Genome-wide Analysis and Functional Characterization of the Della Gene Family Associated With Stress Tolerance in B. Napus

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Abstract

Background: *Brassica napus* is an essential crop for oil and livestock feed. Eventually, this crop's economic interest is at the most risk due to anthropogenic climate change. DELLA proteins constitute a significant repressor of plant growth to facilitate survival under constant stress conditions. DELLA proteins lack DNA binding domain, but can interact with various transcription factors or transcription regulators of different hormonal families. Significant progress has been made on *Arabidopsis* and cereal plants. However, no comprehensive study regarding DELLA proteins have been delineated in *B. napus*.

Results: In our study, we have identified 10 *BnDELLA* genes. All of the *BnDELLA*s are closely related to five *AtDELLA* genes, suggesting a relative function and structure. Chromosomal mapping revealed the uneven distribution of *BnDELLA*s on eight chromosomes, and site-specific selection assessment proposes *BnDELLA*s purifying selection. The motifs composition in all *BnDELLA* genes is uneven; however, every *BnDELLA* gene contains 12 highly conserved motifs, encoding DELLA and GRAS domain. The two known miRNAs (bna-miR6029 and bna-miR603) targets, *BnCO7RGA* and *BnA09GAI*, were also predicted. Furthermore, quantitative real-time PCR (qRT-PCR) analysis has exhibited the *BnDELLA* genes' diverse expression patterns in various plant organs. Additionally, cis-acting element prediction shows that all *BnDELLA* genes contain light, stress, and hormone-responsive elements on their promoters. The gene ontology (GO) enrichment report indicated that *BnDELLA* genes mainly regulate stress responses. Through publicly available RNA-seq analysis, we determined the induced expression of *BnDELLA* genes by different biotic and abiotic stress treatments.

Conclusion: The present study provides essential information on the *BnDELLA* gene family in *B. napus*, illustrates the significance of *BnDELLA*s in stress adaptation, and suggests modulating *BnDELLA*s expression is a promising way to intensify *B. napus* stress tolerance and harvest index.

Background

Brassica napus is becoming the world's most economically important crop since the 1970s [1]. Significant progress has been made in the advancement of this crop selective breeding. The genetic alteration resulted in an improved 40% of high-quality vegetable oil, which has been used in the cooking and pharmaceutical application with the feed after oil isolation utilized as animal fodder. Unfortunately, *B. napus* horticulture is under constant pressure by anthropogenic climate change. This has led to severe loss in harvest index and oil production in many regions of the world and scarce its geographical distribution [2, 3]. Consequently, the effects of environmental stresses on *B. napus* will eventually lose their economic significance. Therefore, there is an imperative need to outline and specify critical components that can accommodate crops to the expected climate conditions.

Plants as sessile organisms evolved varied strategies to modulate their physiology to cope with fluctuating environmental conditions, such as higher salinity, drought, high/low temperature, and pathogen infections [4]. Tremendous work has been done to understand the role of plants' biochemical, molecular, and cellular response to these abiotic and biotic stresses [5, 6]. These studies suggest that phytohormones mediate the plants' internal and external response to facilitate plant vitality under stress conditions. Among these hormones, Gibberellins (GAs) are considered one of the most vital hormones that dramatically affect plant physiology by cross-talking with multiple hormones [7, 8]. However, under external pressure, plants maintain GAs homeostasis by a family of coregulator DELLAs, which interact with a wide variety of transcriptional factors and transcriptional regulators of multiple hormones, to inhibit growth in allocating resources for plant survival [9, 10]. Previously, it proposed that mutation in 17 amino acids of the DELLA N-terminal region resulted in severe dwarf transgenic plant *gai-1* with dark green leaves, much like GA-deficient mutant *ga1-3* [11, 12]. Later it was demonstrated that the N-terminal region of DELLA domain is responsible for DELLAs stability, which is employed by a GAs receptor Gibberellin insensitive Dwarf 1 (GID1) in GAs dependent and independent manner to lift DELLAs repression to stimulate plant growth under natural environment [13–15].

Rice, barley, tomato, contains only one DELLA gene *SLR1* (SLENDER RICE1), *SLN1* (SLENDER1), and *PROCERA*, respectively [9, 16, 17], while *LA*, *CRY* in pea [18], *d8*, *d9* in maize [19] harbor two highly conserved *DELLA* genes. Additionally, researches on *Arabidopsis thaliana* revealed the presence of five *AtDELLA* genes GA-insensitive (*GAI*), Repressor of *ga1-3* (*RGA*), RGA-Like1 (*RGL1*), (*RGL2*), (*RGL3*). Molecular cloning of the single and multiple loss of the *AtDELLA* genes in GA deficient mutant *ga-1* suggested the overlapping and dissimilar roles of *AtDELLA*s in regulating GAs stimulated plant growth. For instance, *AtGAI* and *AtRGA* have been denoted as notable repressors of the plant vegetative growth [9, 20, 21], whereas *AtRGL1* and *AtRGL2*, respectively, are involved in repressing floral augmentation and seed germination [22–25]. *AtRGL3* was recently got attention in plant defense by positively regulating the JA and SA mediated response against pathogen infections [10, 26].

Work over the last decade, the impact of DELLAs on seeded plants productivity has been progressively investigated. Apart from the exogenous splattering of the GAs to increase plant growth by repressing the repressor, one key factor was to alter GAs synthesis by fine-tuning the DELLAs activity for the amelioration of semi-dwarf varieties [17, 27, 28]. This results in enhanced plant tolerance to abiotic stresses, which ultimately increases crops harvest index and survival [29–31]. In addition to this, a recent study identifies a DELLA loss of function semi-dwarf mutant *ds-3* in oilseed rape, which confers a similar phenotype to previously reported semi-dwarf varieties, with resistance to lodging stress [32]. However, the molecular mechanism and characterization of the *DELLA* gene family in *B. napus* have not been well reported. Moreover, diversification of *BnDELLA*s during *B. napus* polyploidization would be of interest. In this study, 10 members of the DELLA protein were systematically characterized and analyzed by their phylogenetic and syntenic relationship, subcellular localization, protein motifs, gene structure, and cis-elements in the *B. napus* genome. Furthermore, expression profiles of the *BnDELLA*s in eight different tissues, Root, Mature-Silique, Leaf, Flower, Flower-Bud, Stem, Tips, Seed, were analyzed using the qRT-PCR. *BnDELLA* genes expression under varied stress conditions, including cold, heat, drought, ABA, NaCl, and *Sclerotinia sclerotiorum* were also predicted by publicly available RNA-Seq data. In addition, Gene Ontology (GO) and miRNAs targeting *BnDELLA* genes were evaluated to designate *BnDELLA*s roles. These results can provide useful insights to illustrate the multiple functions of the DELLA proteins in *B. napus* and provide a basis for further genetic manipulation toward developing *B. napus* variant with increased stress tolerance to environmental fluctuation.

Results

Identification and characterization of *BnDELLAs*

We have identified 10 *BnDELLA* genes in *B. napus* using the known 5 *A. thaliana* *DELLAs* (*GAI*, *RGA*, *RGL1*, *RGL2*, *RGL3*) peptide sequences as queries and performed BLASTP search in the *B. napus* genome database (GENOSCOPE <http://www.genoscope.cns.fr/brassic/napus/>) [33]. To further confirm the *BnDELLA* proteins integrity in the *B. napus*, we analyze the retrieved sequences in different *B. napus* cultivar genome browser (BnPIR, <http://cbi.hzau.edu.cn/bnapus>) and manually corrected the redundant sequence information of the *BnDELLAs* and named them according to their loci. Based on these methods, we found that each member in 5 *AtDELLAs* corresponds to multiple homologs in *B. napus* (Table 1). At the same time, 5 *DELLA* genes in *B. rapa*, 4 in *B. oleracea*, 9 in *B. juncea*, and 5 in *B. nigra* were identified by using the same methods. Furthermore, we found that 10 *BnDELLAs* members are derived from its progenitor *B. rapa* and *B. oleracea*. The chemical properties of the *DELLA* proteins in *B. napus* were identified by using the ExpASY (Table 1). The genomic sequence length of the *BnDELLAs* ranged from the 1524-1740bp, with a molecular weight varying from 55827.07 D to 63323.35 D. Moreover, the pI values of the *BnDELLA* proteins varied from 4.69 to 5.94, which shows that these proteins are highly acidic. Besides, all *BnDELLA* proteins showed a negative value of the grand average of hydrophobicity (GRAVY), indicating that *BnDELLAs* are hydrophilic. Subcellular localization signals of the 10 *BnDELLA* proteins were detected in the nucleus. The names of the *BnDELLA* genes and their locus id are also shown in (Table1).

Evolutionary relationship and gene structure analysis of *BnDELLAs*

The *DELLAs* evolutionary history among six *Brassicaceae* species *A. thaliana*, *B. napus*, *B. rapa*, *B. oleracea*, *B. juncea* and *B. nigra* were deduce using the neighbor-joining method. Based on the phylogenetic analysis, 38 *DELLA* genes in which 5 *AtDELLAs*, 10 *BnDELLAs*, 5 *BrDELLAs*, 4 *BoDELLAs*, 9 *BjDELLAs*, and 5 *BniDELLAs* were cluster into three groups according to the topologies and bootstrap support (Figure 1). Group I contain *GAI* and *RGA* clade, Group II holds *RGL1* clade, Group III includes *RGL2* and *RGL3* clade. *B. napusDELLA* genes were relatively closer to the *A. thaliana*. However, *B. napus* and *B. rapaDELLAs* shows 100% similarity between each other. In addition, a homolog of *AtRGL1* was not identified in the *B. oleracea* compared to those of *B.napus*, *B.rapa*, *B. juncea*, and *B. nigra*, which might be due to gene loss during the evolution process or maybe the emerging of genome gaps in *B. oleracea*. However, in *B. napus*, Group I, II, III had 4, 2, 4 *DELLA* members, respectively. *DELLA* genes grouped into the same subfamily are previously known to have distinct or overlapping functions [12, 22, 26, 34]. To recognize the *DELLA* genes family diversification in *B. napus*, we have implemented the GSDS web analysis by comparing the CDS and corresponding genomic sequences of *AtDELLAs*, *BnDELLAs*, *BrDELLAs*, *BoDELLAs* and *BjDELLAs*. As shown in (Figure 2), members of the *DELLA* genes among denoted species are highly conserved and intron-less with only one exon, which indicates *BnDELLAs* are the retrogenes. Moreover, the exon location of *DELLAs* among different phylogenetic related species is conserved, suggesting a similar evolutionary relationship. However, the length of exon among *DELLA* subfamily was different. For example, *BnRGL1* exon length was smaller than other *BnDELLAs* members in Group I and Group III, indicating gene structure diversification. In summary, the gene structure of the *DELLA* genes from different *Brassicaceae* species are highly conserved with some difference in the exon length (Figure 2).

Multiple sequence alignment and analysis of *BnDELLAs* motifs

The putative sequences of the *DELLA* proteins from *B. napus*, *A. thaliana*, *B. oleracea*, *B. rapa*, *B. juncea*, and *B. nigra* were aligned to explore amino acid conservation in *B. napus*. Based on the alignment, we found five homologues *DELLA* proteins from *A. thaliana* show higher percent amino acid similarity with *B. napus* (Table S1). Similar to *A. thaliana*, the *B. napus* and other denoted species contain highly conserved *DELLA* and GRAS domain at the N-terminal and C-terminal region, respectively. The N-terminal *DELLA* domain is shown to be involved in stabilizing the *DELLA* genes activity [35, 36], while GRAS domain acts as coregulators to interact with several transcriptional factors and regulators [28, 37, 38] (Figure S1). Moreover, the *BnDELLAs* amino acid sequence's alignment showed a highly conserved consensus sequence at the C-terminal region, which possibly suggests an evolutionary relationship between *BnDELLA* genes (Figure S1). In addition, the presence of the (VHIID-PFYRE-SAW) and two leucine heptad repeats LHRI and LHRII on the C-terminal of the GRAS domain are responsible for the protein interaction [39, 40]. However, some studies have also proposed *DELLA* domain lower-affinity with intrinsically unstructured proteins [37, 41]. Overall, the domain arrangements of the *DELLA* members in *B. napus* are equivalent to other species. In addition, the secondary structure feature (alpha-helix and Beta sheets) from the *AtRGL1* accessions number (*At1G66350.1*) was displayed in (Figure S1). However, the predicted secondary structures of all *DELLA* genes from the denoted plant species were relatively different.

Furthermore, to gain more insights into the putative roles of *BnDELLAs* diversity in *B. napus*, we generated a graph showing domains and their position on *AtDELLAs* and *BnDELLA* protein members. We found that the *DELLA* and GRAS domains are conserved in all *DELLA* proteins of *A thaliana* and *B.napus*, but motifs were unevenly distributed (Figure 3). Every *BnDELLA* member contains four to sixteen conserved motifs, and their length ranged from six to fifty amino acids. Motif 1 to 13 were identified in all Groups except *AtRGL3*, *BnA10RGL3*, and *BnC09RGL3* lacking motif 12. In which motif 7 and 8 annotated as *DELLA* domain (Figure S2). Moreover, Motif 14 and 15 were not detected in *AtRGL2*, *BnA05RGL2*, *BnA05RGL2-2*, and Group II, respectively. Motif 16 was detected in *AtRGL3*, *BnA10RGL3*, *BnC09RGL3*, and in *BnA09GAI*, *BnC09GAI*. Furthermore, Motif 17 was only present in the N-terminal of the *AtRGA*, *BnC07RGA*, *BnA06RGA*, and in *BnA05RGL2*, *BnC05RGL2* genes. Motif 18 was detected in *AtRGA* and *BnA09GAI*, *BnC09GAI*, *BnC07RGA*, *BnA06RGA*. In contrast, Group I and *AtRGL2*, *BnA05RGL2*, and *BnC05RGL2* had an extra motif 19 and 20, respectively. A schematic diagram of all *BnDELLAs* motifs logos was shown in (Figure S2). These results exhibit that the *BnDELLAs* subfamilies differ in motif arrangements, indicating the functional divergence of *BnDELLA* genes during duplication events. However, proteins with similar motifs arrangements specified the functional similarities among *BnDELLA* members.

Chromosome location and collinearity analysis

Chromosomal mapping determined that 10 *BnDELLAs* distributed on 8 *B. napus* scaffolds, which has not yet been assembled into a chromosome. Furthermore, no distribution of *BnDELLA* genes were observed in the scaffoldA01, scaffoldA03, scaffoldA04, scaffoldA07, scaffoldA08, scaffoldC01,

scaffoldC03, scaffoldC04, scaffoldC05, scaffoldC06, and scaffoldC08 (Figure 4). However, six *BnDELLA* genes, including, *BnA02RGL1*, *BnA05RGL2*, *BnA05RGL2-2*, *BnA06RGA*, *BnA09GAI*, and *BnA10RGL3* located on the AA subgenome. While four *BnDELLA*s, including *BnC02RGL1*, *BnC09GAI*, *BnC07RGA*, and *BnC07RGL3* located on the CC subgenome. In contrast, *BnA05RGL2* and *BnA05RGL2-2* have formed a cluster in the same scaffoldA05, which might result from tandem duplication (Figure 4). These results indicate that *BnDELLA* genes are unevenly distributed in the *B. napus* genome.

Furthermore, by using the BLAST and MCScanX methods, we investigate the 6 segmental duplication pairs such as, *BnA06RGA/BnC09GAI*, *BnC07RGA/BnA09GAI*, *BnA02RGL1/BnC09RGL3*, and one tandem duplication *BnA05RGL2/BnA05RGL2-2* were determined (Figure 5), which exhibits that during evolution segmental duplication events were the main reason for the divergence of *DELLA* genes in *B. napus*. In addition, comparative synteny of *DELLA* gene pairs between *B. napus* and *A. thaliana* was conducted (Figure 6). The results show that 10 *BnDELLA* genes of *B. napus* are associated with *AtDELLA*s, which are more than one ortholog's copies in *BnDELLA*s. For instance, *AtGAI* and *AtRGA* show synteny relationships with *BnA09GAI*, *BnC09GAI*, *BnA06RGA* and *BnC07RGA*, suggesting that *AtDELLA*s genes might contribute to the evolution of the *BnDELLA*s family. Moreover, we evaluate the pressure of selective constrains on each pair of duplicated *BnDELLA* genes, and calculated the nonsynonymous (Ka) and synonymous (Ks) ratio (Table S2, Figure S3). Our findings showed that all of the *BnDELLA* pairs had the Ka/Ks ratio lower than 1, implying that *BnDELLA* genes experienced robust purifying selective pressure.

Prediction of the bn-microRNAs putative targets sites of *BnDELLA*s

The regulatory purpose of *DELLA*s and their interacting proteins have been characterized widely in various plant species; however, a possible underlying post-transcriptional modification of *DELLA*s in response to environmental stresses is still unclear [42-44]. It has been reported that miRNAs play a significant role in transcriptional and post-transcriptional level to modulate gene expression under stresses [45, 46]. To identify miRNAs interaction with *BnDELLA*s isoforms, we obtained the bn-miRNAs data from *B. napus* comprehensive study to predict the targeted *BnDELLA* genes sites. We found that 10 *BnDELLA* genes from *B. napus* were unambiguously complementary to the 18 known *B. napus* miRNAs. These miRNAs length reached from 1-24 base pairs, with 11 nt being the most frequent in all *BnDELLA*s (Table 2). Target prediction shows that *BnDELLA*s *BnA07RGA* and *BnA09GAI* are targeted by 2 well-known miRNAs, bna-miR6029 and bna-miR6031, respectively. Among the other bn-miRNAs identified in our analysis, bna-miR2111a, bna-miR166a are found to be involved in targeting the *BnRGL1*. In contrast, bna-miR172b targets *BnA02RGL1* and *BnA05RGL2*. Additionally, bna-miR390a and bna-miR168a are found to target *BnRGL3*. Based on this analysis, we perceived that *B. napus* miRNA strongly targets *B. napus* both A and C genome to regulate *BnDELLA*s gene expression under constant stress to stabilize plant growth and defense tradeoff.

cis-element analysis in promoter regions of *BnDELLA*s and their distribution

Physiological and molecular studies on *DELLA*s suggested their role in multiple hormonal signaling pathways by interacting with a wide variety of transcriptional regulators and transcriptional factors. However, the molecular mechanism of interaction and regulation of *DELLA* genes are quite unclear. To gain more insights into the potential function and regulatory mechanism of the *BnDELLA*s, we analyzed the *cis*-regulatory elements in the 1500bp upstream promoter region of the *BnDELLA*s by using the Plant-CARE database and divided them into four categories (Figure 7A). We found that the individual *DELLA* gene in *B. napus* contains multiple *cis*-acting elements (Table S3), nearly all of the *BnDELLA* genes promoters have CAAT-box, TATA-box, light, stress, hormone, and development related responsive *cis*-elements. However, the distribution and numbers differed significantly between the *BnDELLA* genes (Figure 7B). In detail, *BnA09GAI*, *BnA02RGL1* has a higher number of light and hormone-responsive elements. In contrast, *BnA06RGA*, *BnA05RGL2*, and *BnA10RGL3* carried a higher number of stress and development related *cis*-elements, respectively. However, some of the *cis* core elements were only found in some *BnDELLA*s. For example, GC-motif (enhancer-like element involved in anoxic specific inducibility), DRE-core (*cis*-acting regulatory element regulate cold stress induce dehydration), and 3-AF binding site (part of a conserved DNA module array CMA3) were found in *BnA06RGA* and *BnC07RGA* *BnDELLA* members. Similarly, GATA-motif (*cis*-acting regulatory element involved in light-responsive floral, hypocotyl, and seed development), AT-Rich sequence (*cis*-element for maximal elicitor-mediated activation) were present in *BnC09RGA* and *BnA10RGA*. ATCT-motif (Part of a conserved DNA module involved in light responsiveness), Gap-Box (*cis*-acting element related to light-responsive GapA gene) were present in *BnC02RGL1* and *BnA02RGL1*, respectively. Moreover, AuxRR-Core (*cis*-acting regulatory element involved in auxin responsiveness) was only found in *BnA05RGL2* and *BnA05RGL2-2*. In contrast, O2-site (*cis*-regulatory element involved in zein metabolism regulation) was absent in all *BnDELLA*s except *BnC09RGL3* and *BnA10RGL3* (Figure 7A). These results showed that *BnDELLA* genes contain a wide variety of stress and defense-related *cis*-elements compared to development, light, and hormone-responsive *cis*-elements, suggesting the *BnDELLA*s diverse function in response to various biotic and abiotic conditions.

Transcriptomic and qRT-PCR analysis of *BnDELLA*s in different tissues

The candidate *BnDELLA* genes expression transcriptomic data from the different tissues of the *B. napus* variety "Zhongshuang 11" (ZS11) were obtained from the BnTIR database <http://yanglab.hzau.edu.cn/BnTIR>. The extracted data normalized by log2 fold change and heatmap were generated. As shown in (Figure 8), the expression pattern of the 10 *BnDELLA* genes was different among various tissues, which points out that the additional copies of the homologs *BnDELLA* genes show variations in expression during plant development. This can provide important insight into these genes' distinct roles in *B. napus*. To better understand the expression pattern of the *BnDELLA* genes, we performed qRT-PCR in 8 primary tissues (Root, Mature-Silique, Leaf, Flower, Flower-Bud, Stem, Tips, Seed) of *B. napus* variant ZS11. We found a strong correlation between the transcriptomic and our qRT-PCR results (Figure 9). On the whole, *BnGAI* and *BnRGA* are highly expressed in the stem and tips, while *BnRGL1* and *BnRGL2* were mainly expressed in the floral organs and seed, respectively. Conversely, in our qRT-PCR analysis, *BnRGL3* show minimal expression in any tissues. However, based on the transcriptomic data analysis, *BnRGL3* expression was highly observed in the silique. The contradiction between the qRT-PCR and transcriptomic data, especially in the *BnRGL3* expression, might be due to the harvesting of silique at 6 and 28 days after flowering, which show the complex variation of the *BnDELLA* genes expression at various stages of

plant development. These results indicate that the expression of *BnDELLA* genes at multiple plant tissues might play an indispensable role in regulating gibberellins and other phytohormones signals to mediate plant growth and survival tradeoff under constant stress conditions.

Expression analysis of *BnDELLAs* under different stress

To further explore and gain more insights into possible *BnDELLAs* function. We used the publicly available RNA-seq data to analyzed the *BnDELLA* genes expression under different stress conditions, such as MA, CA, FA, DT, HT, ABA, NaCl, and *Sclerotinia sclerotiorum*. Our predicted RNA-seq data show that the *BnDELLAs* gene expression significantly changed upon different stress treatments. For instance, *BnRGL2* was up-regulated by all denoted stresses except in drought and salt (Figure 10). Whereas, *BnGAI* show induced expression in response to MA, HT, DT, and salinity. In contrast, *BnA10RGL3*, *BnC09RGL3* almost exhibits reduced expression in response to heat, drought, ABA, and salt treatment. However, induced expression was observed during cold and *Sclerotinia sclerotiorum* treatment. Many previous studies on *AtDELLA* genes had provided evidence of their distinct and fundamental role in regulating plant physiology under abiotic and biotic stress [24, 47-49], suggesting the importance of *BnDELLA* genes in improving stress tolerance.

Gene Ontology

In order to understand the functional regulatory mechanism, we used the *AtDELLA* orthologous pairs of the *A. thaliana* to performed GO enrichment analysis. Three common categories of GO terms were observed including, biological process (BP), cellular component (CC), and molecular function (MF). In the MF category, DELLA genes are highly enriched in binding (GO:0003700), (GO:0005515), and transcriptional regulation activity (GO:0140110). CC is enriched in the nucleus (GO:0005634), which exhibits that DELLAs are nuclear proteins. Similarly, most of the GO terms (GO:0009737, GO:0009739, GO:0009740, GO:0009753, GO:0042538, GO:0009863, GO:0072593, GO:0009651, GO:0009908, GO:2000033, GO:0030154, GO:0010187, GO:0009938, GO:0006355, GO:0010218, GO:0009723) were abundant in biological process, which show response to hormones and stresses (Figure 11, Table S7). This GO enrichment results suggested that the *BnDELLAs* gene plays a crucial role in regulating hormonal signaling in response to stresses, which is consistent with previous studies [9, 50-52].

Discussion

In recent years, the cultivation of the *B. napus* declining due to the versatility of the environment. DELLAs are well known as the master negative coregulators to mediate cross-talk between GAs and multiple hormonal signals to modulate plant growth and survival tradeoff, responding to abiotic and biotic conditions [8, 10]. Previous reports on seeded plants had identified 1, 2, and 5 *DELLA* genes in *Oryza sativa* [16], *Pisum sativum* [18], and *A. thaliana*, respectively. Cloning and modulation of DELLA proteins in these plants resulted in increased harvest index, seed quality, tillering, flower timing, and stress tolerance. For example, overaccumulation of the DELLA protein enhances the submergence tolerance [53], salt stress [54], and shade avoidance [55, 56], which significantly improve plant fitness. In contrast, reduced DELLA protein expression reduces tillering [57, 58] and seed dormancy [59], suggesting increase seed weight and germination. However, despite the impressive insights gained from these studies, no previous comprehensive analysis of *DELLA* genes had carried out in *B. napus*. In this study, a total of 10 *BnDELLA* genes have been identified in *B. napus*. We analyzed phylogenetic relation, gene structure, motif composition, physicochemical properties, gene duplication, miRNA prediction, and *cis*-element analysis in the promoters. Moreover, qRT-PCR and published RNA-seq data were analyzed to disclose the expression profiling of the *BnDELLA* genes' and their roles under different stress conditions.

Characterization, and evolution of the *B. BnDELLAs* in *B. napus*

B. napus is an allotetraploid (AACC) crop that originated from the hybridization of two diploid progenitors *B. rapa* (AA) and *B. oleracea* (CC) [60]. In our study, we identified 10 full-length putative sequences of *BnDELLA* genes in *B. napus*, which are approximately equal to 5 in *B. rapa*, and 4 in *B. oleracea*. According to phylogenetic analysis, *BnDELLA* genes are classified into three groups (Figure 1), Group I contain *BnGAI* and *BnRGA*; Group II contains *BnRGL1*; Group III contains *BnRGL2*, and *BnRGL3*, which are based upon the identified *AtDELLAs* (Figure1). This suggests the possibility of similar evolutionary trajectories and genome duplication. As a result, one *AtDELLA* was duplicated into two homologs genes in *B. napus* (Figure 6). Additionally, we also find that the expansion of *BnDELLA* genes in *B. napus* is drive by the tandem and segmental duplication during the evolutionary process (Figure 5). However, the number of tandem duplications is much lower than the segmental duplication, suggesting that segmental duplication plays a higher role in expanding *BnDELLA* genes. Additionally, the calculated Ka/Ks ratios of *BnDELLA* gene pairs were less than <1 (Table S2), suggesting *BnDELLAs* are under strong purifying selection during the duplication process.

Furthermore, we also identify that coding regions of the *A. thaliana*, *B. napus*, *B. rapa*, *B. oleracea*, and *B. juncea* *DELLA* genes contain a single exon and does not have any introns (Figure 2), indicating the lower expression of *DELLA* genes in plants. However, previous studies indicated that genes with compact structures maybe expressed rapidly in response to stresses [61, 62]. For instance, the intronless gene *BnRGL3* show reduced expression in most tissues (Figure 9), but expressed rapidly in response to *Sclerotinia sclerotiorum* infection (Figure 10, Table S6). Moreover, exon composition exhibited the higher evolutionary conservation of *DELLA* genes among *Brassicaceae* species. In addition, chromosomal mapping indicated that 5 and 4 *BnDELLA* genes are located on the proximal or the distal ends of AA and CC subgenome, respectively (Figure 4), which exhibits homologs of *BnDELLAs* might play a similar role in biological function as *AtDELLAs*. Significant progress has been made to characterize the distinct roles of the *DELLA* gene family in *Arabidopsis* and other plant species; however, there is little progress in understanding the role of *BnDELLAs* in *B. napus*. Our comparative analysis of the sequence diversification suggests that *BnDELLA* genes contain two highly conserved N-terminal DELLA and C-terminal GRAS domain (Figure S1). The N-terminal DELLA domain is involved in the DELLA genes' stability [63]. In contrast, the C-terminal GRAS domain interacts with a wide variety of transcriptional factors and transcriptional regulators in response to stresses [39, 40]. *DELLA* genes are localized in the nucleus, suggesting their transportation regulation role [39]. In addition, motif numbers and

their composition between *BnDELLA* genes are unevenly distributed, indicating the domain shuffling in the protein structure of the *BnDELLA* genes (Figure 3). However, we observe that motif 1-11 and 13 are common in all *BnDELLA* genes.

A recent study has hypothesized that the OsmiR396 putatively regulated by the rice DELLA gene *SLR1*, targeting GA-responsive growth-regulating factors (GRFs) to inhibit growth promotion in rice [64]. In this study, a total of 18 bn-miRNAs were predicted to putatively regulate all *BnDELLA* genes (Table 2). In which, *BnC07RGA* and *BnA09GAI* are potentially regulated by the two-known miRNAs bna-miR6029 and bna-miR6031, respectively. In compliance with this, a recent study has shown that the increased expression of the bna-miR6029 regulates fatty acid biosynthesis to mediate seed development [65]. Thus, we speculate that the *BnDELLA* genes were the most likely targeted genes by the predicted bn-miRNAs to regulate plant growth and survival tradeoff under constant exogenous or endogenous stimuli. However, further investigation is needed to elucidate the miRNA process with *BnDELLA* genes.

Gene Expression profiling disclose the potential role of the *BnDELLA* genes

DELLA proteins are previously well described as master repressors of GAs signaling to modulate plant physiology [66, 67]. In recent studies, they have been identified for interacting with various transcriptional factors and transcriptional regulators to regulate crosstalk between GAs and multiple phytohormones in favor of plant survival under constant stress conditions [9, 68, 69]. However, the effect of DELLA proteins on growth and productivity is much larger than is widely known. Researches on *A. thaliana* have identified five *AtDELLA* genes GA-Insensitive (*GAI*), Repressor of *ga1-3* (*RGA*), RGA-Like1 (*RGL1*), (*RGL2*), (*RGL3*). Cloning and sequencing of these *AtDELLA* genes reported the distinct and overlapping role in regulating GAs stimulated plant growth. For instance, *AtGAI* and *AtRGA* control hypocotyl cell division and floral induction [20, 21, 70]. *AtRGL1* and *AtRGL2* are involved in modulating leaf senescence, male sterility, and seed germination [23, 24]. While *AtRGL3* has been reported to contribute plant defense in response to biotic stresses [10, 26, 71]. Consistent with this, our qRT-PCR and predicted RNA-Seq data (Table S4.1) indicated that the individual *BnDELLA* gene subfamily exhibits significant varied expression and response to biotic and abiotic stress. For instance, *BnRGL2* was up-regulated by all tested stresses except in drought, salinity, and *Sclerotiniasclerotiorum* (Figure 10). Whereas, *BnGAI* is highly expressed in stems and shows a response to MA, HT, DT, and salinity. In contrast, *BnRGL3* almost exhibits reduced expression in response to heat, drought, and ABA treatment. However, induced expression was observed during cold and NaCl treatment. These findings are consistent with studies that have also been found on their homologs in *A. thaliana* [72, 73]. Moreover, previous studies also confirmed the increased expression of the *AtRGL3* in response to the biotic stresses in *A. thaliana* [10, 26, 71]. As expected, increased expression of the *BnA09RGL3* and *BnC09RGL3* was observed after 24 hours of *Sclerotinia sclerotiorum* infection (Figure 10), suggesting the *BnRGL3* vital role in mediating *B. napus* survival under constant stress condition.

Furthermore, *BnRGL2* homolog in *A. thaliana* *AtRGL2* is denoted as an essential component to positively regulate ABA responses to promote seed dormancy [74-76]. In our qRT-PCR and RNA-seq analysis, we found that *BnRGL2* was mainly expressed in the seeds and showed induced expression after 4 hours of ABA treatment, but eventually reduced after 24 hours of ABA treatment. However, further experimental studies are required to gain more insights into the *BnDELLA*s in the ABA signal transduction pathway. In addition, during salt stress, the transcripts of the *BnA09GAI*, *BnC09GAI*, and *BnC09RGL3* were up-regulated, whereas the rest of the *BnDELLA* genes were down-regulated (Figure 10), suggesting the importance of *BnGAI* in susceptibility to severe salt stress. Importantly the link of *AtGAI* with salt stress has been identified, which confirmed the enhanced salt tolerance by restraining the plant growth [77, 78]. These results indicate the functional diversification of the *BnDELLA* genes under varying environmental conditions.

Moreover, in our study, we found diverse *cis*-elements in *BnDELLA*s promoter, including light-responsive, hormones responsive, and stress-related elements (Figure 7), but their distribution is uneven. For instance, *BnA02RGL1*, *BnC02RGL1*, and *BnA09RGL3*, *BnC09RGL3* had 2 ABREs in their promoter regions, while *BnA05RGL2* and *BnA05RGL2-2* had no ABREs, although they were considered to induced ABA response differently. Additionally, *BnA05RGL2* and *BnA05RGL2-2* had 1 MBS *cis*-element in their promoter regions. Intriguingly, the *BnRGL2* gene relative expression was not observed in the drought treatment (Figure 10). Thus, these findings indicate the presence of unidentified *cis*-elements and signify that the expression of *BnDELLA*s might be regulated through post-transcriptional modification [42, 44], which determines the antagonistic and synergistic effects of *BnDELLA*s in response to environmental fluctuations. To further investigate the biological process associated with *BnDELLA* genes, we had performed the GO enrichment analysis. As shown in (Figure 11), *BnDELLA*s involved in protein and DNA-binding and were located in the nucleus. Additionally, the majority of the *BnDELLA*s participated in hormonal regulation in response to biotic and abiotic stresses. To better understand the various roles of the *BnDELLA*s genes under biotic and abiotic stress conditions, further experimental studies are needed, which will help consolidate our understanding of plant ontogenesis and facilitate the enhancement of agronomic techniques to improve *B. napus* yield.

Conclusions

In our study, we identified and characterized the *BnDELLA* genes in *B. napus*. A total of 10 *BnDELLA* genes are identified in *B. napus* genome, which were classified into three groups. Group I carry *BnGAI*, and *BnRGA*. Group II contains *BnRGL1*. *BnRGL2*, *BnRGL3* belong to Group III. All of the *BnDELLA* genes are closely related to the *Arabidopsis thaliana* five *DELLA* genes, suggesting a comparable function and structure. The motifs composition in all *BnDELLA* genes is uneven; however, every *BnDELLA* gene contains 12 highly conserved motifs, encoding DELLA and GRAS domain. In addition, no introns insertion patterns were predicted in the DELLA and GRAS domains of the *BnDELLA* genes. Furthermore, phylogenetic and synteny study of the *DELLA* genes between *B. napus* and *A. thaliana* provides useful hints or evolutionary features of the *BnDELLA* genes. Moreover, *cis*-element analysis and miRNAs targets were also predicted. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis has exhibited the *BnDELLA* genes' diverse expression patterns in various plant development stages. Furthermore, induced expression of the *BnDELLA* genes by different stress treatments were also determined by RNA-seq analysis. Overall, these results proved useful clues into the evolutionary relationship and potential functions of the *BnDELLA*s, which will be useful for further genetic manipulation toward developing *B. napus* variant with increased stress tolerance to environmental fluctuation.

Methods

Identification and protein sequence analysis of *BnDELLAs*

In order to search *DELLA* gene family in *B. napus*, peptide sequence of the 5 *DELLA* genes from *A. thaliana* genome database (<http://www.arabidopsis.org/>) with corresponding Gene ID (*At1G14920.1*, *At2G01570.1*, *At1G66350.1*, *At3G03450.1*, *At5G17490.1*) were retrieved and used as queries to perform BLAST P search in *B. napus* Genome browser (BnPIR, <http://cbi.hzau.edu.cn/bnapus>), and (GENOSCOPE, <http://www.genoscope.cns.fr/brassicana>). Those from *B. oleracea*, *B. rapa*, *B. juncea*, and *B. nigra* were downloaded from *Brassica* Database (BRAD, <http://brassicadb.org/brad>). The sequences with 80% similarity were selected, and incorrectly or repeated sequences were manually re-annotated for *DELLA* domain analysis in the scan ScanProsite (<http://www.expasy.ch/tools/scanprosite/>) and InterProScan (<http://www.ebi.ac.uk/Tools/InterProScan/>). The protein sequences were then used to calculate the isoelectric point (pI), molecular weight (MW) and number of amino acids by the ProtParam tool (<http://web.expasy.org/>). Furthermore, prediction of subcellular location pattern of each *BnDELLA* was carried out using the web-server Plant-mPLoc (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>) [79], and ProtComp v.9.0 in softberry (<http://linux1.softberry.com/>).

Phylogentic and gene structure assessment of the *DELLA* in *B. napus*, *A. thaliana*, *B. rapa*, *B. oleracea*, *B. juncea*, *B. nigra*

Putative peptide sequences from the six *Brassicaceae* species *B. napus*, *A. thaliana*, *B. rapa*, *B. oleracea*, *B. juncea*, and *B. nigra* aligned using the MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle>) with default parameters. Aligned sequences were then used to construct the evolutionary tree with the MEGA 10.2 software by the neighbor-joining (NJ) method [80]. The authenticity of the tree was tested by performing 1000 bootstrap replications. The Newick format of the phylogenetic tree was then uploaded to iTOL web server (<http://itol.embl.de/>) for better visualization. Furthermore, genomic and coding sequences of the *B. napus*, *B. oleracea*, *B. rapa*, *B. juncea* and *A. thaliana* *DELLA* genes were rendered in Gene Structure Display Server (GSDS2.0) (<http://gsds.cbi.pku.edu.cn>) to predict gene structure and exon/intron location.

Sequence alignment and evaluation of *BnDELLAs* motifs

To classify the *DELLAs* characteristic domains in the *B. napus*, we have aligned the 38 *DELLAs* coding sequence from *B. napus*, *A. thaliana*, *B. rapa*, *B. oleracea*, *B. juncea*, and *B. nigra* by using the Muscle option in the MEGA 10 with default parameters. Furthermore, Motif Elicitation version 5.1.1 (MEME <http://meme-suite.org/tools/meme>) was used to identify the conserved motifs in the *BnDELLAs* with the maximum motif search set to 20, and other parameters are set to default. Any repetitions were considered motifs sites that spread throughout the sequence [81]. Further annotation of the identified motifs was implemented by the InterProScan (InterPro ebi.ac.uk). The conserved motifs were visualized by using the TBtools software [82]. Additionally, the secondary structure of the *BnDELLA* proteins is carried by PSIPRED (<http://bioinf.cs.ucl.ac.uk/PSIPRED>).

Chromosome location, collinearity analysis, and site-specific selection assessment and testing

BnDELLAs detailed chromosome location was acquired from the GFF genome file downloaded from *Brassica napus* genomic database (BnPIR, <http://cbi.hzau.edu.cn/bnapus>), and mapped the predicted location on the chromosome by using the TBtools software with colored gene names indicated as relative position. Gene duplication events were identified by aligning the *BnDELLA* genes sequences using BLASTP, and MCScanX was used to characterize the *BnDELLAs* into a tandem and segmental duplication [83]. Furthermore, the synteny map of *DELLAs* orthologous among *B. napus* and *A. thaliana* were obtained by the custom python script. For examining the site-specific selection, a Bayesian inference approach Selecton Server (<http://selecton.tau.ac.il/>) [84] was used to predict the positive and purifying selection. Besides this, we also calculated the synonymous (*Ks*) and Non-synonymous mutation (*Ka*) at each codon by *KaKs-Calculator* 2.0 [85]. In addition, *BnDELLA* gene pairs' divergence time was presumed using the formula $T = Ks / 2r$ with $r (1.5 \times 10^{-8})$ representing neutral substitution per site per year [86].

miRNA target prediction and *cis*-acting elements regulatory analysis

To predict the miRNAs regulating *BnDELLA* genes. We obtained the *B. napus* stem-loop and mature miRNA sequences from the PNRD (<http://structuralbiology.cau.edu.cn/PNRD/index.php>) [87] and miRbase (<http://www.mirbase.org/>) database. Then we submitted *bn-miRNAs* identified sequences and Genomic segment sequences of the *BnDELLA* genes into psiRNATarget server <http://plantgrn.noble.org/psiRNATarget> with default parameters. For *cis*-element analysis, 1500kb upstream promoter sequence from the translation start site of the *BnDELLAs* were scrutinized in the plant care database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [88], and distribution of the *cis*-acting elements visualized by TBtools software [82].

RNA extraction and quantitative Real-time PCR analysis of *BnDELLAs* in different tissues

To explore the expression pattern of the *BnDELLA* genes, we extracted the RNA of *B. napus* variety ZS11 different tissues (Roots, Mature-silique, Leaf, Flower, Flower-Bud, Stem, Tips, Seed) using Trizol (Invitrogen, Carlsbad, CA). Total RNA was then employed to produce cDNA with HiScript III-RT SuperMix for qPCR (Vazyme, China) according to the manufacturer's instructions. Real-time fluorescence quantitative analysis (qRT-PCR) was performed by Thermo Fisher Scientific QuantStudio 5 Real-Time PCR system with three independent replicates, and Actin was used as an internal control. The $2^{-\Delta\Delta C_t}$ method was implemented to measure the relative gene expression level of *BnDELLA* genes, and the results were visualized using GraphPad Prism8.0 software [89]. All of

the gene-specific primers used in this study were designed by the Beacon primer design program (Primer Biosoft International, Palo Alto, CA) and listed in (Table S5).

Gene ontology and expression pattern analysis of *BnDELLAs* under abiotic biotic stress

The *BnDELLA* genes functional properties were analyzed using the online web server DAVID (<https://david.ncifcrf.gov/>) and panther (<http://go.pantherdb.org/web/services/go/overrep.jsp>) to conduct Gene Ontology enrichment analysis. The predicted GO terms were annotated using the TBtools software. In addition, expression profiles of *BnDELLA* genes under heat, drought, cold, ABA induce, salt and *Sclerotinia sclerotiorum* stress condition were obtained from the publicly available transcriptomic data sets (GSE156029, GSE129220) [90], (CRA001775) [91], and (GSE81545) [92]. The differential expression analysis was performed using DSEeq2 package in R. The predicted values were normalized by log2 fold change, and heatmap was generated via TBtools.

Abbreviations

B. napus: *Brassica napus*; *A. thaliana*: *Arabidopsis thaliana*; *B. rapa*: *Brassica rapa*; *B. oleracea*: *Brassica oleracea*; *B. nigra*: *Brassica nigra*; *B. juncea*: *Brassica juncea*; *AtDELLA*: *Arabidopsis thaliana* DELLA; *BnDELLA*: *Brassica napus* DELLA; GAs: Gibberellins; GO: Gene ontology; BLASTP: Basic local alignment search tool-protein; MW: Molecular weight; MEME: Motif elicitation; bn-miRNAs: *Brassica napus* microRNA; ZS11: Zhongshuang 11; GID1: Gibberellin insensitive Dwarf 1; GRAVY: Grand average of hydrophobicity; pI: Isoelectric point; ABA: Absciscic acid; NaCl: Sodium chloride.

Declarations

Author Contributions

RS and ZKM designed the experiment. RS, TJ, PD and YG carried out the data analysis. RS wrote the manuscript draft and ZKM revised and edit the manuscript. ZKM and RS provided the RNA-seq data. RS, TJ, PD and YG provided the experimental materials. XLT supervised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Availability of the data material

BnDELLA genes expression transcriptomic data from the different tissues of the *B. napus* variety “Zhongshuang 11” (ZS11) were obtained from the BnTIR database <http://yanglab.hzau.edu.cn/BnTIR>. RNA-seq data of *B. napus* under heat, drought, cold; ABA induce, salt and *Sclerotinia sclerotiorum* stress condition were obtained from the publicly available transcriptomic data sets GSE156029, GSE129220; CRA001775, and GSE81545, respectively. All additional datasets supporting the results of this article are included within the article and supplementary tables.

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Tables

Table 1. Characterization of BnDELLA family proteins

Group	Gene Name	Gene LOCUS ID	Chromosome Number	Location			Protein					
				Start	End	Orientation	ORF	AA	PI	Mw (Da)	GRAVY	
I	<i>BnA09GAI</i>	<i>BnaA09G0218400ZS</i>	scaffoldA09	15333875	15336195	Reverse	1740	579	5.23	63323.35	-0.247	8
	<i>BnC09GAI</i>	<i>BnaC09G0254100ZS</i>	scaffoldC09	23759020	23761368	Reverse	1710	569	5.32	62327.38	-0.232	8
	<i>BnA06RGA</i>	<i>BnaA06G0409200ZS</i>	scaffoldA06	46345733	46347952	Forward	1722	573	5.58	62511.61	-0.203	8
	<i>BnC07RGA</i>	<i>BnaC07G0269400ZS</i>	scaffoldC07	41375597	41377853	Reverse	1734	577	5.41	62782.98	-0.177	8
II	<i>BnC02RGL1</i>	<i>BnaC02G0205300ZS</i>	scaffoldC02	17476351	17477874	Forward	1524	507	5.37	55827.07	-0.159	9
	<i>BnA02RGL1</i>	<i>BnaA02G0160500ZS</i>	scaffoldA02	9357771	9367517	Forward	1527	508	5.94	56584.09	-0.215	8
III	<i>BnA05RGL2</i>	<i>BnaA05G0486300ZS</i>	scaffoldA05	44153415	44155599	Forward	1635	544	4.71	59184.6	-0.189	8
	<i>BnA05RGL2-2</i>	<i>BnaA05G0485400ZS</i>	scaffoldA05	44093973	44096162	Forward	1641	546	4.69	59456.9	-0.188	8
	<i>BnC09RGL3</i>	<i>BnaC09G0489900ZS</i>	scaffoldC09	60072165	60073742	Forward	1578	525	4.8	57604.95	-0.211	8
	<i>BnA10RGL3</i>	<i>BnaA10G0194400ZS</i>	scaffoldA10	21533732	21535309	Forward	1578	525	4.78	57634.01	-0.203	8
ORF (Open reading frame); AI (Alphetic index); pI (Isoelectric point); GRAVY (Grand average of hydropathicity)												

Table 2. bn-miRNA targets BnDELLA genes

miRNA_Acc.	Gene symbol	Target_Acc.	Target start	Target end	miRNA aligned fragment	alignment length	e-value
bn-miR6029	<i>BnC07RGA</i>	BnaC07G0269400ZS	590	610	UGGGGUUGUGAUUUCAGGCUU	21	5
bn-miR6031	<i>BnA09GAI</i>	BnaA09G0218400ZS	1667	1690	AAGAGGUUCGGAGCGGUUGAAGC	24	5
bn-miR6036	<i>BnA09GAI</i>	BnaA09G0218400ZS	1581	1591	AUAGUACUAGUACUUGCAUGAUCA	11	5.5
	<i>BnC09GAI</i>	BnaC09G0254100ZS	1551	1561		11	5.4
	<i>BnA06RGA</i>	BnaA06G0409200ZS	1563	1573		11	5.4
	<i>BnC07RGA</i>	BnaC07G0269400ZS	566	578		13	0.35
bn-miR6028	<i>BnC09GAI</i>	BnaC09G0254100ZS	1635	1645	UGGAGAGUAAGGACAUUCAGA	11	5.4
	<i>BnA06RGA</i>	BnaA06G0409200ZS	1647	1657		11	5.4
	<i>BnC07RGA</i>	BnaC07G0269400ZS	1659	1669		11	5.4
bn-miR390a	<i>BnA06RGA</i>	BnaA06G0409200ZS	275	285	AAGCUCAGGAGGGAUAGCGCC	11	5.4
bn-miR171f	<i>BnA06RGA</i>	BnaA06G0409200ZS	888	898	UGAUUGAGCCGCGCCAAUAUC	11	5.4
bn-miR156b	<i>BnC07RGA</i>	BnaC07G0269400ZS	108	123	UUGACAGAAGAUAGAGAGCAC	16	1.4
bn-miR168b	<i>BnC07RGA</i>	BnaC07G0269400ZS	1079	1089	UCGCUUGGUGCAGGUCGAGAA	11	5.4
bn-miR160a	<i>BnC07RGA</i>	BnaC07G0269400ZS	1530	1540	UGCCUGGCUCUUGUAUGCCA	11	5.4
bn-miR2111a	<i>BnC02RGL1</i>	BnaC02G0205300ZS	1375	1391	GUCCUCGGGAUGCGGAUUACC	17	0.31
bn-miR172b	<i>BnC02RGL1</i>	BnaC02G0205300ZS	1038	1048	GGAAUCUUGAUGAUGCUGCAU	11	4.8
bn-miR166e	<i>BnC02RGL1</i>	BnaC02G0205300ZS	612	623	UCGGACCAGGCUUCAUUCUCCC	12	1.2
bn-miR166a	<i>BnA02RGL1</i>	BnaA02G0160500ZS	1187	1198	UCGGACCAGGCUUCAUUCUCCC	12	1.2
bn-miR6030	<i>BnC02RGL1</i>	BnaC02G0205300ZS	736	746	UCCACCAUACCAUACAGACCC	11	4.8
bn-miR2111c	<i>BnC02RGL1</i>	BnaC02G0205300ZS	1288	1298	UAAUCUGCAUCCUGGGGUUUA	11	4.8
bn-miR172b	<i>BnA02RGL1</i>	BnaA02G0160500ZS	1041	1051	GGAAUCUUGAUGAUGCUGCAU	11	4.8
	<i>BnA05RGL2</i>	BnaA05G0486300ZS	745	755		11	5.1
bn-miR390a	<i>BnA10RGL3</i>	BnaA10G0194400ZS	1442	1452	AAGCUCAGGAGGGAUAGCGCC	11	4.9
bn-miR168a	<i>BnC09RGL3</i>	BnaC09G0489900ZS	404	414	AAGCUCAGGAGGGAUAGCGCC	11	4.9

Figures

Figure 1

Figure. 1 The cladogram of DELLA proteins from *A. thaliana* (At:5), *B. napus* (Bn: 10), *B. rapa* (Br: 5), *B. oleracea* (Bo: 4), *B. juncea* (Bj: 9), *B. nigra* (Bni: 4) were conducted in MEGA X [80] using the neighbor-joining method, missing data with gaps were eliminated by complete deletion option. The DELLA proteins are cluster into three groups, which are indicated by the different colors. The bootstrap test (1000 replicates) is shown next to the branches.

Figure 2

Figure. 2 Exon and intron location of the BnDELLA genes; Blue double-sided wedge represents exon, and upstream/downstream regions are indicated as cyan-colored boxes. The scale can estimate the length of the exon at base.

Figure 3

The length of the 20 motifs ranged from 6 to 50 amino acid residues and represent by different colors and numbers, p-values of the motifs on each protein is less than 1e-5

Figure 4

Schematic representation of DELLA genes distribution on *B. napus* chromosomes. Chromosome number is indicated on the side of each chromosome.

Figure 5

Synteny analysis of the BnDELLA family in *B. napus*. Cyan-colored genes belong to group I, and green colored genes belong to group II, yellow-colored genes belong to group III. These colored genes indicate duplicated BnDELLA gene pairs, while gray lines represent synteny blocks in the *B. napus* genome. The distribution density of BnDELLA genes present at the bottom of each chromosome.

Figure 8

Heat map showing BnDELLA genes expression in different *B. napus* tissues. Expression data were analyzed with log2 normalization. Relative expression levels from high (dark-colored) to low (light color) are represented by the color scale bar (listed in Table S4.1).

Figure 9

qRT-PCR analysis of the selected BnDELLA genes expression in different organs. The x-axis corresponds to different organs, values on the y axis are denoted as the mean $\pm$ SD of three independent replicates (listed in Table S4.2).

Figure 10

Heat map of the expression profile of BnDELLA genes under different abiotic and biotic conditions including, MA (Cold shock at chilling 4°C and freezing -4°C temperatures), CA (4 degree Celsius 12 h following cold acclimation 14 days 4 degree Celsius), FA (-4 degree Celsius 12 h following cold acclimation 14 days 4 degree Celsius), DT (Drought treated), HT (Heat treatment), ABA, NaCl, and Sclerotinia sclerotiorum. The color scale reflects the data of the expression being processed with normalization of log₂ (listed in Table S6), and different colors denote different expression levels.

Figure 11

Gene ontology (GO) analysis. Three categories represent as designated colors, Blue (Biological Process), Red (Cellular Component), Orange (Molecular Function), were used to perform annotation on BnDELLA proteins.

Supplementary Files

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