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The modulation of light quality on carotenoids in maize (*Zea mays* L.) sprouts

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ABSTRACT

The present study aimed to identify the regulatory mechanisms of red, blue, and white light on carotenoid biosynthesis in maize sprouts. Determinations of carotenoid, chlorophyll and phytohormone profiles, as well as relative gene expression, were explored. The results identified enhancement of carotenoid and chlorophyll production as well as gene expression. Most notably, the expression levels of *CRY*, *HY5*, and beta-carotene 3-hydroxylase genes peaked under blue light. Photomorphogene-related hormone, auxins and strigolactone production was also altered under different lights and might have a role in carotenoid metabolism. Gibberellins competed with carotenoids for the precursor geranylgeranyl diphosphate and were hindered by certain light characteristics, probably via DELLA-PIF4 signalling. *ERF021* and *MYB68* were negative regulators of carotenoid biosynthesis in maize sprouts. These findings provide new insights into the light-regulated mechanism and biofortification of carotenoids in maize sprouts.

1. Introduction

Plant sprout foods, generated through germination of seeds, have been investigated widely and found to have high nutritional value, good bioavailability and putative health benefits as part of a healthy diet (Geng et al., 2021). For example, broccoli sprouts were detected early with anticancer capacity owing to the inducible function of either glucoraphanin or sulforaphane on detoxication enzymes (Fahey, Zhang, & Talalay, 1997). In addition, sprouting could increase the levels of vitamins and phenolic compounds in wheat (Zilic et al., 2014) and raise the contents of gamma amino butyric acid, phenolics and carotenoids in maize sprouts (Chalorcharoenying, Lomthaisong, Suriharn, & Lertrat, 2017). Therefore, sprouting is an effective means to accumulate bioactive compounds in plants. Maize is one of the staple crops in the world, and its sprouts are studied for maintaining or increasing the output of maize by enhancing the viability and resistance of maize plants. With prominent health-benefit compounds, maize sprouts have also been studied for facilitating specific nutritional fortification in the human daily diet (He et al., 2021).

Carotenoids, mainly synthesized in higher plants, endow color variation from yellow to red and resistance towards unfavorable growing conditions in plants (Rodriguez-Concepcion et al., 2018). Instead of synthesis, carotenoids are accessible to humans via the daily diet. Apart from the irreplaceable function of lutein and zeaxanthin as

Abbreviations: LED, light-emitting diode; cry, cryptoxanthin; PHOT1, phototropin 1; UVR, ultraviolet-B receptor; FC, fold change; FKF1, flavin-binding kelch repeat F-box protein 1; CRY, cryptochrome; PRR, pseudo-response regulator; COP1, constitutive photomorphogenic 1; PIF, phytochrome-interacting factor; HY5, elongated hypocotyl 5; GI, gigantean; ELF, early flowering; IAA, Auxin; JA, Jasmonic acid; CTK, Cytokinin; GA, Gibberellin; SA, Salicylic acid; SL, Strigolactone; OxIAA, 2-oxindole-3-acetic acid; SAG, salicylic acid 2-O-β-glucoside; 5DS, 5-deoxystrigol; IPM, indole-3-pyruvate monooxygenase; AUX, auxin influx carrier; LOX, lipoxygenase; AOS, allene-oxide synthase; 12-OPDA, (15Z)-12-oxophyto-10,15-dienoate; ACA, acetyl-CoA acyltransferase; JAZ, jasmonate ZIM domain-containing protein; ZOG, cytokinin-O-glucosyltransferase; CISZOG, *cis*-zeatin O-glucosyltransferase; AHP, histidine-containing phosphotransfer peotein; G2D, gibberellin 2beta-dioxygenase; MVA, mevalonate; MEP/DOXP, methylerythritol phosphate/deoxyxylulose phosphate; DXPR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; PSY, 15-*cis*-phytoene synthase; PDS, 15-*cis*-phytoene desaturase; Z-ISO, zeta-carotene isomerase; ZDS, zeta-carotene desaturase; CRTISO, carotenoid isomerase; LCYE, lycopene epsilon-cyclase; LCYB, lycopene beta-cyclase; CHYE, carotenoid epsilon hydroxylase; CHYB, beta-carotene 3-hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; LUT5, LUTEIN DEFICIENT 5.

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macular pigments in the eye retina, as well as the protective performance of carotenoids containing β-rings such as provitamin A in vision, carotenoids also serve as antioxidants that prevent chronic disease in humans (Rodriguez-Concepcion et al., 2018). The biosynthesis of carotenoids in plants is regulated by environmental impacts, including temperature, humidity, and light (Stanley & Yuan, 2019). However, as a family of natural pigments, carotenoids are strongly influenced by light, especially light quality (Stanley & Yuan, 2019).

In wheat sprouts, light-emitting diode (LED) technology was used to elevate the richest carotenoid content (Pham Anh et al., 2013). In addition, both chlorophyll and carotenoid pigments in alfalfa sprouts performed differently according to various light qualities (Fiutak, Michalczyk, Filipczak-Fiutak, Fiedor, & Surowka, 2019). Moreover, Ye et al. detected the impacts of light quality on phytohormone signal transduction in tea plants (Ye et al., 2021). Therefore, light quality might perform a regulatory network on carotenoids, involving several metabolites as well as regulatory proteins. As previously reported, light can be perceived by photosensory receptors, including red and far-red (phytochrome), blue (phototropins, cryptochromes, and Zeitlupes) and UV light receptors (UVR8) (Galvao & Fankhauser, 2015). Receptors then interact with ubiquitin E3 ligases (e.g. COP1/SPA, DDB1/DET1) and regulate downstream transcription factors (Legris, Ince, & Fankhauser, 2019). Among these genes, PIF and HY5, separately served as negative and positive regulators, conversely responding in dark and light conditions and antagonistically regulating carotenoid accumulation via the *PSY* (15-*cis*-phytoene synthase) gene, which encodes the initial key enzyme in carotenoid biosynthesis (Toledo-Ortiz, Huq, & Rodriguez-Concepcion, 2010; Toledo-Ortiz et al., 2014). Aside from those findings, the regulatory mechanism of light quality on carotenoids is still under investigation.

However, the light regulatory mechanism of carotenoids in edible maize sprout food has not yet been clearly studied. Therefore, aiming at fortifying the nutritional diet for consumers, this study, by combining determinations of carotenoids, chlorophyll and phytohormones with validations of relative gene expression profiles, could enlarge the theoretical knowledge of light quality regulation on carotenoids and thus facilitate the enhancement of carotenoids in maize sprouts.

2. Methods and materials

2.1. Maize sprout preparation

Maize seeds B73 were first soaked in 7 % NaClO solution for 10 min sterilization. After clear removal of the chemical residue with distilled water, maize seeds were soaked in distilled water for 10 h. Then, >30 seeds were evenly placed in 48 identical transparent plastic boxes with moist absorbent cotton and filter paper. Sets of 12 boxes were separately placed under 4 artificial incubators (l:w:h = 530 mm:470 mm:1000 mm). Except for the identical temperature and humidity conditions (28 °C, 95 %), the LED light qualities were set as red (λ = 620–625 nm, 6–7 μmol m^{-2} s^{-1}, R group), blue (λ = 460–465 nm, 15–16 μmol m^{-2} s^{-1}, B group) and white (6000 K, 24–25 μmol m^{-2} s^{-1}, W group) with a 24 h/0h photoperiod, and constant dark (D group) was set as a control. After 72 h of cultivation, maize sprouts were evenly collected from each box, quickly frozen in liquid nitrogen and stored at −80 °C until analysis. Sample profiles are shown in Fig. 1.

2.2. Carotenoid extraction and determination

Carotenoids in maize sprouts were extracted following previously reported method with modification (Xiang et al., 2019). Briefly, 1 g maize sprout powder was used for extraction. The extracted supernatant was collected and concentrated with nitrogen after purification. Then, 1 mL methyl *tert*-butyl ether (with 0.1 % 2,6-di-*tert*-butyl-4-methylphenol) solvent was added to dissolve extracts for determination.

Ten microlitres of filtered extracts was used for determination via reversed-phase high-performance liquid chromatography (HPLC). A column (YMC™ carotenoid 30, 5 μm packing, 4.5 × 250 mm, YMC CO., ltd., Bafan, Japen) at 25 °C and an HPLC system (Agilent 1260, Agilent Technologies, Inc. Palo Alto, USA) with a photodiode array detector were applied for measurement. The mobile phases and their gradient, as well as the detection conditions, were set according to a previous study (Xiang et al., 2019). Carotenoid isomers were identified and quantified via an external standard method. Standards were purchased from CaroteNature (Münsingen, Switzerland). The results are shown as the means ± SD μg g^{-1} FW in triplicate.

2.3. Chlorophyll extraction and determination

The extraction and determination were performed following the

Fig. 1. Morphological profiles of maize sprouts.

published method (Arnon, 1949) with modification. Briefly, 0.5 g maize sprout powder was weighed and added to 4 mL acetone. The mixture was extracted under ultrasonication for 30 min and centrifuged for 10 min. The supernatant was collected and measured for absorbance at both 646 nm and 663 nm wavelengths. The contents of chlorophyll *a* and *b* were calculated following the formula:

C_a (Chlorophyll *a*, mg L^{-1}) = $12.7A_{663nm}$-$2.69A_{646nm}$.

C_b (Chlorophyll *b*, mg L^{-1}) = $22.9A_{646nm}$-$4.68A_{663nm}$.

The total chlorophyll content was the sum of chlorophyll *a* and *b*. The results are presented as the mean ± SD μg g^{-1} FW (n = 3).

2.4. Phytohormone extraction and determination

Maize sprouts were powdered with liquid nitrogen. Fifty milligrams of the powder was weighed and blended with 10 μL standard solution (Olchemim/isoReag, methanol solvent, 100 ng mL^{-1}) and 1 mL methanol/water/formic acid (15:4:1, v/v/v) for extraction. The mixture was fully mixed with vortexing for 10 min and centrifuged at 12000 rpm for 5 min at low temperature. The supernatant was concentrated and redissolved in 10 μL 80 % methanol–water solution. Then, it was filtered through a 0.22 μm membrane for determination.

The determination was conducted by liquid chromatography-mass spectrometry (LC-MS). Ultra-performance liquid chromatography (ExionLC™ AD, SCIEX, Boston, USA) equipped with a 40 °C C18 column (1.8 μm, 100 mm × 2.1 mm i.d., Waters ACQUITY UPLC HSS T3, Milford, USA) was linked to a tandem mass spectrometer (MS/MS, QTRAP®6500+, SCIEX, Boston, USA). The mobile phase was A: 0.04 % acetic acid/ultrapure water, B: 0.04 % acetic acid/acetonitrile. The injection volume was 2 μL. The flow rate was 0.35 mL min^{-1} with the gradient elution as follows: 0 to 1.0 min A/B = 95:5(v/v); 1.0 to 8.0 min A/B changed from 95:5(v/v) to 5:95(v/v); 8.0 to 9.0 min, A/B = 5:95(v/v); 9.0 to 9.1 min, A/B changed from 5:95(v/v) to 95:5(v/v); 9.1 to 12.0 min, A/B = 95:5(v/v). The temperature of electrospray ionization was 550 °C. The voltages of positive ion mode and negative ion mode were 5500 V and 4500 V, respectively. The results were analyzed by Analyst 1.6.3 (SCIEX, Boston, USA) and MultiQuant 3.0.3 (SCIEX, Boston, USA) according to the standard curve. The results are shown as the mean ± SD ng g^{-1} FW (n = 3).

2.5. RNA sequencing and real-time quantitative PCR (RT-qPCR) analyses

Total RNA of maize sprouts was extracted, and RNA sequencing was conducted by BioMarker (Beijing, China). Total RNA was then reverse transcribed to cDNA using a FastKing RT kit with gDNase (Tiangen Biotech, Beijing, China). RT-qPCR was performed in a LightCycler® 480 Real Time PCR System (Roche ltd., Basel, Switzerland) with a SuperReal PreMix Plus Kit (Tiangen Biotech, Beijing, China). *ZmADF* was used as a reference gene, and primers were designed as follows: forward primer: 5′ - GACTTGGTGCTGCGAAAGAC – 3′; reverse primer: 5′ - GTCTTCTGGAAGCCATGAGGAT –3′. The other primers involved in this study are listed in Table A1. Relative expression levels were calculated according to the Ct value via the $2^{-\Delta\Delta Ct}$ method and were reported as the mean ± SE (n = 3).

2.6. Statistical analyses

Figures were depicted by Origin 2018 (OriginLab, Northampton, USA) and an online metabolic analytical website (MetaboAnalyst 5.0). The Pearson correlation analysis was performed by Origin 2018 (OriginLab, Northampton, USA). The one-way ANOVA was accompanied with Tukey's comparison post tests to identify the significant differences, which were conducted on IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, USA) ($p < 0.05$). All the measurements were taken three times, and the results are shown as the mean ± SD (n = 3).

3. Results

3.1. Carotenoid profiles and relative gene expression in maize sprouts

Generally, 10 kinds of carotenoids were detected in maize sprouts, as depicted in Fig. 2A ($p < 0.05$, Tukey's comparison post tests). Lutein was the most abundant carotenoid in maize sprouts, followed by neoxanthin, antheraxanthin and β-carotene. Different from the light quality-treated samples, sprouts growing in the dark only contain 6 kinds of carotenoids, including violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin and β-carotene, with lutein comprising approximately 44 % of the total. The varied light qualities induced the accumulation of carotenoids, especially the enhancement of blue and white light qualities. The W group enjoyed the highest content of carotenoids among those four groups at 46.69 ± 0.578 μg g^{-1} FW, while the B group ranked second ($p < 0.05$). When compared with the control, red, blue and white light qualities increased lutein content to 6.3-, 14- and 16-folds, respectively, with the highest lutein content detected in the W group as 20.65 ± 0.38 μg g^{-1} FW ($p < 0.05$). The enhancements of β-carotene by light qualities were largest among the detected components, as the contents in the R, B and W groups were 7.7, 21 and 22 times higher, respectively, than that in the D group. Aside from α-cryptoxanthin (α-cry), which showed the highest content in the B group, the other compounds exhibited the same patterns, as their contents were relatively lower in the R group but were similar, with higher values in both the B and W groups ($p < 0.05$).

The relative expression results of genes are depicted in Fig. 2B. Generally, geranylgeranyl diphosphate, which is generated via the mevalonate (MVA) and methylerythritol phosphate/deoxyxylulose phosphate (MEP/DOXP) pathways under the catalysis of several enzymes, including 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXPR), is the precursor of carotenoids. Then, following the functions of PSY1, 15-*cis*-phytoene desaturase (PDS), zeta-carotene isomerase (Z-ISO), zeta-carotene desaturase (ZDS) and carotenoid isomerase (CRTISO), lycopene was created. With the catalysis of lycopene epsilon-cyclase (LCYE) or lycopene beta-cyclase (LCYB), the synthesis of carotenoids was divided into two branches. Both carotenoid epsilon hydroxylase (CHYB) and LUTEIN DEFICIENT 5 (LUT5) promoted the production of β-cryptoxanthin (β-cry) from β-carotene and zeaxanthin from β-cry. Zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) participate in the xanthophyll cycle and modulate the transformations among zeaxanthin, antheraxanthin and violaxanthin. On the other hand, the catalysis of LCYE on lycopene gradually synthesized δ- and ε-carotene. LCYB then transformed carotene from the δ- to the α-configuration. Lutein was generated from α-carotene in two ways under the catalysis of carotenoid epsilon hydroxylase (CHYE), CHYB and LUT5. As investigated, the abundances of the genes *DXPR*, *PSY*, *LCYE* and *ZEP* were relatively high in maize sprouts. Most of the genes were upregulated under light qualities compared to the D group. Among these, the majority expressed the same high levels in both the B and W groups ($p < 0.05$, Tukey's comparison post tests). Notably, the upstream genes *PDS*, *ZDS* and *CRTISO* showed their highest expression values in the B group, whereas the relative expression levels of *CHYE* and *VDE* were high in the W group. In addition, light quality significantly upregulated *CHYB* by approximately 2.4-, 7.2- and 6.3-fold in the R, B and W groups, respectively, compared to the D group. Overall, the various gene expression levels were consistent with the carotenoid content.

3.2. Chlorophyll profiles and genes in the light signal transduction pathway in maize sprouts

The chlorophyll contents in maize sprouts are shown in Fig. 3A. The total chlorophyll contents in the D, R, B and W groups were 1.800 ± 0.240, 110.5 ± 1.69, 251.8 ± 6.64 and 292.2 ± 5.21 μg g^{-1} FW, respectively ($p < 0.05$, Tukey's comparison post tests). Chlorophyll *a* was more abundant in maize sprouts than chlorophyll *b*, comprising a majority of the total. In general, changes in chlorophylls showed that

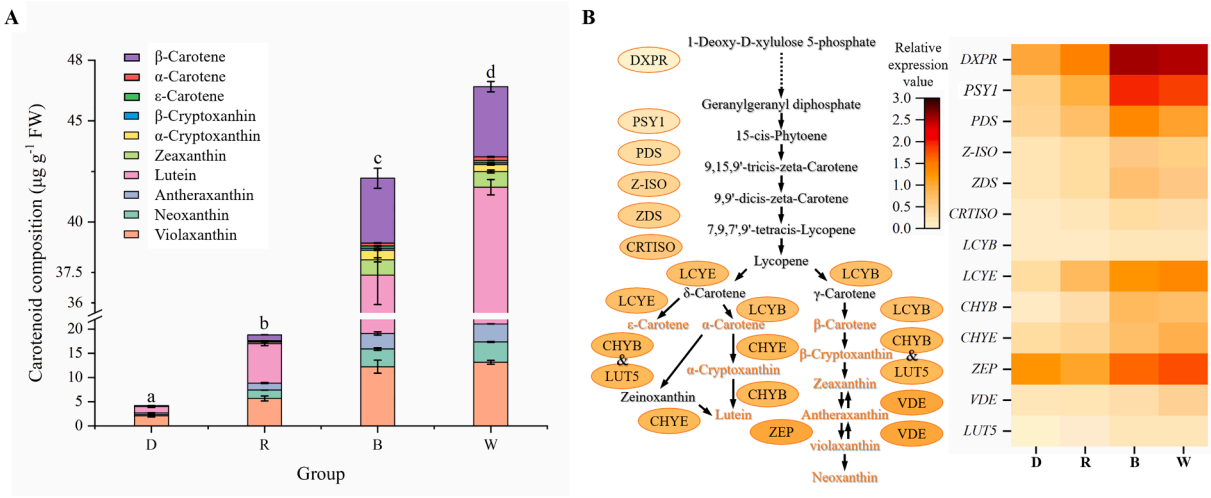

Fig. 2. Carotenoids profiles of maize sprouts. A: Carotenoid compositions in maize sprouts. Bars with no letters in common are significantly different ($p < 0.05$). B: The biosynthesis pathway of carotenoids and expressional patterns of relative genes. DXPR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase, PSY: 15-*cis*-phytoene synthase, PDS: 15-*cis*-phytoene desaturase, Z-ISO: zeta-carotene isomerase, ZDS: zeta-carotene desaturase, CRTISO: carotenoid isomerase, LCYE: lycopene epsilon-cyclase, LCYB: lycopene beta-cyclase, CHYE: carotenoid epsilon hydroxylase, CHYB: beta-carotene 3-hydroxylase, ZEP: zeaxanthin epoxidase, VDE: violaxanthin de-epoxidase, LUT5: LUTEIN DEFICIENT 5.

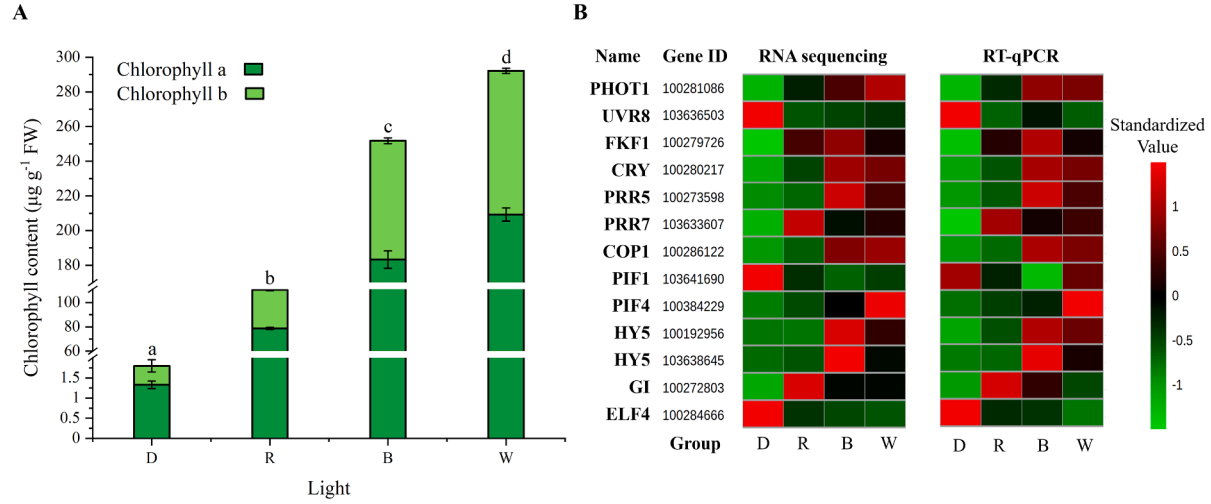

Fig. 3. Chlorophyll and light relative genes profiles in maize sprouts. A: Chlorophyll profiles of maize sprouts. Bars with no letters in common are significantly different ($p < 0.05$). B: The comparison of light relative gene expressions results from RNA sequencing and RT-qPCR. PHOT1: phototropin 1, UVR: ultraviolet-B receptor, FKF1: flavin-binding kelch repeat F-box protein 1, CRY: cryptochrome, PRR: pseudo-response regulator, COP1: constitutive photomorphogenic 1, PIF: phytochrome-interacting factor, HY5: elongated hypocotyl 5, GI: gigantea, ELF: early flowering.

light qualities significantly enhanced chlorophyll synthesis; in particular, the W group had the highest contents of chlorophyll *a*, *b* and total.

To identify the light quality regulatory mechanism on carotenoids in maize sprouts, apart from the determination of chlorophylls, the expression of genes involved in the light signal transduction pathway was also examined, as shown in Fig. 3B. The corresponding genes of light receptors as well as relative regulators were filtered with FPKM values among groups according to RNA sequencing results (fold change (FC) > 1.5, $p < 0.05$). In total, 13 genes were verified by RT-qPCR. *PHOT1* was upregulated, whereas *UVR8* was downregulated under light qualities compared to the control. *FKF1* and *CRY* were found to have higher expressional values under light qualities than in the D group, and their expressional levels were superior in the B group. In addition, the expression level of the *COP1* gene was enhanced when light quality varied from red to white. *PRR5* and *PRR7* separately exhibited dramatic increases in their expression values in the B and R groups. *PIF1* was highly expressed in the D group, while the expression of *PIF4* was enhanced in the W group. Two *HY5* genes were consistently upregulated in both the B and W groups, especially the remarkable increases in the B group. The expression of *ELF4* peaked in the D group. Distinctly, *GI* was light-enhanced, and the highest expression value was found in the R group.

3.3. Phytohormones and relative gene expression in maize sprouts

In total, 44 phytohormones were detected in maize sprouts and were classified into 8 kinds of plant hormones, including abscisic acid (ABA), auxin (IAA), cytokinin (CTK), ethylene (ETH), gibberellin (GA), jasmonic acid (JA), salicylic acid (SA), and strigolactone (SL) (Table 1, $p < 0.05$, Tukey's comparison post tests). As investigated, ABA content was enhanced in the W group (19.80 ± 1.271 ng g^{-1} FW) compared to the control. In addition, 13 kinds of IAA were included, among which L-tryptophan, indole-3-acetic acid, tryptamine and indole-3-acetyl-L-aspartic acid were reduced when light was present compared to the D

Table 1
The detected phytohormones in maize sprouts. (ng g^{-1} FW, mean ± SD, n = 3).

NO.	Compound (ng g^{-1})	Class	D	R	B	W
1	Abscisic acid	ABA	15.81 ± 0.833a	16.72 ± 0.121a	15.86 ± 0.192a	19.80 ± 1.271b
2	L-tryptophan	IAA	7366 ± 163.3c	7337 ± 21.12c	3847 ± 88.57a	5616 ± 226.3b
3	2-oxindole-3-acetic acid	IAA	1551 ± 120.6c	1013 ± 14.99b	803.0 ± 32.719a	735.8 ± 31.60a
4	1-O-indol-3-ylacetylglucose	IAA	467.5 ± 21.36b	357.0 ± 23.38a	487.3 ± 23.21b	450.4 ± 21.80b
5	Indole-3-acetic acid (IAA)	IAA	25.89 ± 0.537b	15.69 ± 0.729a	14.82 ± 0.572a	14.48 ± 0.156a
6	Tryptamine	IAA	16.95 ± 1.359b	5.670 ± 0.153a	5.961 ± 0.036a	7.490 ± 0.404a
7	Indole-3-carboxaldehyde	IAA	12.45 ± 0.692ab	11.65 ± 0.526a	13.872 ± 0.735bc	14.484 ± 0.621c
8	Indole-3-acetyl-L-aspartic acid	IAA	4.792 ± 0.224c	2.131 ± 0.080ab	1.907 ± 0.035a	2.448 ± 0.048b
9	Methyl indole-3-acetate	IAA	1.251 ± 0.091b	0.741 ± 0.017a	1.110 ± 0.043b	0.881 ± 0.068a
10	Indole-3-acetyl glutamic acid	IAA	1.181 ± 0.105bc	1.024 ± 0.013ab	1.238 ± 0.070c	0.985 ± 0.042a
11	Indole-3-carboxylic acid	IAA	0.671 ± 0.079	0.607 ± 0.016	0.622 ± 0.046	0.757 ± 0.077
12	3-Indoleacetonitrile	IAA	0.304 ± 0.036a	0.322 ± 0.020ab	0.366 ± 0.006b	0.298 ± 0.017a
13	N-(3-Indolylacetyl)-L-valine	IAA	0.214 ± 0.002a	0.220 ± 0.007ab	0.287 ± 0.016c	0.253 ± 0.023bc
14	Indole-3-acetyl-L-valine methyl ester	IAA	0.013 ± 0.001a	0.017 ± 0.001ab	0.019 ± 0.004b	0.015 ± 0.001ab
15	N6-Isopentenyl-adenine-7-glucoside	CTK	18.66 ± 0.305b	17.06 ± 0.934b	14.74 ± 0.823a	15.130 ± 0.416a
16	*trans*-Zeatin-O-glucoside	CTK	8.293 ± 0.537a	8.766 ± 0.575a	12.05 ± 0.654b	11.61 ± 0.626b
17	*cis*-Zeatin-O-glucoside riboside	CTK	3.824 ± 0.306b	3.202 ± 0.016a	4.526 ± 0.129c	4.891 ± 0.197c
18	*ortho*-Topolin-9-glucoside	CTK	2.056 ± 0.113c	1.738 ± 0.089b	1.267 ± 0.152a	1.215 ± 0.050a
19	*ortho*-Topolin	CTK	0.955 ± 0.039c	0.688 ± 0.049a	0.907 ± 0.069c	0.715 ± 0.062a
20	Kinetin riboside	CTK	0.860 ± 0.046a	2.219 ± 0.128b	2.395 ± 0.147b	3.102 ± 0.092c
21	N6-Benzyladenine-9-glucoside	CTK	0.454 ± 0.017c	0.233 ± 0.021b	0.151 ± 0.022a	0.165 ± 0.019a
22	6-Benzyladenine	CTK	0.328 ± 0.023c	0.277 ± 0.009bc	0.221 ± 0.024ab	0.170 ± 0.025a
23	*cis*-Zeatin riboside	CTK	0.184 ± 0.017a	0.269 ± 0.020a	0.510 ± 0.020b	0.518 ± 0.056b
24	2-Methylthio-*cis*-zeatin riboside	CTK	0.136 ± 0.015a	0.223 ± 0.01b	0.256 ± 0.019b	0.258 ± 0.020b
25	*para*-Topolin	CTK	0.125 ± 0.007	0.127 ± 0.007	0.130 ± 0.011	0.133 ± 0.010
26	*cis*-Zeatin-9-glucoside	CTK	0.078 ± 0.003a	0.074 ± 0.003a	0.081 ± 0.001a	0.096 ± 0.008b
27	*cis*-Zeatin	CTK	0.075 ± 0.013ab	0.051 ± 0.006a	0.081 ± 0.010b	0.090 ± 0.013b
28	N6-isopentenyladenine	CTK	0.044 ± 0.004b	0.026 ± 0.002a	0.030 ± 0.002a	0.034 ± 0.003a
29	N6-isopentenyladenosine	CTK	0.035 ± 0.004	ND	ND	ND
30	1-Aminocyclopropanecarboxylic acid	ETH	650.6 ± 59.65ab	740.6 ± 46.12b	555.3 ± 54.16a	571.4 ± 43.89a
31	Gibberellin A19	GA	57.17 ± 3.495c	31.95 ± 0.257a	59.89 ± 1.079c	47.83 ± 5.195b
32	Gibberellin A53	GA	9.666 ± 0.464c	4.025 ± 0.339a	11.83 ± 0.474d	6.864 ± 0.086b
33	Gibberellin A20	GA	2.260 ± 0.274c	1.633 ± 0.072b	ND	1.098 ± 0.092a
34	Gibberellin A15	GA	0.239 ± 0.071c	0.043 ± 0.013a	0.104 ± 0.018ab	0.158 ± 0.020bc
35	cis(+)-12-Oxophytodienoic acid	JA	32.15 ± 2.883a	27.45 ± 4.630a	51.33 ± 1.260b	67.10 ± 7.323c
36	Jasmonic acid	JA	12.89 ± 1.131c	8.500 ± 0.597b	5.548 ± 0.342a	13.52 ± 0.250c
37	3-oxo-2-(2-(Z)-Pentenyl) cyclopentane-1-butyric acid	JA	7.928 ± 0.140d	4.839 ± 0.221b	1.931 ± 0.076a	7.243 ± 0.073c
38	Jasmonoyl-L-isoleucine	JA	1.154 ± 0.026d	0.511 ± 0.034b	0.420 ± 0.044a	0.821 ± 0.010c
39	Dihydrojasmonic acid	JA	0.317 ± 0.018b	0.236 ± 0.044ab	0.215 ± 0.043a	0.207 ± 0.019a
40	Methyl jasmonate	JA	0.289 ± 0.026a	0.482 ± 0.011b	0.474 ± 0.031b	0.483 ± 0.043b
41	N-[(-)-Jasmonoyl]-(L)-valine	JA	0.050 ± 0.004c	0.022 ± 0.004a	0.017 ± 0.002a	0.041 ± 0.004b
42	Salicylic acid	SA	66.91 ± 0.482d	60.125 ± 1.210c	42.19 ± 2.704a	54.11 ± 2.748b
43	Salicylic acid 2-O-β-glucoside	SA	0.721 ± 0.116a	1.125 ± 0.203a	2.096 ± 0.006b	1.999 ± 0.273b
44	5-Deoxystrigol	SL	0.608 ± 0.069a	1.246 ± 0.186b	2.179 ± 0.220c	1.203 ± 0.191b

*No letters in common in each line stands for significant differences ($p < 0.05$).
** ND: No detected.

group. Compared to the D group, the inhibitory effect of blue light quality on L-tryptophan was dramatic, as the content dropped to 3847 ± 88.57 ng g^{-1} FW; red light quality uniquely decreased the content of 1-O-indol-3-ylacetylglucose. Indole-3-carboxaldehyde and N-(3-indolylacetyl)-L-valine slightly increased under light qualities compared to the D group. Six of the 15 detected CTKs had lower contents under light quality than under dark conditions. In contrast, another 6 compounds were abundant in the light quality groups. Moreover, red light quality decreased the content of *cis*-zeatin-O-glucoside riboside, while blue and white light qualities increased it. In contrast to the B group, both the R and W groups declined *ortho*-topolin when compared to the dark condition. The only detected 1-aminocyclopropanecarboxylic acid classified into ETH exhibited low values in both the B and D groups. Four gibberellins were detected, including GA15, 19, 20 and 53. They responded differently to light qualities. When compared to the D group, red and white light qualities had negative effects on their accumulation, whereas blue light quality exerted accumulation in GA53 content to 11.83 ± 0.474 ng g^{-1} FW but resulted in the loss of GA20 ($p < 0.05$, Tukey's comparison post tests). JA was reduced in the R and B groups, while methyl JA was increased in the light quality groups compared to the D group. Other phytohormones belonging to the JA group generally possessed the highest values in the D group. Meanwhile, SA showed its highest value in the D group and was strongly hindered by blue light quality. In contrast, salicylic acid 2-O-β-glucoside accumulated in both the B and W groups. 5-Deoxystrigol was significantly increased from 0.608 ± 0.069 ng g^{-1} FW in the dark to 2.179 ± 0.220 ng g^{-1} FW in blue light, approximately 3-fold ($p < 0.05$, Tukey's comparison post tests).

Fold change analysis of phytohormones was performed to identify the strong effects of light quality, and the outcomes are shown in Fig. 4A (FC > 2, $p < 0.05$). Generally, light qualities had negative influences on IAAs, GAs and the majority of JAs. Two CTKs positively responded to light qualities, whereas another CTK was reduced when lights were present. Red light quality significantly decreased GA15 and GA53. Blue and white light qualities obviously increased SAG content, and as mentioned above, blue light quality indeed dramatically improved the accumulation of SL, a carotenoid-derived phytohormone in maize sprouts, which at the same time indicated the regulatory function of blue light on carotenoids.

Viewing from the significant differences in phytohormones among groups, the biosynthesis pathways of the majority of the hormones in Fig. 4B are depicted, which are involved in tryptophan metabolism, linolenic acid metabolism, zeatin biosynthesis, diterpenoid biosynthesis and phenylalanine metabolism, and the signal transduction pathways are also included. In addition, the relative genes in those pathways with

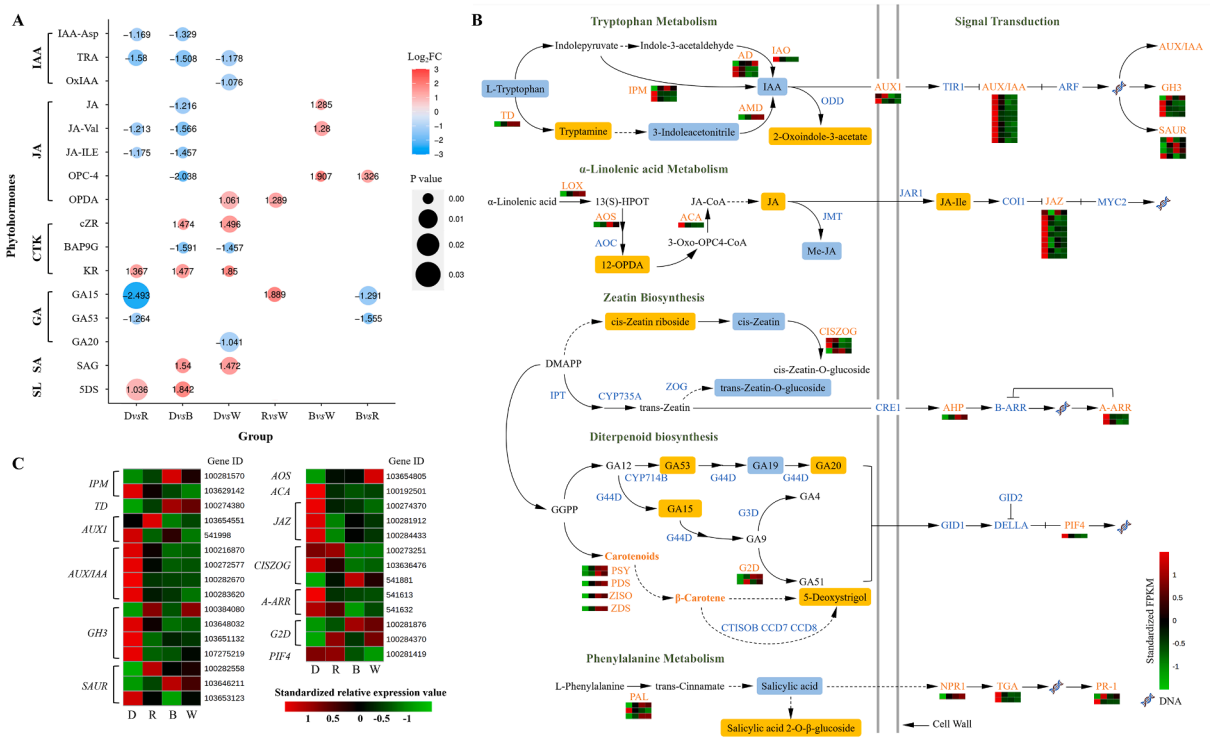

Fig. 4. Phytohormones and relative genes profiles in maize sprouts. A: The significantly varied phytohormones in maize sprouts. IAA: Auxin, JA: Jasmonic acid, CTK: Cytokinin, GA: Gibberellin, SA: Salicylic acid, SL: Strigolactone, IAA-Asp: Indole-3-acetyl-L-aspartic acid, TRA: Tryptamine, OxIAA: 2-oxindole-3-acetic acid, JA-Val: N-[(-)-Jasmonoyl]-(L)-valine, JA-ILE: Jasmonoyl-L-isoleucine, OPC-4: 3-oxo-2-(2-(Z)-Pentenyl) cyclopentane-1-butyric acid, OPDA: cis(+)-12-Oxophytodienoic acid, cZR: *cis*-Zeatin riboside, BAP9G: N6-Benzyladenine-9-glucoside, KR: Kinetin riboside, SAG: Salicylic acid 2-O-β-glucoside, 5DS: 5-Deoxystrigol. B: Biosynthesis and signal transduction pathways of phytohormones and the relative gene expression from RNA-seq results. The components in blue frame were detected with no significant changes among groups, whereas the components in orange frame changed among groups (FC > 2, $p < 0.05$). The genes in blue were detected with no significant changes among groups, whereas the genes in orange changed among groups (FC > 2, $p < 0.05$). TD: L-tryptophan decarboxylase, IPM: indole-3-pyruvate monooxygenase, AD: amidase, IAO: indole-3-acetaldehyde oxidase, AMD: amidase, ODD: 2-oxoglutarate-dependent dioxygenase, AUX1: auxin influx carrier, TIR1: transport inhibitor response 1, ARF: auxin response factor, LOX: lipoxygenase, 13(S)-HPOT: (9Z,11E,15Z)-(13S)-Hydroperoxyoctadeca-9,11,15-trienoate, AOS: allene-oxide synthase, AOC: allene-oxide cyclase, 12-OPDA: (15Z)-12-Oxophyto-10,15-dienoate, ACA: acetyl-CoA acyltransferase, Me-JA: Methyl jasmonate, JMT: jasmonate O-methyltransferase, JAR1: jasmonic acid-amino synthetase, COI1: coronatine-insensitive protein 1, JAZ: jasmonate ZIM domain-containing protein, DMAPP: Dimethylallyl diphosphate, GGPP: Geranyl diphosphate, IPT: isopentenyl transferase, ZOG: cytokinin-O-glucosyltransferase, CISZOG: *cis*-zeatin O-glucosyltransferase, CRE1: histidine kinase, AHP: histidine-containing phosphotransfer protein, B-ARR: two-component response regulator ARR-B family, A-ARR: two-component response regulator ARR-A family, G44D: gibberellin-44 dioxygenase, G3D: gibberellin 3beta-dioxygenase, G2D: gibberellin 2beta-dioxygenase, CTISOB: beta-carotene isomerase, CCD: carotenoid cleavage dioxygenase, GID: gibberellin receptor, PAL: phenylalanine ammonia-lyase, PR-1: pathogenesis-related protein 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

different expressional performances among groups according to the RNA sequencing results (FC > 2, $p < 0.01$) are shown in Fig. 4B as the standardized FPKM value. Changes in the relative expression values of 29 genes were verified by RT-qPCR, and the results are shown in Fig. 4C. The gene encoding L-tryptophan decarboxylase was apparently upregulated when light was present. An *IPM* (indole-3-pyruvate monooxygenase) gene was proven to have a light-inhibited pattern, while another was light enhanced. The expression values of all the *AUX/IAA* genes were lower in the light quality groups than in the D group. Generally, *GH3* genes were expressed at low levels under light qualities. *LOX* (lipoxygenase) and *AOS* (allene-oxide synthase), which encode enzymes participating in 12-OPDA synthesis, were upregulated by light quality. In contrast, *ACA* (acetyl-CoA acyltransferase) exhibited its highest expression value in the D group. Eight of the *JAZ* (jasmonate ZIM domain-containing protein) genes were inhibited by light qualities, while another one was dramatically downregulated in the R group compared to the D group. In zeatin biosynthesis, three *cis*-zeatin O-glucosyltransferase (CISZOG) genes were identified, but no differential expression occurred in *ZOG* genes. An *AHP* (histidine-containing phosphotransfer protein) gene in the signal transduction pathway was enhanced in the B group. Correspondingly, the genes encoding the two-component response regulator ARR-A family were downregulated by light quality. Concerning the biosynthesis of GAs, only *G2D* (gibberellin 2beta-dioxygenase) genes were found to have different expression patterns, as the two genes were highly expressed in light quality groups. A *PIF4* gene involved in signal transduction of zeatin was downregulated in the light quality groups. As depicted, the synthesis of carotenoids is closely related to the formation of 5-deoxystrigol. Five genes involved in carotenoid biosynthesis significantly increased their expression in light qualities (FC > 2, $p < 0.01$). For the biosynthesis of salicylic acid, the corresponding genes of phenylalanine ammonia-lyase were uniquely identified, and two of them were light-enhanced, while another one was light-inhibited. In the signal transduction pathway, *NPR1* was highly expressed, while *TGA* was expressed at low levels in the light quality groups.

3.4. The relative expression of transcription factors and correlation analysis

Thirty-one transcription factors and three transcription receptors (TRs) that were differentially expressed among groups (FC > 2, $p < 0.01$) were verified by RT-qPCR (Fig. 5A). The majority of the verified *AP2/ERFs* and *bHLHs* were inhibited by light quality. In contrast, blue light quality dramatically increased the expressional value of a *bZIP*

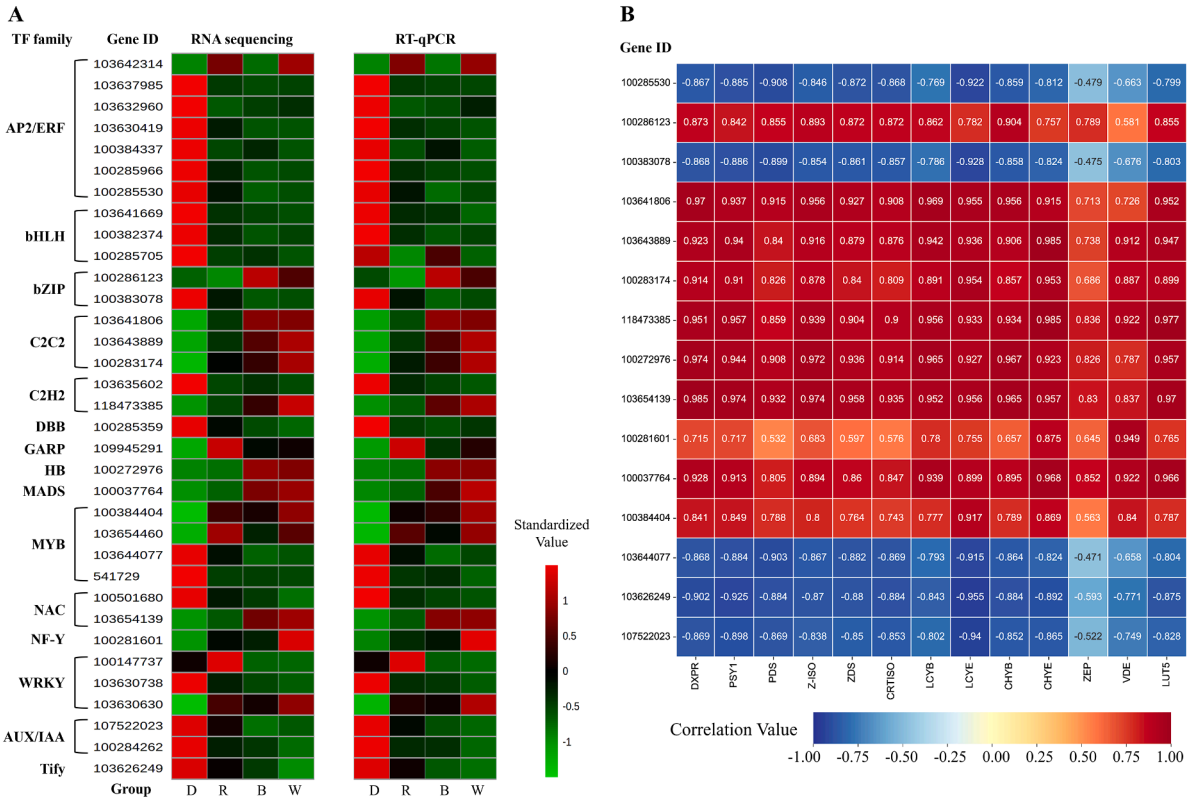

Fig. 5. The validated transcription factors profiles in maize sprouts. A: The comparison of transcription factors expressions results from RNA sequencing and RT-qPCR. B: The correlation values among TFs and relative genes in carotenoid biosynthesis pathway.

(GenBank ID: 100286123). In addition, light qualities exerted positive effects on the expression of *C2C2s*, especially the compound white light. Two genes from the C2H2 family exhibited disparity in expressional values among groups. *DBB* was inhibited by light quality, whereas *GARP* was distinctly highly expressed in the R group. Both the verified *HB* and *MADS* were obviously upregulated by blue and white light qualities. TFs from the MYB, NAC and WRKY families exhibited discrepant expression patterns among groups, while *NF-Y* increased its expression in light qualities, especially in white light. The two *AUX/IAA* and *Tify* TRs were downregulated in light quality treatments.

The correlation results of TFs and carotenoid biosynthesis genes (Fig. 5B, $p < 0.05$, Pearson correlation analysis), an *AP2/ERF* (preferred name: *ERF021*, GenBank ID: 100285530) and two TRs showed a negative correlation with upstream genes, including *DXPR*, *PSY1*, *PDS*, *Z-ISO*, *ZDS*, and *CRTISO*, and with three downstream genes, *LCYE*, *CHYB* and *CHYE*, in the carotenoid biosynthesis pathway. In contrast, the general positive correlations were among TFs and carotenoid biosynthetic genes except *ZEP*. A *bZIP* (preferred name: *HY5*, GenBank ID: 100286123) showed an apparent correlation value with the *CHYB* gene of 0.904. Distinctly, the *NF-Y* gene had a high correlation value with the *VDE* gene of 0.949. *MYB68* (GenBank ID: 100384404) was significantly correlated with *LCYE*, with a value of 0.917.

4. Discussion

4.1. Blue light quality was obviously perceived by photoreceptors and regulated downstream genes

Plants take advantage of a series of photoreceptors to accurately detect and respond to different light qualities over a broad range of wavelengths (Galvao & Fankhauser, 2015). Among the reported photoreceptors, we detected four genes that were differentially expressed under light quality treatments, including *UVR8*, which encodes UV-B photoreceptors, and three genes *PHOT1*, *CRY* and *FKF1*, which encode the blue light photoreceptors. As reported previously, *UVR8* was expressed in nonirradiated *Arabidopsis* and maize leaves, but the expression was reduced after irradiation (Fernández, Lamattina, & Cassia, 2020). Similarly, *UVR8* was exposed to light qualities and was downregulated in our results. In contrast, the expression of *PHOT1* was enhanced by irradiation. Evidently, PHOT1 in maize, participating in phototropic responses to blue light, could be activated by light irradiation and autophosphorylation (Suzuki et al., 2019). In addition, the expression levels of *CRY* and *FKF1* were enhanced by light quality in the present study, especially blue light quality. CRY can be activated by blue light and oligomerized for essential signalling roles on downstream genes (Shao et al., 2020). In addition, blue light-induced conformational and functional changes at the molecular level were well elucidated in FKF1, an F-BOX protein from the zeitlupe family with its light-sensing LOV domain (Ito, Song, & Imaizumi, 2012).

Aside from the investigation of blue light-activated photoreceptor genes, the differentially expressed downstream genes in the light-signal transduction pathway, including *COP1*, *PRR*, *GI*, *ELF*, *PIF* and *HY5*, were also detected. As a previous study investigated, CRY mediated GI accumulation by modulating the proteins that participated in regulating GI stability, COP1 and the clock regulator ELF3 (Suetsugu & Wada, 2012). Similarly, under the possible modulation of CRY, the *GI* gene in our study exhibited high relative expression values under light qualities, while the expression of *ELF4* was inhibited. Additionally, FKF1 could interact with GI under blue light treatment and thus sequester the interaction with PRR5; in contrast, FKF1 degraded PRR5 in a dark environment (Ito et al., 2012). In the analysed maize sprouts, *PRR5* reached its highest and lowest expression values under blue light quality and dark conditions, respectively, indicating the regulatory effect of *FKF1* on *PRR5*. There is long-standing evidence on the CRY-mediated regulation and stabilization of HY5 by interacting with the G-protein β subunit AGB1 (Lian et al., 2018) and the widely studied COP1/SPA

complex (Legris et al., 2019), supporting the highly expressed *HY5* in light qualities. In particular, blue light was suggested to positively regulate the accumulation of HY5/HYH via transcriptional and post-transcriptional mechanisms (Hajdu et al., 2018). Therefore, in this work, the dramatic upregulation of the *HY5* gene in blue light quality was probably regulated by the blue light photoreceptor CRY, which was proven by the same high expression values in the B group. Moreover, the antagonistic HY5-PIF regulatory module on photosynthetic pigment was well discussed (Toledo-Ortiz et al., 2014). Correspondingly, our results identified a downregulated *PIF1* gene in light qualities, and the reduction was more obvious in blue light quality. In general, blue light quality was distinctly perceived by CRY and transduced the light signal by mediating the up- and downregulation of *HY5* and *PIF1*, respectively, in maize sprouts.

4.2. The functions of light signal-related genes in carotenoid biosynthesis

As a kind of pigment that expands the range of light absorption during photosynthesis, carotenoids have been studied for their various patterns under light quality. By applying different light qualities to buckwheat sprouts, Tuan et al. investigated the accumulative trends of carotenoids, as the highest contents were identified in white light-treated samples, with lutein and β-carotene being the most abundant (Pham Anh et al., 2013). Previously, Zhang et al. (Zhang et al., 2012) demonstrated that, in contrast to red light, blue light had significant impacts on both the transcriptional level and contents of carotenoids in citrus. As detected in our study, α-cry and its downstream gene *CHYB* were enhanced by blue light quality, which indicated the potential regulatory mechanism of blue light quality on carotenoid biosynthesis.

According to published works, studies of light-signal transduction regulation of carotenoid biosynthesis have extensively focused on the synthetic gene *PSY*. As such, the negative regulatory effect of PIFs on *PSY* expression has been well studied, as PIFs directly bind to the promoter of *PSY* and inhibit its expression in dark conditions, triggering the degradation of HY5; in contrast, when exposed to light, the released HY5 accumulates and activates *PSY* as well as carotenoid biosynthesis (Quian-Ulloa & Stange, 2021). PIFs also served as regulators in inhibiting chlorophyll biosynthesis and promoting the elongation of hypocotyls to search for light (Quian-Ulloa & Stange, 2021). In the present findings, the various patterns of chlorophyll, carotenoids and related genes involved in light-signal transduction (*PIFs* and *HY5s*) and carotenoid synthetic pathways (*PSY*) were consistently expressed according to published essays. However, the investigated superiority of blue light quality in triggering carotenoid biosynthesis has not been clearly studied before. Previously, two separately located *cis*-acting elements on the *PSY* promoter induced by red and blue lights were detected (Welsch, Medina, Giuliano, Beyer, & von Lintig, 2003), which indicated the differential regulation of carotenoid biosynthesis by the two wavelengths of light. In addition, Tuan et al. witnessed a 27.35 % increase in total carotenoid content as well as the greatest increase in the expression of *CHYB* (*SbCHXB* in the cited work) in blue light-exposed *Scutellaria baicalensis* (Tuan et al., 2017). In our work, the higher content of α-cry as well as the highest expression value of *CHYB* in the B group indicated an increasing flux to generate lutein. Statistically, the lutein content in the B group was 88.56 % of that in the W group. Together with the findings in the light-signal transduction pathway, we inferred that the blue light photoreceptor CRY might play a predominant role in perceiving monochromatic blue light and transduced light signals to regulate the expression of *HY5* and *PIF1* in maize sprouts, hence resulting in the increased synthesis of carotenoids by activating the *PSY* gene and prominently enhancing lutein biosynthetic flux by modulating *CHYB*. Therefore, the application of blue light to cultivate maize sprouts could serve as an energy-saving strategy for relatively high lutein supplementation.

4.3. The potential regulatory roles of phytohormones on carotenoid accumulation in light qualities

Phytohormones are essential for plant growth and development and serve as important regulators of the photomorphogenesis of chloroplasts in illuminated plants (Müller & Munné-Bosch, 2021). Moreover, light often influences hormone levels, such as the reported effects of light intensity and spectral composition on phytohormones in tea plants (Ye et al., 2021). Hence, the study of phytohormones in light quality-treated maize sprouts would enrich the knowledge of their profiles under illumination as well as their regulatory roles on maize sprout metabolism.

Carotenoids are a group of isoprenoids that are generated from the MVA and MEP/DOXP pathways. They not only serve as precursors for the synthesis of ABA and SL in plants but also under the regulation of phytohormones (Stanley & Yuan, 2019). In addition, as depicted in Fig. 4B, the biosynthesis of carotenoids is alternatively related to the generation of GAs and CTKs, as they share the same precursor, dimethylallyl diphosphate. As previously observed in tomato, IAA acted antagonistically to ETH in regulating fruit ripening, while the silencing of auxin response factor 2 altered the expression patterns of *SlPSY1*, *SlPDS*, *SlZDS*, *SlLCYB* and *SlCHYB* (Stanley & Yuan, 2019). In addition, the key enzyme in CTK biosynthesis, IPT4, mediates the expression of several genes in lycopene biosynthesis (Stanley & Yuan, 2019). In maize, the modulatory roles of phytohormones on carotenoids were previously focused on (Battal, Erez, Turker, & Berber, 2008), which initiated the investigation of the relationships between phytohormones and carotenoids; however, the inner network has not yet been discussed; hence, the regulation of phytohormones on carotenoid accumulation in maize sprouts was discussed in the present study.

Our results demonstrated the enhancement of carotenoids in light quality-treated maize sprouts, along with the significant reductions of three IAAs and increments on a SL phytohormone. On the one hand, it was reported in *Arabidopsis* that the genes in the light signal-transduction pathway, *PIF4* and *PIF5*, could directly combine with the promoter of the *YUC* gene (IPM in Fig. 4B) (Hornitschek et al., 2012), thus exhibiting light regulatory roles in IAA synthesis. On the other hand, it is well known that both carotenoid-derived SL and IAA can act in lateral shoot branching and root development (Yu, Chen, Wang, & Lou, 2021). In our results, along with the low expressional value of *PIF1* under light qualities, two *IPMs* were downregulated and resulted in the reduction of IAA and its downstream product, OxIAA. In particular, the various patterns were more obvious in the B group than in the other light-treated groups. Consistently, the precursor of SL, 5DS, was most greatly enhanced by blue light quality. In addition, at the morphological level, hypocotyl elongation in the light quality groups was correspondingly inhibited compared to that in the D group (Fig. 1). As transcriptionally studied, SL are potential positive regulators of light harvesting and can interact with IAA in tomato seedlings (Yu et al., 2021). Therefore, we depicted a possible light regulatory network involving carotenoids, IAA and SL, as *PIF1* was depleted in light qualities and hindered the biosynthesis of IAA by modulating the *YUC* gene; moreover, carotenoids accumulated under the regulation of the HY5-PIF module and consequently generated SL. IAA and SL together performed in morphogenesis of maize sprouts. Importantly, the regulation might be specifically enhanced by blue light quality.

Previously, an apparent decrease in carotenoid content was detected in GA-treated citrus, which indicated the potential negative modulatory role of GA (Zhang et al., 2012). In addition, a reduction in GA in exposed *Arabidopsis* seedlings was detected and indicated the importance of light in repressing GA signalling (Achard et al., 2007). Furthermore, under light conditions, as low GA levels, DELLA protein, which localizes to the signalling pathway of GA, accumulates and sequesters PIF4 and PIF5 from their target genes (Lau & Deng, 2010). It was reported that PIF4 could interact with the DELLA protein and participate in modulating GA signal transduction in maize (Shi et al., 2018). Practically, the present study detected the downregulation of *PIF4* (Fig. 4B) in maize sprouts

under both blue and white light qualities, as well as the consistent expression profiles of genes that encoded DELLA. Hence, we inferred that DELLA protein was stimulated by light qualities and consumed in hindering PIF4 function on its downstream genes, provoked the biosynthesis of carotenoids, which competed for the precursor geranylgeranyl diphosphate with GA, thus resulting in the low level of GA in the currently studied maize sprouts.

4.4. Possible functional TFs involved in carotenoid accumulation in maize sprouts

Many studies have identified the transcriptional regulation of carotenoid accumulation by TFs. ERFs often participate in ETH signalling and regulate ethylene-responsive genes for fruit ripening. Carotenoid accumulation is relatively associated. Previously, Lee et al. (Lee et al., 2012) reduced the expression of *SlERF6* via the RNAi method and reported the accumulation of both carotenoid and ETH in tomato, thus demonstrating the possible negative regulatory role of *SlERF6* on carotenoid biosynthesis. This is similar to the negative correlation of *ERF021* with the carotenoid biosynthetic genes found in our results. In addition, negative correlations between *MYB68* and related genes in the carotenoid biosynthesis pathway were also detected in our results. Evidently, *CrMYB68* was identified to have a repressive role on *CrBCH2* and could regulate the formation of α- and β-branch carotenoids (Zhu et al., 2017). Apart from the abovementioned results, the possible regulatory role of *bZIP* TF (preferred name HY5, GenBank ID: 100286123) on *CHYB* expression in carotenoid metabolism was identified in our results as the high correlation value 0.904 shown and particularly provided evidence for the regulatory role of *HY5* on *CHYB*. Therefore, the possible regulatory role of blue light on lutein biosynthesis via the light-signal transduction pathway in maize sprouts was elucidated in the present work and requires further investigation and verification.

5. Conclusion

In general, apart from investigating the improvements in carotenoid content and biosynthesis by light quality, the results indicated the regulation of blue light quality on enhancing lutein biosynthesis by modulating the light signal-transduction pathway involving *CRY* and *HY5*, as well as a gene in the synthetic pathway, *CHYB*. In addition, the increasing carotenoid derivative SL might be involved in carotenoid metabolism with the decreased IAAs. The biosynthesis of carotenoids was provoked under light qualities and competed for the precursor geranylgeranyl diphosphate with GA. Correlation analyses indicated the negative regulatory roles of *ERF021* and *MYB68* on carotenoid biosynthesis in maize sprouts. Overall, the given results could enlarge the knowledge of the carotenoid regulatory mechanism and facilitate nutritional biofortification in maize sprouts. Further studies could be performed at the protein and molecular levels to verify the exact regulatory mechanism.

CRediT authorship contribution statement

Nan Xiang: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Investigation, Visualization. **Yihan Zhao:** Methodology, Validation, Software, Investigation, Data curation. **Siyun Wang:** Writing – review & editing, Data curation. **Xinbo Guo:** Conceptualization, Supervision, Project administration, Writing – review & editing, Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100128.

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