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L-Tryptophan synergistically increased carotenoid accumulation with blue light in maize (*Zea mays* L.) sprouts

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ABSTRACT

In the present study, L-tryptophan was applied in combination with blue light to modulate carotenoid biosynthesis in maize sprouts. The profiles of carotenoids, chlorophylls, and relative genes in carotenoid biosynthesis and light signaling pathways were studied. L-tryptophan and blue light both promoted the accumulation of carotenoids, and their combination further increased carotenoid content by 120%. L-tryptophan exerted auxin-like effects and stimulated *PSY* expression in blue light exposure maize sprouts, resulting in increased α- and β- carotenes. L-tryptophan could also play a photoprotective role through the xanthophyll cycle under blue light. In addition, *CRY* in the light signaling pathway was critical for carotenoid biosynthesis. These findings provide new insights into the regulation of carotenoid biosynthesis and L-tryptophan could be used in conjunction with blue light to fortify carotenoids in maize sprouts.

1. Introduction

Maize (*Zea mays* L.) is an important cereal for human daily intake and a major source of global food supply (Palacios-Rojas et al., 2020). In addition to nutritious carbohydrates such as starch and dietary fiber, maize contains functional phytochemical including polyphenol and carotenoids that are beneficial to consumer's health (Sheng et al., 2018). Cost-saving and effective sprouting techniques that enrich nutrients have been widely applied to cereal grains for a period of time (Singh et al., 2015). Germinated maize sprouts and seedlings were identified to have enhanced carotenoid and total phenolic contents (Chalorcharoenying et al., 2017).

Carotenoids, a group of pigments that endow plant tissues with diverse color, are produced by the terpenoid backbone pathway (Rodriguez-Concepcion et al., 2018). In addition to the provitamin A activity of β-ring-containing carotenoids, the antioxidant activity of carotenoids also plays an important role in reducing the risk of degenerative chronic diseases (Rodriguez-Concepcion et al., 2018). During crop cultivation, environmental factors, especially lights, usually play a crucial role in modulating the compositions and contents of carotenoids, which are perceived by photoreceptor and transduced to downstream regulators to initiate carotenoid biosynthesis (Saini & Keum, 2018). As in a recent study, a larger proportion of blue light is considered to be a better condition for carotenoid accumulation in pak choi sprouts (Frede & Baldermann, 2022). In addition, the application of external hormones such as ethylene and auxins may be involved in the light-mediated regulation of carotenogenesis during tomato ripening (Cruz et al., 2018). Thus, it is speculated that the combination of hormones and light

Abbreviations: OCP, Orange Carotenoid Protein; GGDP, Geranylgeranyl diphosphate; 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; NXS, neoxanthin synthase; NXD1, NEOXANTHIN-DEFICIENT 1; HPLC, high-performance liquid chromatography; PHOT1, phototropin 1; FKF1, flavin-binding kelch repeat F-box protein 1; CRY, cryptochrome; COP1, constitutive photomorphogenic 1; PIF, phytochrome-interacting factor; FAD, flavin adenine dinucleotide; HY5, protein long hypocotyl 5.

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conditions has an extraordinary effect on carotenoids accumulation.

In our previous work, maize sprouts exposed to blue light significantly accumulated carotenoids and retained a significant low level of L-tryptophan as compared to dark-grown maize sprouts (Xiang et al., 2022). L-tryptophan is an essential amino acid for human and is a biologically active auxin precursor (Mustafa et al., 2018). With its pivotal role in regulating plant growth and development, L-tryptophan has been widely applied exogenously to improve crop growth and productivity (Mustafa et al., 2018). It has been reported that different concentration of L-tryptophan applied by seed priming, foliar applying or soil applying have varying degrees of increase in shoot and root lengths, yields, moisture, protein, and chlorophyll contents of maize (Mustafa et al., 2018). The enhanced effect of L-tryptophan on carotenoid content in snapdragon was also found in a previous study (Aziz et al., 2009). In addition, the tryptophan residue in Orange Carotenoid Protein (OCP) is required for its stability in the dark state, and OCP is involved in the light response of keto-carotenoid (Maksimov et al., 2020).

Basing on these findings, the exogenous application of L-tryptophan is supposed to influence the biosynthesis of carotenoids in maize sprouts, and may synergize with blue light to play a positive role in carotenoid accumulation. Furthermore, how L-tryptophan and blue light modulate carotenoid biosynthesis remains to be explored. Therefore, this study combined blue light treatment and L-tryptophan, applied to maize sprouts, with the aim of identifying possible modulatory mechanisms and providing an efficient sprouting technique to produce nutritious maize sprouts.

2. Methods and materials

2.1. Sample preparation

Maize seeds B73 were used for germination. Seeds were sterilized with 7 % NaClO solution for 10 min and then washed with distilled water. After soaking in distilled water for 12 h, seeds were evenly placed into transparent plastic boxes with moist absorbent cotton and filter paper. The absorbent cotton in each box was infiltrated with an equal volume of distilled water, 20 $mg{\cdot}L^{-1}$ L-tryptophan solution or 100 $mg{\cdot}L^{-1}$ L-tryptophan solution. The plastic boxes were placed in two identical artificial incubators at a temperature of 28 °C and a humidity of 95 %. The light conditions of the artificial incubators were separately set as dark for 24 h a day and blue light (λ = 460–465 nm, 15–16 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$) for 24 h a day. Spray the sprouts after 24 h and 48 h of cultivation. After culturing for 72 h, maize sprouts were collected and labelled as D0, D20, D100, B0, B20 and B100 according to the light conditions and the concentrations of treated L-tryptophan solution (D: dark grown sprouts, B: blue light exposure sprouts; 0: distilled water treated, 20: 20 $mg{\cdot}L^{-1}$ L-tryptophan solution treated, 100: 100 $mg{\cdot}L^{-1}$ L-tryptophan solution treated). Each treatment contained eight boxes as biological replications. Maize sprouts were quickly frozen by liquid nitrogen and stored at −80 °C until analysis. L-tryptophan was purchased from Shanghai yuanye Bio-Technology Co., ltd (Shanghai, China) and solution was made before use.

2.2. The extraction and determination of carotenoids

Carotenoids were extracted according to the previously reported method (Xiang et al., 2019). Maize sprouts were ground into powder for extraction. Two grams of powder was weighted and mixed with the extract solution. After saponification, the mixture was extracted three times, and the supernatant was collected and concentrated with nitrogen. Extracts were dissolved in 1 mL of methyl *tert*-butyl ether (containing 0.1 % 2,6-di-*tert*-butyl-4-methylphenol) solvent and stored at −20 °C until determination.

A reported reversed-phase high-performance liquid chromatography (HPLC) system was used for the determination of carotenoids (Xiang et al., 2022). Twenty microliters of filtered extract were injected for determination. The gradient elution method was set according to the previous study (Xiang et al., 2019). The identification and quantification of carotenoid isomers were carried out by external standard method, and the standards were purchased from CaroteNature (Münsingen, Switzerland). The results were presented as the mean ± SD $\mu g{\cdot}g^{-1}$ FW in triplicate.

2.3. The extraction and determination of chlorophylls

Chlorophylls were extracted and determined according to the published study (Xiang et al., 2022). Briefly, 0.1 g of maize sprouts powder was weighted for extraction. After being thoroughly mixed with acetone, the mixture was centrifugated to measure absorbances at wavelengths of 646 and 663 nm. The contents of chlorophyll *a* and *b* were calculated according to the following equations:

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

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

Results were expressed as mean ± SD $\mu g{\cdot}g^{-1}$ FW in triplicate.

2.4. RNA extraction, reverse transcription and real-time quantitative PCR

The total RNA of maize sprouts was extracted using the RNA Easy Fast Plant Tissue Kit (TIANGEN BIOTECH Co., ltd., Beijing, China). Reverse transcription was completed with a FastKing gDNA Dispelling RT SuperMix (TIANGEN BIOTECH Co., ltd., Beijing, China) in a Bio-Rad Thermal Cycler (T100, Bio-Rad Laboratories Inc., Hercules, USA). The generated cDNA was used to operate real-time quantitative PCR (RT-qPCR) with a Master qPCR Mix (SYBR Green I) kit (Tsingke Biotechnology Co., ltd., Beijing, China) in a Bio-Rad CFX96 PCR System (Bio-Rad Laboratories Inc., Hercules, USA). The reference gene and primers used were listed in Table 1. The relative expression levels of genes were calculated vby the $2^{-\Delta\Delta Ct}$ method and were reported as the mean ± SE in triplicate.

2.5. Statistical analysis

The one-way ANOVA method was accompanied with Tukey's comparison post-tests to analyze the significant differences of contents and gene expression levels among groups on IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, USA) ($p < 0.05$). The Pearson correlation analysis was performed by Origin 2018 (OriginLab, Northampton, USA). Figures were depicted by Origin 2018 (OriginLab, Northampton, USA) and an online website (www.bioinformatics.com.cn) with modification.

3. Results

3.1. The effects of L-tryptophan and blue light on the compositions of carotenoids and chlorophyll in maize sprouts

The carotenoid compositions in maize sprouts were listed in Table 2. As shown, nine carotenoid compositions were detected in the present study. Unlike maize sprouts grown under blue light, those grown in the dark contained only four to five carotenoid compositions, with lutein being the highest. The content of lutein and β-carotene ranked the top two respectively in the maize sprouts irradiated with blue light. Among all experimental groups, the carotenoid content was peaked at the B100 group as 21.88 ± 0.88 $\mu g{\cdot}g^{-1}$ FW. By comparing the total carotenoid content of maize sprouts grown under different light conditions, it was found that the content of B0, B20, and B100 groups was 13, 10, and 11 times higher than that of D0, D20, and D100 groups, respectively. Under both dark and blue light conditions, spraying L-tryptophan had a significant effect on the accumulation of carotenoid compositions, as shown in Table 2. Under dark condition, treatment with 20 $mg{\cdot}L^{-1}$ L-tryptophan uniquely raised the content of antheraxanthin, while 100 $mg{\cdot}L^{-1}$ L-tryptophan led to the accumulation of zeaxanthin. In addition, treatments with both concentrations of L-tryptophan increased lutein

Table 1
The sequence of primers used.

Gene Name	Gene ID	Primer	Sequence (5′-3′)
ADF	542445	Forward	GACTTGGTGCTGCGAAAGAC
		Reverse	GTCTTCTGGAAGCCATGAGGAT
PSY1	100136882	Forward	GTCCGAGCAGAAGGTCTACG
		Reverse	TCCGAGGTAAAACGTCTTGG
PSY2	542686	Forward	CAGGTCTCACGGAAGAGGAC
		Reverse	CACGCTTGGTGAAGTTGTTG
PSY3	109940986	Forward	ACCAGCTCACCAACATCCTCA
		Reverse	GGAGCAGAGAAGACCACACCG
PDS	103633372	Forward	TCTGTTTGGCGAGCTTAGGA
		Reverse	AGGTGCTGGCAAAGTTTCTG
Z-ISO	100194203	Forward	ACTTCGCCGGGATACTAGGT
		Reverse	GTAAACACGCTCCCCCACTA
ZDS	542604	Forward	TCCGCCTCATGAAGAAGGTT
		Reverse	AGCCCGAACAACTGGACTAA
CRTISO	100281747	Forward	GGGGAAGCTCTGGGTACTAC
		Reverse	CCCAACTGCTTCCAATGCTT
LCYB	100383002	Forward	CTCGTCCCTCCTCATCTGTG
		Reverse	CGTAGAGGAAGGTGGGGATG
LCYE	100280448	Forward	TGGCGTGACATACCTGAACT
		Reverse	AGCCTGCAAATTATCTCGCG
CHYB	100281693	Forward	CTGCCGCTCACAAGATACAC
		Reverse	TCCTTTTCCAGCTCGTCCAG
CHYE	103637620	Forward	CGGCGTGCAATAGTTGATGA
		Reverse	CAACACATTTCCGAGGACCC
LUT5	103627895	Forward	AGACTATATCCACAGCCGCC
		Reverse	CTTCTGCATCATCCCAGTGC
ZEP	100285076	Forward	AAGCGAACATGGTACCTGGA
		Reverse	TTCACCCGGAACATAGCCTT
VDE	100281366	Forward	GTCCTGAGCCTCCTCTTGTG
		Reverse	TCAGTCGCTTCCATGTTGAG
NXD1	100217307	Forward	GCCTGCTAGGATCTGGAGTG
		Reverse	TGGACGAGTACCCGACTTTC
PHOT1	100281086	Forward	GTTCTCCCGCACCTGTCCTA
		Reverse	GAACGCCATCCACTTCTCCA
FKF1	100279726	Forward	AAGGGATGCCACTGTCAAGC
		Reverse	AAGGACCAAGCGGTTACCAA
CRY	100280217	Forward	GAGCTTGGGTACGGCGATAT
		Reverse	TCACAAAGGCAGAGGGGTCA
COP1	100286122	Forward	TGTCCAGTTGTTTCCTTTCGTG
		Reverse	GTCAGCTGACCATCTGCAAAAC
PIF1	103641690	Forward	CGTGGTCCACCGCTATTCAA
		Reverse	CAGGGTCCAAACCACCATCAT
PIF4	100384229	Forward	TGCCAAGGCCAGTCGGTTTA
		Reverse	TGCCATCCGCTCCTCATCAC

content in maize sprouts compared with distilled water. Diversely, under blue light condition, the antheraxanthin of both the B20 and B100 groups were enhanced, while the increment of β-carotene was only found in the B100 group as compared to the B0 group. In addition, L-tryptophan treatment had no effect on the lutein content in maize sprouts. Zeaxanthin unexpectedly reduced under the exertion of L-tryptophan, while the content of α-carotene slightly increased with the treatment of high concentration of L-tryptophan. Overall, blue light increased carotenoid level in maize sprouts as compared to dark condition. L-tryptophan acted as a positive inducer of carotenoid accumulation in maize sprouts regardless of the applied light condition. And the combination of L-tryptophan and blue light exerted a synergistic enhancement on carotenoid biosynthesis in maize sprouts.

As shown in Fig. 1, both chlorophyll *a* and b were detected in maize sprouts. In maize sprouts grown under dark condition, chlorophyll *a* accounted for the majority of the total, about 70 %. The application of high concentration of L-tryptophan significantly decreased the contents of chlorophyll *a* and total chlorophyll. As for the maize sprouts illuminated by blue light, the ratio of chlorophyll *a* and *b* were comparable. Although the chlorophyll content in the B20 group did not increase significantly, the chlorophyll *a*, *b* and total chlorophyll contents in the B100 group reached 331.8 ± 23.2, 340.5 ± 20.1 and 672.3 ± 43.3 $\mu g \cdot g^{-1}$ FW, respectively, which were 1.29-, 1.44- and 1.36-fold as compared to the B0 group. Thus, L-tryptophan treatment had differential effects on maize sprouts grown under dark and blue light conditions, as it inhibited chlorophyll accumulation under dark condition but accrued chlorophyll under blue light.

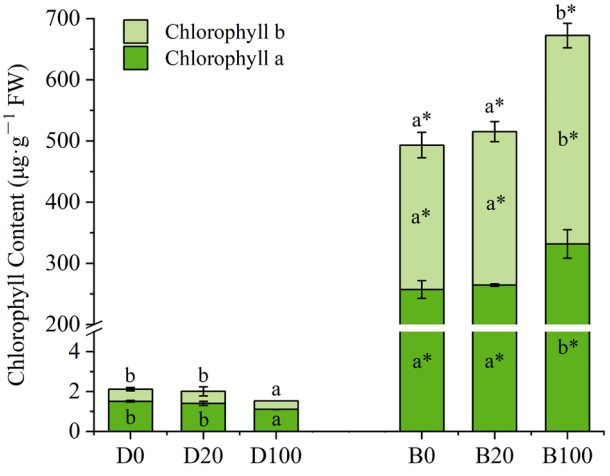


Fig. 1. Chlorophyll content of maize sprouts. The significant difference analysis was operated separately in the D and B groups. No letters in common stands for the significant differences ($p < 0.05$) in columns of each composition. '*' in the B group stands for the significant differences as compared to the D group at the same concentration of L-tryptophan ($p < 0.05$). D0, D20, D100 separately stand for distilled water, 20 $mg \cdot L^{-1}$ L-tryptophan, and 100 $mg \cdot L^{-1}$ L-tryptophan treatment under dark condition while B0, B20, B100 separately stand for distilled water, 20 $mg \cdot L^{-1}$ L-tryptophan, and 100 $mg \cdot L^{-1}$ L-tryptophan treatment under blue light condition.

Table 2
Carotenoid composition of maize sprouts (mean ± SD, n = 3).

Contents ($\mu g \cdot g^{-1}$ FW)	D0	D20	D100	B0	B20	B100
Violaxanthin	0.286 ± 0.011abα	0.302 ± 0.011bα	0.270 ± 0.008aα	0.781 ± 0.006ABβγ	0.731 ± 0.004Aβγ	0.803 ± 0.044Bγ
Neoxanthin	ND	ND	ND	0.816 ± 0.038AB	0.926 ± 0.077B	0.763 ± 0.032A
Antheraxanthin	0.050 ± 0.008aα	0.285 ± 0.043bα	0.099 ± 0.005aα	0.352 ± 0.004Aα	3.977 ± 0.175Bβγ	4.221 ± 0.408Bβ
Lutein	0.898 ± 0.039aα	1.308 ± 0.059bα	1.274 ± 0.061bα	11.13 ± 1.08β	10.96 ± 0.64β	10.40 ± 0.43β
Zeaxanthin	ND	ND	0.178 ± 0.019α	0.337 ± 0.028Bβ	0.221 ± 0.015Aα	0.211 ± 0.008Aα
α-Cryptoxanthin	ND	ND	ND	0.125 ± 0.007	0.131 ± 0.015	0.128 ± 0.013
α-Carotene	ND	ND	ND	0.065 ± 0.001A	0.068 ± 0.010A	0.106 ± 0.001B
β-Carotene	0.153 ± 0.007α	0.153 ± 0.001α	0.153 ± 0.023α	4.288 ± 0.119Aβ	4.662 ± 0.267Aβ	5.242 ± 0.256Bγ
Total	1.387 ± 0.043aα	2.047 ± 0.019bα	1.974 ± 0.039bα	17.90 ± 1.08Aβ	21.68 ± 0.75Bγ	21.88 ± 0.88Bγ

a-b: Different letters in the same row denote significant difference ($p < 0.05$) in dark-grown maize sprouts.
A-B: Different letters in the same row denote significant difference ($p < 0.05$) in blue light exposure maize sprouts.
α-γ: Different Greek letters in the same row denote significant difference ($p < 0.05$) between dark-grown and blue light exposure maize sprouts.
D0, D20, D100 separately stand for distilled water, 20 $mg \cdot L^{-1}$ L-tryptophan, and 100 $mg \cdot L^{-1}$ L-tryptophan treatment under dark condition while B0, B20, B100 separately stand for distilled water, 20 $mg \cdot L^{-1}$ L-tryptophan, and 100 $mg \cdot L^{-1}$ L-tryptophan treatment under blue light condition.

3.2. The effects of L-tryptophan and blue light on the expression profiles of relative genes in carotenoid biosynthesis

Fig. 2 exhibited the biosynthesis pathway of carotenoids and relative expression levels of structural genes. Carotenoids are synthesized from geranylgeranyl diphosphate (GGDP). With the catalyzation of 15-*cis*-phytoene synthase (PSY), 15-*cis*-phytoene desaturase (PDS), zeta-carotene isomerase (Z-ISO), zeta-carotene desaturase (ZDS), and carotenoid isomerase (CRTISO), lycopene is created. It can then be cyclized into two configurations, δ- and γ-carotenes, via lycopene beta-cyclase (LCYB) and lycopene epsilon-cyclase (LCYE), respectively. β-Carotene, synthesized by the sequential action of LCYB, is the precursor to synthesize β-cryptoxanthin and zeaxanthin under the catalyzation of LUTEIN DEFICIENT 5/ beta-carotene 3-hydroxylase (LUT5/CHYB). Zeaxanthin, antheraxanthin, and violaxanthin can be converted by zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) to establish the xanthophyll cycle in plants. ε-Carotene and α-carotene are produced from δ-carotene under the catalyzation of LCYE and LCYB, respectively. Lutein is then created from α-carotene through two pathways involving LUT5/CHYB and carotenoid epsilon hydroxylase (CHYE).

Among the three detected *PSY* genes, the expression value of *PSY1* was the highest. Expression levels of *PSY1/2/3* showed consistent trends among groups, as they were downregulated by L-tryptophan treatment in dark-grown maize sprouts, but were upregulated by L-tryptophan treatment in blue light-exposed maize sprouts. Compared with the D0 group, the relative expression levels of *Z-ISO*, *LCYE*, *CHYB*, *VDE* and *NXD1* (NEOXANTHIN-DEFICIENT 1) in the D20 and D100 groups were all lower, while the *PDS* and *ZEP* genes were uniquely downregulated by high concentration of L-tryptophan. However, gene expression patterns in maize sprouts exposed to blue light were diverse. Compared with the B0 group, L-tryptophan treatment enhanced the expression levels of *LCYE*, *ZEP* and *NXD1*. Distinctively, 20 $mg{\cdot}L^{-1}$ L-tryptophan treatment led to a decrease in the expression level of *CRTISO*, but an increase in the expression levels of *CHYE* and *VDE*. Overall, the expression level of structural genes was higher in maize sprout exposed to blue light than in the dark-grown one. L-tryptophan negatively and positively affected gene expression in maize sprouts grown under dark and blue light conditions, respectively. For *PDS*, *Z-ISO*, and *CHYB*, the effect of L-tryptophan disappeared in blue light-exposed maize sprouts as compared to the dark-grown one. In contrast, L-tryptophan had a unique influence on the expression profiles of *CRTISO* and *CHYE* in blue light-exposed maize sprouts. Furthermore, *LCYE*, *ZEP*, *VDE* and *NXD1* in maize sprouts grown under blue light dark conditions responded oppositely to L-tryptophan treatment.

3.3. The effects of L-tryptophan and blue light on the expression profiles of genes in light signal transduction pathway

In order to verify the mechanism by which L-tryptophan modulates carotenoid metabolism via the light signal transduction pathway, the expression levels of relative genes were evaluated by RT-qPCR, and the results are shown in Fig. 3 and listed in Table A.1. Based on the previous study (Xiang et al., 2022), six genes validated by transcriptomic results, including three genes encoding blue light receptors, phototropin 1 (PHOT1), flavin-binding kelch repeat F-box protein 1 (FKF1) and cryptochrome (CRY), *COP1* (constitutive photomorphogenic 1), and two *PIF*s (phytochrome-interacting factor) were selected in the present study. As depicted, *CRY* had the highest expression abundance among the three genes encoding blue light receptors. Blue light activated the expression of both *PHOT1* and *CRY*, and the expression of *CRY* was uniquely influenced by L-tryptophan. L-tryptophan treatment downregulated *CRY* in dark-grown maize sprouts, and conversely, it significantly upregulated *CRY* in blue light-exposed maize sprouts, but high concentration resulted in only a slight enhancement. The varied expression profile of *COP1* in blue light-exposed maize sprouts was similar to that of *CRY*, and notably, no variation was detected in dark-grown one. L-tryptophan negatively affected the expression levels of *PIF*s. In general, blue light increased the expression of *CRY* and *COP1*. L-tryptophan negatively regulated the expressions of *CRY*, *COP1*, and *PIF1* in the dark-grown maize sprouts. Diversely, L-tryptophan played positive roles on the expressions of *CRY* and *COP1*, but negatively influenced the expressions of *PIF*s when the blue light was exerted on maize sprouts.

4. Discussion

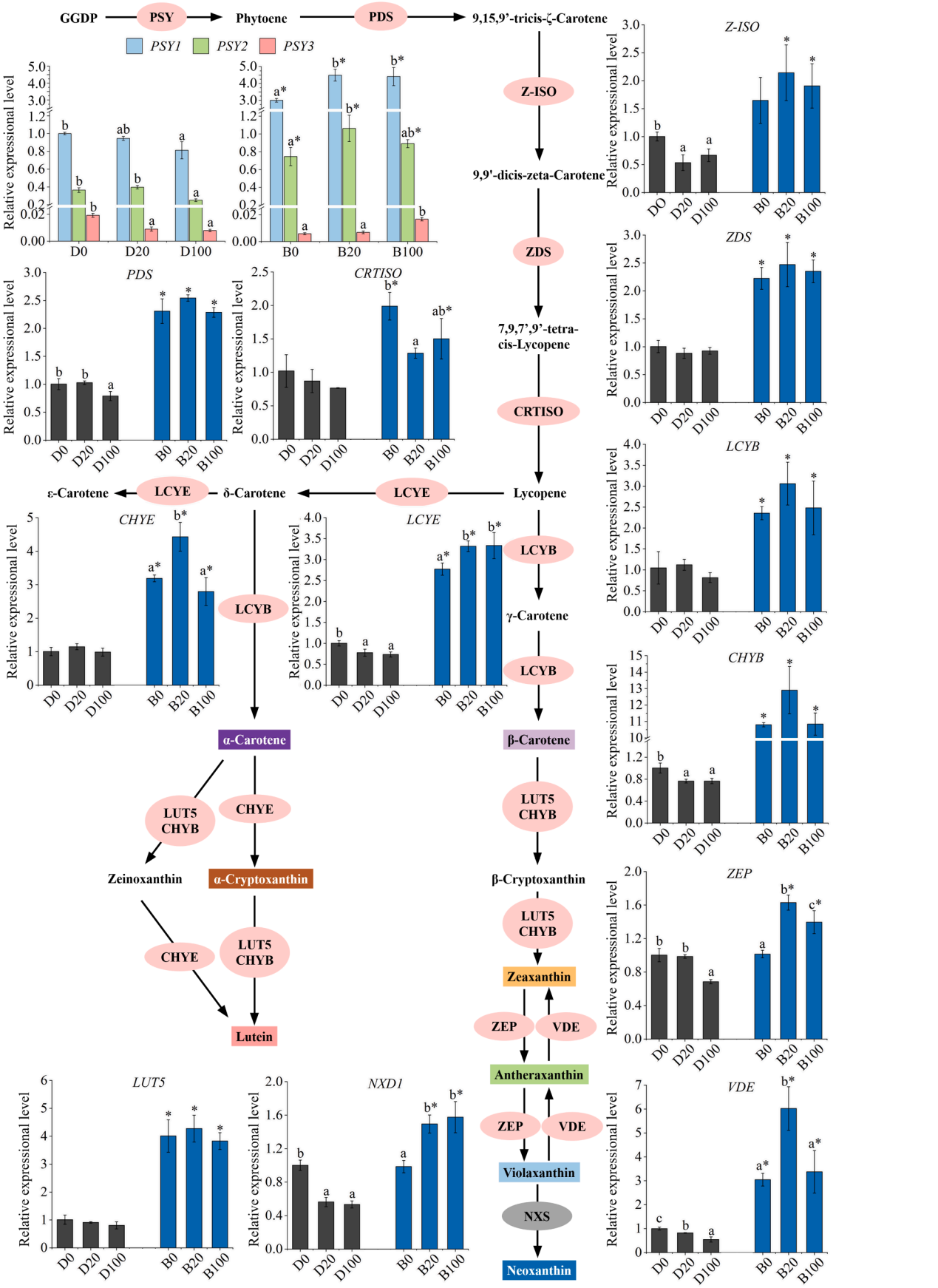
4.1. The enhanced photosynthetic efficiency of maize sprouts treated with L-tryptophan and blue light

Light has long been considered as a principal component of plant growth and development. As natural pigments, carotenoids are mainly synthesized in photosynthetic tissues of plants, such as sprouts, leaves, and fruits, and are produced in large quantities under light conditions (Quian-Ulloa & Stange, 2021). Notably, blue light around 400–480 nm can positively affect carotenoid biosynthesis, which has been previously reported in maize sprouts (Xiang et al., 2022), pak choi (Frede & Baldermann, 2022), etc. Along with the upregulation of structural genes, the total carotenoid content in maize sprouts was significantly increased by blue light as compared to dark condition in this study.

In addition, spraying with L-tryptophan resulted in prominent increases of α- and β- carotenes in the B100 group, which was accompanied by the upregulations of *PSY*s and the constant expression patterns of *CHYE* and *CHYB* compared with the B0 group. In contrast, *CHYE* and *CHYB* were highly expressed in the B20 group, consuming α- and β-carotenes, resulting in unchanged contents. Considered to be the rate-limiting and key enzyme for carotenoid biosynthesis in a wide range of plant species, PSY has been practically applied in transgenic plants to produce surprising amounts of β-carotene (Beyer et al., 2002). Pearson correlation analysis were applied on results of expressional levels of upstream structural genes and carotenoid compositions under blue light (Fig. 4A). It showed that *PSY* genes performed higher correlation values with both α- and β-carotenes as compared to other genes. In addition, *PSY1* had a high correlation value with total carotenoid content ($r = 1.00$). Therefore, high concentration of L-tryptophan contributed to the upregulation of *PSY* and the accumulation of carotenes in maize sprouts irradiated with blue light. Correspondingly, blue light conspicuously accumulated chlorophylls in maize sprouts compared to the dark-grown one. Compared with the B0 group, L-tryptophan (100 $mg{\cdot}L^{-1}$) significantly increased the chlorophyll content. Both chlorophyll and carotenoids are key photosynthetic pigments that absorb light energy, and their increments can improve photosynthetic efficiency by absorbing light energy and self-protecting the photosynthetic apparatus (Liu et al., 2020). Therefore, the application of L-tryptophan under blue light condition was considered to increase the photosynthetic efficiency of maize sprouts.

4.2. Regulation of carotenoids biosynthesis by L-tryptophan via PSY gene

Although there is no evidence for the direct interaction between L-tryptophan and carotenoids, exogenous L-tryptophan can significantly alter carotenoid accumulation in plants. For example, foliar application of L-tryptophan increased total carotenoid content and drought stress tolerance in wheat (Jamil et al., 2018). To the best of our knowledge, L-tryptophan is a biologically active auxin precursor, that is involved in auxin production in plants under the single or multiple catalysis of tryptophan aminotransferase, tryptophan decarboxylase, aldehyde oxidase, and YUC family of flavin-containing monooxygenase through four pathways, including the indole-3-pyruvic acid pathway, indole-3-acetamide pathway, tryptamine pathway, and indole-3-acetaldoxime pathway (Mustafa et al., 2018). According to Muneer et al. (Muneer et al., 2009), the application of L-tryptophan had positive effects on maize appearance such as cell expansion and shoot growth, exerting an



(caption on next page)

Fig. 2. The biosynthesis pathway of carotenoid and relative expression profiles of structural genes. The significant difference analysis was operated separately in the D and B groups. No letters in common stands for the significant differences ($p < 0.05$) in columns. '*' in the B group stands for the significant differences as compared to the D group at the same concentration of L-tryptophan ($p < 0.05$). GGDP: Geranylgeranyl diphosphate, 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, NXS: neoxanthin synthase, NXD1: NEOXANTHIN-DEFICIENT 1. D0, D20, D100 separately stand for distilled water, 20 mg·L^{-1} L-tryptophan, and 100 mg·L^{-1} L-tryptophan treatment under dark condition while B0, B20, B100 separately stand for distilled water, 20 mg·L^{-1} L-tryptophan, and 100 mg·L^{-1} L-tryptophan treatment under blue light condition.

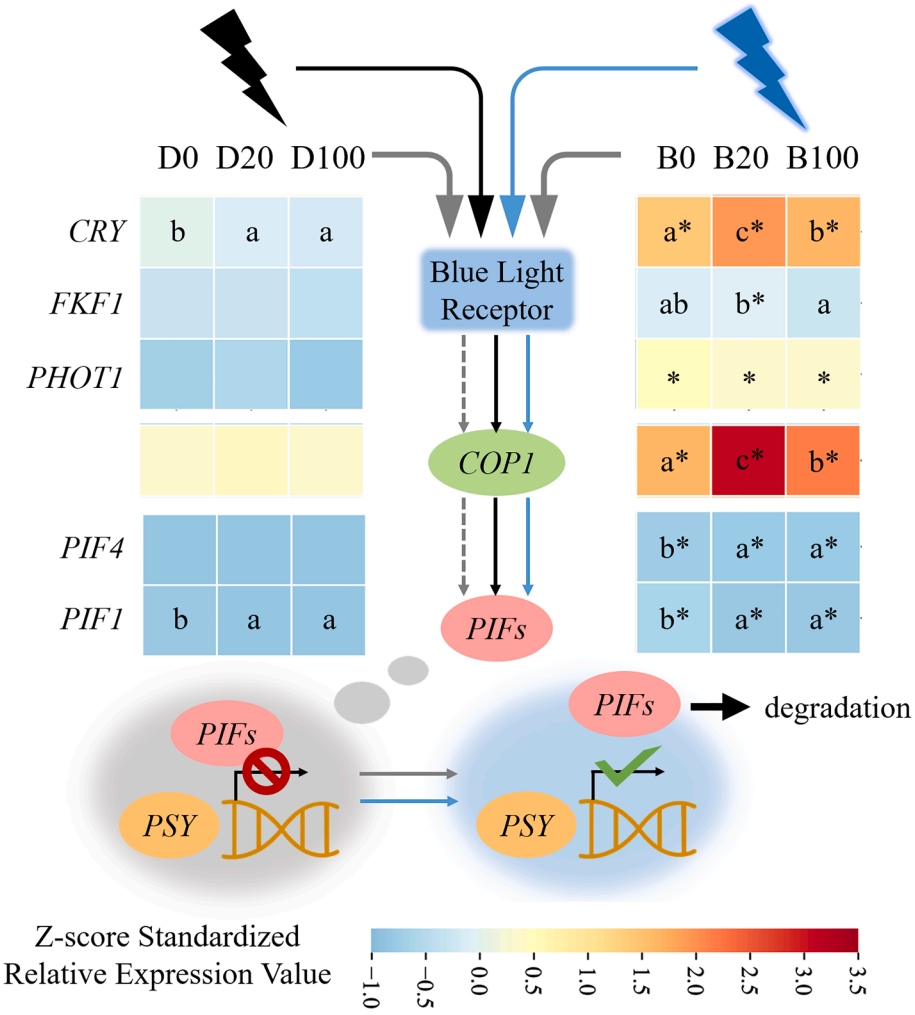


Fig. 3. The selected light signaling pathway. The grey, blue, and black arrowed line separately refer to the signal transduction of L-tryptophan, blue light and dark condition, while the discontinuous line was the speculation in the present study. The significant difference analysis was operated separately in the D and B groups and shown as different lower case letters ($p < 0.05$). '*' in the B group stands for the significant differences as compared to the D group at the same concentration of L-tryptophan ($p < 0.05$). PHOT1: phototropin 1, FKF1: flavin-binding kelch repeat F-box protein 1, CRY: cryptochrome, COP1: constitutive photomorphogenic 1, PIF: phytochrome-interacting factor. D0, D20, D100 separately stand for distilled water, 20 mg·L^{-1} L-tryptophan, and 100 mg·L^{-1} L-tryptophan treatment under dark condition while B0, B20, B100 separately stand for distilled water, 20 mg·L^{-1} L-tryptophan, and 100 mg·L^{-1} L-tryptophan treatment under blue light condition.

auxin-like function in regulating plant growth. The direct modulation of auxins on carotenoid accumulation is still lacking in identification, but the crosstalk between auxin and carotenoid derivative strigolactones has been well-elucidated (Faizan et al., 2020). Auxin treatment could compensate and increase carotenoid synthesis in citrus treated with a combination of gibberellin and prohydrojasmon, which could only exhibit poor color at the mature stage (Ma et al., 2021). In addition, auxin could induce a novel transcription factor *CsULT1* in *Crocus sativus*, whose overexpression led to the enhanced expressions of *PSY*, *PDS*, *BCH* and *CCDs* (carotenoid cleavage dioxygenases) in *Crocus calli* (Ashraf et al., 2015). Furthermore, enrichment of putative brassinosteroids-auxin response elements in the promoter of *PSY* supports a role for auxin in regulating *PSY* expression (Meier et al., 2011). Hence, L-tryptophan could regulate *PSY* and accumulated carotenoids by acting as an auxin-like hormone in maize sprouts.

4.3. L-Tryptophan could alleviate light damage via the xanthophyll cycle

The xanthophyll cycle mainly consist of zeaxanthin, violaxanthin, and antheraxanthin, which has been well-studied to dissipate light energy in excess of photosynthetic requirements through non-photochemical quenching in higher plants (Fernandez-Marin et al., 2021). However, there is also de-epoxidation of xanthophylls in the dark, which is regulated by both VDE and ZEP (Fernandez-Marin et al., 2021). Under dark condition, uniquely downregulated *ZEP* allowed zeaxanthin to be detected in the D100 group. Evidently, ZEP has been reported to degrade under stress and lead to the accumulation of zeaxanthin (Bethmann et al., 2019; Schwarz et al., 2015). Compared with the D0 group, a downregulation of *VDE* was detected in the D20 group, thus reducing the conversion from antheraxanthin to zeaxanthin, resulting in an increased antheraxanthin content. Therefore, although the exogenous L-tryptophan decreased the relative expressions of upstream genes, it enhanced the de-epoxidation of xanthophylls, resulting in the

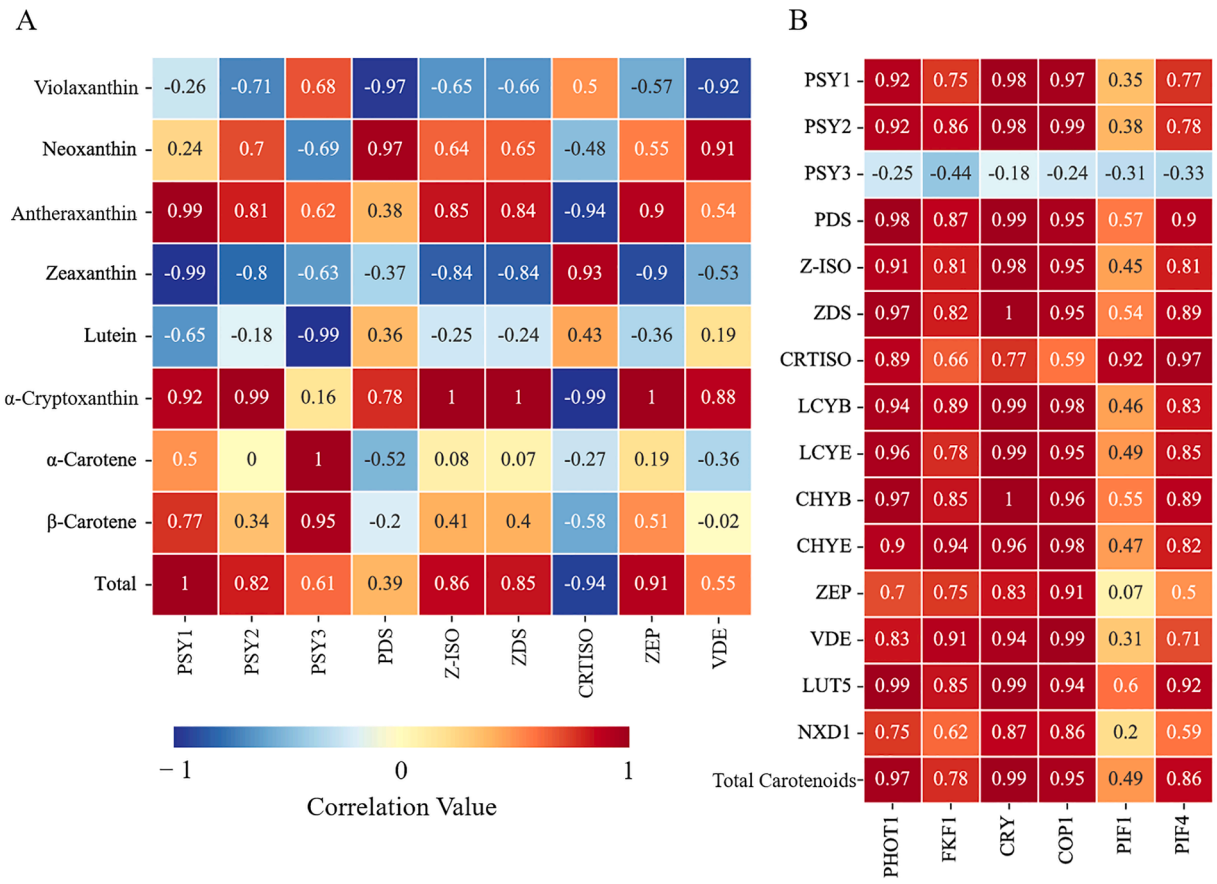


Fig. 4. Correlation analysis ($p < 0.05$). A: The correlation analysis between structural genes and carotenoid compositions (including total carotenoid) in blue light-exposed maize sprouts. B: The correlation analysis among structural genes, total carotenoid and light signal relative genes in all groups of maize sprouts.

accumulation of carotenoids in dark-grown maize sprouts.

Under blue light condition, *ZEP* and *VDE* were upregulated by L-tryptophan, which indicated that the flow of xanthophyll cycle was stimulated. Consequently, compared with the B0 group, the antheraxanthin of the B20 group was significantly increased, and the zeaxanthin of the B100 group was significantly decreased. Pearson correlation results also indicated a high positive value between *ZEP* and antheraxanthin as well as a negative correlation between *VDE* and violaxanthin (Fig. 4A, $r = 0.90$ and $r = -0.92$). Xanthophyll cycle pigments has been studied with superior de-epoxidation state under blue light compared with other spectrum of light qualities (Costa et al., 2013). Therefore, the application of L-tryptophan accelerated the conversion of zeaxanthin to antheraxanthin, indicating that the damage caused by the excessive energy of blue light was alleviated.

4.4. Regulation of carotenoids biosynthesis by L-tryptophan via light signaling pathway

Existing studies have elucidated the regulatory role of light on carotenoid biosynthesis. Typically, light signals are perceived and signaled by photoreceptors, including phytochromes (for red and far-red light), phototropins, cryptochromes and zeitlupes (for blue light), as well as UV light receptors that can interact with ubiquitin E3 ligases (such as COP1/SPA, DDB1/DET1) (Legris et al., 2019). Downstream transcription factors *PIF* and *HY5* (Protein long hypocotyl 5) act antagonistically to regulate the expression of structural genes in carotenoid biosynthesis, especially *PSY* (Toledo-Ortiz et al., 2014). Under dark condition, PIF1 binds to the G-box in the *PSY* promoter to repress its expression, or the expressions of other structural genes in the carotenoids biosynthesis pathway. When photoactivated phytochromes interact with and degrade PIFs, these structural genes are derepressed, and at the same time, HY5 binds to the G-box in the *PSY* promoter, enhancing its expression and increasing carotenoid biosynthesis (Toledo-Ortiz et al., 2014).

Cryptochromes are defined by a common two-domain structure containing the chromophore flavin adenine dinucleotide (FAD). When light is present, FAD receives electrons relayed through three evolutionarily conserved tryptophan residues from its oxidized and inactive state in the dark, thereby reducing, these changes morphologically affect the CRY molecules and increase the affinity of CRYs for other proteins (Wang & Lin, 2020). In the present study, the addition of L-tryptophan had a significant effect on the gene expression of CRY, which may reflect the changes in the protein configuration of CRY. Correlation analysis indicated high correlation values between *CRY* and structural genes in the carotenoids biosynthesis pathway, including *PSY1*, *PSY2*, *PDS*, *Z-ISO*, *ZDS*, *LCYB*, *LCYE*, *CHYB*, and *CHYE* (Fig. 4B, $r >= 0.96$). In addition, the correlation value between *CRY* and total carotenoid content was 0.99. Previously, the *cry1a* mutants showed that CRY played an important role in the accumulation of carotenoid compositions including phytofluene, phytoene, lycopene, and β-carotene in tomato fruits (C.-C. Liu et al., 2018). Furthermore, light-regulated genes including *DcCRY2* were supposed to be involved in the regulation of carotenoid synthesis during storage root development of carrots in the dark. (Arias et al., 2020). Therefore, the accumulation of carotenoids may be affected by the expression of *CRY* in maize sprouts under the application of L-tryptophan. In addition, regulatory signals might be transferred to PIFs, resulting in decreased expression of *PIFs*, and the binding of PIFs to the *PSY* promoter was released, thereby increasing the expression of *PSY* in the B20 and B100 groups.

Moreover, the change trend of chlorophylls under L-tryptophan

treatment was similar to that of the relative genes in carotenoid biosynthesis and *CRY*. GGDP is the precursor of both carotenoids and chlorophylls. And both chlorophyll *a* and *b*, as well as carotenoids are located in the chloroplasts of the photosynthetic active organs of plants, in order to fulfill the important roles in the process of photosynthesis (Quian-Ulloa & Stange, 2021). It has been identified in *Physcomitrella patens* that blue light was involved in the regulating the biosynthesis of *ent*-kaurenoic acid from GGDP (Miyazaki et al., 2014). Therefore, in this study, the blue light receptor CRY may be involved in modulating the GGDP flux under blue light, thereby enhancing the accumulations of both chlorophyll and carotenoid. Besides, in addition to binding to *PSY*, PIF1 has also been shown to directly bind to the promoter of pchlide oxidoreductase, thus regulating chlorophyll synthesis in *Arabidopsis thaliana* (Moon et al., 2008). During dark-induced senescence, auxin accelerated the loss of chlorophyll and carotenoids in the leaves of *Tropaeolum majus* (Karatas et al., 2010). These studies have shown that carotenoids and chlorophylls are similarly regulated, and there are differences in dark and light conditions, which also help to explore the inductive effect of L-tryptophan on carotenoid accumulation under different light conditions.

In general, the present study suggests that L-tryptophan is a positive inducer on carotenoid biosynthesis, and it can synergistically regulate carotenes accumulation with blue light. L-tryptophan modulates *PSY* and stimulates carotenoid synthesis by acting as an auxin-like hormone. In addition, it may affect the light signaling pathway by changing the configuration of CRY, thereby modulating carotenoids accumulation. The application of L-tryptophan also helps enhance photosynthetic efficiency and regulates xanthophyll cycle as a photoprotector. These findings increase our knowledge of the modulation of carotenoid biosynthesis and develop a new means to enhance nutritional fortification of maize sprouts by combing L-tryptophan and blue light.

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CRediT authorship contribution statement

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

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2023.100161.

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