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Integrated transcriptome and metabolome analysis reveals the impacts of prolonged light exposure on starch and protein content in maize kernels

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Abstract

Background The light environment significantly influences crop growth, development, quality, and yield, particularly in controlled-environment agriculture. Recent advances in artificial lighting technology have allowed growers to precisely control the light environment in terms of duration, spectrum, and intensity. Starch and protein are the most significant nutritional constituents of maize kernels. However, little is known about the effects of the light environment on starch and protein content in maize kernels. Therefore, we investigated the effects of natural light and supplemental exposure to blue (B), far-red (FR), and red (R) light on starch and protein content in kernels of the inbred maize line B73.

Results Exposure to supplemental B, FR, or R light resulted in significant increases in starch content but decreases in protein content. Notably, protein content was lowest under B light. Substantial proportions of genes (5.03–75.23%) and metabolites (46.89–85.64%) were regulated by different wavelengths of light. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, as well as weighted gene co-expression network analysis (WGCNA), revealed that differentially expressed genes (DEGs) under B, FR, and R light were involved in pathways related to starch and protein synthesis. KEGG metabolomic analysis showed that differentially abundant metabolites (DAMs) were primarily associated with histidine, D-amino acid, cysteine, and methionine metabolism. Nine DEGs related to starch synthesis were identified as potential candidates for investigating the effects of light quality on starch synthesis, and 14 DEGs related to protein synthesis provided evidence for the influence of light quality on protein synthesis in maize.

Conclusions This study identified the regulatory network governing starch and protein content in B73 maize kernels under different light conditions, contributing to a deeper understanding of how light quality affects the nutritional components of maize kernels.

Keywords Light quality, Maize, Metabolome, Protein, Starch, Transcriptome

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Background

Maize (*Zea mays* L.) is among the most widely cultivated cereal crops due to its high yield potential. The starch and protein contents of maize kernels directly impact yield and quality [1, 2]. However, breeding to improve both the quality and yield of maize has proven challenging. Multiple studies have reported negative correlations between yield and quality, mainly in relation to starch and protein content [3–5]. Such negative correlations arise mainly from the competition between energy and matter during the grain-filling stage of maize kernel development, as starch and protein are accumulated [6, 7].

The light environment influences plant growth by providing energy for photosynthesis and modulating various physiological, developmental, and metabolic processes through photoreception [8, 9]. R, FR, and B wavelengths activate different photoreceptors, including phytochromes, cryptochromes, and phototropins, and thereby trigger distinct physiological and biochemical responses [8, 10, 11]. Light also influences the synthesis of diverse metabolites such as dry matter, sugars, and phenolic compounds [12].

Artificial exposure to specific light spectra can significantly impact plant physiology and biochemistry, thereby affecting crop yield and quality. For example, exposing birch trees to B light significantly increases leaf photosynthetic capacity [13]. In tomatoes, combining R and B light can enhance yield and substantially increase sugar content [14, 15]. Similar results were observed in bilberry, where a combination of R and B light was observed to activate sugar metabolism, thereby improving fruit quality [16]. In lettuce, exposure to R, B, or white light results in increased soluble sugar content, whereas exposure to R light decreases yield, and exposure to B light increases both yield and leaf area [17, 18]. Exposing Chinese cabbage to monochromatic B light significantly enhances the soluble sugar content of leaves [19]. These results suggest that artificial lighting can be strategically employed to optimise the growth and yield of different crop plants. The light environment significantly influences crop yield and quality throughout the growth process. Spectral composition appears to be a crucial determinant of biomass accumulation [20]. In wheat, monochromatic R light decreases yield, whereas a higher R:FR ratio significantly increases yield [21, 22]. In rice, exposure to B light increases leaf amino acid content [23]. In addition, rice yield is increased by both R and B light exposure [24]. In barley, the intensity of photosynthesis under B light treatment surpasses that under R light; however, B light inhibits the growth of spring wheat while increasing chlorophyll content [25, 26]. Although spectral composition effects have been studied in many crops, similar research in maize has been limited. Investigating the impacts of different light spectra on kernel starch and protein contents holds significant theoretical and practical significance. Specifically, the rational application of artificial lighting is a promising approach for enhancing the yield and nutritional quality of maize.

Therefore, we conducted transcriptomic and metabolomic analyses of the inbred maize line B73 under both natural light and artificial illumination (supplemental R, FR, and B light) during the grain-filling stage. Our primary objective was to investigate the effects of different light spectra on the starch and protein contents of maize kernels. We also investigated the light-driven regulation of starch and protein biosynthesis and accumulation. We performed GO and KEGG enrichment analyses, as well as WGCNA, to identify significant DEGs and DAMs. Our results provide a theoretical foundation that will support the identification of gene loci related to light-regulated starch and protein synthesis in maize inbred line B73 for use in future breeding projects.

Materials and methods

Plant materials and growth conditions

The inbred maize line B73 was grown at the experimental farm of Henan Agricultural University (Jiaozuo, Henan, China) during summer 2022. Control (CK) plants were exposed only to natural light, while the treatment groups were exposed to an additional 8 h of supplemental light (B: 400–500 nm, FR: 700–750 nm, and R: 600–700 nm) daily during the periods 4:00–8:00 and 18:00–22:00 (Fig. S1). Light supplementation commenced at anthesis and continued for 21 days until the end of the grain-filling period, when the maize kernels were harvested. Following harvest, the maize kernels were rapidly frozen in liquid nitrogen and transferred to a –80 °C freezer for storage. Subsequently, the maize kernels were processed by Biomarker Technologies Co., Ltd. (Beijing, China) for transcriptomic and metabolomic analyses. For each treatment group, we conducted two and six transcriptome and metabolome biological replicates, respectively, to ensure the reliability of the results. Each biological replicate is composed of grains from three different plants.

Determination of starch and protein contents

The starch content was quantified using a commercial starch content detection kit (AKSU015 C, BOXBIO, Beijing, China). Briefly, 20 mg of each sample was placed into a centrifuge tube, and 1 mL of eluent was added. The sample was thoroughly mixed and then heated in an 80 °C water bath for 30 min. The heated mixture was then centrifuged at 3,000 ×g at room temperature for 5 min. Next, the supernatant was discarded, and the pellet was retained. The pellet was resuspended in 500 µL of sterile deionised water and gelatinised in a 95 °C water bath

for 15 min. After cooling to room temperature, 1 mL of extraction solution was added, and the mixture was continuously shaken at room temperature for 15 min to ensure complete extraction. Once the extraction was complete, the mixture was centrifuged at 3,000 ×g at room temperature for 10 min, and the supernatant was collected. The supernatant was diluted 100-fold and mixed with a chromogenic reagent (pre-mixed with H_2SO_4), generating a blue-green derivative. The absorbance value of the diluted reaction product was measured at 620 nm.

To construct a standard curve, glucose solutions with different concentration gradients (0.12, 0.10, 0.08, 0.04, 0.02, and 0.01 mg/g) were prepared, and their absorbance values at 620 nm were measured. The standard curve was plotted using these values. The starch content of the sample was calculated using the formula: $(1.35 \times \mathcal{X} \times 100/W)$ where $\mathcal{X}$ is the concentration determined from the standard curve based on the sample's absorbance value, and W is the sample weight.

Prior to protein content determination, lysis buffer containing protease inhibitors was prepared as a pre-mixed solution. The pre-mixed solution was added to the samples in a 1:10 (sample:pre-mix) ratio; all procedures were carried out on ice. After 30 min of incubation on ice, the samples were centrifuged at 12,000 ×g for 5 min. The resulting supernatant was used to quantify total protein content according to the bicinchoninic acid method [27, 28].

Illumina library construction and transcriptome sequencing

Total RNA was extracted from eight samples using the Eastep Super Total RNA Extraction Kit (Promega, Madison, WI, USA). Subsequently, RNA purity and concentration were quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was evaluated using an Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Once the samples passed the quality assessment, library construction began. After construction, preliminary quantification was conducted using a Qubit v3.0 fluorometer, with a required concentration of ≥1 ng/μL. Quality, concentration, and inserted fragment size were evaluated using the Agilent Bioanalyzer 2100 system. Transcriptomic sequencing (RNA-seq) was conducted on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) [29] by Biomarker Technologies Co., Ltd.

Transcriptomic analysis

RNA-seq sequencing of eight samples was conducted on an Illumina platform. Subsequently, clean reads were mapped to the B73 maize RefGen_v4 reference genome (https://www.maizegdb.org/assembly/). HISTA2 [30] was used to derive positional information and mapped reads were assembled using StringTie [31]. To more accurately reflect transcriptional expression levels, we normalized the number and length of reads mapped to the reference genome and standardized gene expression levels using FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) [32]. To assess the reliability of the RNA-seq data, Pearson correlation coefficients (R^2) [33] were calculated for each gene based on the FPKM values. Principal component analysis (PCA) was performed using BMKCloud (www.biocloud.net). DEGs were identified based on thresholds of $\log_2|\text{fold change (FC)}| > 1.5$ and false discovery rate (FDR) < 0.5. UpSet plots of DEGs were generated using the UpSetR package.

Metabolite extraction

Metabolites were extracted from six biological replicates representing four treatments according to the methods of Want [34] and Dunn [35]. Briefly, each 50-mg sample was combined with 1 mL of a methanol–acetonitrile–water extraction solvent containing an internal standard (20 mg/L L- 2-chlorophenylalanine). The mixtures were vortexed for 30 s and then ground with a steel ball at a frequency of 45 Hz for 10 min. The ground samples were placed in an ice water bath for 10 min and then centrifuged at 4 °C and 12,000 rpm for 5 min. For each sample, 500 μL of the supernatant was transferred to a new Eppendorf tube and dried using a vacuum concentrator. Next, the dried samples were reconstituted in 160 μL of extraction solvent (1:1, acetonitrile:water), vortexed for 30 s, and incubated for 10 min in an ice water bath. The samples were centrifuged again at 4 °C and 12,000 rpm for 5 min. Finally, 120 μL of each supernatant was transferred into a new 2-mL vial. To generate quality control samples, we pooled 10 μL from each sample. Metabolites were analysed using an Acquity I-Class PLUS ultra-high-performance liquid chromatography (UPLC) system coupled to a Watern Xevo G2-XS quadrupole-time-of-flight mass spectrometer (QTOF-MS), utilising a Waters Acquity UPLC HSS T3 column (1.8 μm, 2.1 ×100 mm). Metabolomic analysis was conducted by Biomarker Technologies Co., Ltd.

Metabolomic analysis

Metabolites were identified by comparing their mass-to-charge ratios (m/z), fragment ions, and isotopic patterns to those in the LipidMaps database (https://lipidmaps.org/). The raw data were explored using the METLIN database within Progenesis QI (https://hmdb.ca/), the KEGG database (http://www.genome.jp/kegg/), and an in-house library developed by Biomarker Technologies

Co., Ltd. Theoretical fragment ions were identified according to a parent ion mass deviation within 100 ppm and a fragment ion mass deviation within 50 ppm [36]. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was employed to identify DAMs, with the thresholds of variable importance in projection (VIP) ≥ 1, FC ≥ 1.5, and $P < 0.05$.

WGCNA analysis

The identified DEGs were subjected to WGCNA using the WGCNA [37] package in R (R Core Team, Vienna, Austria). The adjacency matrix weight parameter was set to a power of 30. The Pearson correlation algorithm was used to calculate R^2 and P values associated with module eigengenes and traits. Modules associated with each trait were identified according to thresholds of $R^2 \geq 0.3$ and $P < 0.05$. For each module–trait linkage, correlations (i.e. gene significance [GS]) were calculated between module gene expression and both the corresponding trait and the module eigengene. Scatter plots were generated based on these values, and hierarchical clustering was conducted using the dynamic tree cutting algorithm (mergeCutHeight = 0.25). Modules were considered significantly enriched according to thresholds of $R^2 > 0.5$ and $P < 0.05$.

GO and KEGG enrichment analysis

To identify significantly enriched GO terms, a hypergeometric test was performed for each group of DEGs using the *ClusterProfiler* package in R [38]. The results were visualised with the TreeMap tool in REVIGO (http://revigo.irb.hr/). Functional annotations of DEGs and DAMs were then mapped to KEGG pathways (http://www.genome.jp/kegg/). Significantly enriched pathways were evaluated at a threshold of $P < 0.05$.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted using an Eastep Super Total RNA Extraction Kit (Promega). Following quality evaluation, complementary DNA (cDNA) was synthesised using the GoScript Reverse Transcription Mix (Promega). qRT-PCR was performed using a Light Cycler 480 II instrument, the associated SP4 v1.5.0 software (Roche, Basel, Switzerland), and a SYBR Green I detection system for real-time fluorescence quantification. Primers were designed based on sequences retrieved from the National Center for Biotechnology Information (NCBI) database. The *ZmUBQ* (*Zm00001 d015327*) gene was used as an internal control to normalise gene expression levels. Gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method.

Results

Supplementary lighting treatment enhances starch content and reduces protein content in maize kernels

To investigate the influence of different light spectra on the accumulation of starch and protein in maize kernels, we applied supplemental lighting to maize inbred line B73 during anthesis. Experimental plants were exposed to R, FR, or B light for 4 h each morning and evening for 21 days. Maize kernels were harvested at the dough stage to quantify their total starch and protein contents. After 2% I_2-KI staining, kernels from plants exposed to supplemental R, FR, and B light exhibited deeper coloration than CK kernels (Fig. 1A–D). These results, together with our starch content analysis results (Fig. 1E), indicate that supplemental lighting significantly increased the starch content. Notably, supplemental lighting (R, FR, and B) significantly reduced the protein content (Fig. 1F), and a negative correlation was observed between starch and protein contents (Fig. 1E). These results underscore the substantial impact of supplemental lighting on the nutritional content of B73 maize kernels.

Supplementary lighting shapes gene expression in maize kernels

To explore the impact of different light environments on gene expression, we analysed gene expression levels in kernels of maize inbred line B73 under natural and supplemental light conditions. A total of 53.46 GB of clean data were obtained, with a Q30 $\geq$ 93.52% (Table S1). Over 83.99% of clean reads were successfully aligned to the B73 RefGen_v4 reference genome (https://www.maizegdb.org/assembly/) [39]. Correlation analysis of the FPKM values of all identified genes revealed that the treatment groups were well-clustered. The average R^2 values of the two biological replicates ranged from 0.901 to 0.993 (Fig. 2A), indicating high repeatability. Significant differences were observed among the gene expression patterns of the treatment groups according to PCA, with PC1 and PC2 accounting for 26.59% and 19.01% of the total variance, respectively. Notably, the gene expression profiles of the R and FR groups were similar (Fig. 2B). To verify the RNA-seq results, 15 genes were randomly selected for quantitative validation (Table S2), and the results supported the credibility of the RNA-seq data ($R^2 = 0.9061$; Fig. 2C).

Distinct light environments yield unique DEG regulatory networks

Using thresholds of $\log_2|FC| > 1.5$ and FDR < 0.05, differential gene expression analysis was performed on the three treatment groups, resulting in the identification of 4,271 DEGs (Table S3). Compared to CK, 3,213,

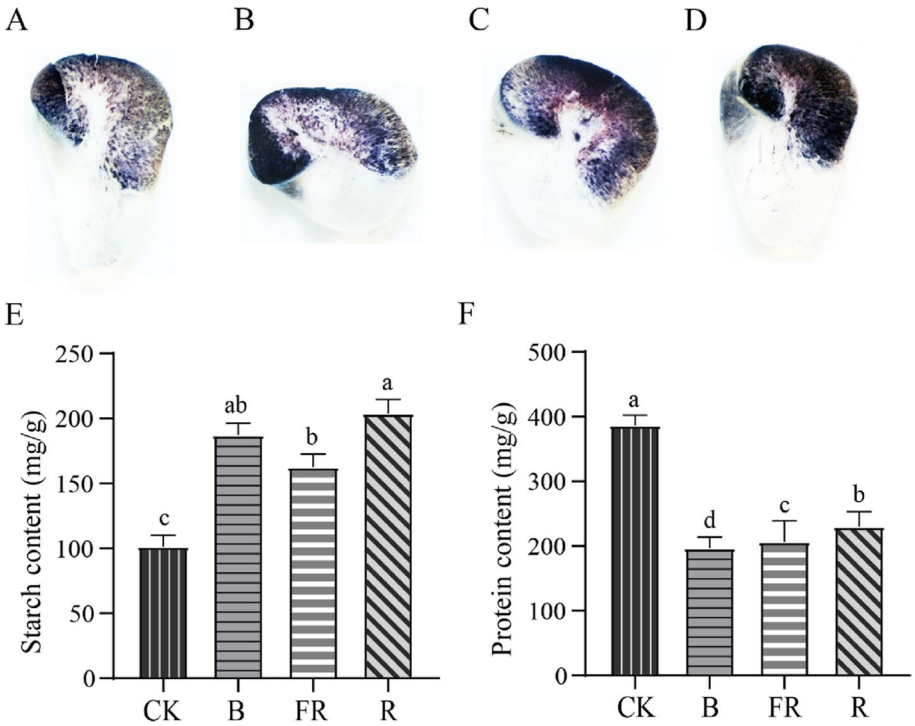

Fig. 1 Starch and protein contents of maize kernels exposed to different light environments. **A**–**D** Cross-sections of I_2-KI-stained maize kernels. A, natural light (CK); B, blue (**B**) light supplementation; C, far-red (FR) light supplementation; D, red (R) light supplementation. **E** Starch content of maize kernels. **F** Total protein content of maize kernels

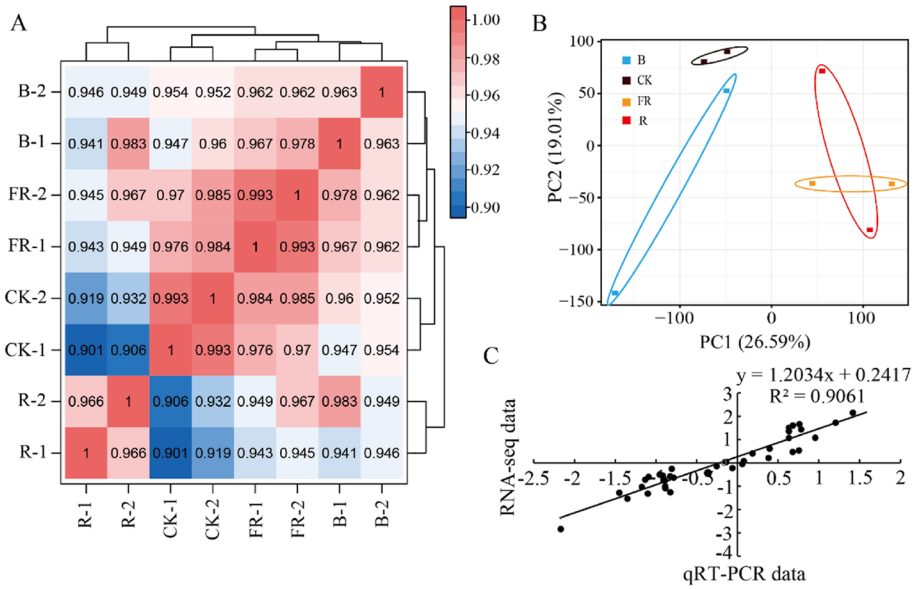

Fig. 2 Transcriptomic analysis of maize kernels exposed to different light environments. **A** Correlation coefficients (R^2) between two biological replicates. **B** Principal component analysis results. **C** Correlation between RNA sequencing (RNA-seq) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) data for 15 candidate genes across four different lighting treatments. PC, principal component

1,743, and 215, DEGs were identified in the R, FR, and B treatment groups, respectively (Fig. 3A). UpSet analysis showed that kernels from plants exposed to supplementary R light harboured the most DEGs (1,566), followed by those exposed to supplemental FR light (1,015) and supplemental B light (82). In addition, 642

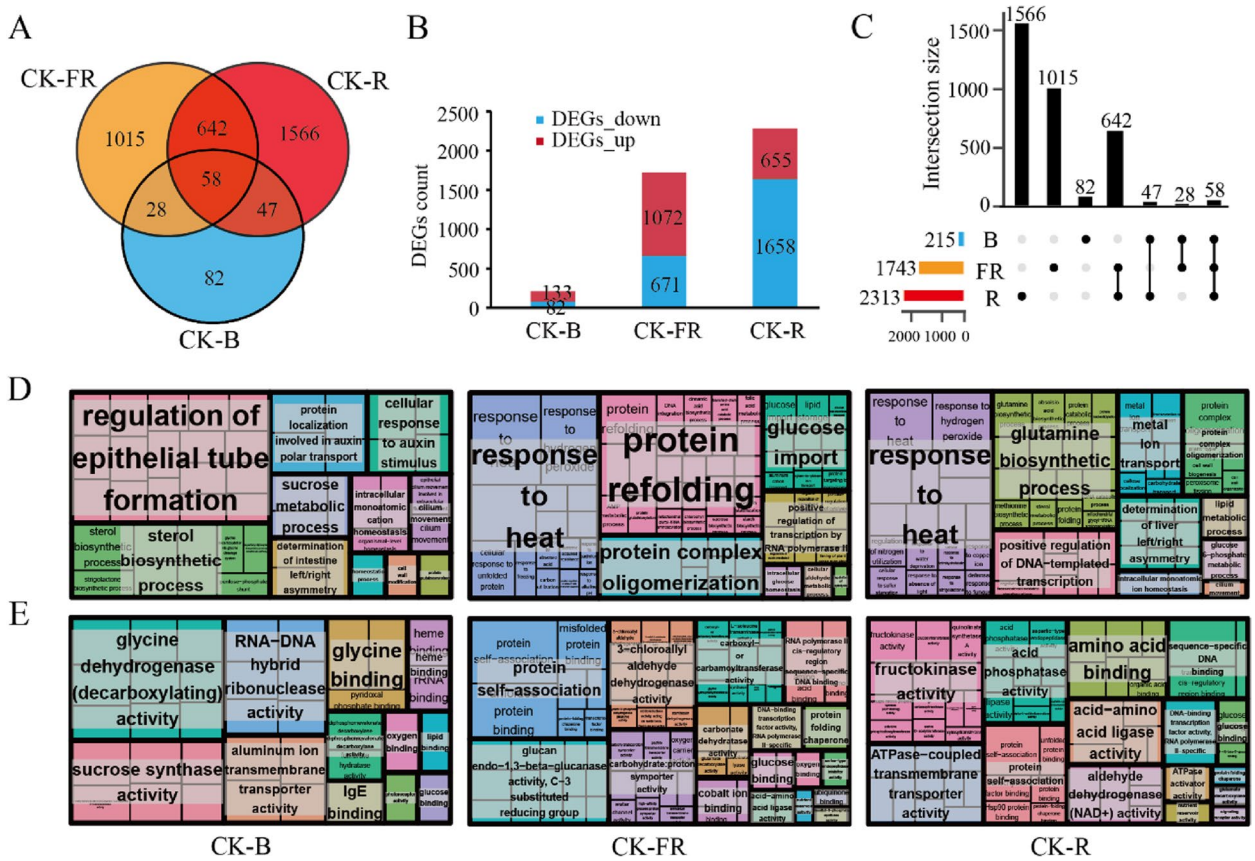

Fig. 3 Differential gene expression in maize kernels exposed to different light environments. **A** Numbers of differentially expressed genes (DEGs) under various light conditions. **B** Up- and downregulated DEGs under various light conditions. **C** UpSet plot for DEGs under various light conditions. **D** Biological processes and (**E**) molecular functions associated with DEGs according to Gene Ontology (GO) enrichment analysis. Significance criteria: $\log_2$|fold change (FC)|> 1.5, false discovery rate (FDR) < 0.05, $P < 0.05$

DEGs were regulated by both R and FR light, 47 were regulated by R and B light, 28 were regulated by FR and B light, and 58 were regulated by all three light conditions (Fig. 3C). Kernels from plants exposed to B and FR light had more upregulated DEGs (133 and 1,072, respectively) than downregulated DEGs (82 and 671, respectively). In contrast, kernels from plants exposed to R light had more downregulated DEGs (1,658) than upregulated DEGs (655) (Fig. 3B). These results suggest that different light spectra exert unique effects on gene expression in maize inbred line B73.

Kernels from plants exposed to supplemental B, FR, and R light exhibited distinct responses related to biological processes (Table S4). Specifically, 79 biological processes were associated with exposure to B light, including sucrose metabolic processes (2 genes, 2.15%) and protein glutathionylation (1 gene, 1.07%). Protein refolding (14 genes, 1.85%), protein complex oligomerisation (15 genes, 1.98%), and glucose import (10 genes, 1.32%) were associated with exposure to FR light; and"protein complex oligomerisation (12 genes, 1.12%) and glucose- 6-phosphate metabolic processes (4 genes, 0.37%) were associated with exposure to R light (Fig. 3D).

Exposure to supplemental B, FR, and R light was associated with 39, 93, and 90 molecular functions, respectively. Exposure to B light was associated with glycine levels (2 genes, 1.64%) and sugar transport (3 genes, 2.46%). Exposure to FR light was associated with protein self-association (17 genes, 1.57%), carbohydrate:proton symporter activity (10 genes, 0.92%), glucose binding (3 genes, 0.28%), protein folding chaperoning (9 genes, 0.83%), and nutrient reservoir activity (8 genes, 0.74%); and exposure to R light was associated with glycolysis and starch synthesis (10 genes, 0.66%), cellular energy conversion (24 genes, 1.58%), and protein synthesis pathways (21 genes, 1.38%) (Fig. 3E).

These results suggest that exposure to supplemental light of different wavelengths alters carbohydrate and protein metabolism in distinct ways. Supplemental exposure to B light appears to affect glycine levels, indirectly influencing starch and protein synthesis. Exposure to supplemental FR light primarily modulates the

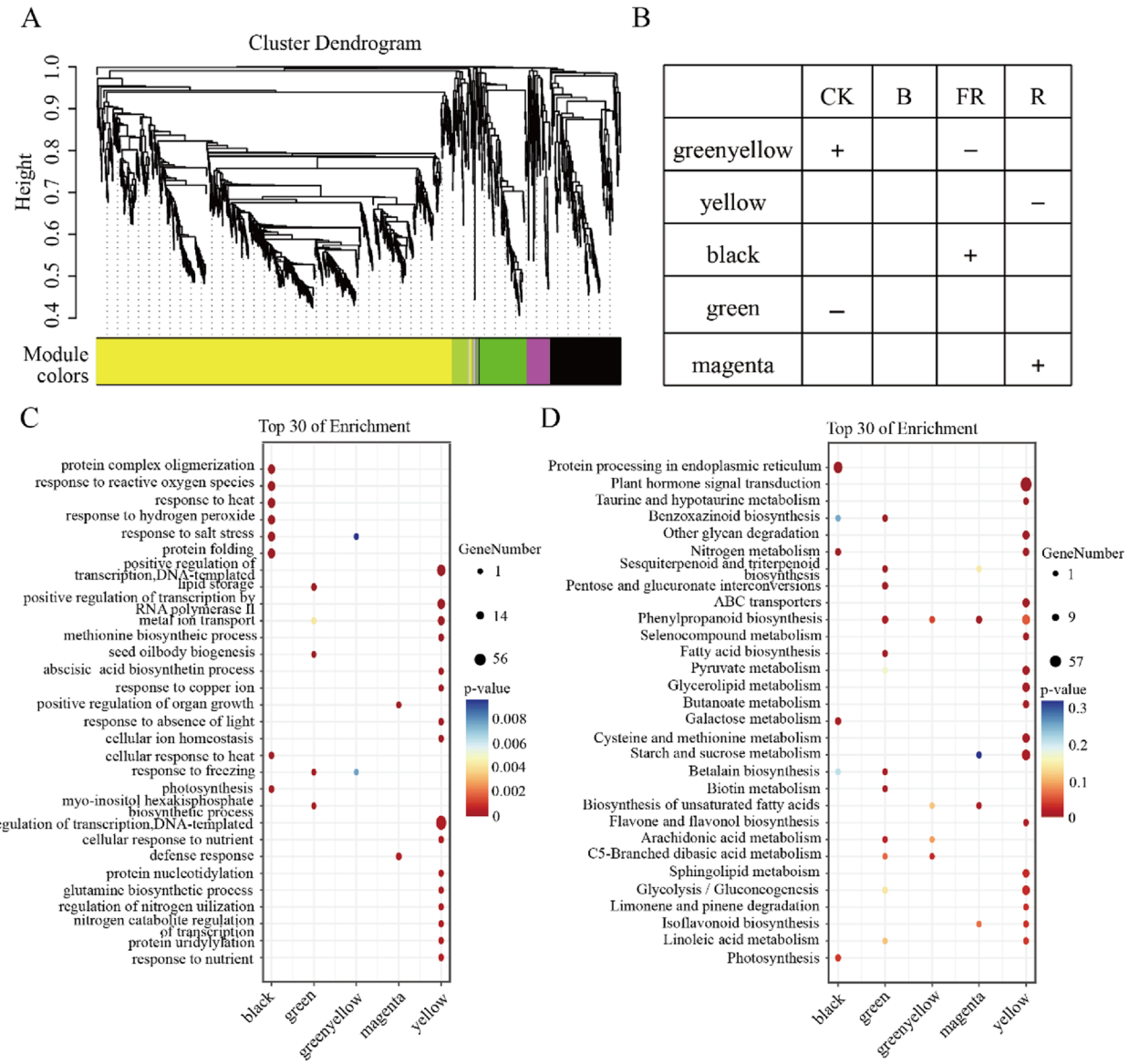

Fig. 4 Weighted gene coexpression network analysis (WGCNA). **A** Dendrogram of gene co-expression modules. **B** Module–trait correlation analysis between gene expression patterns in specific modules and starch content under distinct light conditions. Starch content was averaged under the four light conditions. + and – indicate positive and negative correlations, respectively. **C** Gene Ontology (GO) and (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis results

accumulation of carbohydrates and proteins. In contrast, supplemental R light regulates glycolysis, as well as starch and protein synthesis, thereby altering the nutritional content of B73 maize kernels.

WGCNA analysis of DEGs under different supplementary lighting conditions

We conducted WGCNA on the 2,471 identified DEGs to investigate the effects of different light conditions on the expression trends of core genes associated with nutritional substances [37]. Five distinct modules were identified, along with a grey module that did not demonstrate significant clustering (Fig. 4A). Module–trait correlation analysis was performed to identify significant positive or negative correlations between gene expression patterns in specific modules and starch content under distinct light conditions. Gene expression in the greenyellow, black, and magenta modules was significantly positively correlated with starch content in the CK ($r=$ 0.77, $P=$ 0.003), FR ($r=$ 0.97, $P=$ 2×10^{-7}), and R ($r=$

0.97, $P = 1 \times 10^{-7}$) groups. Conversely, gene expression in the green-yellow, yellow, and green modules was significantly negatively correlated with starch content in the FR ($r = -0.8$, $P = 0.002$), R ($r = -0.79$, $P = 0.002$), and CK ($r = -0.67$, $P = 0.02$) groups (Figs. 4B and S2).

DEGs in each module were subjected to GO and KEGG enrichment analyses to understand their biological roles. According to the GO enrichment analysis (Fig. 4C, Table S5), DEGs in the black module (positively correlated with FR light) were primarily associated with metabolic and biosynthetic functions including protein complex oligomerisation, response to reactive oxygen species, response to hydrogen peroxide, protein folding, and photosynthesis. DEGs in the yellow module (negatively correlated with R light) were predominantly associated with transcriptional regulation and nitrogen metabolism. Conversely, DEGs in the green module (negatively correlated with CK light) were primarily associated with lipid synthesis and metal ion transport. Notably, both the green and magenta modules contained DEGs related to abiotic stress responses.

KEGG enrichment analysis (Fig. 4D) indicated that DEGs in the black module were primarily associated with protein processing and photosynthesis-related pathways, including protein processing in the endoplasmic reticulum, nitrogen metabolism, benzoxazinone biosynthesis, and photosynthesis. DEGs in the yellow module were related to stimulus-associated carbohydrate and protein metabolism pathways such as plant hormone signal transduction and pyruvate, cysteine, methionine, starch, and sucrose metabolism. DEGs in the magenta module were primarily associated with carbohydrate metabolism, including phenylpropanoid and isoflavonoid biosynthesis, and starch and sucrose metabolism. Notably, DEGs in the green-yellow, yellow, green, and magenta modules were all involved in phenylpropanoid biosynthesis. In summary, different light conditions appear to have profound effects on starch and protein metabolism in maize inbred line B73.

Metabolite profiling under different supplementary lighting conditions

To explore the impact of supplemental light on B73 kernel metabolism, we conducted an untargeted metabolomic analysis of kernel endosperm samples using an LC-QTOF platform. Each experimental group consisted of six biological replicates, with R^2 values ranging from 0.9723 to 0.9868 (Fig. S3 A), underscoring the high quality of the metabolomic data. The PCA results showed that PC1 accounted for 37.77% of the total variance, whereas PC2 accounted for 16.96%. Consistent with the transcriptomic PCA, PC1 exhibited clear dispersion corresponding to different light environments, suggesting that the application of different light spectra results in significant variation in kernel metabolism (Fig. S3B).

Among the 2,916 detected metabolites (Table S7), 1,337 were differentially expressed (Table S8), 1,205 of which were annotated in the Human Metabolome Database (HMDB) (Table S9). The 1,205 annotated metabolites were broadly categorised into eight classes, among which lipids were the most common (314 metabolites, 26.06%), followed by acids (187 metabolites, 15.52%), oxylipins (128 metabolites, 10.62%), organic heterocyclic compounds (100 metabolites, 8.30%), phenylpropanoids (100 metabolites, 8.30%), benzenoids (71 metabolites, 5.89%), and nucleosides (41 metabolites, 3.40%) (Fig. S3 C).

DAM expression network under different supplementary lighting conditions

Among the 1,337 identified DAMs, 1,032 were detected under B light, 873 under FR light, and 565 under R light, and 319 were common to all three light conditions (Fig. 5A). There were consistently more downregulated DAMs than upregulated DAMs across all three light conditions; the highest number of downregulated DAMs was observed under B light (3.01-fold higher), followed by FR light (2.56-fold higher) and R light (1.22-fold higher) (Fig. 5B). UpSet analysis showed that kernels from plants exposed to supplementary B light harboured the most DAMs (286), followed by those exposed to supplemental FR light (183) and supplemental R light (54). In addition, 303 DAMs were regulated by both B and FR light, 124 by B and R light, and 68 by FR and R light (Fig. 5C). These results stand in contrast to the transcriptomic data, suggesting that the same metabolites may be regulated by multiple genes. The top 20 metabolites were associated with several metabolic pathways, including histidine, vitamin B6, D-amino acid, and cysteine and methionine metabolism, oxidative phosphorylation, and pantothenate and CoA biosynthesis (Fig. 5D). Histidine plays a significant role in amino acid and protein biosynthesis, thereby indirectly affecting the nutritional quality of maize kernels [40, 41]. Consistent with the transcriptomic data, the metabolomic data were also enriched in cysteine and methionine metabolism. These results further demonstrate that the light environment significantly affects starch and protein metabolism in B73 maize kernels.

Light-regulated DEGs and DAMs participate in starch and protein biosynthesis

The primary storage proteins in maize endosperm are α, β, γ, and δ-zeins [42–45]. We identified 14 DEGs involved in zein synthesis, most of which were associated with the synthesis of α- zeins (9 of 14), followed by γ- (2), δ- (2),

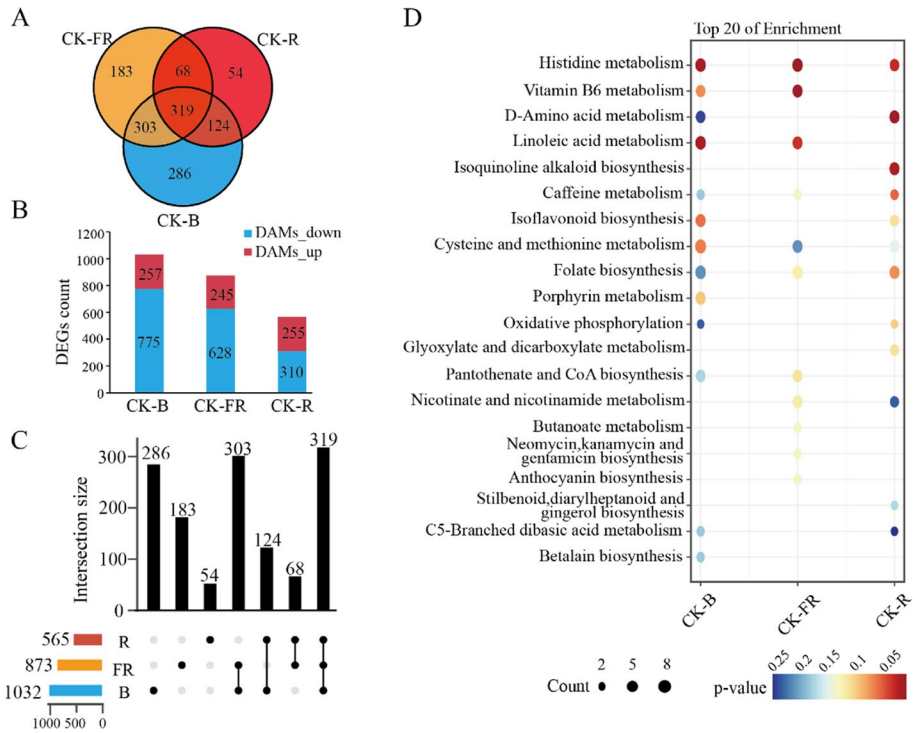

Fig. 5 Differential metabolite expression in maize kernels exposed to different light environments. **A** Number of differentially abundant metabolites (DAMs) under various light conditions. **B** Up- and downregulated DAMs under various light conditions. **C** UpSet plot for DAMs under various light conditions. **D** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DAMs under various light conditions. Significance criteria: $\log_2|FC| > 1$, $P < 0.05$, VIP = 1

and β-zeins (1). These DEGs significantly influence nutrient reservoir activity (GO:0045735) (Table S10). The qRT-PCR results were mainly consistent with the RNA-seq data. Overall, the majority of zein-related DEGs exhibited lower expression levels under R, FR, and B light compared to CK, with the exception of 18-kDa δ-zein under R light conditions (Fig. 6A, B). These findings suggest that supplemental light negatively regulates zein synthesis in B73 maize endosperm, which aligns with our protein content analysis (Fig. 1F).

Among the 2,471 DEGs, nine were associated with starch metabolism: two sucrose synthase genes (*Zm00001 d029087* and *Zm00001 d029091*), four hexokinase genes (*Zm00001 d035037*, *Zm00001 d037573*, *Zm00001 d042536*, and *Zm00001 d044813*), one AGPase gene (*Zm00001 d039131*), and two starch synthase genes (*Zm00001 d026337* and *Zm00001 d051976*) (Fig. 6C). Notably, the hexokinase gene *Zm00001 d042536*, the AGPase gene *Zm00001 d039131*, and the starch synthase gene *Zm00001 d051976* were significantly upregulated ($\log_2|FC| > 1.5$) under R, FR, and B light. Furthermore, two hexokinase genes, *Zm00001 d035037* and *Zm00001 d037573*, were significantly upregulated under B light, the hexokinase gene *Zm00001 d044813* was significantly upregulated under FR light, and the starch synthase gene *Zm00001 d026337* was significantly upregulated under FR light. Other genes were significantly downregulated. For example, the sucrose synthase genes *Zm00001 d029087* and *Zm00001 d029091* were downregulated under B light, the hexokinase gene *Zm00001 d044813* was downregulated under B and FR light, the hexokinase gene *Zm00001 d037573* was downregulated under FR and R light, and the starch synthase gene *Zm00001 d026337* was downregulated under FR light (Fig. 6D). These results underscore the critical importance of the light spectrum on carbohydrate biosynthesis and generally suggest that supplemental light exposure can increase the starch content of B73 maize kernels.

Discussion

Supplemental light significantly impacts starch and protein content in maize kernels of B73

Light is essential for plant growth and development, and the light environment directly influences the nutritional quality of maize kernels. Under low light, starch and protein synthesis are compromised due to reduced enzyme activity [46, 47]. However, providing additional light at night has been shown to enhance grain weight and yield in wheat [48]. Our results indicate that supplemental B, FR, and R light increases starch content while reducing

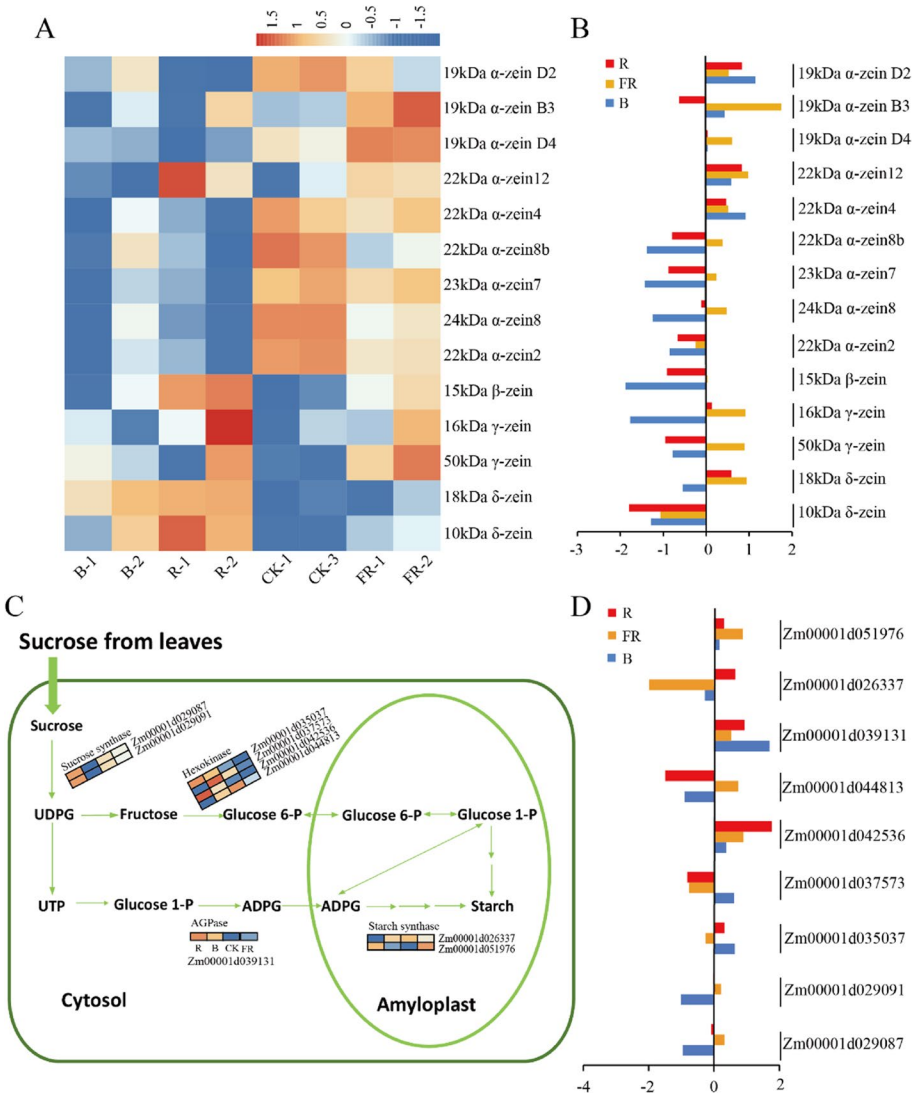

Fig. 6 The effects of supplemental light exposure on protein and starch synthesis in maize kernels. **A** Heatmap of fragments per kilobase of transcript per million fragments mapped (FPKM) reads for 14 zein-related genes. **B** qRT-PCR analysis of 14 zein-related genes. **C** Effects of supplemental lighting on starch synthesis in maize kernels. **D** qRT-PCR analysis of starch biosynthesis-related genes

protein content in maize inbred line B73. This effect may be attributed to extended light exposure, as we functionally extended the photosynthetic period into the night. Overall, the light environment (spectrum and duration) appears to significantly influence starch and protein content in B73 maize kernels.

Through comprehensive transcriptomic and metabolomic analyses, this study has elucidated the regulatory networks of DEGs and DAMs under varying light qualities. Specifically, nine DEGs associated with starch synthesis and 14 DEGs linked to protein synthesis were identified as potential candidate genes. These discoveries significantly enhance our understanding of how light quality regulates the pathways involved in starch and protein synthesis. As technology advances and costs decline, there may be increased opportunities for applying supplemental lighting in maize production. It is possible to enhance both the nutritional quality and yield of maize through the optimisation of light conditions.

Phytochromes play a crucial role in starch and protein biosynthesis

Phytochromes are photoreceptive molecules that detect changes in R, FR, and B light. Phytochromes influence plant growth and development through a series of signal transduction processes, including the downstream

biosynthesis of starch and protein [49, 50, 51]. In plants, R and B light are the two most photosynthetically active wavelengths [52], exerting differential effects on growth, development, and nutritional composition. R light appears to enhance soluble sugar content in plants [12], whereas B light increases protein content [53]. However, we found that B light exposure decreased protein content in B73 maize kernels; this discrepancy could be related to either the experimental conditions or the maize variety used in these studies.

Phytochromes modulate the expression of starch biosynthesis genes by regulating the activity of associated transcription factors, increasing starch content. In addition, phytochromes can directly or indirectly influence the starch and protein metabolic pathways, thereby regulating their contents in maize kernels [24, 54, 55]. We found that B, FR, and R light regulated the expression of 5.03–75.23% of genes and 46.89–85.64% of metabolites (Table S3 and S8). These findings suggest that phytochromes play a significant role in starch and protein biosynthesis, thereby affecting the nutritional quality of B73 maize kernels.

Starch and protein synthesis are negatively correlated in B73 maize kernels

The transcription factors ZmNAC128 and ZmNAC130 play crucial roles in grain filling in maize kernels by directly regulating the expression of γ-zein genes and several key starch biosynthesis enzyme-encoding genes. These transcription factors coordinate with Opaque2 to regulate starch and protein synthesis, thereby influencing the accumulation of filling substances in maize kernels [56]. Starch is the primary component of maize kernels, followed by protein. Starch content is significantly negatively correlated with the contents of crude protein, crude fat, and crude ash [6], which is consistent with our findings.

Our research indicates that supplemental B, FR, and R light not only affect starch content but also significantly reduce protein content, with the lowest protein content observed under B light. This balance shift between starch and protein content provides a new perspective on understanding the comprehensive impact of the light environment on the nutritional composition of maize kernels. This negative correlation may be attributed to competition for the same precursor molecules during starch and protein synthesis under resource limitation, or perhaps to shared regulatory mechanisms. Together, while we have a foundational theoretical understanding of how light conditions affect the nutritional quality of the maize inbred line B73, these insights require validation and application across a broader range of varieties and environmental contexts. Further research will enhance our comprehension of these mechanisms and explore their potential for improving the nutritional quality of crops.

Conclusions

This study presents a comprehensive analysis of the effects of different light conditions on the nutritional quality of maize kernels in the inbred line B73. Providing supplemental B, FR, and R light significantly influences both starch and protein biosynthesis of B73 maize kernels. Specifically, supplemental B, FR, and R light appear to promote starch synthesis while inhibiting protein synthesis. Through combined transcriptomic and metabolomic analyses, we identified 21 genes and four metabolites that appear to be involved in starch and protein synthesis. These results offer a theoretical foundation for enhancing the nutritional value of crops by manipulating light conditions and provide insights for optimizing lighting strategies in controlled-environment agriculture. Our study fills the gap in understanding the comprehensive impact of light quality on maize kernel starch and protein content.

Abbreviations

GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
WGCNA	Weighted gene co-expression network analysis
DEGs	Differentially expressed genes
PCA	Principal component analysis
FC	Fold change
FDR	False discovery rate
VIP	Variable importance in projection
qRT-PCR	Real-time quantitative reverse transcription PCR

Supplementary Information

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Additional file 1.

Additional file 2.

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Authors' contributions

J, Y, and S designed the study. K, L, J, and Y prepared experimental materials and figure 1. K, W, Y, and Y analyzed the data. K, Y, J, and S wrote and revised the manuscript. All authors read and approved the final manuscript.

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Data availability
Transcriptome raw data in this study have been uploaded to the National Center for Biotechnology Information Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra; accession no. PRJNA1203886).

Declarations

Ethics approval and consent to participate
We declare that all experimental studies and field experiments, including the collection and use of maize seeds, are in compliance with relevant institutional, national and international guidelines and legislation. All experimental protocols were approved by the Academic Committee of the College of Agriculture, Henan Agricultural University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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