**Supporting Information**

**Article Title: *Brassica napus* mediator subunit16 induces BnMED25- and BnWRKY33-activated defense signaling to confer *Sclerotinia sclerotiorum* resistance**

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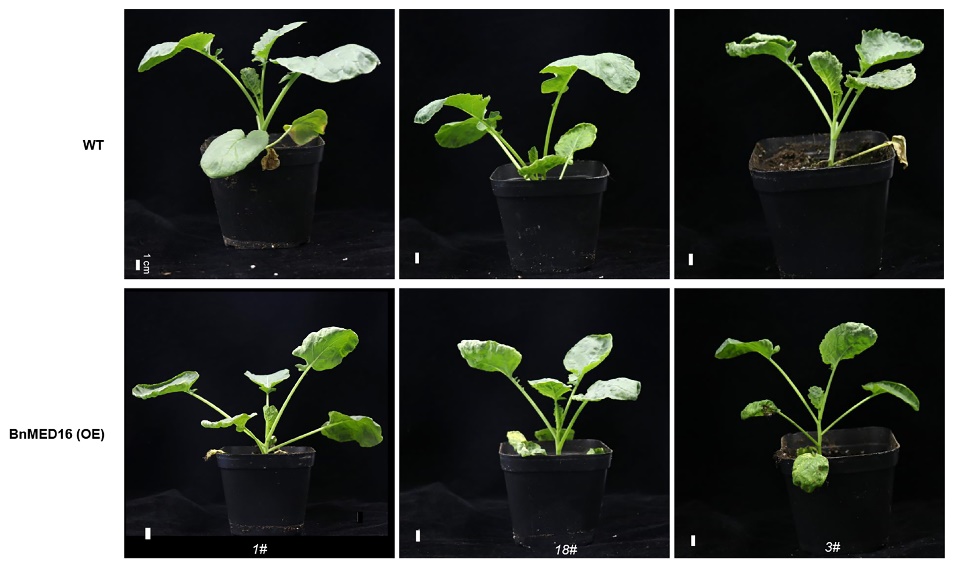
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**Supplementary Table 1** Primers used in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Purpose** | **Genes/Vectors** | **Primer name** | **Primer sequence (5’-3’)** | **Restriction Enzyme** | **Length of amplification (bp)** |
|
| qRT-PCR | *BnMED16* | Q-BnMED16-F | CTTAAACAGCCCAACTCCAATC | / | 228 |
| BnaA09g20140D | Q-BnMED16-R | AGAGTCTGCAACAACATTGAAC |
| *BnMED14* | Q-BnMED14-F | CAGCGACTTCAAGACAATCAAA | / | 219 |
| BnaAnng10600D | Q-BnMED14-R | CACGGAGTACTACAGAGACATC |
| *BnMED25* | Q-BnMED25-F | CTCCAAAGGACTTGCGATTATG | / | 160 |
| BnaA09g28640D | Q-BnMED25-R | GTTCCTCTCTCCGTTTAACTCA |
| *BnMED8* | Q-BnMED8-F | ATCAAATGCCATATTCGCAGTC | / | 208 |
| BnaA09g53670D | Q-BnMED8-R | GACATGTTTGGTATCATCTGGC |
| *BnMED21* | Q-BnMED21-F | TTGTCGGAGAATCAGTAGATCG | / | 158 |
| BnaA09g20250D | Q-BnMED21-R | CGTATTGCGAAATCCCAGTAGA |
| *BnMED19a* | Q-BnMED19a-F | GCGTGATAAAGAAGATGGAACC | / | 147 |
| BnaC09g44480D | Q-BnMED19a-R | AATCTGATAGAGACGCGGTAAG |
| *BnWRKY33* | Q-BnWRKKY33-F | GTACTTTCCCAAACTGTCCAAC | / | 131 |
| BnaC04g06800D | Q-BnWRKKY33-R | CGAAGAAGACGACGATCTTCTA |
| *BnPR1*  BnaC01g04530D | Q-BnPR1-F | ATCTCCGAAAAGGCCAGGGA | / | 118 |
| Q-BnPR1-R | TACCATTTACCGCATCGCCC |
| *BnPR2* | Q-Bn13EG-F | GTAACGAAGTTGTACCGTCCAACG | / | 127 |
| BnaA01g17540D | Q-Bn13EG-R | CAAAGTCATATCAACCGCTGTAGAC |
| *BnPR3*  *BnaA05g26640D* | Q-BnChitinase-F | GGTAACACCGAACCTTACTGTA | / | 194 |
| Q-BnChitinase-R | GACTTAGCGGCATTGATAAAGG |
| *BnPDF1.2*  BnaC02g23620D | Q-BnPDF1.2-F | GCATGAATCAGTGCATTAACCT | / | 173 |
| Q-BnPDF1.2-R | AATCCACACACAATTAAGCACC |
| *BnJAZ1* | Q-BnJAZ1-F | TACGGCGGGCAAGTGATT | / | 175 |
| BnaA06g13250D | Q-BnJAZ1-R | TTGGTTCGGGGTAGGAGC |
| *BnMYC2* | Q-BnMYC2-F | TTATGGGTCGGATCAGTTAACC | / | 152 |
| BnaC05g28450D | Q-BnMYC2-R | TCATAAGATCCGAACTCTGTCG |
| *BnCOI1* | Q-BnCOI1-F | GCATTCTATCTCAGACAAAGCG | / | 166 |
| BnaA03g56600D | Q-BnCOI1-R | CTCTCATCTCCAGCTTCTGTAG |
| *BnEIN3* | Q-BnEIN3-F | GAGGATCTGGGATAACTGTGAG | / | 201 |
| BnaA05g20160D | Q-BnEIN3-R | CATCCATCGTTCCTACTACTCC |
| *BnORA59*  BnaC08g44670D | Q-BnORA59-F | TCATACAGAGGAGTGAGGAAGA | / | 242 |
| Q-BnORA59-R | GGAGACTCTCCGTTATCGAAAT |
| *BnERF1*  BnaA01g23940D | Q-BnERF1-F | ATGCTTCTCTACGGACTAATCG | / | 271 |
| Q-BnERF1-R | CCGGAAAATTTAATATCGCCGA |
| *BnPGIP1*  BnaC09g48690D | Q-BnPGIP1-F | TCATTTGGATCATTCCCAGGAA | / | 123 |
| Q-BnPGIP1-R | CTTGTTCCGTGAGAAATCGATC |
| *BnCYP71B7*  BnaC08g40600D | Q-BnPAD3-F | TCGCGTTTGGGATAGATATTCA | / | 162 |
| Q-BnPAD3-R | GTGTCTTGTTCTGTCCTGAAAC |
| *BnCSD1* | Q-BnCSD1-F | TCGATCATTGACATGTTTGCTC | / | 126 |
| BnaA06g05150D | Q-BnCSD1-R | AGACACAACCGGATATGAGATC |
| *BnFSD2*  BnaA03g13310D | Q-BnFSD2-F | GGTCTCATCTGACTAGGTAAGC | / | 211 |
| Q-BnFSD2-R | GCTCAGACTTTTATTAGCGCAA |
| *BnCAT2* | Q-BnCAT2-F | CAAAGCTCACTACGTGAAGTTC | / | 206 |
| BnaA03g53180D | Q-BnCAT2-R | GGTCAAAGTCGAACTTGTCTTC |
| *BnCAT3* | Q-BnCAT3-F | CACGCAACTAAAGATCTCCATG | / | 131 |
| BnaA08g21730D | Q-BnCAT3-R | CATATCTTGGTCACATCAAGCG |
| *BnPER21* | Q-BnPER21-F | TTGCCCAAGTCCAAACCC | / |  |
| BnaC03g20530D | Q-BnPER21-R | GACCAGGAGCCCTCTATG |
| *BnPRX34* | Q-BnPRX34-F | CATCGTACGAGAAACCATTGTC | / | 252 |
| BnaA01g20660D | Q-BnPRX34-R | ATATCTGCGCATGAAACAGTTC |
| *BnGPX7* | Q-BnGPX7-F | ACATAGTATCGACCACGTGTTT | / | 191 |
| BnaA03g51760D | Q-BnGPX7-R | TGTCCGTATGTAGAACACAAGT |
| *BnAPX1*  BnaA09g49190D | Q-BnAPX1-F | TGAAATCTTCTGTTGGTTGCAG | / | 134 |
| Q-BnAPX1-R | ACCGCCTAAAAGAACACAAATC |
| *BnActin7* | Q-BnActin7F1 | TCTTCCTCACGCTATCCTCCG | / | 181 |
| Q-BnActin7R1 | AGCCGTCTCCAGCTCTTGC |
| OE vector | *pCAMBLA1300-2301-D35S:BnMED16* | BnMED16-F | CGGAATTCTTGCTCTCTCGCTCGACAACGATCAG | EcoR I | 3741 |
| BnMED16-R | ACGCGTCGACATCCCACGCAATTTTGTCATCAAAC | SalI |
| Y2H vector | *pGBKT7-BnMED16* | BD-BnMED16-F | GAATTCATGAATCAGCCCCAAGTTTCTC | EcoRI | 3744 |
| BD-BnMED16-R | GGATCCCTATACAACACGGACCCACGTT | BamHI |
| *pGBKT7-BnMED25* | BD-BnMED25-F | GAATTCATGTCGTCGGAGTTGAAACAG | EcoRI | 2466 |
| BD-BnMED25-R | GGATCCTTATCCCATGAAGCCCGC | BamHI |
| *pGADT7-BnMED25* | AD-BnMED25-F | GAATTCATGTCGTCGGAGTTGAAACAG | EcoRI | 2466 |
| AD-BnMED25-R | GGATCCTTATCCCATGAAGCCCGC | BamHI |
| *pGADT7-BnMYC2* | AD-BnMYC2-F | GAATTCATGACGGAGCCGACGATG | EcoRI | 1827 |
| AD-BnMYC2-R | GGATCCTTAACCAATCTTTGAGATTAAACTCG | BamHI |
| *pGADT7-BnCOI1* | AD-BnCOI1-F | GAATTCATGGAGGATCCAGACATCAAGA | EcoRI | 1809 |
| AD-BnCOI1-R | GGATCCTCATGTCTCCTCCTTTAGTACTTTAAC | BamHI |
| *pGADT7-BnERF1* | AD-BnERF1-F | GAATTCATGGATCCATATTTTGTTCAGTCA | EcoRI | 633 |
| AD-BnERF1-R | GGATCCTCACCAGGCCCCACTGTT | BamHI |
| *pGADT7-BnORA59* | AD-BnORA59-F | GAATTCATGGAGTTTCAAACTAACTTCTTTTCT | EcoRI | 735 |
| AD-BnORA59-R | GGATCCTCATGAACATAATCTCATAAGCTCTTC' | BamHI |
| *pGADT7-BnEIN3* | AD-BnEIN3-F | GAATTCATGATGTTTAACGAGATGGGAATG | EcoRI | 1881 |
| AD-BnEIN3-R | GGATCCTTAGAACCATATGGATACATCTTGCT | BamHI |
| *pGADT7-BnWRKY33* | AD-BnWRKY33-F | GAATTCATGGCTGCTTCTTCCCTCC | EcoRI | 1473 |
| AD-BnWRKY33-R | GGATCCTCACGACAAGAACGAATCAAAA | BamHI |
| *pGADT7-BnMED14* | AD-BnMED14-F | GAATTCATGGCGGAATTAGGGCAAC | EcoRI | 4893 |
| AD-BnMED14-R | GGATCCCTATATGGTAAACTCCTTTTGAAGAGTG | BamHI |
| *pGADT7-BnWRKY15* | AD-BnWRKY15-F | GAATTCATGGCGGTGGAGCTCATGAC | EcoRI | 897 |
| AD-BnWRKY15-R | GGATCCTCAAGACGATTCCAAAATGAGAT | BamHI |
| *pGADT7-BnWRKY75* | AD-BnWRKY75-F | GAATTCATGGAGGGATATCAAAATGGATC | EcoRI | 444 |
| AD-BnWRKY75-R | GGATCCTTAATTAAAAGAAGAGTAGATTTGCATT | BamHI |
| BIFC vector | *35s-BnMED16-Myc-nYFP* | BnMED16-nYFP-F | TCTAGAATGAATCAGCCCCAAGTTTCTC | XbaI | 3744 |
| BnMED16-nYFP-R | GGATCCTACAACACGGACCCACGTT | BamHI |
| *35s-BnMED25-Myc-nYFP* | BnMED25-nYFP-F | TCTAGAATGTCGTCGGAGTTGAAACAG | XbaI | 2466 |
| BnMED25-nYFP-R | GGATCCTCCCATGAAGCCCGC | BamHI |
| *35s-BnMED25-HA-cYFP* | BnMED25-cYFP-F | TCTAGAATGTCGTCGGAGTTGAAACAG | XbaI | 2466 |
| BnMED25-cYFP-R | GGTACCTCCCATGAAGCCCGC | KpnI |
| *35s-BnWRKY33-HA-cYFP* | BnWRKY33-cYFP-F | TCTAGAATGGCTGCTTCTTCCCTCC | XbaI | 1473 |
| BnWRKY33-cYFP-R | GGTACCCGACAAGAACGAATCAAAA | KpnI |
| *35s-BnMYC2-HA-cYFP* | BnMYC2-cYFP-F | TCTAGAATGACGGAGCCGACGATG | XbaI | 1827 |
| BnMYC2-cYFP-R | GGTACCACCAATCTTTGAGATTAAACTCG | KpnI |
| *35s-BnCOI1-HA-cYFP* | BnCOI1-cYFP-F | TCTAGAATGGAGGATCCAGACATCAAGA | XbaI | 1809 |
| BnCOI1-cYFP-R | GGTACCTGTCTCCTCCTTTAGTACTTTAAC | KpnI |
| *35s-BnEIN3-HA-cYFP* | BnEIN3-cYFP-F | TCTAGAATGATGTTTAACGAGATGGGAATG | XbaI | 1881 |
| BnEIN3-cYFP-R | GGTACCGAACCATATGGATACATCTTGCT | KpnI |

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**Supplementary Figure S1** The expression level of *BnMED16* gene in T0 transgenic lines and wild type. WT, wild type; BnMED16(OE) was the transgenic plants that overexpressed *BnMED16* genes in *B. napus*; 1#, 3#, 7#, 10#, 18#, 19#, 22# indicates the individual T0 transgenic lines. *BnActin7* was used as the internal control. The data are the mean ± SD of three independent biological replicates and the asterisks denote statistical significance at *P* < 0.01 (\*\*) between the WT and BnMED16(OE) lines by Student’s t-tests.

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**Supplementary Figure S2** Phenotype of T4 homozygous BnMED16(OE) lines and WT seedlings. Bars, 1 cm.

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**Supplementary Figure S3** Analysis of defense protease activities of WT and *BnMED16* overexpression seedling leaves before and after inoculation with *S. sclerotiorum.* **(A)** Determination of β-1,3-glucanaseactivities; **(B)** Determination of chitinase activities. The data are the mean ± SD of three independent biological replicates. Least-significant difference (LSD) tests were used for multiple comparisons. Different letters above bars indicate that the means differ according to ANOVA and LSD tests (*P* < 0.01).

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**Supplementary Figure S4** Clustered heatmap and signaling pathway enrichment analysis of differentially expressed genes (DEGs) of *B. napus* genes responsive to *S. sclerotiorum* between BnMED16 overexpression and WT lines. **(A)** All DEGs were clustered in the heatmap of based on FPKM (fragments per kilobase of exon per million fragments mapped) levels in BnMED16(OE) and WT seedling leaves in response to *S. sclerotiorum* infection after 6 hours. The DEGs are clustered on the Y-axis according to hierarchical agglomerative clustering. The FPKM was normalized using the *log10 (fpkm+1)* to generate the heatmap. Red and blue represent high and low gene expression, respectively; **(B,C)** KEGG pathway and gene ontology (GO) classification of DEGs as shown in **(A)**.

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**Supplementary Figure S5** DEGs between transgenic and WT *B. napus* lines in response to *S. sclerotiorum* infection. Heatmap of DEGs related to *S. sclerotiorum* infection *B. napus* seedling leaves of transcription factor WRKY genes **(A)**, camalexin synthesis genes **(B)**, MAP kinases pathway genes **(C)**, and receptor-like protein kinases (RLKs) related genes**(D)**. DEGs were considered statistically significant if *q*-value <0.005 and |log2-fold change| >1. The log2-fold change is indicated according to the scale bar. The color from yellow to magenta represents the DEGs from down-regulated to up-regulated. The intensity of the color indicates the multiple of change for DEGs, with a darker color meaning higher fold change. The common up-regulated DEGs are highlighted by red boxes. Details of the genes are given in **Supplementary Dataset 1**.



**Supplementary Figure S6** qRT-PCR confirmation of the DEGs between BnMED16 overexpression and WT lines after inoculation. Expression pattern of *BnMED25* (*BnaA09g28640D*) **(A)**, *BnJAZ1* (*BnaA06g13250D*) **(B)**, *BnMYC2* (*BnaC05g28450D*) **(C)**, *BnCOI1* (*BnaA03g56600D*) **(D)**, *BnEIN3* (*BnaA05g20160D*) **(E)**, *BnORA59* (*BnaC08g44670D*) **(F)**, *BnERF1* (*BnaA01g23940D*) **(G)**, *BnPAD3* (*BnaC08g40600D*) **(H)**, and *BnPGIP1* (*BnaC09g48690D*) **(I)**in BnMED16(OE) and WT seedling leaves at different time points after inoculation with *S. sclerotiorum*. *BnActin7* was used as the internal control; Data are means ± SD of three biological replicates and the asterisks denote statistical significance at *P* < 0.01 (\*\*) or *P* < 0.05 (\*) between the WT and BnMED16(OE) lines at each time point by Student’s t-tests.