregulation of POR1 in potato tubers exposed to light. Interestingly, chlorophyllide *a* oxygenase (CAO), which catalyses the oxygenation of Chlide *a* producing Chlide *b* showed very low expression levels in the dark and light in all three varieties tested. In contrast, CAO expression was rapidly induced by light in *Arabidopsis* (Espineda *et al.* 1999, Matsumoto *et al.* 2004) and was classified as one of the key regulatory genes in the pathway together with HEMA1, CHLH, and CRD1 (Matsumoto *et al.* 2004).

Our results showed that light downregulated the expression of PAO, which negatively correlated with Chl synthesis during tuber greening. Stronger downregulation was found in the susceptible variety compared to the resistant one, although the differences were not always significant. In Wang *et al.* (2025), PAO expression increased in potato tubers with storage time (0–12 d) but was not light-induced and dark-stored tubers had greater transcript levels after 12 d supporting our findings. Previous studies revealed a role of these genes in Chl retention during leaf senescence or yellowing, where mutations in PAO (Pružinská *et al.* 2005) in *Arabidopsis* lead to a stay-green phenotype. It was suggested that the low expression levels of Chl-biosynthesis genes and the high expression levels of Chl-catabolic genes lead to the absence of Chl in non-green carnation petals (Ohmiya *et al.* 2014) and white chrysanthemum petals (Ohmiya

et al. 2017). Thus, we hypothesise that the constitutively higher expression of PAO promotes an improved resistance to greening.

Conclusions: We propose that, upon exposure of tubers to light, the increasing expression of key chlorophyll-biosynthesis genes and decreasing expression of key chlorophyll-degradation genes drive tuber greening. Furthermore, the extent of up- and downregulation of these genes varies between varieties depending upon their relative resistance to tuber greening. Greening in the susceptible variety was associated with greater upregulation of chlorophyll-synthesis genes and greater downregulation of chlorophyll-degradation and cycling genes than in the greening-resistant variety. Several studies have identified and tested the potential of tuber-specific promoters to drive tuber-specific gene expression (Diretto *et al.* 2007, Li *et al.* 2013, Ha *et al.* 2015), therefore future investigations, manipulating the expression of the identified key genes, specifically in tubers, could assist in the development of greening resistant varieties.

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