Transcriptional Regulation of Photomorphogenesis in *Brassica Napus* Under Different Light Quality

Xin Zhang

Zhejiang University

Yang Liu

Zhejiang University

Yuqing Huang

Hangzhou Academy of Agricultural Sciences

Dezhi Wu

Zhejiang University

Shengguan Cai (✉ caisg@zju.edu.cn)

Zhejiang University <https://orcid.org/0000-0001-6029-7385>

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Abstract

Background: Plants undergo skotomorphogenesis or photomorphogenesis upon the absence or presence of light. Although the light signaling pathway has been well documented in *Arabidopsis*, relatively little is known in other plant species including *Brassica napus*.

Results: In this study, we examined the response of hypocotyl elongation to different light quality (white, blue, red and far-red light) in *B. napus*. The result showed each of tested light quality promoted photomorphogenesis, with less effect of red light than blue/far-red light. Subsequently, RNA-sequencing was performed to obtain the transcriptomic profile in *B. napus* in response to dark and different light quality. A total of 9748 genes were differentially expressed among treatments. Correlation analysis and hierarchical cluster analysis of differentially expressed genes (DEGs) indicated the transcriptomic profile in red light exhibited an analogous pattern with that in dark to some extent, while blue/far-red light induced transcriptional profiles showed distinct patterns. Moreover, we found the differential expressions of *PHYB*, *COP1* and *BBXs* homologs between two subgenomes, which may affect their interactions with up/downstream pathway. Finally, we constructed a transcriptional network including light signaling, phytohormone and cell elongation/modification, which explained the observed hypocotyl phenotype under different light quality.

Conclusions: We partially explained why red light has reduced effect on photomorphogenesis in *B. napus* by RNA-seq and displayed gene expression of the key regulators in light signaling pathway and their contribution to photomorphogenesis under different light quality in *B. napus*. The result could improve new sight into understanding on transcriptional regulation of photomorphogenesis of *B. napus*.

Background

Plants have evolved complex and comprehensive transcriptional networks to sense various environmental changes and adjust themselves to growth better and survival flexible. Upon light irradiation, plants regulate multiple developmental processes including seed germination, photomorphogenesis, photoperiod and flowering via monitoring the light quantity, quality, direction and duration [1]. When seedling emerges from soil, it perceives light signal to transfer plant growth from skotomorphogenesis to photomorphogenesis [2]. Under shade conditions, neighbor plants usually accelerate elongation of internodes and petioles, elevate leaf angles from the horizontal, and reduce shoot branching to struggle for light [3, 4]. These processes confer plants distinct ability to complicatedly response to diverse light conditions.

As photosensory switches, photoreceptors play crucial roles to response to light and are tightly controlled by light in multiple ways [5]. So far, 13 photoreceptors have been identified in *Arabidopsis thaliana* [1]. Among these, phytochrome (PHY), cryptochrome (CRY), Phototropin (PHOT) and UVR8 are major photoreceptors. In *Arabidopsis thaliana*, phytochromes consist of five members (phyA to phyE). They can be classified into light-labile type I (phyA) and light-stable type II (phyB to phyE) species, among which

phyA is responsible for sensing far-red (FR) light, and phyB to phyE play roles in red (R) light-mediated photomorphogenic development [6]. Two cryptochromes (cry1 and cry2) have been well characterized to sense blue and UV-A light [5], and function in blue light-stimulated expansion of cotyledons and inhibition of hypocotyl elongation [7]. It is worth noting that many transcription factors (TFs) are induced to regulate downstream genes expression in light signaling. HY5, a member of the basic leucine zipper (bZIP) transcription factor family [8], with its homologue HY5-HOMOLOG (HYH), positively regulates seedling photomorphogenesis by inhibiting hypocotyl growth and promoting pigment accumulation under light [9]. In Arabidopsis, HY5 is a major regulator and usually directly binds to cis-regulatory elements of targeting genes, which approximately consist of one-third genes among whole genome, to control gene expression in transcriptome [9, 10]. Meanwhile, HY5 integrates major branches downstream of all photoreceptors in a light-dependent manner [5]. Phytochrome interacting factor 3 (PIF3), a basic helix-loop-helix (bHLH) protein [11], accumulates in the dark to promote hypocotyl elongation, and it serves as a key negative regulator in seedling photomorphogenesis. Although the molecular mechanism of light signaling and downstream pathway was well documented in Arabidopsis, it is still largely unknown in other plant species.

Despite the fact rapeseed (*Brassica napus*) is an important oilseed crop worldwide, relatively little is known about photomorphogenic development at the physiological and molecular levels. *Brassica napus* is an allopolyploid species (AACC, $2n = 38$), hybridized by *Brassica rapa* (AA = 20) and *Brassica oleracea* (CC = 18) between 6800 and 12,500 years ago [12–14]. Even though they belong to same genus *Brassica*, the photomorphogenic response to different light exhibited a significant difference among them (Fig. S1). It is of interest to study the effect of polyploidization or gene duplication on photomorphogenesis in *B. napus*. In behalf of the rapid development of sequencing technology and reduction of costs, RNA-sequencing becomes a common tool to acquire the overall transcriptional profile [15]. In this study, we determined the transcriptome of rapeseed seedling in five different light conditions using RNA-sequencing. The results displayed a global transcriptional profile of photomorphogenesis in *B. napus*, and provide clues for future research.

Results

Red-light mediated inhibition of hypocotyl elongation in *Brassica napus* was less effective than in other tested species and other light conditions.

Although the effects of light quality on photomorphogenesis and the underlying molecular mechanism has been well studied in Arabidopsis [16–18], it is poorly understood in other plant species. We tested the effects of light quality on the hypocotyl elongation in eight eudicot species. All tested plant species showed longest and shortest hypocotyl under dark and white light (Fig. S1). However, the extent in inhibition of hypocotyl elongation showed wide variations among plant species and among light quality. The red light induced the inhibition of hypocotyl elongation was in a less extent in *B. napus* (cultivar ZD622) than other tested plant species, especially *Brassica rapa* (Fig. S1), even though they shared the most closely kinship. Moreover, ZD622 showed longer hypocotyl under red light than other light quality

(Fig. 1). We also tested the effect of different light quality on hypocotyl length in 267 rapeseed genotypes collected from worldwide, and we found that the overall hypocotyl length was longer in red light than that in blue or far-red light (data not shown). These results suggested that red light has less influence on promotion of photomorphogenesis in *B. napus*.

The overview of transcriptomic profiles in *B. napus* under different light quality.

Transcriptional reprogramming, including signal transduction and activation or inhibition of downstream pathways (phytohormone and plant growth), plays a vital role in photomorphogenesis [1, 5]. In order to investigate the transcriptional regulation in response to different light quality, we performed transcriptome analysis of seedling under dark and four light quality. To validate the reliability of transcriptome data, we performed qRT-PCR analysis of 12 genes. The qRT-PCR result was in accordance with that in RNA-seq result, indicating the high reliability of transcriptome data (Fig. S2). Our transcriptional data shows that there are 9748 genes, approximately 15% portion of total genes, differential expressed in seedling under different light quality. To visualize the overall transcriptional patterns in different light conditions, we conducted the principle cluster analysis (PCA) of all differentially expression genes (DEGs). The transcriptional profiles under dark/red light and blue/far-red light was clearly separated by principle component 1 (PC1), which explained 49.6% of variation. This result indicates that dark/red light regulated transcriptional profiles was distinct from that in blue/far-red light conditions. In addition, strong negative correlations were found in gene expression under dark vs blue light ($r=-0.77$), dark vs far-red light ($r=-0.76$), red light vs blue light ($r=-0.7$) and red light vs far-red light ($r=-0.71$), which support the opinion above from another perspective (Fig. 2B). Furthermore, significant positive correlations were observed between gene expression under far-red and blue light ($r=0.71$), and between dark and red light ($r=0.38$). It is consistent with the observed phenotypes that red light treated plants showed longer hypocotyl than blue and far-red light treated plants. In a word, red light mediated transcriptional profile exhibited an analogous pattern to dark to some extent, and displayed a distinct pattern with that under blue and far-red light.

Hierarchical cluster and gene ontology (GO) analysis of DEGs

Hierarchical cluster analysis of DEGs divided all DEGs into 12 clusters. It is worthy noted that approximately 75% of DEGs exhibiting the analogous tendency between dark and red light, or between blue and far-red light. These genes includes 2789 genes in Group 1 (high expression in dark/red and low expression in blue/far-red), 4480 genes in Group 2 and Group 3 (low expression in dark/red and high expression in blue/far-red). We performed gene ontology (GO) analysis of these DEGs. Group 1 associated GO terms include "circadian rhythm", "photoperiodism", "shade avoidance", "plant growth", "cell wall" (Fig. 4A). Group 2 and 3 associated GO terms include "red/far-red/blue/UV-A/UV-B light signal response", "circadian rhythm", "de-etiolation", "photomorphogenesis", "photoperiodism", "shade avoidance", "chloroplast movement" and "photosynthesis" (Fig. 4C). Additionally, 23.67% of blue and far-red light activated genes were directly associated with light signaling and light-dependent biological

processes, while only 3.8% genes induced by dark and red light were enriched in those processes (Fig. 4B, 4D).

Key regulators in light signaling pathway

Light signaling and its downstream pathway was displayed according to our knowledge on photomorphogenesis in plants, mainly in *Arabidopsis*. PHYA and PHYB perceive far-red and red light, respectively, to be converted to active Pfr form. Previous studies showed that PHYA, as a type I phytochrome, accumulates abundantly in the dark, and it is degraded or repressed under both red and far-red light exposure. PHYB belongs to Type II phytochrome, and it is stable in the light [1, 5]. In this study, two *PHYA* transcripts were accumulated in dark, and were significantly decreased in all light conditions (Fig. 5). This result indicates that light not only represses PHYA in protein level, but also inhibits its gene expression. Although the active Pfr form of PHYB can be activated by red light, the gene expression of *PHYB* didn't change in response to red light, indicating the influence of red light on PHYB was in protein level rather than in transcriptional level.

HY5 is one of the key positive regulators in seedling photomorphogenesis [10] and plays central role in integrating branches of all photoreceptors in light signaling pathway. In *Arabidopsis*, light enhances both *HY5* expression and HY5 protein level, thereby promoting photomorphogenesis [19]. HYH, a homolog of HY5, interacts with HY5 to promote photomorphogenesis [20]. Our result showed that the *HY5* and *HYH* was in low expression under red light whereas their expressions were upregulated in response to blue and far-red light (Fig. 5 and S2C). HY5 physically interacts with HFR1 to promote PHYA-mediated photomorphogenesis [21]. In this study, HFR1 expression was strongly repressed in dark and red light, while it was highly upregulated in blue and far-red light. We speculated that the low expression of these three positive regulators may lead to enhance hypocotyl elongation under red light.

On the contrary, COP1 is regarded as a central negative factor on plant photomorphogenesis [22, 23]. It has been reported that COP1-SPAs complex not only target positive regulators (HY5, HFR1, etc) for ubiquitination and degradation, but also promote the accumulation of negative factors such as PIFs [17, 24, 25]. We found four *COP1* transcripts, among which only one transcript had relative high expression. Due to conserved and fundamental function of COP1 from animals to plants [26], we speculated that single homologous gene may function properly, and two or more COP1s may cause confusion in regulation of downstream pathway. In dark and red light condition, hypocotyl length was relative long, while *COP1* was in relative low expression (Fig. 5 and S2D). The result indicated that the gene expression level of COP1 can't explain phenotypic difference of hypocotyl elongation. Actually, COP1 functions in post-translational level [27], rather than in transcriptional level.

PIFs and BBXs transcription factors

PIFs, the basic helix-loop-helix (bHLH) transcription factor, are negative regulators of light responses by repressing photomorphogenesis [2]. All of PIF members contains APB domain, while only PIF1 and PIF3 contain APA motifs (PHYA-Pfr interacting site). Intriguingly, PIF1 and PIF3 had analogous transcriptional

pattern, in which their expressions were repressed by dark and red light, and were upregulated by blue and far-red light (Fig. 5 and S3A). PIF3 is a major negative regulator, and it is deduced that its high expression and following accumulation in protein level helps to repress photomorphogenesis. PIF1 was reported as a key factor regulating red/far-red light mediated seed germination. Although PIF1 had a similar transcriptional pattern with PIF3, whether PIF1 have a putative role in regulation of photomorphogenesis remains elusive.

PIF4 is responsible for high temperature-induced hypocotyl elongation [28], and both PIF4 and PIF5 function in shade avoidance-mediated hypocotyl elongation. Overexpression of PIF4 or PIF5 caused constitutively long hypocotyl [29]. In this study, we found the PIF4 and PIF5 were highly expressed in red light (Fig. S4), which may partially explain the long hypocotyl under red light.

BBXs, B-BOX domain proteins, are one of the most important groups of transcription factors related with light signal. It has been identified BBX21/22/23 promotes photomorphogenesis [30–32], whereas BBX24/25/28/30/31/32 negatively regulate light signaling [33, 34]. These BBXs interact with HY5 via distinct regulatory ways in regulation of photomorphogenesis [34]. However, unlike PIFs, the transcriptional pattern of many BBXs genes can't explain the hypocotyl phenotypes under different light conditions (Fig. S3B). Previous study suggested that BBXs function in post-translational level in regulation of HY5 expression [32, 35].

Phytohormone

Previous studies reported that abscisic acid (ABA) promotes photomorphogenesis whereas gibberellin (GA), brassinosteroid (BR), ethylene, and auxin promote skotomorphogenesis [20, 36–41]. These multiple hormonal pathways interact with a key signal integration center HY5 to regulate photomorphogenesis [9]. *ABA INSENSITIVE 5 (ABI5)*, a positive regulator in ABA signaling, is activated through binding with HY5, thereby promoting photomorphogenesis [9]. Both of *INDOLE ACETIC ACID 7 (IAA7)* and *INDOLE ACETIC ACID 14 (IAA14)*, auxin signaling inhibitors, are promoted by HY5 and negatively regulate hypocotyl elongation [9]. *GA2ox2*, an important GA catabolic gene, is induced via HY5 to negatively regulate GA level and inhibited hypocotyl elongation [9]. Overexpression of *GA2ox8* reduces bioactive GA level and causes growth retardation, flowering delay and lignification decrease [42]. Taken together, we can conclude that *ABI5*, *IAA7/IAA14*, *GA2ox2/GA2ox8* serves as positive regulators which indirectly promotes photomorphogenesis. In this study, the gene expression of *ABI5*, *IAA7/IAA14*, *GA2ox2/GA2ox8* was promoted by blue/far-red light rather than red light (Fig 5), which can explain the phenotypic difference between blue/far-red light and red light. *BZR1* (BRASSINAZOLE RESISTANT 1), a positive regulator in BR signaling pathway, suppressed by HY5 to promote photomorphogenesis [9]. Our results showed the expression of *BZR1* was induced in dark while it was repressed in light condition (Fig 5), which can explain the light regulated photomorphogenesis in transcriptional level.

It has been reported that EIN3 plays an important role in the balance of ethylene and light signaling in hypocotyl growth [43]. *ETHYLENE INSENSITIVE 3 (EIN3)* has been shown to repress cotyledon development and promote hypocotyl elongation in the dark, and EIN protein is rapidly degraded by light

exposure. Consistently, our result showed EIN3 showed higher expression level in dark, which helps to promote skotomorphogenesis [18]. Previous study showed that ethylene can promote hypocotyl growth in high intensity of red light via activation of *PIF3* expression by EIN3/EIL1 [44]. It can be an explanation for the observation of high *PIF3* expression and long hypocotyl in red light (Fig. 5).

Genes related with cell elongation and cell wall modification

In dark, PIFs activate the gene expressions of many genes responsible for cell elongation/division to promote hypocotyl elongation [37, 38]. For instance, Xyloglucan endotransglycosylase/hydrolase (XTH) and expansin (EXP) are two classes of vital enzymes in cell wall loosening and cell expansion [45, 46]. Pectin methylesterases (PME) plays pivotal role in plant cell wall formation [47]. In this study, *EXP3/EXP9*, *XTH9* and *PME31* exhibited high expression in dark/red light and low expression in blue/far-red light. These genes may function in dark/red light mediated hypocotyl elongation via promoting cell elongation and cell wall development.

Discussion

In the past few years, our understanding of the light-signaling-regulated transcriptional networks have been much improved. Many studies revealed various interaction mechanisms between key regulators (COP1, PIFs and HY5) and downstream cascade genes in signaling pathways, even though the deep molecular mechanism remains elusive. However, most of research was performed on *Arabidopsis thaliana*. It is largely unknown whether the light signaling and downstream pathway is conserved in other plant species, since many genes are not evolutionarily conserved with diverse function. In this study, we found all of light quality have positive effect on promoting photomorphogenesis in eight dicotyledonous plants including *B. napus*, with the extent of promotion varied among plant species and light qualities. *B. napus* (cultivar ZD622) showed relative longer hypocotyl than other seven dicotyledonous plants (in particular, *B. rapa*) under red light. This phenotype was partially explained by transcriptome data, in which dark and red light treated seedling exhibited an analogous transcriptional pattern to some extent.

Since *Brassica* with *A. thaliana* divided from *Brassicaceae* approximately 14–20 million years ago [48], *Brassica* species experienced an extra whole genome triplication (WGT) event before long [49, 50]. Followed by massive genome fractionation and chromosome reduction, stable diploid species formed gradually [51]. Subsequently, two important *Brassica* species, *Brassica oleracea* and *Brassica rapa*, occurred about 4 million years ago [52]. As an allopolyploidy, *B. napus* was formed ~ 7500 years ago, with hybridized from *B. oleracea* and *B. rapa* and followed by chromosome doubling [13]. Together with more ancient polyploidizations, most higher plants undergone genome multiplications [13]. Thus, for homologous genes, one gene in *A. thaliana* refers to two to six homologous genes in *B. napus*. It is a question which one plays a major role in the pathway, or whether these homologs function redundantly. Many key regulators, including *PHYA*, *PHYB*, *HY5*, *HYH*, *HFR1* and *PIF3* (Fig. 5) showed an analogous transcriptional pattern among homologs, suggesting that these homologs may have redundant function. For each gene (except *PHYB*), 2–3 homologs were in relative high expression, with one homolog from

subgenome A and one from subgenome C (Fig. 5F), suggesting that the significance of these homologs in photomorphogenesis.

It is worthy noted that both of two *PHYB* homologs (BnC03g0582300 and BnC05g0721960) in relative high expression were from subgenome C. As the vital role of *PHYB* in red/far-red light signaling, the *PHYB* is highly structural and functional conserved in land plants including *B. rapa* [53]. Genes may interact well with genes from same species but not other species. For example, *COP1* and *SPAs* interact with each other, and they co-evolved gradually within the plant species. The moss *PpSPA* can't rescue the phenotype of *spa* mutant in *Arabidopsis*, indicating that moss *PpSPAs* can't interact with *Arabidopsis AtCOP1* [54]. Analogously, the two interacting proteins from the same subgenome may display better interactions than those from different subgenome. The gene silence of *PHYB* from subgenome A may interfere with its interaction with downstream pathway from subgenome A. Among eight tested species, *B. rapa* showed lowest relative hypocotyl length under red light (Fig. S1), indicating the importance of red light on promotion of photomorphogenesis in *B. rapa*. The gene silence of *PHYB* from subgenome A may abolish the effect of red light on photomorphogenesis in *B. napus*.

As known, *COP1* is evolutionary conserved from plants to animals [55], and plays a central role in skotomorphogenesis/photomorphogenesis. For instance, Although there are four *COP1* homologs in *B. napus* genome, we found only one gene (*BnA05g0197770*) was in relative high expression, while other three were hardly detected. *COP1* interacts with many key downstream proteins (*HY5*, *HYH*, *HFR1*, etc.) to degrade them. The presence of two or more homologs may cause confusion in its regulation on downstream pathway. Therefore, it is reasonable for plant to keep only one homolog expressed. It is widely known that *PHYB* can phosphorylate *COP1* and degrade it in light condition [6]. The transcripts of *PHYB* (subgenome C) and *COP1* (subgenome A) from different subgenome in *B. napus* may have reduced interaction between them, which may contribute to the relative long hypocotyl under red light. This hypothesis need be examined by future work.

Recently, many studies revealed the importance of *BBXs* in photomorphogenesis in *Arabidopsis* (Xu et al., 2016b; Zhang et al., 2017b; Zhao et al., 2020). Previous study reported that *BBX21* expression decreased in transcriptome at first and then gradually increased after 6 to 48 h of light exposure. Surprisingly, *BBX21* protein level performed opposite manner compared with transcriptional level [56]. *BBX23* have an analogous expression pattern to *BBX21*. The mRNA level of *BBX23* was gradually reduced when exposed to light for up to 3 h while *BBX23* protein accumulated simultaneously [57]. The converse pattern in mRNA and protein level suggested the complex regulatory roles of these *BBXs* in photomorphogenesis. On the contrary, *BBX11* accumulated both in transcript level and protein level when dark-grown seedlings transferred to light for 24 h [58]. In addition, *BBX22* and *BBX25* accumulated at transcriptional level when treated with light conditions for 10 h [59]. Coincidentally, *BBXs* expression, especially *BBX21/22/23/25* in *A. thaliana* is consistent with our transcriptional result in *B. napus* (Fig. 6). Those results suggested that light induced transcriptional regulation of these *BBXs* was conserved in *A. Thaliana* and *B. napus*. Moreover, we also found transcriptional content of some major *BBXs*, such as *BBX21*, *BBX24*, *BBX25* from subgenome C distinctly exceeded those from subgenome A (Fig. 6). Photoreceptors *PHYA*, *PHYB*,

CRY1, and CRY2 promoted *BBX11* transcripts expression under light [58]. Therefore, PHYA, PHYB, CRY1 and CRY2 may play important roles in regulating *BBXs* transcriptional level. Our results revealed *PHYA*, *CRY1*, *CRY2* gene expression exists both in subgenome A and subgenome C (Fig. 5A and Fig. S3), whereas *PHYB* expression only from subgenome C. We suspected that loss-function of PHYB from subgenome A may affect regulation to *BBXs* expression from subgenome A. PHYB from subgenome C may interact tightly and weakly with *BBXs* transcripts from subgenome C and subgenome A, respectively, which maybe partially explained why some *BBXs* expression from subgenome C surpassed those from subgenome A. However, the molecular mechanism of transcriptional regulation of *BBXs* was still largely unknown. Hence, our data provided a global description in transcriptional profile of *BBXs* in response to different light quality, but the underlying mechanism remains elusive.

Conclusion

In conclusion, our results displayed a comprehensive overview of transcriptional profile in response to different light quality in seedling morphogenesis of *B. napus*, and it can provide useful information for future research. It is worthy noted that some signaling modules function in protein level via protein modification or protein interaction, rather than in transcriptional level. The response of proteome profile to different light quality is of significance and need be further explored in the future.

Methods

Plant material and light treatments

Eight dicotyledon plants species seeds, including *Brassica napus* (cultivar ZD622; semi-winter type), *Brassica rapa* (Jinguan56), *Brassica oleracea* (Zhonggan15), *Solanum lycopersicum* (Zhongshu4), *Cucumis sativus* (Xiangcuitian), *Medicago sativa* (Gannong3), *Glycine max* (Zhonghuang37) and *Fagopyrum esculentum* (Lvobao) were used in this study. *Brassica napus* (ZD622) was provided by Prof. Zhou Weijun in Zhejiang University. The other seeds of seven species were commercial varieties obtained from local shop (Jinzhongzi Nongzi Company, Beijing, China). The imbibed seeds were sowed into soil and incubated under white light (W), red light (R), far-red light (FR), blue light (B), and dark (D) conditions respectively. The hypocotyl length was measured after two days treatment in a growth chamber (14 h light/10 h dark). Monochromatic red, far-red and blue light was provided by light-emitting diodes with maximum wavelengths of 660 nm, 730 nm and 460nm, respectively.

Library preparation for RNA sequencing

After two days treatments (6 h of light exposure in the daytime), the seedlings (including hypocotyl and cotyledon) were sampled and frozen stored (-80 °C) for transcriptome analysis. Total RNA was extracted using the Quick RNA Isolation Kit. The sequencing libraries were generated using NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) and index codes were added to attribute sequences to each

sample. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v4-cBot-HS (Illumina).

Sequencing and read mapping

After cluster generation, the library preparations were sequenced on an Illumina platform and paired-end reads were generated. The genome of ZS11 [52] was used as reference genome in following analysis. The spliced reads were mapped to reference genome using HISAT2 (<http://ccb.jhu.edu/software/hisat2/index.shtml>) and the transcript was assembled using String Tie (<https://ccb.jhu.edu/software/stringtie/index.shtml>). The gene functional annotation was performed based on the following databases: Nr (NCBI non-redundant protein sequences); Nt (NCBI non-redundant nucleotide sequences); Pfam (Protein family); KOG/COG (Clusters of Orthologous Groups of proteins); Swiss-Prot (A manually annotated and reviewed protein sequence database); KO (KEGG Ortholog database); GO (Gene Ontology).

Data analysis of RNA-sequencing

Differential expression analysis among treatments was conducted using DEseq R package. FDR (false discovery rate) adjusted P-value <0.01 was used as a threshold for screening of differentially expressed genes (DEGs). Gene Ontology (GO) enrichment analysis of DEGs was carried out using Goseq R package, which is based on Wallenius non-central hyper-geometric distribution [60]. Correlation coefficient was implemented by CORREL function among DEGs in group Ⅰ, group Ⅱ, group Ⅲ and the significance test of correlation was performed using SPSS Statistics v24 (IBM, USA). Hierarchical cluster analysis of DEGs was conducted using heatmap R package.

Validation of transcriptome data by quantitative real-time PCR

In order to validate the reliability of transcriptome data, the gene expressions of 12 selected genes were analyzed by quantitative real-time PCR (qRT-PCR). The qRT-PCR was conducted using SYBR Green fluorescence and *PerfectStart*TM Green qPCR SuperMix on a Roche LightCycler 480 sequence detection system. *BnACT1* (BnA04g0180440) was used as reference gene.

Abbreviations

B. napus: *Brassica napus*; *A. thaliana*: *Arabidopsis thaliana*; DEGs: Differentially expressed genes; qRT-PCR: Quantitative real time polymerase chain reaction

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the raw data supporting the results of this article have been deposited into the NCBI Sequence Read Archive under accession number SRR12488723, SRR12488724, SRR12488725, SRR12488726, SRR12488727, SRR12488728, SRR12488729, SRR12488730, SRR12488731, SRR12488732, SRR12488733, SRR12488734, SRR12488735, SRR12488736, SRR12488737.

Competing interests

The authors declare that they have no competing interests.

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Author contribution

S.C. designed and supervised this study. X.Z. performed the experiments. X.Z., Y.L. Y.H. and D.W. analyzed data. X.Z. and S.C. wrote the manuscript. All authors have read and approved the manuscript.

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Figures

Figure 1

The effects of light quality on hypocotyl length of photomorphogenesis in *Brassica napus*. a. Morphology of seedling in different light conditions (W, B, FR, R, D) in *Brassica napus*; b. Hypocotyl length of seedling under different light quality (W, B, FR, R, D) in *Brassica napus*. Bar: 1 cm

Figure 2

Principle cluster analysis (PCA) and correlation analysis under different light conditions in *Brassica napus*. a. Principle component analysis (PCA) of all differentially expression genes (DEGs) under different light quality (W, B, FR, R and D) in *Brassica napus*; b. Correlation analysis between two of different light quality in *Brassica napus*. *** and ** represents significance at $P < 0.001$ and $P < 0.01$ level, respectively.

Figure 3

Hierarchical cluster analysis of DEGs of transcriptome in response to different light quality in *Brassica napus*. The scale bar represents the log10 of the ratio.

Figure 5

The transcriptional expression level of major genes in light signaling pathway in *Brassica napus* under different light quality (D, R, W, B, FR). a. PHYA and PHYB; b. HY5 and HYH; c. HFR1; d. COP1; e. PIF3; f. The gene number of highly expressed genes in two subgenomes.

Figure 6

Gene expression of BBXs gene family in Brassica napus upon various light conditions. The number in the box represents the FPKM value. The scale bar represents the log2 of the ratio.

Figure 7

Transcriptional regulation of key regulators and downstream pathway in light signaling networks. The scale bar represents the log2 of the ratio. Pointed-head arrow and flat-head arrow represents promotion and repression, respectively.

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