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Genome-Wide Analysis of Auxin Receptor Family Genes in Brassica juncea var. tumida

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Abstract: Transport inhibitor response 1/auxin signaling f-box proteins (TIR1/AFBs) play important roles in the process of plant growth and development as auxin receptors. To date, no information has been available about the characteristics of the TIR1/AFB gene family in Brassica juncea var. tumida. In this study, 18 TIR1/AFB genes were identified and could be clustered into six groups. The genes are located in 11 of 18 chromosomes in the genome of B. juncea var. tumida, and similar gene structures are found for each of those genes. Several cis-elements related to plant response to phytohormones, biotic stresses, and abiotic stresses are found in the promoter of BjuTIR1/AFB genes. The results of qPCR analysis show that most genes have differential patterns of expression among six tissues, with the expression levels of some of the genes repressed by salt stress treatment. Some of the genes are also responsive to pathogen Plasmoodiophora brassicae treatment. This study provides valuable information for further studies as to the role of BjuTIR1/AFB genes in the regulation of plant growth, development, and response to abiotic stress.

Keywords: Brassica juncea var. tumida; auxin receptor; TIR1; AFB; gene expression pattern; abiotic stress

1. Introduction

Auxin is one of most important phytohormones, playing a crucial role in most physiological and biochemical processes of plant growth and development, such as embryo and fruit development, tissue differentiation, root initiation, plant gravitropism, and plant response to biotic and abiotic stresses [1–4]. The transport inhibitor response 1/auxin signaling f-box protein (TIR1/AFB) family genes perceive the auxin signal as auxin receptors, which then trigger the auxin response signaling pathway [5,6].

It has been reported that TIR1 and the AFBs are located in the nucleus and bind auxin [5,6]; the nuclear localization of GmTIR1 and GmAFB3 was also reported in soybean [7]. After auxin molecules bind to TIR1/AFB proteins, the activated TIR1/AFB proteins react with auxin/indole-3-acetic acid (Aux/IAA) family proteins [8]. The Aux/IAA family genes play important roles in the regulation of auxin response factor genes (ARFs). These ARF family genes are notable for their ability to directly activate or inhibit auxin responsive genes at the transcription level [9–11]. This TIR1/AFB–Aux/IAA–ARF cascade comprises a typical auxin signaling pathway.

At conditions of low auxin concentration in the plant, the Aux/IAA proteins could bind to ARFs, which results in the inhibition of the transcriptional activity of ARFs. When auxin molecules accumulate...
in plant cells, the reaction between the auxin molecules and TIR1/AFBs triggers a combination of TIR1/AFBs and Aux/IAAs, leading to degradation of the Aux/IAA proteins through the ubiquitin-26S proteasome system. After the degradation of Aux/IAA proteins, the released ARFs could effectively activate a form of auxin response system gene, which attempts to correctly regulate the plant’s response to auxin signaling [12].

TIR1 was the first identified in Arabidopsis, which includes an F-box domain in the N terminus and several Leucine-rich repeat (LRR) domains in the C terminus [13]. The tir1 loss-of-function mutants are deficient in some auxin-regulated growth processes, such as hypocotyl elongation and lateral root formation [13]. Overexpression of AtTIR1 results in inhibited primary root growth, agravitropic root tips, and promoted lateral root development [14]. The TIR1 functions as the substrate receptor of SCF E3 complex, which could specially degrade Aux/IAA proteins through the ubiquitin-26S proteasome system. Auxin molecules could promote the interaction between the Aux/IAA and SCF

Recently identified TIR1/AFB receptors also exist in tumorous stem mustard; however, no related report has been found for the function of those genes in this crop. Here, we have identified the TIR1/AFB family members in tumorous stem mustard, and analyzed their genes and the protein characteristics of those genes, as well as their expression.
patterns during plant growth and development. This study will be useful as a starting point for more functional investigations of TIR1/AFBs in tumorous stem mustard.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

In this study, tumorous stem mustard cultivar Yong An XiaoYe was used for gene expression pattern analysis. The seeds were sowed into 2:1 vermiculite:turfy soil, cultured under constant light at 22 °C with a 16/8 h light/dark regime in culture room. For the salt stress assay, the seeds of Yong An Xiao Ye were sowed in pots in a culture room, and two-week-old seedlings were irrigated by nutrient solution with or without 200 mM NaCl for 3, 6, 12, 24 h, respectively, and the roots were collected for real-time quantitative PCR analysis. For pathogen treatment, two-week-old seedlings were then inoculated with a 5 mL resting spore suspension of *Plasmodiophora brassicae* (OD_{600} = 0.07) for the indicated time points.

2.2. The Identifications of Transport Inhibitor Response 1/Auxin Signaling F-Box Protein in Tumorous Stem Mustard and Phylogenetic Analysis

The TIR1, AFB1, AFB2, AFB3, AFB4, and AFB5 sequence of *A. thaliana* were obtained from The Arabidopsis Information Resource website (https://www.arabidopsis.org/). Using the TIR1/AFB genes as the query sequence to blast relative homologs in the *B. juncea* genome database (http://brassicadb.org/brad/). The gene structure diagram was drawn using the online software of the GSDS2.0 server (http://gsds.cbi.pku.edu.cn/), the phylogenetic analysis was done using MEGA5 software [26] with the neighbor-joining method. The bootstrap value was 1000 replicates.

2.3. The Analysis of Protein Structures and Characters

The amino acid isoelectric point and molecular weight were predicted by online ExPASy software (https://web.expasy.org/compute_pi/), and the protein secondary structure was analyzed by SMART (http://smart.embl-heidelberg.de/) and CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The diagram of the protein domain was drawn by ExPASy. The cis-elements in the promotor of TIR1/AFBs were predicted by SOGO online software (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en&pj=640&action=page&page=newplace).

2.4. RNA Extraction and Real-Time Quantitative PCR Analysis

The total RNA was extracted from different plant materials using Trizol reagent (Tiangen Biotech Co., Ltd., Beijing, China). The total RNA samples were treated with DNase I (Invitrogen, Carlsbad, CA, United States) to remove contaminating genomic DNA. First-strand cDNA was synthesized from the total RNA using a FastQuant RT Kit (Tiangen Biotech (Beijing) Co., Ltd.). Real-time qPCR (qRT-PCR) was performed using SuperReal PreMix Plus (SYBR Green; Tiangen Biotech (Beijing) Co., Ltd.). *BjuACTIN3* was used as the internal reference gene for qRT-PCR [27], and the gene-specific primers are listed in Supplementary Table S1.

2.5. Statistical Analysis

All data were analyzed using SigmaPlot 10.0 (Systat Software, Inc., Chicago, IL, United States) and SPSS 16.0 software. The averages and standard deviations of all results were calculated, one-way ANOVA followed by the Dunnett test was used.
3. Results

3.1. The Identification of TIR1/AFB Homologs in Brassica juncea var. tumida

Eighteen genes were identified as homologs of TIR1/AFB genes in B. juncea var. tumida; they were named BjuTIR1 and BjuAFBs, and the CDS sequences of those genes can be found in Supplementary File S2. The open reading frame of BjuTIR1/AFBs varied between 1566 bp to 1830 bp, coding 521 to 609 amino acids; the molecular weight was 58.58–68.13 and the isoelectric point (pI) were 5.22–8.19 (Table 1). With the exception of BjuO006283 contig, which could not be anchored in the chromosome of B. juncea var. tumida, the left 17 BjuTIR1/AFBs genes located are in 11 of 20 B. juncea chromosomes. Each of the chromosome A03, A09, and B06 contains three genes, and the other eight genes were located at different chromosomes (Figure 1). BjuTIR1A, BjuTIR1D, and BjuTIR1F were located at the same chromosome of B06 (Figure 1).

| Group | Gene Name | Locus | Sequence ID | Exon | Genomics (bp) | CDS (bp) | Protein (aa) | pI  | MW (kD) |
|-------|-----------|-------|-------------|------|---------------|----------|--------------|-----|--------|
| TIR1  | BjuTIR1A  | B06   | BjuB022928  | 3    | 2360          | 1788     | 595          | 6.80| 66.87  |
|       | BjuTIR1B  | A09   | BjuA036398  | 3    | 2340          | 1785     | 594          | 7.42| 66.75  |
|       | BjuTIR1C  | A07   | BjuA026836  | 3    | 2318          | 1785     | 594          | 7.70| 66.72  |
|       | BjuTIR1D  | B06   | BjuB006272  | 3    | 2302          | 1785     | 594          | 7.96| 66.86  |
|       | BjuTIR1E  | A04   | BjuA014423  | 3    | 2428          | 1776     | 591          | 7.41| 66.57  |
|       | BjuTIR1F  | B06   | BjuB022182  | 3    | 2667          | 1566     | 521          | 8.13| 58.58  |
| AFB1  | BjuAFB1A  | A09   | BjuA031307  | 3    | 2746          | 1755     | 584          | 8.14| 66.24  |
|       | BjuAFB1B  | A03   | BjuA042044  | 2    | 1873          | 1758     | 585          | 5.22| 65.24  |
|       | BjuAFB1C  | A03   | BjuA010943  | 3    | 2540          | 1758     | 585          | 5.49| 65.52  |
|       | BjuAFB1D  | B08   | BjuB018573  | 4    | 2489          | 1680     | 559          | 8.19| 63.57  |
| AFB2  | BjuAFB2   | B04   | BjuB027337  | 3    | 2230          | 1731     | 576          | 6.30| 64.74  |
| AFB3  | BjuAFB3A  | A08   | BjuA031011  | 3    | 2266          | 1779     | 592          | 5.94| 66.60  |
|       | BjuAFB3B  | B03   | BjuB023204  | 3    | 1917          | 1773     | 590          | 6.13| 66.41  |
|       | BjuAFB3C  | A09   | BjuA036983  | 3    | 2269          | 1734     | 577          | 6.49| 65.04  |
|       | BjuAFB3D  | B02   | BjuB048292  | 4    | 2136          | 1659     | 552          | 5.68| 62.07  |
| AFB4  | BjuAFB4   | B05   | BjuB014241  | 3    | 1987          | 1830     | 609          | 5.58| 68.13  |
| AFB5  | BjuAFB5A  | Contig| BjuO006283  | 3    | 2017          | 1818     | 605          | 5.52| 67.81  |
|       | BjuAFB5B  | A03   | BjuA011137  | 3    | 2167          | 1821     | 606          | 5.54| 67.84  |

pl: Isoelectric point; MW: molecular weight.

![Figure 1](image-url) Figure 1. The gene locations of BjuTIR1 and BjuAFBs. Seventeen identified BjuTIR1/AFBs homologous genes were mapped to 11 out of 18 chromosomes. The chromosome name is at the top of each bar. The scale of the chromosome is in millions of bases (Mb).
3.2. The Phylogenetic Analysis and Gene Structures of BjuTIR1/AFBs

The TIR1/AFBs protein sequences of *A. thaliana* and *B. juncea* var. *tumida* were used for phylogenetic analysis, and six clades were obtained in the neighbor-joining phylogenetic tree (Figure 2). According to the phylogenetic analysis, six TIR1 homologs were identified, and named BjuTIR1A to BjuTIR1F; four AFB1 homologs were identified, and named BjuAFB1A to BjuAFB1D; one AFB2 homolog was identified, and named BjuAFB2; four AFB3 homologs were identified, and named BjuAFB3A to BjuAFB3D; one AFB4 homolog was identified, and named BjuAFB4; and two AFB5 homologs were identified, and named BjuAFB5A to BjuAFB5B (Figure 2, Table 1). We also analyzed the phylogenetic relationships of TIR1/AFBs genes between *B. juncea* var. *tumida* and *Brassica rapa*, and the results showed that there was high sequence similarity between those genes (Figure S3). Interestingly, many TIR1/AFB genes are located at same chromosomes in each genome, respectively. For example, BjuTIR1C had highest sequence identity with Bra003518, and they were both located at chromosome A07 in their genome, respectively (Figure S3). According to gene structure analysis, most of the BjuTIR1/AFBs genes had similar gene structure to *Arabidopsis TIR1/AFBs*, which includes three exons and two introns. The exceptions were BjuAFB1D and BjuAFB3D, both of which contain four exons and three introns, as well as BjuAFB1B with two exons and one intron (Figure 2).

![Figure 2](image_url)

**Figure 2.** The phylogenetic analysis and gene structure of TIR1/AFBs in *Arabidopsis thaliana* and *Brassica juncea*. The protein sequence of each gene was used for the alignment, and the phylogenetic neighbor-joining tree was constructed using MEGA5 phylogenetic analysis software.

3.3. The Alignment of BjuTIR1/AFB Proteins and Secondary Domain Prediction

The BjuTIR1/AFBs protein sequences were aligned by Clustalx [28], and the results showed that the sequences of those proteins were conserved (Figure 3). The sequence identification was more than 83.67% among the BjuTIR1 proteins. Similar results were found for BjuAFBs proteins: there was more than 90.77%, 89.21%, and 98.36% sequence identification among BjuAFB1, BjuAFB3, and BjuAFB5 family proteins, respectively. In particular, the peptide sequences were highly conserved in the F-box domain region (Figure 3).
CRTDREHVCBF2, and ABREATRE22 (Figure 5). This result suggests that those four cis-elements might specifically play roles in *B. juncea* var. *tumida*.

The secondary domains of *BjuTIR1* and *BjuAFBs* were predicted, the results showed that all the *BjuTIR1*, *BjuAFB1*, *BjuAFB2*, and *BjuAFB3* proteins contained an F-box domain in the N-terminus, indicating that those genes might function as E3 ubiquitin ligases. However, no F-box domains were found in the *BjuAFB4* and *BjuAFB5* proteins, suggesting that their genes function in a different manner. There were several LRR domains in all the *BjuTIR1* and *BjuAFB* proteins; those LRR domains were related to the interaction between the *BjuTIR1*/AFBs with other proteins (Figure 4).
3.5. The Transcriptional Expression Pattern of BjuTIR1/AFBs in Different Tissue

The seedlings of Yong An Xiao Ye were cultivated in a field for six months, and the root, stem, swollen stem, leaf, flower, and pod were harvested for RNA extraction. The real-time quantitative PCR were performed to detect the expression pattern of \textit{BjTIR1/AFB} family genes; the raw data of the qPCR results can be found in the Supplementary Materials (File S4). The results showed that \textit{BjuTIR1A}, \textit{BjuTIR1D}, and \textit{BjuAFB1B} were highly expressed in most tissues, and moderate expression levels were found for \textit{BjuTIR1C}, \textit{BjuAFB1B}, \textit{BjuAFB3A}, and \textit{BjuAFB3C} (Figure 6). \textit{BjuAFB1D}, \textit{BjuAFB2}, \textit{BjuAFB3D}, and \textit{BjuAFB5A} demonstrated lower expression levels in all tissues, suggesting that those four genes had limited function during plant growth and development (Figure 6). As shown in Figure 6, \textit{BjuTIR1A} was expressed at much higher levels in the flower than in any other tissues, indicating this gene might be involved in flower development. Many genes, such as \textit{BjuTIR1B}, \textit{BjuTIR1C}, \textit{BjuTIR1D}, \textit{BjuTIR1F}, \textit{BjuAFB1B} and \textit{AFB3C}, showed their highest expression levels in pods, suggesting that those genes might be responsible for pod formation. All the \textit{BjuTIR1} genes were highly expressed in the pod except \textit{BjuTIR1A}, which indicates that \textit{BjuTIR1} family genes mainly function in pod formation and development (Figure 6). Besides that, the high expression of \textit{BjuTIR1A}, \textit{BjuTIR1D}, \textit{BjuAFB1B}, and \textit{AFB3A} in the stem as well as the observed swollen stem suggests that those genes might play important roles in the function of stem development (Figure 6).

3.4. The Cis-Element Prediction of BjuTIR1/AFB Promoters

The 2 kb DNA region upstream \textit{BjuTIR1/AFB} sequences were chosen as promoters of those genes, and \textit{cis}-elements were predicted by the PLACE software. Some auxin-responsive elements were found in most of \textit{BjuTIR1/AFBs'} promoters, such as ARFAT (TGTCTC) [29] and GMSAUR (CATACT) [30] (Figure 5). There were also some \textit{cis}-elements related to plant response to biotic-stresses, such as GT1GMSCAM4 (GAAAAA) [31] and GCCCORE (GCCGCC) [32]. The \textit{cis}-elements MYCCONSENSUSAT (CANNNTG) [33], MYB1AT (WAACCA) [33], and MYBATRD22 (CTAACCA) [34] were also found in the promoter region of most \textit{BjuTIR1/AFBs} genes. Those \textit{cis}-elements were involved in plant response to dehydration stress. Some \textit{cis}-elements responsive to low temperatures were also found, such as LTRE1HVBLT49 (CCGAAA) [35], CRTDREHVCBF2 (GTGAC) [36], and LTRECOREATCOR15 (CCGAC) [37]. Besides that, some \textit{cis}-elements responsive to ABA (ABRELATERD1 [38] and ABREATRE22 [39]) and salicylic acid (SA) (ASF1MOTIFCAMV) [40] were also included in the promoter regions of those genes (Figure 5). Together, several \textit{cis}-elements responsive to auxin, ABA, SA, biotic stresses, and abiotic stresses existed in the promoter of \textit{BjuTIR1} and \textit{BjuAFBs} family genes. We also analyzed those \textit{cis}-elements in the promoters of \textit{AtTIR1/AFB} genes. The results showed that most of the \textit{cis}-elements could be found in the promoters of \textit{Arabidopsis TIR1/AFB} genes, except GMSAUR, GCCCORE, CRTDREHVCBF2, and ABREATRE22 (Figure 5). This result suggests that those four \textit{cis}-elements might specifically play roles in \textit{B. juncea} var. \textit{tumida}.
Figure 5. The cis-element prediction of BjuTIR1/AFBs promoters. The promoter sequences (2000 bp) upstream of the genes were chosen for cis-elements analysis using the software at http://www.dna.affrc.go.jp/PLACE/.
3.5. The Transcriptional Expression Pattern of BjuTIR1/AFBs in Different Tissue

The seedlings of Yong An Xiao Ye were cultivated in a field for six months, and the root, stem, swollen stem, leaf, flower, and pod were harvested for RNA extraction. The real-time quantitative PCR were performed to detect the expression pattern of BjuTIR1/AFB family genes; the raw data of the qPCR results can be found in the Supplementary Materials (File S4). The results showed that BjuTIR1A, BjuTIR1D, and BjuAFB1B were highly expressed in most tissues, and moderate expression levels were found for BjuTIR1C, BjuAFB1B, BjuAFB3A, and BjuAFB3C (Figure 6). BjuAFB1D, BjuAFB2, BjuAFB3D, and BjuAFB5A demonstrated lower expression levels in all tissues, suggesting that those four genes had limited function during plant growth and development (Figure 6). As shown in Figure 6, BjuTIR1A was expressed at much higher levels in the flower than in any other tissues, indicating this gene might be involved in flower development. Many genes, such as BjuTIR1B, BjuTIR1C, BjuTIR1D, BjuTIR1F, BjuAFB1B and AFB3C, showed their highest expression levels in pods, suggesting that those genes might be responsible for pod formation. All the BjuTIR1 genes were highly expressed in the pod except BjuTIR1A, which indicates that BjuTIR1 family genes mainly function in pod formation and development (Figure 6). Besides that, the high expression of BjuTIR1A, BjuTIR1D, BjuAFB1B, and AFB3A in the stem as well as the observed swollen stem suggests that those genes might play important roles in the function of stem development (Figure 6).

![Figure 6](image-url)  
*Figure 6.* The expression patterns of BjuTIR1/AFB genes in different tissues. The tissue-specific expression pattern of BjuTIR1/AFB homologous genes were analyzed by qPCR. BjuActin3 was used as the internal control. The boxes displayed the gene expression levels using the 2−ΔΔCT method, and different colors represent different expression levels under the marked threshold values above the ruler.
3.6. The Transcriptional Expression Pattern of *BjuTIR1*/AFBs Under Salt Stress Treatment

To investigate candidate *BjuTIR1*/AFB genes’ interaction with plant response to salt stress, the gene expression levels were tested. Two-week-old seedlings of Yong An Xiao Ye were treated by 200 mM NaCl for 3, 6, 12, and 24 h, respectively, and the roots were collected for RT-qPCR analysis. The results showed that *BjuTIR1A* was significantly induced by salt treatment, especially after 3 and 6 h treatment, as the transcriptional expression levels of *BjuTIR1B* and *BjuAFB1C* increased at 3 and 6 h, respectively (Figure 7). In contrast, the expression of most *BjuTIR1*/AFB family genes were inhibited by salt treatment, including *BjuTIR1C*, *BjuTIR1D*, *BjuTIR1E*, *BjuAFB1A*, *BjuAFB1B*, *BjuAFB3A*, *BjuAFB3B*, *BjuAFB3C*, and *BjuAFB4* (Figure 7). Those results suggest that most of the *BjuTIR1*/AFB genes were repressed by salt treatment, and might be involved in plant responses to salt stress.

![Figure 7](image-url)
3.7. The Transcriptional Expression Pattern of BjuTIR1/AFBs under Plasmodiophora brassicae Treatment

Plasmodiophora brassicae is a main pathogen of B. juncea var. tumida, which could lead to club root of cruciferous plants. To test which gene may be involved in the plant response to pathogen attack, the expression patterns of BjuTIR1/AFB family genes were detected under P. brassicae treatment. Two-week-old seedlings of Yong An Xiao Ye were treated by P. brassicae (OD600 = 0.07) for 6, 12, 24, 48, and 72 h, respectively, and the roots were collected for (RT-qPCR) analysis. The results showed that BjuTIR1A and BjuAFB3B were severely induced by P. brassicae treatment; BjuTIR1D, BjuTIR1E, BjuAFB1A, BjuAFB1B, BjuAFB2, BjuAFB3D, BjuAFB4, and BjuAFB5B were induced moderately by P. brassicae treatment (Figure 8). Other genes showed similar expression levels during P. brassicae treatment, and nearly no gene was inhibited by this biotic stress treatment. Experiment results show the BjuTIR1/AFB family genes induced by P. brassicae could be involved in plant response to this pathogen—especially BjuTIR1A (Figure 8).

![Figure 8. The expression patterns of BjuTIR1/AFB genes under the pathogen Plasmodiophora brassicae. The expression pattern of BjuTIR1/AFB homologous genes were analyzed by qPCR. BjuActin3 was used as the internal control. Three independent biological repeats were performed, and all data points were the means of three biological replicates ± SE. Significant differences: *, p < 0.05; **, p < 0.01.](image)
4. Discussion

*B. juncea* var. *tumida* is an allotetraploid species formed by hybridization between the diploid ancestors of *B. rapa* and *Brassica nigra*, followed by genome duplication [41]. In *A. thaliana*, six auxin receptor genes were identified [5,6,16-18]. According to our results, 18 putative auxin receptors were found in the genome of *B. juncea* var. *tumida*. Except for *AtAFB2* and *AtAFB4*, each *Arabidopsis* auxin receptor was homologous to 2–6 genes in the *B. juncea* var. *tumida* genome (Table 1). The multiple homologs of auxin receptors in *B. juncea* var. *tumida* may be from the result of genome duplication. Among the 18 auxin receptors, nine genes were located in the A sub-genome and eight in the B sub-genome (*BjuAFB5A* could not be mapped to a genome). The comparable auxin receptor gene numbers in the A or B sub-genome indicates that the genome of *B. juncea* var. *tumida* experienced co-linearity in the gene duplication process. Most of the auxin receptor genes contained three exons and two introns, the exceptions being *BjuAFB1D* and *BjuAFB1D*, both of which contained four exons and three introns (Figure 2). The gene structure showed that more exons attributed to a partial exon coding sequence converted to a non-coding sequence. This exon–intron splicing arrangement is present in other species, such as rice and maize [42,43].

Abnormal phenotypes of enlarged flag leaf inclination, more tillers, early flowering and a lower tolerance to salt were found due to reduction of *OsTIR1* and *OsAFB2* in rice [44,45]. However, the expression levels of *BjuAFB2* was low in all tissues of *B. juncea* var. *tumida* (Figure 8), suggesting that *BjuAFB2* might not play an important role as a main auxin receptor. In contrast, the high expression levels of *BjuTIR1A* and *BjuTIR1D* indicate that these two genes might be the main auxin receptors in *B. juncea* var. *tumida*. According to the results of the gene expression pattern, tissue-specific gene expressions were identified, such as *BjuTIR1A* demonstrating influence on the regulation of flower development, and *BjuTIR1F* may specifically influence pod development regulation.

It has been reported that auxin signaling plays an important role in plant resistance to abiotic stress, especially at high salinity [3]. In this study, the expression levels of most of auxin receptor genes was inhibited under salt treatment (Figure 7). In tobacco, overexpression of *AtmiR393a* could repress the expression levels of auxin receptors like *AtTIR1*, leading to the inhibition of plant growth and enhanced resistance to salt stress [46]. The reduced expressions of *BjuTIR1/AFB* family genes under salt treatment were consistent with the previous study. The exception was *BjuTIR1A*—the expression of this gene was significantly induced by salt treatment, which might result from feedback of the signaling transduction.

The auxin signaling pathway mediated by TIR1/AFBs is usually involved in plant resistance or tolerance to pathogen attacks, such as the anthracnose disease in cassava [47], *Zea mays* defense against *Rhizoctonia solani* [48], or *Arabidopsis* resistance to the bacterium *Pseudomonas syringae* [25]. In *Arabidopsis*, *AtTIR1* and *AtAFB1* are transcriptionally upregulated in clubroots, and the mutants of *tir1*, *afb1-3*, and *afb1-3* *afb2-3* all resulted in more susceptible reactions to the root pathogen *P. brassicae* [49]. In this study, similar results were found. The transcriptional level of *BjuTIR1A* was severely induced by *P. brassicae* treatment, especially at 6 and 12 h after inoculation. Besides that, all the *BjuTIR1* were induced by this pathogen treatment, except for *BjuTIR1B* (Figure 8). For *BjuAFB1* genes, the expression levels of *AFB1A*, *AFB1B*, and *AFB1C* also increased at 48 h after pathogen inoculation (Figure 8). The induction of those genes suggested that *BjuTIR1s* and *BjuAFB1s* might play similar roles to *AtTIR1/AFB1s* in the processes of plant defense against *P. brassicae* invasion.

5. Conclusions

In this study, a total of 18 *BjuTIR1/AFB* genes were identified, and they have similar gene structures and protein domains to the auxin receptors of *Arabidopsis*. Several cis-elements related to plant response to phytohormones, biotic stresses, and abiotic stresses were found in the promoter of *BjuTIR1/AFB* genes, indicating those genes may play a role in the regulation of plant development and defense against biotic and abiotic stresses, which could benefit from a more targeted follow-up study. Gene expression analysis showed that some *BjuTIR1/AFB* family genes exhibit a special expression pattern,
and most genes are responsive to salt stress treatment. Together, our data provides a useful foundation for future research regarding the function of auxin receptors in B. juncea var. tumida.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4425/10/2/165/s1, Table S1: Primers used for qPCR in this study; File S2: The CDS sequences of TIR1/AFBs; Figure S3: The phylogenetic analysis of TIR1/AFBs in A. thaliana, B. rapa and B. juncea var. tumida. The protein sequence of each gene was used for the alignment, and the phylogenetic neighbor-joining tree was constructed using MEGA5 phylogenetic analysis software. File S4: The raw data of qPCR assays.

**Author Contributions:** D.W. and Z.C. conceived the project and designed the experiments. Z.C., D.-e.Z., J.L., C.C., Z.S., M.X., M.F., and Y.C. performed the experiments and analyzed the data; D.W. and Z.C. prepared the manuscript.

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