



Review

The Flavonoid Biosynthesis and Regulation in *Brassica napus*: A Review

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Abstract: *Brassica napus* is an important crop for edible oil, vegetables, biofuel, and animal food. It is also an ornamental crop for its various petal colors. Flavonoids are a group of secondary metabolites with antioxidant activities and medicinal values, and are important to plant pigmentation, disease resistance, and abiotic stress responses. The yellow seed coat, purple leaf and inflorescence, and colorful petals of *B. napus* have been bred for improved nutritional value, tourism and city ornamentation. The putative loci and genes regulating flavonoid biosynthesis in *B. napus* have been identified using germplasms with various seed, petal, leaf, and stem colors, or different flavonoid contents under stress conditions. This review introduces the advances of flavonoid profiling, biosynthesis, and regulation during development and stress responses of *B. napus*, and hopes to help with the breeding of *B. napus* with better quality, ornamental value, and stress resistances.

Keywords: *Brassica napus*; flavonoid; plant development; stress response; regulation



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1. Introduction

Flavonoids are a group of secondary metabolites that broadly exist in plants and play important roles in plant development and stress responses by participating in various physiological processes [1,2]. Flavonoids are mainly compounds with C6-C3-C6 framework, including chalcones, flavans, flavones, flavonols, anthocyanins, and proanthocyanidins (PAs, or condensed tannins) that are synthesized through the flavonoid biosynthetic pathway [3]. Aside from these, stilbenes have also been reported as a group of flavonoids with C6-C2-C6 framework [4]. Hitherto, over 8000 flavonoids have been identified in plants [5]. As reported, flavonoids are major contributors for red, pink, blue, and purple pigmentations in plant organs (i.e., seeds, fruits, flowers, and leaves), and are also involved in plant adaptations to various abiotic and biotic stresses [6–8]. For example, the endoplasmic reticulum (ER)-derived flavonoids in *B. napus* anthers are mainly accumulated in tapetum cells to protect pollens from light damage and water stress [9]. Furthermore, these compounds are valuable to the quality of plant products, and have been intensively studied for their health and medical benefits against different diseases (i.e., cancers, cardiovascular diseases, and inflammatory diseases) [10–13].

Rapeseed (*Brassica napus* L.), as the second leading oil crop in the world, is a natural allotetraploid widely cultivated for its vegetable value, edible oil production, and animal foraging value [14]. Previously, breeding rapeseed with a yellow seed coat has been preferred for better oil and protein content, and less anti-nutrients (i.e., lignin, proanthocyanidins). Lately, rapeseed germplasms with colorful flowers have been bred for flower-based tourism and city ornamentation in China [15,16]. Moreover, rapeseed with purple leaves and inflorescence is rich in anthocyanins and provides valuable nutrients for human health [17]. These traits are closely related to flavonoid biosynthesis, and many research papers focusing on the gene screening and molecular regulation of flavonoid

3. Flavonoid Profiles Tentatively Identified in Rapeseed

In *B. napus*, the flavonoid profiles in seeds, roots, leaves, and petals have been reported. Farag et al. (2013) identified 68 phytochemical compounds in different organs (root, leaf, stem, inflorescence, and seed) of *B. napus* via UPLC-QTOF-MS, including 13 hydroxycinnamic acids/sinapoyl cholines, 25 flavonols, and one flavanone [38]. Shao et al. (2014) reported a comprehensive profile of flavonoids and hydroxycinnamic acid derivatives in black seeds of *B. napus*, including 39 kaempferols, 11 isorhamnetins, 5 quercetins, 6 flavanols, and 30 hydroxycinnamic acid derivatives [39]. Qu et al. (2013) identified 35 flavonols in the seed coat of *B. napus*, including 13 and 2 compounds specific to yellow and black seeds, respectively. They found the flavonoid biosynthetic genes, anthocyanin- and proanthocyanidin-specific genes, as well as regulators in the flavonoid synthetic pathway were repressed in yellow seeds compared with that in black seeds [40]. Li et al. (2009) created yellow-seeded rapeseed germplasms from *B. napus*-*Sinapis alba* somatic hybrids, and Jiang et al. (2013) revealed the 16 flavonoids differentially accumulated between yellow and black seeds, which might be due to the repressed flavonoid biosynthetic genes in yellow seeds compared with black seeds [41,42]. Later, Wang et al. (2018) compared the content of 56 phenolic components between yellow- and black-seeded *B. napus*, including 15 hydroxycinnamic acid derivatives, 21 kaempferols, 10 isorhamnetins, 5 quercetins, 5 epicatechins and derivatives, which might be correlated with the seed coat color variation in the progeny of *B. napus*-*S. alba* hybrids [43]. Yin et al. (2019) identified 15 phenolic acids and 28 flavonoids in rapeseed accessions with pale white, yellow, pink, and red petals, which might be responsible for the petal color variation [44]. Recently, 39 flavonoids including 35 anthocyanins were identified in three rapeseed cultivars with purple, reddish-green, and green leaves. A total of 22 anthocyanins that exhibited different accumulation might contribute to the leaf color variations, including cyanidin and kaempferol derivatives. The differentially expressed genes among three rapeseed cultivars were most enriched in metabolic pathways (i.e., flavonoid biosynthesis, secondary metabolite biosynthesis). Many regulatory genes (i.e., *MYB*, *ERF*, *NAC*, and *bHLH*) and structural genes (i.e., *BnDFR*, *BnANS*, *BnUGTs*, and *BnGSTs*) related to anthocyanin biosynthesis were up-regulated in purple and reddish-green leaves more than that in green leaves [17]. The flavonoid profiles have also been reported in rapeseed under different growth conditions. Shen et al. (2022) comprehensively analyzed the metabolic profiles in roots and leaves of rapeseed grown under normal nitrogen (HN) and nitrogen-deficient (LN) conditions. They identified that three anthocyanins and 22 flavonols were increased, while one anthocyanin, three flavonols, and five flavanones were decreased in leaves under LN compared to HN conditions. In roots of rapeseed, 13 flavonoids were increased and 12 were decreased in plants grown under LN compared to HN conditions [45]. Although many flavonoids have been characterized in different organs of rapeseed and stressed plants, the regulation of flavonoid accumulation during rapeseed development and in response to various stresses has not been fully elucidated. Hitherto, only a few genes and loci related to flavonoid regulation in *B. napus* have been reported (Table 1).

Table 1. The functionally characterized genes regulating flavonoid synthesis in *B. napus*.

Gene	Origin	Function	Phenotype	Reference
<i>BnTT8</i>	<i>Brassica napus</i>	<i>BnTT8</i> mutation suppressed the phenylpropanoid and flavonoid biosynthetic gene expression, and inhibited proanthocyanidin accumulation in seed coat of rapeseed.	Seed color	[20]
<i>BnTT1</i>	<i>Brassica napus</i>	Silencing of <i>BnTT1</i> reduced flavonoid accumulation and fatty acid biosynthesis through altering gene expression in flavonoid and fatty acid biosynthesis.	Seed color	[46]
<i>BnTT10</i>	<i>Brassica napus</i>	Silencing of <i>BnTT10</i> increased soluble proanthocyanidins, decreased extractable lignin, and retarded pigmentation in seed coat of <i>B. napus</i> .	Seed color	[47]

Table 1. Cont.

Gene	Origin	Function	Phenotype	Reference
<i>BnTT2</i>	<i>Brassica napus</i>	Mutation of <i>BnTT2</i> reduced flavonoids and improved fatty acid composition in seed of <i>B. napus</i> .	Seed color	[19]
<i>OvPAP2</i>	<i>Orychophragmus violaceus</i>	Ectopic expression of <i>OvPAP2</i> led to red anthers and petals in <i>B. napus</i> .	Petal color	[48]
<i>BnaA03.ANS</i>	<i>Brassica napus</i>	RNA interference of <i>BnaA03.ANS</i> repressed anthocyanin accumulation in red petal rapeseed.	Petal color	[16]
<i>BnaA07.PAP2</i>	<i>Brassica napus</i>	The insertions in –184 and –371 bp were responsible for the transcriptional activation of <i>BnaA07.PAP2</i> and anthocyanin-related genes, and resulted apricot petal color in rapeseed.	Petal color	[49]
<i>BnaA09.ZEP/BnaC09.ZEP</i>	<i>Brassica napus</i>	<i>BnaA09.ZEP</i> and <i>BnaC09.ZEP</i> negatively regulated the orange color in rapeseed petals by affecting the carotenoid and flavonoid content, as well as the expression of carotenoid and flavonoid biosynthetic genes.	Petal color	[15]
<i>BnaCRTISO</i>	<i>Brassica napus</i>	<i>BnaCRTISO</i> mutation reduced chalcone content and increased carotene content, thus changing the petal and leaf color of rapeseed.	Petal/leaf color	[50]
<i>BnaA.PL1</i>	<i>Brassica napus</i>	A QTL locus for anthocyanin-rich mutant of rapeseed, including a candidate gene <i>BnAPR2</i> that encoded adenosine 5'-phosphosulfate reductase.	Leaf color	[51]
<i>BnaPAP2.A7</i>	<i>Brassica napus</i>	Three isoforms of <i>BnaPAP2.A7</i> identified in rapeseed introgression line were confirmed with different roles in manipulating anthocyanin accumulation in leaves.	Leaf color	[52]
<i>BnGL3-1</i>	<i>Brassica napus</i>	Ectopic expression of <i>BnGL3-1</i> increased the trichome number and anthocyanin accumulation in true leaves of <i>Arabidopsis gl3-3</i> mutant.	Leaf color	[53]
<i>AtPAP1</i>	<i>Arabidopsis thaliana</i>	Overexpression of <i>Arabidopsis PAP1</i> increased flavonoid and sinapic acid accumulation in leaves and stems of rapeseed.	Leaf/stem color	[54]
<i>BnCRY1/BnCRY2</i>	<i>Brassica napus</i>	Overexpression of <i>BnCRY1</i> and <i>BnCRY2a</i> increased anthocyanin content and regulated seedling photomorphogenesis of <i>B. napus</i> .	Seedling development	[55,56]
<i>AtDFR</i>	<i>Arabidopsis thaliana</i>	Overexpression of <i>Arabidopsis AtDFR</i> increased anthocyanin accumulation and improved salt tolerance of <i>B. napus</i> .	Salt tolerance	[57]
<i>BnFLS</i>	<i>Brassica napus</i>	Overexpression of <i>BnFLS</i> recovered the flavonol content in <i>Arabidopsis atfls1-ko</i> mutant.	–	[58]
<i>BnET</i>	<i>Brassica napus</i>	Overexpression of <i>BnET</i> promoted anthocyanin accumulation in <i>Arabidopsis</i> .	–	[59]
<i>BnFLS1-1/1-2</i>	<i>Brassica napus</i>	<i>BnFLS1-1</i> and <i>BnFLS1-2</i> restored the flavonoid content in <i>Arabidopsis ans/fls1</i> and <i>f3h</i> mutants.	–	[60]

4. Regulation of Flavonoid Biosynthesis in Seeds of *B. napus*

Flavonoid accumulation could affect seed coat color, seed germination, and seed meal quality of *B. napus*. Jia et al. (2012) proved that increased proanthocyanidin content inhibited seed germination of *Arabidopsis* and *B. napus*, which was due to the ABA accumulation in seeds. Proanthocyanidin might play a role as a doorkeeper during seed germination [61]. Through a genome-wide association study (GWAS) of 96 *B. napus* accessions, Bhinder et al. (2022) identified 789 significant single nucleotide polymorphisms (SNPs) associated with seed meal quality of rapeseed. Three candidate genes, *SOT12*, *SK1*, and *UGT88A1*,

related to flavonoid biosynthesis were identified, which should be helpful for reducing anti-nutritional components and improving seed meal quality of *B. napus* [62]. In *B. napus*, many studies of flavonoid biosynthesis and regulation related to seed coat color have been reported.

4.1. Gene Expressional Changes in Yellow Seed of *B. napus*

Yellow seed is a quantitative trait preferred by breeders for its advantages of high oil and protein content and less anti-nutritional components (i.e., lignin, fiber, pigments, and polyphenols) compared with its black-seeded counterpart [63]. However, due to the genome complexity of rapeseed and genetic background of yellow-seeded germplasm, yellow seed trait (YST) is sensitive to harvesting time, temperature and fertilizers. The related molecular mechanisms of this phenotype and its connection with other seed traits still need to be elucidated. As reported, *F3'H*, *TT1*, *TT2*, *PAL*, *BAN*, *TTG1*, and *TT10* have been cloned and proven to have functions in flavonoid biosynthesis [46,47,64–69]. Yu (2013) reviewed the molecular mechanism of manipulating seed coat color in *Brassica* species, including the homologous *TTs* cloned in *Brassicaceae* [63]. Homologous *TT* genes related to seed coat variation in *Brassicaceae* have also been analyzed, including 95 copies of 21 *TTs* in *B. napus* [18]. Liu et al. (2016) identified all the proanthocyanidin-associated genes in the genome of *Brassicaceae*, including the 58 homologous genes in *B. napus* [70]. Based on the transcriptome analysis of yellow- and brown-seeded near-isogenic lines (NILs) of *B. napus*, Hong et al. (2017) found that the down-regulated genes in yellow seed coats were enriched in phenylpropanoid and flavonoid biosynthesis [71]. Jiang et al. (2019) compared the gene expression in developing seeds of yellow seeds from *B. napus*-*Sinapis alba* somatic hybrids with black rapeseeds, and found many differentially expressed genes (DEGs) were involved in flavonoid and phenylpropanoid biosynthesis, phenylalanine metabolism, flavone and flavonol biosynthesis, fatty acid (FA) biosynthesis, and metabolism [72]. Lin et al. (2020) analyzed the novel transcripts in yellow- and black-seeded rapeseeds, and identified the alternative splicing profiles at different seed developmental stages. Finally, they screened 24 differentially alternative splicing (DAS) genes (i.e., *BAN*, *CHI*, *DFR*, *AHA10*, *STK*, *TT5/8/10/12/16*) that might be related to the seed coat color variation, and would be valuable to yellow seed breeding [73]. These DEGs would be helpful for the molecular dissection of yellow seed trait in rapeseed.

4.2. Quantitative Trait Loci of Yellow Seed Trait in *B. napus*

Hitherto, many QTLs related to the yellow seed trait of *B. napus* have been reported. Badani et al. (2006) identified a major QTL of seed color on C08, which was co-located with a major QTL of acid detergent fiber (ADF) and confirmed by Zhang et al. (2011) afterwards [74,75]. Similarly, a major QTL on A09 was reported to control seed color and fiber content [76,77]. The locus reported by Liu et al. (2012) was tightly linked to a major QTL for seed acid-detergent lignin (ADL) content, and *BnaA.CCR1.A9* with a functional mutation in the first exon might be the candidate gene for yellow seed with reduced ADL content [76]. Afterwards, they reported 11 QTLs for seed color and fiber traits across four different environments, and constructed a high-density map with considerably improved QTL resolution [78]. Using the recombinant inbred lines (RILs) between *B. napus* cv. Zhongyou 821 and yellow seed line GH06, Qu et al. (2017) constructed a linkage map with 1089 markers, identified 72 eQTLs associated with the 18 flavonoid biosynthetic genes, and found *bZIP25*, *MYC1*, *MYB51*, and *MYB52* were candidate genes in the eQTL hotspots [79]. Wang et al. (2017) also identified two homologous loci controlling seed coat color on C08 and A09 through GWAS analysis [80]. In addition, many minor QTLs of YST were also detected on C02, C05, C06, C07, A01, A04, A07, and A08 [80,81]. Recently, Chao et al. (2022) identified a major yellow-seed QTL, *cqSC-A09*, and found the advantageous allele significantly increased the oil content, and reduced the pigment and fiber content in seeds of *B. napus*. Among the 648 genes in *cqSC-A09*, *BnaA09.JAZ1*, *BnaA09.GH3.3*, and

BnaA09.LOX3 were predicted as major candidates after sequence variation annotation, expression differences and interaction network analysis [82].

4.3. Functionally Characterized Genes Regulating Yellow Seed Trait of *B. napus*

Although many QTLs and DEGs related to YST have been identified in *B. napus*, only a few genes regulating flavonoid biosynthesis and seed coat color of *B. napus* have been proven. Zhai et al. (2020) firstly created yellow-seeded *B. napus* through the CRISPR/Cas9 technique, and confirmed that homozygous mutation of *BnTT8* suppressed the expression of phenylpropanoid and flavonoid biosynthetic genes, inhibited proanthocyanidin accumulation in seed coat, improved seed oil and protein content, and altered FA composition of rapeseed [20]. Xie et al. (2020) also found that mutation of *BnTT2*, another regulator in flavonoid biosynthetic pathway, reduced flavonoids and improved FA composition in rapeseed [19]. Previously, RNAi of *BnTT1* and *BnTT10* revealed their function in regulating PA metabolism, lignin synthesis, seed coat pigmentation, and FA biosynthesis [46,47]. All these genes are homologs of *Arabidopsis* TTs in rapeseed, and none of the reported DEGs or candidate genes in the aforementioned QTLs have been functionally characterized.

5. Regulation of Anthocyanin Biosynthesis in *B. napus* Flowers

Apart from carotenoids, flavonoids such as chalcones, certain flavonols, and anthocyanins are also flower pigments, of which anthocyanins are major contributors for orange, pink, red, purple, and blue flowers [83,84]. In *Arabidopsis*, anthocyanin biosynthesis is regulated by MBW complexes consisting of a MYB protein (i.e., AtMYB113/114, MYBL2, AtPAP1/2), a bHLH protein (i.e., GL3, EGL3, TT8), and a WD40 protein (i.e., TTG1) [85]. Hitherto, only a few studies on anthocyanin-related rapeseed petal color have been reported. Overexpression of *Orychophragmus violaceus* *OvPAP2*, a homolog of *AtPAP2*, resulted in purple and red pigmentation on flower organs of *Arabidopsis* and rapeseed, respectively [48]. Based on the map-based cloning and gene functional analysis, Liu et al. (2020) proved that *BnaA09.ZEP* and *BnaC09.ZEP* negatively regulate the orange color in rapeseed petals by affecting the carotenoid content. Moreover, the expression of flavonoid biosynthetic genes and flavonoid profiles were also changed between the *BnaA09.ZEP/BnaC09.ZEP* mutants and their complementary lines [15]. Metabolomic and RNA-seq analysis on two developmental stages of unopened red, white, and yellow petals of rapeseed revealed that enriched flavonoids and up-regulated anthocyanin biosynthetic genes (i.e., *DFR*, *ANS*, and *UF3GT*) might be responsible for the red pigmentation. Repression of *BnaA03.ANS* in red-petal rapeseed changed the petal color to beige red and zinc yellow, while overexpression of *BnaA03.ANS* did not change the color of yellow petals [16]. Ye et al. (2022) found *BnaA07.PAP2*, *BnaMYBL2*, *BnaTT8* and structural genes (i.e., *DFR*, *ANS*, *UF3GT* and *GST*) in anthocyanin biosynthesis were up-regulated in apricot and pink flowers compared with yellow and white flowers of rapeseed. They confirmed *BnaA07.PAP2* as a key gene regulating anthocyanin pigmentation of rapeseed flowers through map-based cloning and multi-omics analysis. Moreover, two insertions in -184 and -371 bp were responsible for the transcriptional activation of *BnaA07.PAP2* and anthocyanin-related genes. Introducing *BnaA07.PAP2*^{In-184-317} into rapeseed cultivar 'Westar' changed the yellow petals to apricot petals [49]. Mutation of the carotenoid isomerase gene *BnaCRTISO* altered the gene expression in flavonoid biosynthesis, carotenes and xanthophylls synthesis pathway, reduced chalcone content and increased carotene content, thus inducing white petals and yellowish leaves in rapeseed [50].

6. Flavonoid Regulation in Rapeseed Leaves

As mentioned above, anthocyanins are helpful to human health for their antioxidant, chemoprotective, and anti-inflammatory properties. *B. napus* germplasms with increased anthocyanin content have been used as vegetables with better nutritional values. Mapping and gene identification of red and purple Brassicas were mainly reported in *B. rapa*, *B. juncea*, and *B. oleracea* [86–89]. Due to the complicated genome divergence and homologous genes

of *B. napus*, the molecular mechanism of genes controlling the purple leaf phenotype has not been fully elucidated. Only a few genes have been reported with functions in regulating anthocyanin biosynthesis in rapeseed leaves.

Overexpression of *Arabidopsis PAP1* stimulated flavonoid and phenolic biosynthetic gene expression in *B. napus*, thus promoting cyanidin, pelargonidin, quercetin, sinapic acid content, and inducing purple leaves and stems in the transgenic plants [54]. Ectopic expression of *BnGL3-1*, which encoded a nucleus-located transcription factor, increased the trichome number and anthocyanin accumulation in true leaves of *Arabidopsis gl3-3* mutant by manipulating the expression level of genes involved in trichome formation (i.e., *GL2*, *MYB23*) and anthocyanin biosynthesis (i.e., *PAP1*, *LBD37/38*, *F3H*, *F3'H*, *DFR*, *ANS*) [53]. Goswami et al. (2018) created a resynthesized *B. napus* line Rs035 with high cyanidins and malvidins by crossing *B. rapa* and *B. oleracea* with high anthocyanin content. Three EBGs (*CHS*, *CHI*, and *F3H*), four LBGs (*FLS1*, *DFR*, *ANS*, and *UGT75C1*), two regulatory genes (*MYB111* and *TT8*), and a transporter gene (*TT19*) were up-regulated in Rs035 than in the diploid parents, which might be putative candidates for the enhanced anthocyanin content in Rs035 [90]. Chen et al. (2020) identified three alternatively spliced isoforms of *BnaPAP2.A7* in a rapeseed introgression line from the progenies of (*B. rapa* × *O. violaceus*) × *B. napus*, which exhibited purple leaves and petioles. Only the *BnaPAP2.A7-744* isoform promoted anthocyanin accumulation, and changed the green leaves to purple leaves through up-regulation of EBGs, LBGs and transcription factors in the flavonoid biosynthetic pathway, while overexpression of *BnaPAP2.A7-910* or *BnaPAP2.A7-395* repressed anthocyanin-related genes, and could not change the leaf color [52]. He et al. (2021) analyzed the expression pattern of anthocyanin biosynthetic genes in two rapeseed lines with purple and green leaves, and their F1 progenies. They found the up-regulation of *TT8*, *DFR*, *ANS*, *UFGT*, and *TT19* promoted anthocyanin biosynthesis in early development of purple leaves, but these genes were down-regulated and anthocyanin content was decreased at the late developmental stage [91]. Recently, five anthocyanins (mainly cyanidins) were identified in the leaves and stem bark of a purple rapeseed line PR01. A total of 157 anthocyanin biosynthesis genes in rapeseed were screened through comparative analysis with *Arabidopsis* genes, among which *BnaA07.PAP2* and *BnaC06.PAP2* were identified as the key regulators of anthocyanin biosynthetic genes (i.e., *DFR*, *F3'H*, and *ANS*) and high anthocyanin content in PR01 [27]. Schilbert et al. (2021) characterized the gene structure, genomic location, and expression pattern of 13 *FLS* genes in rapeseed. They found the *BnFLS1-1* and *BnFLS1-2* could restore the flavonoid (i.e., kaempferol and quercetin derivatives) contents in *Arabidopsis ans/fls1* and *f3h* mutants, indicating the *FLS*s play a role as bifunctional enzymes in generating dihydrokaempferol and kaempferol [60]. Presently, only one locus *BnaA.PL1* on chromosome A03 was mapped for an anthocyanin-rich mutant of rapeseed, and *BnAPR2* was predicted to be a main candidate gene that encoded adenosine 5'-phosphosulfate reductase [51].

7. Flavonoid Regulation Related to Abiotic and Biotic Stress Responses in Rapeseed

Abiotic stresses, such as salinity, drought, cold, and logging, inhibit rapeseed growth and yield, and affect the seed quality, thus leading to great economic loss and restricting the worldwide cultivation area of *B. napus*. Flavonoids have been reported to have functions in plant response and adaptation to various environmental stresses. Moreover, flavonoids are also helpful for improving plant resistance to different biotic stresses. However, the molecular mechanism of how flavonoid biosynthesis is involved in plant response to different stresses has barely been reported.

7.1. Flavonoid Regulation in Response to Abiotic Stresses

Ellerstrom et al. (2005) found overexpression of *BnET*, a gibberellin-response repressor, promoted anthocyanin accumulation in *Arabidopsis* and might be favorable to balance ABA and GA in plants [59]. Yu et al. (2014) analyzed the gene expression in siliques of rapeseed under heat stress, and found many genes in silique wall metabolic pathways and flavonoid

synthesis (i.e., *PAL1*, *CHI*, *FLS1*, and *GST*) were down-regulated [92]. Anthocyanin content could be induced in rapeseed seedlings under sucrose, ABA, NaCl, and mannitol stress, but not under GA₃ treatment. In the root tip of *B. napus*, flavonols were increased under sucrose, GA₃, NaCl, and mannitol treatments, but not under ABA treatment. *BnFLS*, an early biosynthesis gene in the flavonoid biosynthetic pathway, could be induced by mannitol treatment, and inhibited by sucrose, GA₃, and NaCl treatments. Overexpression of *BnFLS* recovered the flavonol content, and increased the reactive oxygen species (ROS) scavenging activity in an *Arabidopsis atfls1-ko* mutant [58]. Koeslin-Findeklee et al. (2015) analyzed the gene expression of different *B. napus* cultivars under nitrogen starvation, leaf shading, and detaching to screen the genes' response to leaf senescence. They found genes related to flavonoid synthesis were induced by nitrogen starvation and leaf detaching, which would contribute to protect leaves against photooxidative stress [22]. Physiologic and transcriptomic analysis revealed that with high anthocyanin accumulation in rapeseed under salinity stress, the genes in anthocyanin biosynthesis were up-regulated (i.e., *CHS*, *DFR*, *PAP1*, *PAP2*, *bHLH122*, and *WD40*), which might be helpful for protecting leaves from stress-induced damage [93]. Overexpression of *Arabidopsis AtDFR* increased anthocyanin accumulation and improved salt tolerance of *B. napus* [57]. Based on the proteomic analysis of two *B. napus* cultivars with different cold tolerance, Mi et al. (2021) identified 34 common different abundant proteins (DAPs) between two cultivars that had been grown at −4 °C for 12 h and 24 h, including a candidate protein FLS that is involved in flavonoid biosynthesis and might be related to the ROS scavenging under cold stress [94]. Sami et al. (2020) revealed that melatonin significantly enhanced the anthocyanin content in rapeseed seedlings under cadmium or aluminum stresses, which might be helpful to the metal tolerance of *B. napus* [95]. The morphological and physiological parameters of two rapeseed germplasms with a green stem and a purple stem indicated the main function of anthocyanin accumulation in rapeseed under drought stress was to improve antioxidant capability and stress tolerance. Furthermore, the genotype with a purple stem exhibited higher drought tolerance than the green stem genotype, which might be related to the higher expression of anthocyanin biosynthetic genes and antioxidant enzymes [96].

7.2. Flavonoid Regulation in Response to Light Conditions

Aside from the aforementioned abiotic stresses, flavonoid accumulation was also changed in rapeseeds under different light conditions. Rapeseed in the main cultivation area of China, the Qinghai-Tibetan plateau, usually suffers light stress, but the mechanisms underlying rapeseed adaptation to light stress are still unknown. Gerhardt et al. (2008) found flavonoid accumulation in *B. napus* was suppressed by far-red light, while addition of UV-B altered the flavonoid composition [97]. Luo et al. (2021) found the anthocyanin (i.e., cyanidin, delphinidin, and petunidin) contents in rapeseed under high light stress were significantly increased compared to that grown under normal light conditions, and the genes in the anthocyanin biosynthetic pathway (i.e., *BnDFR*, *BnANS*, *BnPAP1*, and *BnGL3*) and the jasmonic acid biosynthetic pathway were also induced by high light stress [98]. Moreover, Groenbaek et al. (2019) found the annual or biennial life cycle and seasonal differences, other than developmental stages, had major effects on the levels of flavonoid glycosides and hydroxycinnamic acids in baby leaf rapeseed [99]. Flavonol and anthocyanin content in microgreens of *B. napus* and *B. juncea* were changed under different blue-red light ratios, which could be used as growth parameters in evaluating the nutritional values of microgreens [100]. UV-B irradiation could enhance the phenylpropanoid, flavonoid, and anthocyanin accumulation in *B. napus* seedlings by affecting the gene expression in secondary metabolite biosynthesis [101]. *BnCRY1* and *BnCRY2a*, two homologs of *Arabidopsis* UV-B photoreceptors *AtCRY1* and *AtCRY2*, were involved in regulating photomorphogenesis and anthocyanin accumulation of rapeseed seedlings. Overexpression of *BnCRY1* and *BnCRY2a* inhibited hypocotyl length and increased anthocyanin content in *B. napus* seedlings grown under blue and white light by affecting the gene expression associated with phytohormone synthesis and signaling, as well as cell wall components [55,56,102].

7.3. Flavonoid Regulation in Response to Biotic Stresses

Presently, only a few research papers on flavonoids involved in biotic stress responses have reported on *B. napus*. A kaempferol derivative, kaempferol 3-*O*-sinapoyl-sophoroside 7-*O*-glucoside (KSSG), has been identified as a flavonoid related to the improved resistance to cabbage seedpod weevil of the BC2 double haploid lines from *B. napus*-*S. alba* intergeneric hybrids. A QTL on linkage group N7 that explained 9.5% of the KSSG variation was identified [103]. Li et al. (2015) found that BnaMAPKKK4 induced ROS, malondialdehyde (MDA), and anthocyanin accumulation by interacting with BnaMPK3 to regulate ROS-induced cell death in tobacco leaves [104]. Islam et al. (2019) reported that cell wall-bound phenolic metabolites were significantly increased in resistant rapeseed cultivar after inoculation of *Xanthomonas campestris* pv. *campestris*, accompanied by increased jasmonic acid content and up-regulation of genes in the phenylpropanoid biosynthetic pathway [105]. An *anthocyanin-more (am)* mutant of rapeseed cultivar 'Zhongshuang11' was identified with improved *Sclerotinia sclerotiorum* resistance and waterlogging tolerance, which might be due to the higher anthocyanin content, and expressional changes of anthocyanin-related structural and regulatory genes in the mutant [106,107].

8. Conclusions and Perspectives

The flavonoid profiles in different organs, especially seeds, leaves, and petals of rapeseed, as well as *B. napus* seedlings under various stresses have been reported. This research clarified the specific compounds presented in different tissues, and the effects of environmental conditions on the accumulation pattern of flavonoids in *B. napus*, which would be helpful for studying the flavonoid regulation related to rapeseed development and stress responses. The molecular regulation of flavonoid biosynthesis in *A. thaliana* has been well characterized, but the genes involved in flavonoid biosynthesis in *B. napus* were not fully identified, mainly due to the genome polyploidization that made the flavonoid regulation more complex in rapeseed. Presently, many genes related to flavonoid biosynthesis in *B. napus* have been screened through transcriptional comparison between germplasms exhibiting different flavonoid content in the seed, petal, leaf, or stem, or under different growth conditions. Only a few QTLs of flavonoid biosynthesis in seed coats, leaves, and petals have been identified. These genes are valuable for the dissection of the regulatory network of flavonoid synthesis and the biological roles of these chemicals in plant adaptation. However, the functions of most candidate genes regulating flavonoid synthesis were not analyzed. Most of the characterized genes were orthologous of *Arabidopsis* *TT* genes, such as *TT1/2/3/8/10*, *FLS*, *ANS*, and *PAP1/2*. Here, we summarized the research on flavonoid biosynthesis and regulation in *B. napus*, and hope these metabolic profiles and candidate genes will facilitate the quality improvement, ornamental value, and stress resistances of rapeseed in the near future.

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References

1. Winkel-Shirley, B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol.* **2001**, *126*, 485–493. [[CrossRef](#)] [[PubMed](#)]
2. Lepiniec, L.; Debeaujon, I.; Routaboul, J.M.; Baudry, A.; Pourcel, L.; Nesi, N.; Caboche, M. Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol.* **2006**, *57*, 405–430. [[CrossRef](#)] [[PubMed](#)]
3. Shen, N.; Wang, T.; Gan, Q.; Liu, S.; Wang, L.; Jin, B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chem.* **2022**, *383*, 132531. [[CrossRef](#)] [[PubMed](#)]
4. Teka, T.; Zhang, L.; Ge, X.; Li, Y.; Han, L.; Yan, X. Stilbenes: Source plants, chemistry, biosynthesis, pharmacology, application and problems related to their clinical Application-A comprehensive review. *Phytochemistry* **2022**, *197*, 113128. [[CrossRef](#)] [[PubMed](#)]
5. Andersen, O.M.; Markham, K.R. *The Flavonoids: Chemistry, Biochemistry and Applications*; CRC Press: Boca Raton, FL, USA, 2006.
6. Alappat, B.; Alappat, J. Anthocyanin pigments: Beyond aesthetics. *Molecules* **2020**, *25*, 5500. [[CrossRef](#)]
7. Laoue, J.; Fernandez, C.; Ormeno, E. Plant flavonoids in Mediterranean species: A focus on flavonols as protective metabolites under climate stress. *Plants* **2022**, *11*, 172. [[CrossRef](#)]
8. Morales-Quintana, L.; Mino, R.; Mendez-Yanez, A.; Gundel, P.E.; Ramos, P. Do fungal-endosymbionts improve crop nutritional quality and tolerance to stress by boosting flavonoid-mediated responses? *Food Res. Int.* **2022**, *161*, 111850. [[CrossRef](#)]
9. Hsieh, K.; Huang, A.H. Tapetosomes in *Brassica* tapetum accumulate endoplasmic reticulum-derived flavonoids and alkanes for delivery to the pollen surface. *Plant Cell* **2007**, *19*, 582–596. [[CrossRef](#)]
10. Cushnie, T.P.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356. [[CrossRef](#)]
11. Rousserie, P.; Rabot, A.; Geny-Denis, L. From flavanols biosynthesis to wine tannins: What place for grape seeds? *J. Agric. Food Chem.* **2019**, *67*, 1325–1343. [[CrossRef](#)]
12. Maleki, S.J.; Crespo, J.F.; Cabanillas, B. Anti-inflammatory effects of flavonoids. *Food Chem.* **2019**, *299*, 125124. [[CrossRef](#)] [[PubMed](#)]
13. Reyes-Farias, M.; Carrasco-Pozo, C. The anti-cancer effect of quercetin: Molecular implications in cancer metabolism. *Int. J. Mol. Sci.* **2019**, *20*, 3177. [[CrossRef](#)] [[PubMed](#)]
14. Chalhoub, B.; Denoeud, F.; Liu, S.; Parkin, I.A.; Tang, H.; Wang, X.; Chiquet, J.; Belcram, H.; Tong, C.; Samans, B.; et al. Plant genetics. Early allopolyploid evolution in the post-neolithic *Brassica napus* oilseed genome. *Science* **2014**, *345*, 950–953. [[CrossRef](#)] [[PubMed](#)]
15. Liu, Y.; Ye, S.; Yuan, G.; Ma, X.; Heng, S.; Yi, B.; Ma, C.; Shen, J.; Tu, J.; Fu, T.; et al. Gene silencing of *BnaA09.ZEP* and *BnaC09.ZEP* confers orange color in *Brassica napus* flowers. *Plant J.* **2020**, *104*, 932–949. [[CrossRef](#)]
16. Hao, P.; Liu, H.; Lin, B.; Ren, Y.; Huang, L.; Jiang, L.; Hua, S. *BnaA03.ANS* identified by metabolomics and RNA-seq partly played irreplaceable role in pigmentation of red rapeseed (*Brassica napus*) petal. *Front. Plant Sci.* **2022**, *13*, 940765. [[CrossRef](#)]
17. Li, H.; Du, Y.; Zhang, J.; Feng, H.; Liu, J.; Yang, G.; Zhu, Y. Unraveling the mechanism of purple leaf formation in *Brassica napus* by integrated metabolome and transcriptome analyses. *Front. Plant Sci.* **2022**, *13*, 945553. [[CrossRef](#)]
18. Qu, C.; Zhao, H.; Fu, F.; Wang, Z.; Zhang, K.; Zhou, Y.; Wang, X.; Wang, R.; Xu, X.; Tang, Z.; et al. Genome-wide survey of flavonoid biosynthesis genes and gene expression analysis between black- and yellow-seeded *Brassica napus*. *Front. Plant Sci.* **2016**, *7*, 1755. [[CrossRef](#)]
19. Xie, T.; Chen, X.; Guo, T.; Rong, H.; Chen, Z.; Sun, Q.; Batley, J.; Jiang, J.; Wang, Y. Targeted knockout of *BnTT2* homologues for yellow-seeded *Brassica napus* with reduced flavonoids and improved fatty acid composition. *J. Agric. Food Chem.* **2020**, *68*, 5676–5690. [[CrossRef](#)]
20. Zhai, Y.; Yu, K.; Cai, S.; Hu, L.; Amoo, O.; Xu, L.; Yang, Y.; Ma, B.; Jiao, Y.; Zhang, C.; et al. Targeted mutagenesis of *BnTT8* homologs controls yellow seed coat development for effective oil production in *Brassica napus* L. *Plant Biotechnol. J.* **2020**, *18*, 1153–1168. [[CrossRef](#)]
21. Khare, S.; Singh, N.B.; Niharika; Singh, A.; Amist, N.; Azim, Z.; Yadav, R.K. Phytochemicals mitigation of *Brassica napus* by IAA grown under Cd and Pb toxicity and its impact on growth responses of *Anagallis arvensis*. *J. Biotechnol.* **2022**, *343*, 83–95. [[CrossRef](#)]
22. Koeslin-Findeklee, F.; Rizi, V.S.; Becker, M.A.; Parra-Londono, S.; Arif, M.; Balazadeh, S.; Mueller-Roeber, B.; Kunze, R.; Horst, W.J. Transcriptome analysis of nitrogen starvation- and cultivar-specific leaf senescence in winter oilseed rape (*Brassica napus* L.). *Plant Sci.* **2015**, *233*, 174–185. [[CrossRef](#)] [[PubMed](#)]
23. Zheng, J.; Wu, H.; Zhu, H.; Huang, C.; Liu, C.; Chang, Y.; Kong, Z.; Zhou, Z.; Wang, G.; Lin, Y.; et al. Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves. *New Phytol.* **2019**, *223*, 705–721. [[CrossRef](#)] [[PubMed](#)]
24. Yang, X.; Wang, J.; Xia, X.; Zhang, Z.; He, J.; Nong, B.; Luo, T.; Feng, R.; Wu, Y.; Pan, Y.; et al. *OsTTG1*, a WD40 repeat gene, regulates anthocyanin biosynthesis in rice. *Plant J.* **2021**, *107*, 198–214. [[CrossRef](#)]
25. Zhang, S.; Sun, F.; Zhang, C.; Zhang, M.; Wang, W.; Zhang, C.; Xi, Y. Anthocyanin biosynthesis and a regulatory network of different-colored wheat grains revealed by multiomics analysis. *J. Agric. Food Chem.* **2022**, *70*, 887–900. [[CrossRef](#)]
26. Lu, N.; Rao, X.; Li, Y.; Jun, J.H.; Dixon, R.A. Dissecting the transcriptional regulation of proanthocyanidin and anthocyanin biosynthesis in soybean (*Glycine max*). *Plant Biotechnol. J.* **2021**, *19*, 1429–1442. [[CrossRef](#)] [[PubMed](#)]
27. Fu, H.; Chao, H.; Zhao, X.; Wang, H.; Li, H.; Zhao, W.; Sun, T.; Li, M.; Huang, J. Anthocyanins identification and transcriptional regulation of anthocyanin biosynthesis in purple *Brassica napus*. *Plant Mol. Biol.* **2022**, *110*, 53–68. [[CrossRef](#)] [[PubMed](#)]

28. Mekapogu, M.; Vasamsetti, B.M.K.; Kwon, O.K.; Ahn, M.S.; Lim, S.H.; Jung, J.A. Anthocyanins in floral colors: Biosynthesis and regulation in *Chrysanthemum* flowers. *Int. J. Mol. Sci.* **2020**, *21*, 6537. [[CrossRef](#)] [[PubMed](#)]
29. Zhang, Y.; Xu, S.; Ma, H.; Duan, X.; Gao, S.; Zhou, X.; Cheng, Y. The R2R3-MYB gene *PsMYB58* positively regulates anthocyanin biosynthesis in tree peony flowers. *Plant Physiol. Biochem.* **2021**, *164*, 279–288. [[CrossRef](#)] [[PubMed](#)]
30. Chiu, L.W.; Li, L. Characterization of the regulatory network of *BoMYB2* in controlling anthocyanin biosynthesis in purple cauliflower. *Planta* **2012**, *236*, 1153–1164. [[CrossRef](#)] [[PubMed](#)]
31. Wang, Q.; Wang, Y.; Sun, H.; Sun, L.; Zhang, L. Transposon-induced methylation of the *RsMYB1* promoter disturbs anthocyanin accumulation in red-fleshed radish. *J. Exp. Bot.* **2020**, *71*, 2537–2550. [[CrossRef](#)]
32. Jiu, S.; Guan, L.; Leng, X.; Zhang, K.; Haider, M.S.; Yu, X.; Zhu, X.; Zheng, T.; Ge, M.; Wang, C.; et al. The role of *VvMYBA2r* and *VvMYBA2w* alleles of the *MYBA2* locus in the regulation of anthocyanin biosynthesis for molecular breeding of grape (*Vitis* spp.) skin coloration. *Plant Biotechnol. J.* **2021**, *19*, 1216–1239. [[CrossRef](#)] [[PubMed](#)]
33. Liu, H.; Liu, Z.; Wu, Y.; Zheng, L.; Zhang, G. Regulatory mechanisms of anthocyanin biosynthesis in apple and pear. *Int. J. Mol. Sci.* **2021**, *22*, 8441. [[CrossRef](#)] [[PubMed](#)]
34. Routaboul, J.M.; Kerhoas, L.; Debeaujon, I.; Pourcel, L.; Caboche, M.; Einhorn, J.; Lepiniec, L. Flavonoid diversity and biosynthesis in seed of *Arabidopsis thaliana*. *Planta* **2006**, *224*, 96–107. [[CrossRef](#)] [[PubMed](#)]
35. Appelhagen, I.; Thiedig, K.; Nordholt, N.; Schmidt, N.; Huet, G.; Sagasser, M.; Weisshaar, B. Update on transparent testa mutants from *Arabidopsis thaliana*: Characterisation of new alleles from an isogenic collection. *Planta* **2014**, *240*, 955–970. [[CrossRef](#)]
36. Xu, W.; Grain, D.; Bobet, S.; Le Gourrier, J.; Thevenin, J.; Kelemen, Z.; Lepiniec, L.; Dubos, C. Complexity and robustness of the flavonoid transcriptional regulatory network revealed by comprehensive analyses of MYB-BHLH-WDR complexes and their targets in *Arabidopsis* seed. *New Phytol.* **2014**, *202*, 132–144. [[CrossRef](#)]
37. Appelhagen, I.; Jahns, O.; Bartelniewoehner, L.; Sagasser, M.; Weisshaar, B.; Stracke, R. Leucoanthocyanidin dioxygenase in *Arabidopsis thaliana*: Characterization of mutant alleles and regulation by MYB-BHLH-TTG1 transcription factor complexes. *Gene* **2011**, *484*, 62–69. [[CrossRef](#)]
38. Farag, M.A.; Sharaf Eldin, M.G.; Kassem, H.; Abou el Fetouh, M. Metabolome classification of *Brassica napus* L. organs via UPLC-QTOF-PDA-MS and their anti-oxidant potential. *Phytochem. Anal.* **2013**, *24*, 277–287. [[CrossRef](#)]
39. Shao, Y.; Jiang, J.; Ran, L.; Lu, C.; Wei, C.; Wang, Y. Analysis of flavonoids and hydroxycinnamic acid derivatives in rapeseeds (*Brassica napus* L. var. *napus*) by HPLC-PDA-ESI(-)-MSⁿ/HRMS. *J. Agric. Food Chem.* **2014**, *62*, 2935–2945. [[CrossRef](#)]
40. Qu, C.; Fu, F.; Lu, K.; Zhang, K.; Wang, R.; Xu, X.; Wang, M.; Lu, J.; Wan, H.; Tang, Z.; et al. Differential accumulation of phenolic compounds and expression of related genes in black- and yellow-seeded *Brassica napus*. *J. Exp. Bot.* **2013**, *64*, 2885–2898. [[CrossRef](#)]
41. Li, A.; Jiang, J.; Zhang, Y.; Snowdon, R.J.; Liang, G.; Wang, Y. Molecular and cytological characterization of introgression lines in yellow seed derived from somatic hybrids between *Brassica napus* and *Sinapis alba*. *Mol. Breed.* **2012**, *29*, 209–219. [[CrossRef](#)]
42. Jiang, J.; Shao, Y.; Li, A.; Lu, C.; Zhang, Y.; Wang, Y. Phenolic composition analysis and gene expression in developing seeds of yellow- and black-seeded *Brassica napus*. *J. Integr. Plant Biol.* **2013**, *55*, 537–551. [[CrossRef](#)] [[PubMed](#)]
43. Wang, Y.; Meng, G.; Chen, S.; Chen, Y.; Jiang, J.; Wang, Y.P. Correlation analysis of phenolic contents and antioxidation in yellow- and black-seeded *Brassica napus*. *Molecules* **2018**, *23*, 1815. [[CrossRef](#)] [[PubMed](#)]
44. Yin, N.W.; Wang, S.X.; Jia, L.D.; Zhu, M.C.; Yang, J.; Zhou, B.J.; Yin, J.M.; Lu, K.; Wang, R.; Li, J.N.; et al. Identification and characterization of major constituents in different-colored rapeseed petals by UPLC-HESI-MS/MS. *J. Agric. Food Chem.* **2019**, *67*, 11053–11065. [[CrossRef](#)] [[PubMed](#)]
45. Shen, X.; Yang, L.; Han, P.; Gu, C.; Li, Y.; Liao, X.; Qin, L. Metabolic profiles reveal changes in the leaves and roots of rapeseed (*Brassica napus* L.) seedlings under nitrogen deficiency. *Int. J. Mol. Sci.* **2022**, *23*, 5784. [[CrossRef](#)] [[PubMed](#)]
46. Lian, J.; Lu, X.; Yin, N.; Ma, L.; Lu, J.; Liu, X.; Li, J.; Lu, J.; Lei, B.; Wang, R.; et al. Silencing of *BnTT1* family genes affects seed flavonoid biosynthesis and alters seed fatty acid composition in *Brassica napus*. *Plant Sci.* **2017**, *254*, 32–47. [[CrossRef](#)] [[PubMed](#)]
47. Zhang, K.; Lu, K.; Qu, C.; Liang, Y.; Wang, R.; Chai, Y.; Li, J. Gene silencing of *BnTT10* family genes causes retarded pigmentation and lignin reduction in the seed coat of *Brassica napus*. *PLoS ONE* **2013**, *8*, e61247. [[CrossRef](#)] [[PubMed](#)]
48. Fu, W.; Chen, D.; Pan, Q.; Li, F.; Zhao, Z.; Ge, X.; Li, Z. Production of red-flowered oilseed rape via the ectopic expression of *Orychophragmus violaceus* *OvPAP2*. *Plant Biotechnol. J.* **2018**, *16*, 367–380. [[CrossRef](#)] [[PubMed](#)]
49. Ye, S.; Hua, S.; Ma, T.; Ma, X.; Chen, Y.; Wu, L.; Zhao, L.; Yi, B.; Ma, C.; Tu, J.; et al. Genetic and multi-omics analysis reveal *BnaA07.PAP2In-184-317* as the key gene conferring anthocyanin-based color in *Brassica napus* flowers. *J. Exp. Bot.* **2022**, *73*, 6630–6645. [[CrossRef](#)]
50. Li, H.; Yu, K.; Amoo, O.; Yu, Y.; Guo, M.; Deng, S.; Li, M.; Hu, L.; Wang, J.; Fan, C.; et al. Site-directed mutagenesis of the carotenoid isomerase gene *BnaCRTISO* alters the color of petals and leaves in *Brassica napus* L. *Front. Plant Sci.* **2022**, *13*, 801456. [[CrossRef](#)]
51. Li, H.; Zhu, L.; Yuan, G.; Heng, S.; Yi, B.; Ma, C.; Shen, J.; Tu, J.; Fu, T.; Wen, J. Fine mapping and candidate gene analysis of an anthocyanin-rich gene, *BnaA.PL1*, conferring purple leaves in *Brassica napus* L. *Mol. Genet. Genomics* **2016**, *291*, 1523–1534. [[CrossRef](#)]
52. Chen, D.; Liu, Y.; Yin, S.; Qiu, J.; Jin, Q.; King, G.J.; Wang, J.; Ge, X.; Li, Z. Alternatively spliced *BnaPAP2.A7* isoforms play opposing roles in anthocyanin biosynthesis of *Brassica napus* L. *Front. Plant Sci.* **2020**, *11*, 983. [[CrossRef](#)] [[PubMed](#)]

53. Gao, C.; Guo, Y.; Wang, J.; Li, D.; Liu, K.; Qi, S.; Jin, C.; Duan, S.; Gong, J.; Li, Z.; et al. *Brassica napus* GLABRA3-1 promotes anthocyanin biosynthesis and trichome formation in true leaves when expressed in *Arabidopsis thaliana*. *Plant Biol.* **2018**, *20*, 3–9. [[CrossRef](#)] [[PubMed](#)]
54. Li, X.; Gao, M.J.; Pan, H.Y.; Cui, D.J.; Gruber, M.Y. Purple canola: *Arabidopsis PAP1* increases antioxidants and phenolics in *Brassica napus* leaves. *J. Agric. Food Chem.* **2010**, *58*, 1639–1645. [[CrossRef](#)] [[PubMed](#)]
55. Sharma, P.; Chatterjee, M.; Burman, N.; Khurana, J.P. *Cryptochrome 1* regulates growth and development in *Brassica* through alteration in the expression of genes involved in light, phytohormone and stress signalling. *Plant Cell Environ.* **2014**, *37*, 961–977. [[CrossRef](#)] [[PubMed](#)]
56. Sharma, P.; Mishra, S.; Burman, N.; Chatterjee, M.; Singh, S.; Pradhan, A.K.; Khurana, P.; Khurana, J.P. Characterization of *Cry2* genes (*CRY2a* and *CRY2b*) of *B. napus* and comparative analysis of BnCRY1 and BnCRY2a in regulating seedling photomorphogenesis. *Plant Mol. Biol.* **2022**, *110*, 161–186. [[CrossRef](#)]
57. Kim, J.; Lee, W.J.; Vu, T.T.; Jeong, C.Y.; Hong, S.W.; Lee, H. High accumulation of anthocyanins via the ectopic expression of *AtDFR* confers significant salt stress tolerance in *Brassica napus* L. *Plant Cell Rep.* **2017**, *36*, 1215–1224. [[CrossRef](#)]
58. Vu, T.T.; Jeong, C.Y.; Nguyen, H.N.; Lee, D.; Lee, S.A.; Kim, J.H.; Hong, S.W.; Lee, H. Characterization of *Brassica napus* flavonol synthase involved in flavonol biosynthesis in *Brassica napus* L. *J. Agric. Food Chem.* **2015**, *63*, 7819–7829. [[CrossRef](#)]
59. Ellerstrom, M.; Reidt, W.; Ivanov, R.; Tiedemann, J.; Melzer, M.; Tewes, A.; Moritz, T.; Mock, H.P.; Sitbon, F.; Rask, L.; et al. Ectopic expression of *EFFECTOR OF TRANSCRIPTION* perturbs gibberellin-mediated plant developmental processes. *Plant Mol. Biol.* **2005**, *59*, 663–681. [[CrossRef](#)]
60. Schilbert, H.M.; Schone, M.; Baier, T.; Busche, M.; Viehover, P.; Weisshaar, B.; Holtgrawe, D. Characterization of the *Brassica napus* flavonol synthase gene family reveals bifunctional flavonol synthases. *Front. Plant Sci.* **2021**, *12*, 733762. [[CrossRef](#)]
61. Jia, L.; Wu, Q.; Ye, N.; Liu, R.; Shi, L.; Xu, W.; Zhi, H.; Rahman, A.N.; Xia, Y.; Zhang, J. Proanthocyanidins inhibit seed germination by maintaining a high level of abscisic acid in *Arabidopsis thaliana*. *J. Integr. Plant Biol.* **2012**, *54*, 663–673. [[CrossRef](#)]
62. Bhinder, G.; Sharma, S.; Kaur, H.; Akhatar, J.; Mittal, M.; Sandhu, S. Genomic regions associated with seed meal quality traits in *Brassica napus* germplasm. *Front. Plant Sci.* **2022**, *13*, 882766. [[CrossRef](#)] [[PubMed](#)]
63. Yu, C.Y. Molecular mechanism of manipulating seed coat coloration in oilseed *Brassica* species. *J. Appl. Genet.* **2013**, *54*, 135–145. [[CrossRef](#)] [[PubMed](#)]
64. Xu, B.B.; Li, J.N.; Zhang, X.K.; Wang, R.; Xie, L.L.; Chai, Y.R. Cloning and molecular characterization of a functional flavonoid 3'-hydroxylase gene from *Brassica napus*. *J. Plant Physiol.* **2007**, *164*, 350–363. [[CrossRef](#)] [[PubMed](#)]
65. Wei, Y.L.; Li, J.N.; Lu, J.; Tang, Z.L.; Pu, D.C.; Chai, Y.R. Molecular cloning of *Brassica napus* TRANSPARENT TESTA 2 gene family encoding potential MYB regulatory proteins of proanthocyanidin biosynthesis. *Mol. Biol. Rep.* **2007**, *34*, 105–120. [[CrossRef](#)] [[PubMed](#)]
66. Auger, B.; Baron, C.; Lucas, M.O.; Vautrin, S.; Berges, H.; Chalhoub, B.; Fautrel, A.; Renard, M.; Nesi, N. *Brassica* orthologs from *BANYULS* belong to a small multigene family, which is involved in procyandin accumulation in the seed. *Planta* **2009**, *230*, 1167–1183. [[CrossRef](#)]
67. Chai, Y.R.; Lei, B.; Huang, H.L.; Li, J.N.; Yin, J.M.; Tang, Z.L.; Wang, R.; Chen, L. TRANSPARENT TESTA 12 genes from *Brassica napus* and parental species: Cloning, evolution, and differential involvement in yellow seed trait. *Mol. Genet. Genomics* **2009**, *281*, 109–123. [[CrossRef](#)]
68. Ni, Y.; Jiang, H.; Li, J.; Chai, Y. Molecular cloning, characterization and expression of two rapeseed (*Brassica napus* L.) cDNAs orthologous to *Arabidopsis thaliana phenylalanine ammonialyase 1*. *Euphytica* **2007**, *159*, 1–16. [[CrossRef](#)]
69. Lu, J.; Li, J.; Lei, B.; Wang, S.; Chai, Y. Molecular cloning and characterization of two *Brassica napus* TTG1 genes reveal genus-specific nucleotide preference, extreme protein-level conservation and fast divergence of organ-specificity. *Genes Genomics* **2009**, *31*, 129–142. [[CrossRef](#)]
70. Liu, X.; Lu, Y.; Yan, M.; Sun, D.; Hu, X.; Liu, S.; Chen, S.; Guan, C.; Liu, Z. Genome-wide identification, localization, and expression analysis of proanthocyanidin-associated genes in *Brassica*. *Front Plant Sci.* **2016**, *7*, 1831. [[CrossRef](#)]
71. Hong, M.; Hu, K.; Tian, T.; Li, X.; Chen, L.; Zhang, Y.; Yi, B.; Wen, J.; Ma, C.; Shen, J.; et al. Transcriptomic analysis of seed coats in yellow-seeded *Brassica napus* reveals novel genes that influence proanthocyanidin biosynthesis. *Front. Plant Sci.* **2017**, *8*, 1674. [[CrossRef](#)]
72. Jiang, J.; Zhu, S.; Yuan, Y.; Wang, Y.; Zeng, L.; Batley, J.; Wang, Y.P. Transcriptomic comparison between developing seeds of yellow- and black-seeded *Brassica napus* reveals that genes influence seed quality. *BMC Plant Biol.* **2019**, *19*, 203. [[CrossRef](#)] [[PubMed](#)]
73. Lin, A.; Ma, J.; Xu, F.; Xu, W.; Jiang, H.; Zhang, H.; Qu, C.; Wei, L.; Li, J. Differences in alternative splicing between yellow and black-seeded rapeseed. *Plants* **2020**, *9*, 977. [[CrossRef](#)] [[PubMed](#)]
74. Badani, A.G.; Snowdon, R.J.; Wittkop, B.; Lipsa, F.D.; Baetzel, R.; Horn, R.; De Haro, A.; Font, R.; Luhs, W.; Friedt, W. Colocalization of a partially dominant gene for yellow seed colour with a major QTL influencing acid detergent fibre (ADF) content in different crosses of oilseed rape (*Brassica napus*). *Genome* **2006**, *49*, 1499–1509. [[CrossRef](#)]
75. Zhang, Y.; Li, X.; Chen, W.; Yi, B.; Wen, J.; Shen, J.X.; Ma, C.Z.; Chen, B.Y.; Tu, J.X.; Fu, T.D. Identification of two major QTL for yellow seed color in two crosses of resynthesized *Brassica napus* line No. 2127-17. *Mol. Breed.* **2011**, *28*, 335–342. [[CrossRef](#)]

76. Liu, L.; Stein, A.; Wittkop, B.; Sarvari, P.; Li, J.; Yan, X.; Dreyer, F.; Frauen, M.; Friedt, W.; Snowdon, R.J. A knockout mutation in the lignin biosynthesis gene *CCR1* explains a major QTL for acid detergent lignin content in *Brassica napus* seeds. *Theor. Appl. Genet.* **2012**, *124*, 1573–1586. [[CrossRef](#)]
77. Stein, A.; Wittkop, B.; Liu, L.Z.; Obermeier, C.; Friedt, W.; Snowdon, R.J. Dissection of a major QTL for seed colour and fibre content in *Brassica napus* reveals colocalization with candidate genes for phenylpropanoid biosynthesis and flavonoid deposition. *Plant Breed.* **2013**, *132*, 382–389. [[CrossRef](#)]
78. Liu, L.; Qu, C.; Wittkop, B.; Yi, B.; Xiao, Y.; He, Y.; Snowdon, R.J.; Li, J. A high-density SNP map for accurate mapping of seed fibre QTL in *Brassica napus* L. *PLoS ONE* **2013**, *8*, e83052. [[CrossRef](#)] [[PubMed](#)]
79. Qu, C.; Zhao, H.; Fu, F.; Zhang, K.; Yuan, J.; Liu, L.; Wang, R.; Xu, X.; Lu, K.; Li, J.N. Molecular mapping and QTL for expression profiles of flavonoid genes in *Brassica napus*. *Front. Plant Sci.* **2016**, *7*, 1691. [[CrossRef](#)]
80. Wang, J.; Xian, X.; Xu, X.; Qu, C.; Lu, K.; Li, J.; Liu, L. Genome-wide association mapping of seed coat color in *Brassica napus*. *J. Agric. Food Chem.* **2017**, *65*, 5229–5237. [[CrossRef](#)]
81. Gacek, K.; Bayer, P.E.; Anderson, R.; Severn-Ellis, A.A.; Wolko, J.; Lopatynska, A.; Matuszczak, M.; Bocianowski, J.; Edwards, D.; Batley, J. QTL genetic mapping study for traits affecting meal quality in winter oilseed rape (*Brassica napus* L.). *Genes* **2021**, *12*, 1235. [[CrossRef](#)]
82. Chao, H.; Guo, L.; Zhao, W.; Li, H.; Li, M. A major yellow-seed QTL on chromosome A09 significantly increases the oil content and reduces the fiber content of seed in *Brassica napus*. *Theor. Appl. Genet.* **2022**, *135*, 1293–1305. [[CrossRef](#)]
83. Tohge, T.; de Souza, L.P.; Fernie, A.R. Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. *J. Exp. Bot.* **2017**, *68*, 4013–4028. [[CrossRef](#)] [[PubMed](#)]
84. Iwashina, T. Contribution to flower colors of flavonoids including anthocyanins: A review. *Nat. Prod. Commun.* **2015**, *10*, 529–544. [[CrossRef](#)] [[PubMed](#)]
85. Xu, W.; Dubos, C.; Lepiniec, L. Transcriptional control of flavonoid biosynthesis by MYB-bHLH-WDR complexes. *Trends Plant Sci.* **2015**, *20*, 176–185. [[CrossRef](#)] [[PubMed](#)]
86. He, Q.; Zhang, Z.; Zhang, L. Anthocyanin accumulation, antioxidant ability and stability, and a transcriptional analysis of anthocyanin biosynthesis in purple heading chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *J. Agric. Food Chem.* **2016**, *64*, 132–145. [[CrossRef](#)] [[PubMed](#)]
87. Zhang, Y.; Hu, Z.; Zhu, M.; Zhu, Z.; Wang, Z.; Tian, S.; Chen, G. Anthocyanin accumulation and molecular analysis of correlated genes in purple kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *J. Agric. Food Chem.* **2015**, *63*, 4160–4169. [[CrossRef](#)]
88. Heng, S.; Cheng, Q.; Zhang, T.; Liu, X.; Huang, H.; Yao, P.; Liu, Z.; Wan, Z.; Fu, T. Fine-mapping of the *BjPur* gene for purple leaf color in *Brassica juncea*. *Theor. Appl. Genet.* **2020**, *133*, 2989–3000. [[CrossRef](#)]
89. Zhang, K.; Yang, D.; Hu, Y.; Njogu, M.K.; Qian, J.; Jia, L.; Yan, C.; Li, Z.; Wang, X.; Wang, L. Integrated analysis of transcriptome and metabolome reveals new insights into the formation of purple leaf veins and leaf edge cracks in *Brassica juncea*. *Plants* **2022**, *11*, 2229. [[CrossRef](#)]
90. Goswami, G.; Nath, U.K.; Park, J.I.; Hossain, M.R.; Biswas, M.K.; Kim, H.T.; Kim, H.R.; Nou, I.S. Transcriptional regulation of anthocyanin biosynthesis in a high-anthocyanin resynthesized *Brassica napus* cultivar. *J. Biol. Res.* **2018**, *25*, 19. [[CrossRef](#)]
91. He, D.; Zhang, D.; Li, T.; Liu, L.; Zhou, D.; Kang, L.; Wu, J.; Liu, Z.; Yan, M. Whole-genome identification and comparative expression analysis of anthocyanin biosynthetic genes in *Brassica napus*. *Front. Genet.* **2021**, *12*, 764835. [[CrossRef](#)]
92. Yu, E.; Fan, C.; Yang, Q.; Li, X.; Wan, B.; Dong, Y.; Wang, X.; Zhou, Y. Identification of heat responsive genes in *Brassica napus* siliques at the seed-filling stage through transcriptional profiling. *PLoS ONE* **2014**, *9*, e101914. [[CrossRef](#)] [[PubMed](#)]
93. Ahmad, Z.; Anjum, S.; Skalicky, M.; Waraich, E.A.; Muhammad Sabir Tariq, R.; Ayub, M.A.; Hossain, A.; Hassan, M.M.; Brestic, M.; Sohiful Islam, M.; et al. Selenium alleviates the adverse effect of drought in oilseed crops camelina (*Camelina sativa* L.) and canola (*Brassica napus* L.). *Molecules* **2021**, *26*, 1699. [[CrossRef](#)] [[PubMed](#)]
94. Mi, W.; Liu, Z.; Jin, J.; Dong, X.; Xu, C.; Zou, Y.; Xu, M.; Zheng, G.; Cao, X.; Fang, X.; et al. Comparative proteomics analysis reveals the molecular mechanism of enhanced cold tolerance through ROS scavenging in winter rapeseed (*Brassica napus* L.). *PLoS ONE* **2021**, *16*, e0243292. [[CrossRef](#)] [[PubMed](#)]
95. Sami, A.; Shah, F.A.; Abdullah, M.; Zhou, X.; Yan, Y.; Zhu, Z.; Zhou, K. Melatonin mitigates cadmium and aluminium toxicity through modulation of antioxidant potential in *Brassica napus* L. *Plant Biol.* **2020**, *22*, 679–690. [[CrossRef](#)] [[PubMed](#)]
96. Chen, W.; Miao, Y.; Ayyaz, A.; Hannan, F.; Huang, Q.; Ulhassan, Z.; Zhou, Y.; Islam, F.; Hong, Z.; Farooq, M.A.; et al. Purple stem *Brassica napus* exhibits higher photosynthetic efficiency, antioxidant potential and anthocyanin biosynthesis related genes expression against drought stress. *Front. Plant Sci.* **2022**, *13*, 936696. [[CrossRef](#)] [[PubMed](#)]
97. Gerhardt, K.E.; Lampi, M.A.; Greenberg, B.M. The effects of far-red light on plant growth and flavonoid accumulation in *Brassica napus* in the presence of ultraviolet B radiation. *Photochem. Photobiol.* **2008**, *84*, 1445–1454. [[CrossRef](#)]
98. Luo, Y.; Teng, S.; Yin, H.; Zhang, S.; Tuo, X.; Tran, L.P. Transcriptome analysis reveals roles of anthocyanin- and jasmonic acid-biosynthetic pathways in rapeseed in response to high light stress. *Int. J. Mol. Sci.* **2021**, *22*, 13027. [[CrossRef](#)]
99. Groenbaek, M.; Tybirk, E.; Neugart, S.; Sundekilde, U.K.; Schreiner, M.; Kristensen, H.L. Flavonoid glycosides and hydroxycinnamic acid derivatives in baby leaf rapeseed from white and yellow flowering cultivars with repeated harvest in a 2-years field study. *Front. Plant Sci.* **2019**, *10*, 355. [[CrossRef](#)]
100. Brazaityte, A.; Miliauskiene, J.; Vastakaite-Kairiene, V.; Sutuliene, R.; Lauzike, K.; Duchovskis, P.; Malek, S. Effect of different ratios of blue and red LED light on Brassicaceae microgreens under a controlled environment. *Plants* **2021**, *10*, 801. [[CrossRef](#)]

101. Lee, J.H.; Shibata, S.; Goto, E. Time-course of changes in photosynthesis and secondary metabolites in canola (*Brassica napus*) under different UV-B irradiation levels in a plant factory with artificial light. *Front. Plant Sci.* **2021**, *12*, 786555. [[CrossRef](#)]
102. Chatterjee, M.; Sharma, P.; Khurana, J.P. *Cryptochrome 1* from *Brassica napus* is up-regulated by blue light and controls hypocotyl/stem growth and anthocyanin accumulation. *Plant Physiol.* **2006**, *141*, 61–74. [[CrossRef](#)] [[PubMed](#)]
103. Lee, R.W.; Malchev, I.T.; Rajcan, I.; Kott, L.S. Identification of putative quantitative trait loci associated with a flavonoid related to resistance to cabbage seedpod weevil (*Ceutorhynchus obstrictus*) in canola derived from an intergeneric cross, *Sinapis alba* × *Brassica napus*. *Theor. Appl. Genet.* **2014**, *127*, 419–428. [[CrossRef](#)] [[PubMed](#)]
104. Li, L.; Ye, C.; Zhao, R.; Li, X.; Liu, W.Z.; Wu, F.; Yan, J.; Jiang, Y.Q.; Yang, B. Mitogen-activated protein kinase kinase kinase (MAPKKK) 4 from rapeseed (*Brassica napus* L.) is a novel member inducing ROS accumulation and cell death. *Biochem. Biophys. Res. Commun.* **2015**, *467*, 792–797. [[CrossRef](#)] [[PubMed](#)]
105. Islam, M.T.; Lee, B.R.; Park, S.H.; La, V.H.; Jung, W.J.; Bae, D.W.; Kim, T.H. Hormonal regulations in soluble and cell-wall bound phenolic accumulation in two cultivars of *Brassica napus* contrasting susceptibility to *Xanthomonas campestris* pv. *campestris*. *Plant Sci.* **2019**, *285*, 132–140. [[CrossRef](#)] [[PubMed](#)]
106. Liu, R.; Ding, L.N.; Li, M.; Cao, W.; Wang, Y.K.; Wang, W.J.; Yu, Y.-K.; Wang, Z.; Zhu, K.M.; Tan, X.L. Characterization of a rapeseed anthocyanin-more mutant with enhanced resistance to *Sclerotinia sclerotiorum*. *J. Plant Growth Regul.* **2019**, *39*, 703–716. [[CrossRef](#)]
107. Ding, L.N.; Liu, R.; Li, T.; Li, M.; Liu, X.Y.; Wang, W.J.; Yu, Y.K.; Cao, J.; Tan, X.L. Physiological and comparative transcriptome analyses reveal the mechanisms underlying waterlogging tolerance in a rapeseed anthocyanin-more mutant. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 55. [[CrossRef](#)]

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