

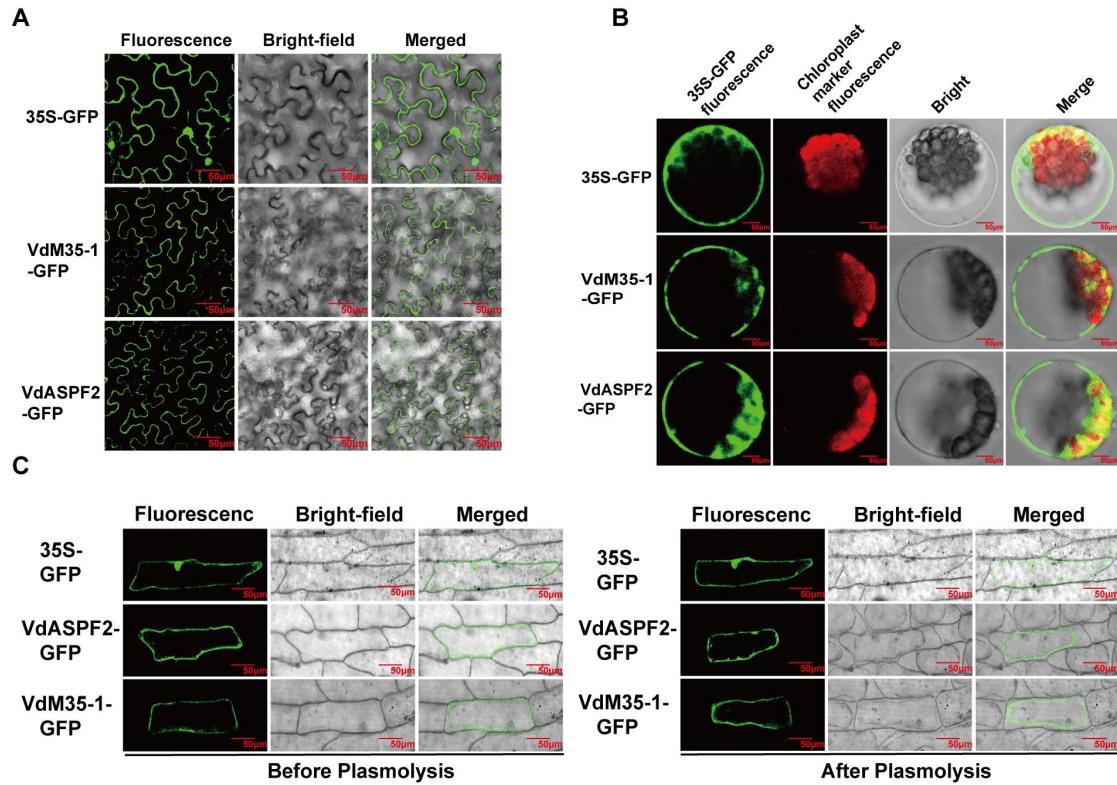


Figure S1: VdM35-1 and VdASPF2 are mainly located in the cell membrane. (A) Subcellular localization of VdM35-1-GFP and VdASPF2-GFP transiently expressed by *Agrobacterium* in *N. benthamiana*. After 48h of injection, the fluorescence was detected by confocal microscope. (B) Subcellular localization of VdM35-1-GFP and VdASPF2-GFP recombinant plasmids in Arabidopsis protoplast expression. After 16 hours of expression, fluorescence was detected by confocal microscopy. (C) Using biological technology, tungsten powder particles wrapped by recombinant plasmids of VdM35-1-GFP and VdASPF2-GFP were directly introduced into onion skin by high pressure gas. After 24 hours of expression, fluorescence was detected by confocal microscopy.

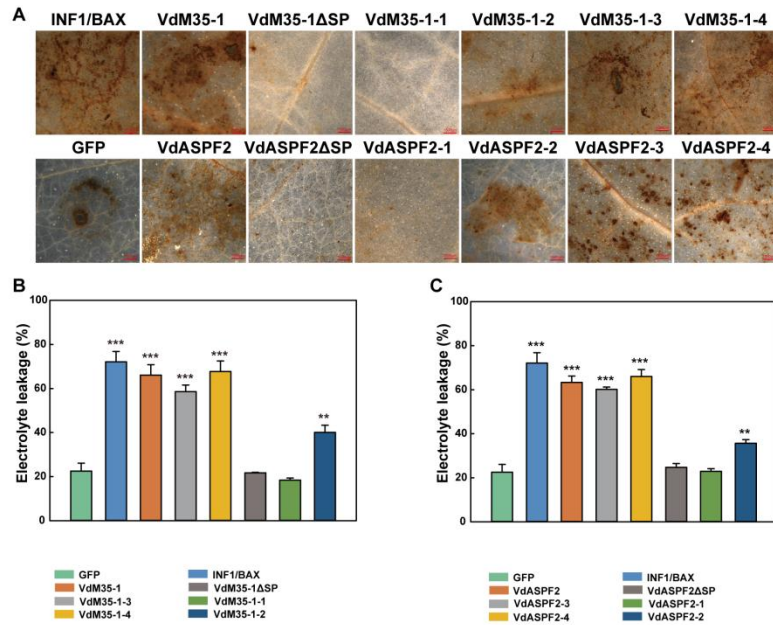


Figure S2: VdM35-1 and VdASPf2 triggered ROS accumulation and electrolyte leakage. (A) ROS accumulation in *N. benthamiana* induced by positive control BAX/INF1, negative control GFP, VdM35-1, VdASPf2 and all mutants. (B, C) Analysis of cell death by electrolyte leakage measurements. Values represent means $\pm$ standard deviation of three replicates. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

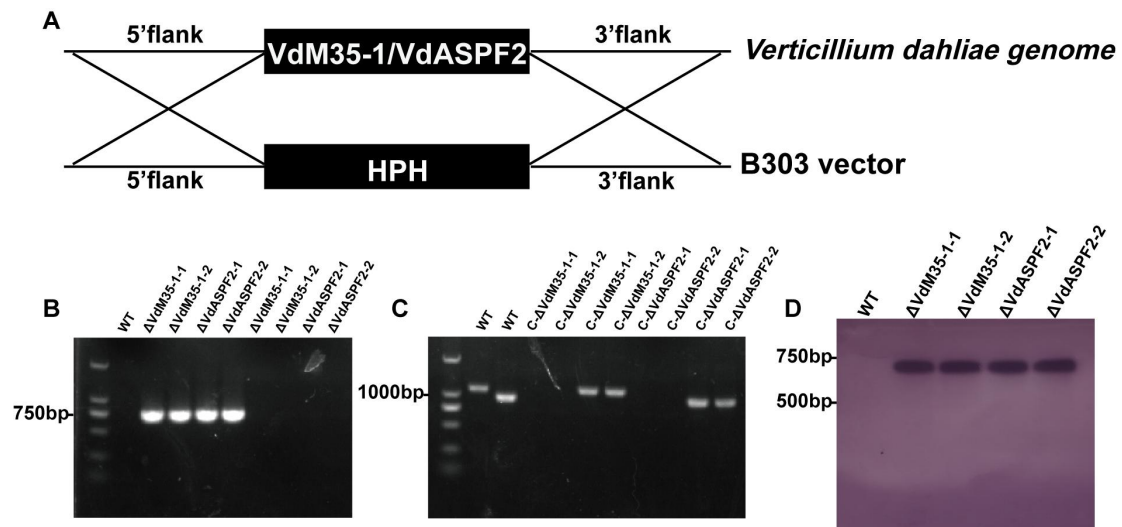


Figure S3: Acquisition of knock-out and complementation mutants. (A) Gene knockout mechanism of *V. dahliae* VdM35-1 and VdASPf2. (B, C) Knockout and complementation strains were determined by PCR. (D) Southern blot was used to identify the knockout strains.

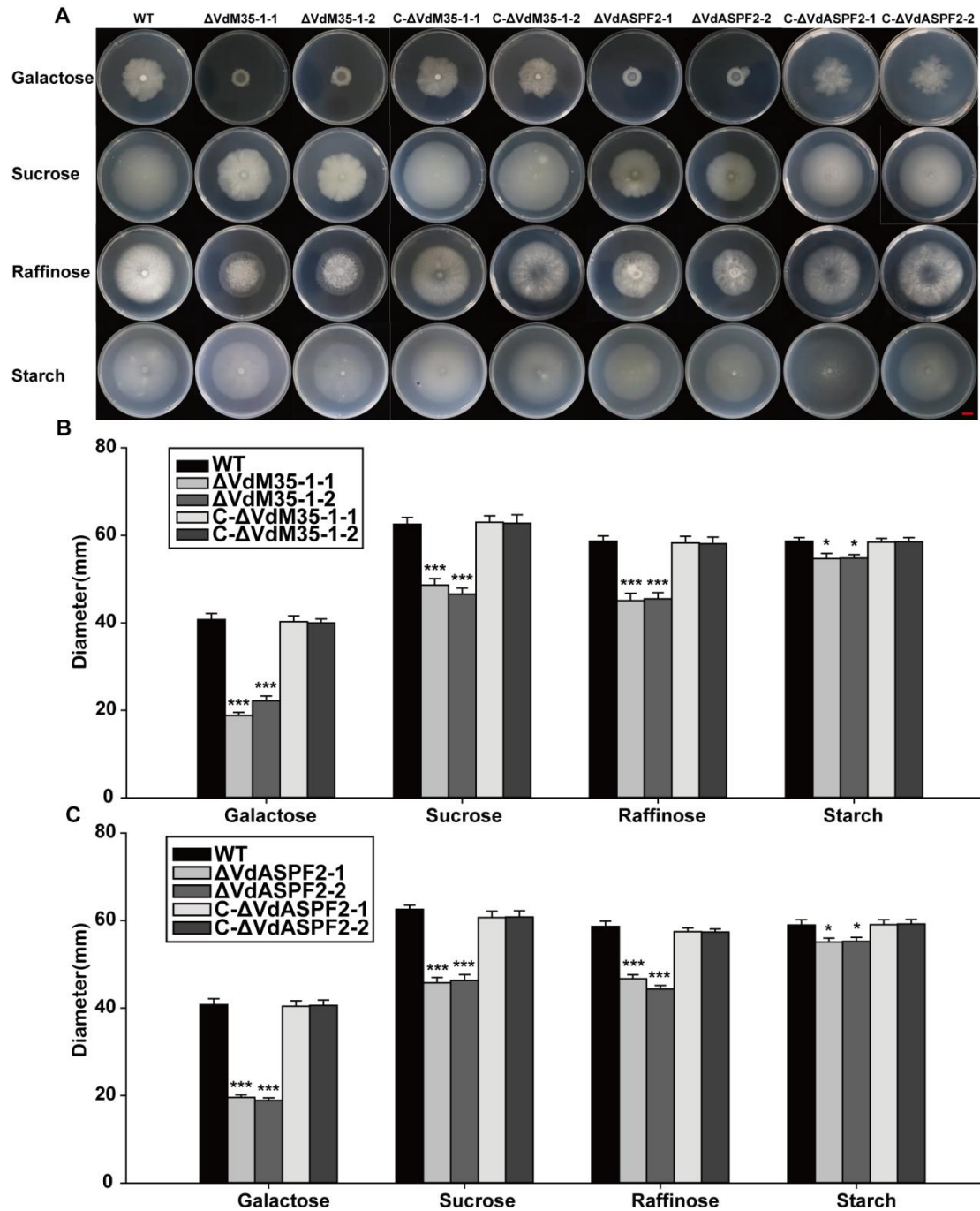


Figure S4: VdM35-1 and VdASP2 were essential for normal vegetative growth and conidial production of *V. dahliae*. (A) Colony morphology of all strains cultured at 25 °C in dark for 14 days on Czapek-Dox Medium with different carbon sources. Scale = 1 cm (B, C) Colony diameter of all strains. Values represent means $\pm$ standard deviation of three replicates. The asterisks represent statistical differences performed by a t-test (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$) in comparison with the wild type strains.

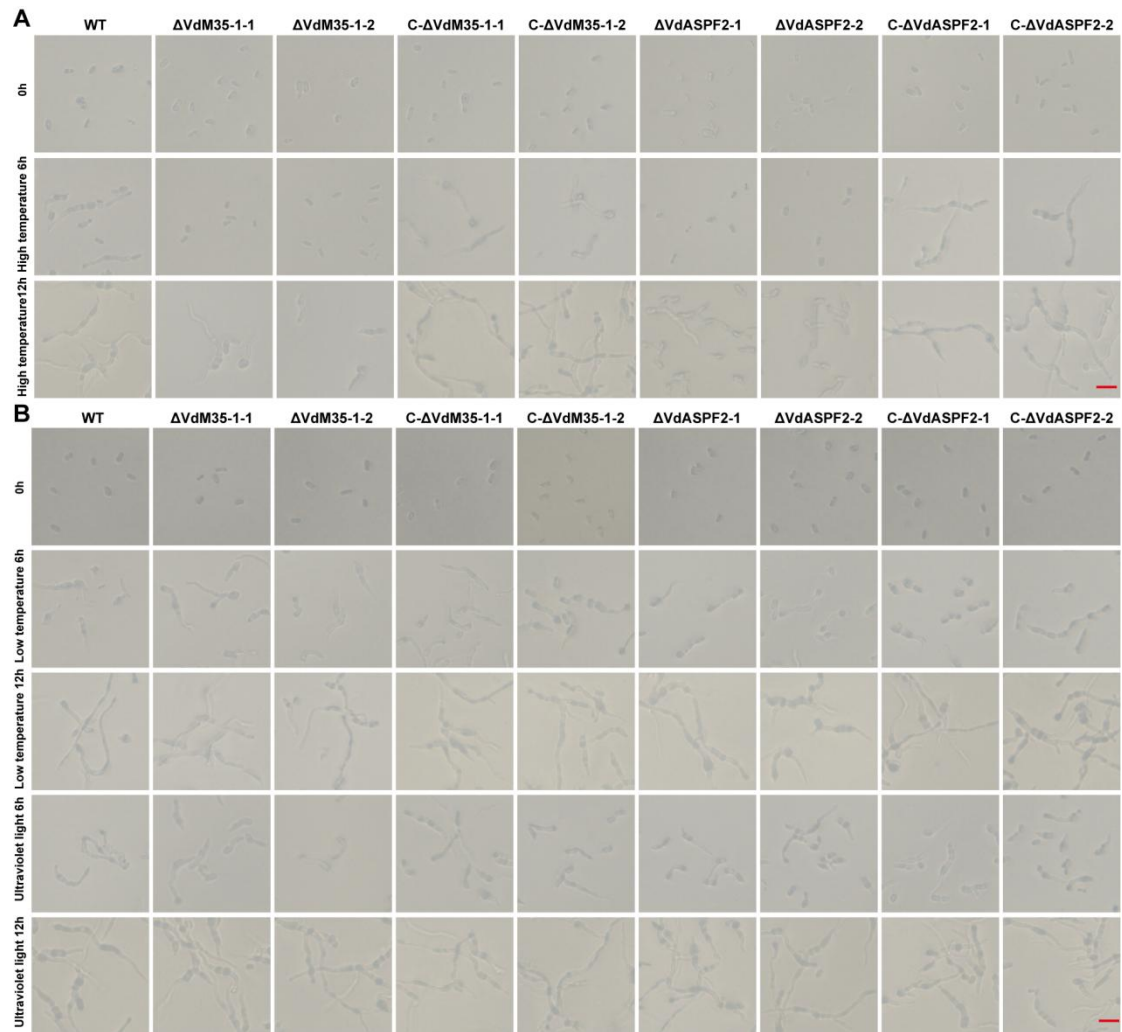


Figure S5: The spore germination of wild, Δ VdM35-1, Δ VdASPF2, C- Δ VdM35-1, and C- Δ VdASPF2 strains under high temperature, low temperature and UV treatment. (A) The conidia of all strains were treated at 45 °C for an hour under fluorescence microscope. (B) The conidia of all strains were treated at 4 °C for an hour and irradiated by ultraviolet light for 3 minutes under fluorescence microscope. Scale = 200 μ m

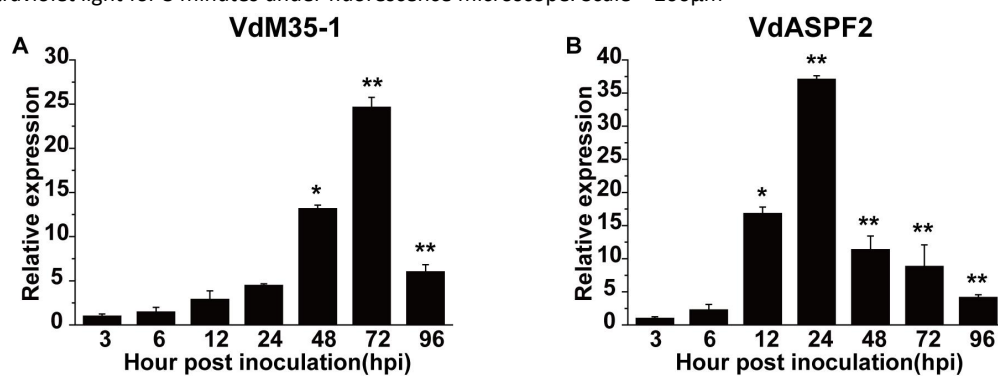


Figure S6: Expression patterns of *VdM35-1* and *VdASPF2*. (A) Expression pattern of *VdM35-1* in cotton infected by *V. dahliae*. (B) Expression pattern of *VdASPF2* in cotton infected by *V. dahliae*. (*, $p < 0.05$, **, $p < 0.01$)

Table S1. Primers used in this study

Primer name	Sequence (5'-3')	Purpose
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VDAG_00129-Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGAAGTTTCTCGTG GCC	Clone VDAG_00129 to PGR107 for expression in <i>N. benthamiana</i>
VDAG_00129-Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAACGGGCGAAGA TGGA	
VDAG_04551-Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGCTTTCTCTCCAG ACC	Clone VDAG_04551 to PGR107 for expression in <i>N. benthamiana</i>
VDAG_04551-Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CGAGCAGTGAACAA AGCCATC	
VdM35-1 ^{ΔSP} -Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGGCCTCGGTCGA CTTG	Clone VdM35-1 ^{ΔSP} to PGR107 for expression in <i>N. benthamiana</i>
VdM35-1 ^{ΔSP} -Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAACGGGCGAAGA TGGA	
NBPR1 ^{SP} +VdM35-1 ^{ΔSP} - Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGGGATACTCCAAAACA TT	
NBPR1 ^{SP} +VdM35-1 ^{ΔSP} - R	CAAGTCGACCGAGGCAGCTTGAGATGAGGGAGACA	Clone NBPR1 ^{SP} +VdM35-1 ^{ΔSP} to PGR107 for expression in <i>N.</i>
NBPR1 ^{SP} +VdM35-1 ^{ΔSP} - F	TGTCTCCCTCATCTCAAGCTGCCTCGGTCGACTTG	<i>benthamiana</i>
VdM35-1-Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAACGGGCGAAGA TGGA	
VdASPF2 ^{SP} +VdM35-1 ^Δ ^{SP} -Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGCTTTCTCTCCAG ACC	
VdASPF2 ^{SP} +VdM35-1 ^Δ ^{SP} -R	CAAGTCGACCGAGGCAGAGGCAGCGACGAG	Clone VdASPF2 ^{SP} +VdM35-1 ^{ΔSP} to PGR107 for expression in <i>N.</i>
VdASPF2 ^{SP} +VdM35-1 ^Δ ^{SP} -F	CTCGTCGCTGCCTCTGCCTCGGTCGACTTG	<i>benthamiana</i>
VdM35-1-Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> GCAACGGGCGAAGA TGGA	
VdASPF2 ^{ΔSP} -Clal-F	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGCCCCGTGGCACG CGCT	Clone VdASPF2 ^{ΔSP} to PGR107 for expression in <i>N. benthamiana</i>
VdASPF2 ^{ΔSP} -Smal-R	TCGCCCTTGCTCACCAT <u>CCCGGG</u> CGAGCAGTGAACAA AGCCATC	
NBPR1 ^{SP} +VdASPF2 ^{ΔSP} -	GGTCAGCACCAGCTAGC <u>ATCGAT</u> ATGGGATACTCCAAAACA	Clone NBPR1 ^{SP} +VdASPF2 ^{ΔSP} to

Clal-F	TT	PGR107 for expression in <i>N. benthamiana</i>
NBPR1 ^{SP} +VdASPF2 ^{ΔSP} -R	AGCGCGTGCCACGGGAGCTTGAGATGAGGGAGACA	
NBPR1 ^{SP} +VdASPF2 ^{ΔSP} -F	TGTCTCCCTCATCTCAAGCTCCCGTGGCACGCGCT	
VdASPF2-Smal-R	TCGCCCTTGCTCACCATCCCGGGCGAGCAGTGAACAA AGCCATC	
VdM35-1 ^{SP} +VdASPF2 ^Δ SP-Clal-F	GGTCAGCACCCAGCTAGCATCGATATGAAGTTTCTCGTG GCC	
VdM35-1 ^{SP} +VdASPF2 ^Δ SP-R	AGCGCGTGCCACGGGAGCCGAGCTAGCAG	Clone VdM35-1 ^{SP} +VdASPF2 ^{ΔSP} to PGR107 for expression in <i>N. benthamiana</i>
VdM35-1 ^{SP} +VdASPF2 ^Δ SP-F	CTGCTAGCTCGGGCTCCCGTGGCACGCGCT	
VdASPF2-Smal-R	TCGCCCTTGCTCACCATCCCGGGCGAGCAGTGAACAA AGCCATC	
VdM35-1 ^{ΔHEXXH} -Clal-F1	GGTCAGCACCCAGCTAGCATCGATATGAAGTTTCTCGTG GCC	
VdM35-1 ^{ΔHEXXH} -R1	CGCAATCTCGGCCAGGAGGATGGTCGTCGT	Clone VdM35-1 ^{ΔHEXXH} to PGR107 for expression in <i>N. benthamiana</i>
VdM35-1 ^{ΔHEXXH} -F2	CAGACGACCATCCTCTGGCCGAGATTGCG	
VdM35-1 ^{ΔHEXXH} -Smal-R2	TCGCCCTTGCTCACCATCCCGGGGCAACGGGCGAAGA TGGA	
VdM35-1 ^{ΔE} -Clal-F1	GGTCAGCACCCAGCTAGCATCGATATGAAGTTTCTCGTG GCC	
VdM35-1 ^{ΔE} -R1	GGCCAGATGAGTCATGTGGAGGATGGTCGT	Clone VdM35-1 ^{ΔE} to PGR107 for expression in <i>N. benthamiana</i>
VdM35-1 ^{ΔE} -F2	ACGACCATCCTCCACATGACTCATCTGGCC	
VdM35-1 ^{ΔE} -Smal-R2	TCGCCCTTGCTCACCATCCCGGGGCAACGGGCGAAGA TGGA	
VdASPF2 ^{ΔHRXXH} -Clal-F1	GGTCAGCACCCAGCTAGCATCGATATGCTTTCTCTCCAG ACC	
VdASPF2 ^{ΔHRXXH} -R1	GATGCCGGGCAGCAGAAGGTCAGT	Clone VdASPF2 ^{ΔHRXXH} to PGR107 for expression in <i>N. benthamiana</i>
VdASPF2 ^{ΔHRXXH} -F2	ACTGACCTTCTGCTGCCCCGCATC	
VdASPF2 ^Δ	TCGCCCTTGCTCACCATCCCGGGCGAGCAGTGAACAA	

HRXXH_Smal-R2	AGCCATC	
VdASPF2 ^{ΔR} -ClaI-F1	GGTCAGCACCAGCTAGCATCGATATGCTTTCTCTCCAGACC	
VdASPF2 ^{ΔR} -R1	CAGGTGGAAGGCGTCAGAAGGTCAGT	Clone VdASPF2 ^{ΔR} to PGR107 for expression in <i>N. benthamiana</i>
VdASPF2 ^{ΔR} -F2	ACTGACCTTCTGCACGCCTTCCACCTG	
VdASPF2 ^{ΔR} -SmaI-R2	TCGCCCTTGCTCACCATCCCGGGCGAGCAGTGAACAAAGCCATC	
VdM35-1SP-EcoR1-F	CCGGAATTCATGAAGTTTCTCGTGGCCAT	Clone VdM35-1 signal peptide to pSUC2 for yeast signal trap assay
VdM35-1SP-Xho1-R	CCGCTCGAGAGCCCGAGCTAGCAGAGCCA	
VdASPF2SP-EcoR1-F	CCGGAATTCATGCTTTCTCTCCAGACCGC	Clone VdASPF2 signal peptide to pSUC2 for yeast signal trap assay
VdASPF2SP-Xho1-R	CCGCTCGAGAGAGGCAGCGACGAGAGGGA	
VdM35-1-Xba1-F	GAGAACACGGGGGACTCTAGAATGAAGTTTCTCGTGGCC	Clone VdASPF2 to p2300 for subcellular localization analysis
VdM35-1-BamH1-R	ACCCATGTTAATTAAGGATCCGCAACGGGCGAAGATGGA	
VdASPF2-Xba1-F	GAGAACACGGGGGACTCTAGAATGCTTTCTCTCCAGACC	Clone VdASPF2 to p2300 for subcellular localization analysis
VdASPF2-BamH1-R	ACCCATGTTAATTAAGGATCCGAGCAGTGAACAAAGCCATC	
TRV2-BAK1-F	CGGGATCCGTGAGGGTGGTGAGCGGGATAAT	
TRV2-BAK1-R	CGGAATTCGCTCATAACTGGGCAAAGGGCTT	
TRV2-SOBIR1-F	CGGGATCCAATCTTTATCCACCAGATCATGC	VIGS in <i>N. benthamiana</i>
TRV2-SOBIR1-R	CGGAATTCAGAAAGTTTCCAATGGCAG	
TRV2-GFP-F	CGGGATCCGTGAGCAAGGGCGAGGAGCTGT	
TRV2-GFP-R	CGGAATTCGTCCTTGAAGAAGATGGTGCGCT	
qRT-NbBAK1-F	GAGGTGGGAGGAATGGCAAA	qRT-PCR analysis for VIGS efficiency
qRT-NbBAK1-R	TTGGCCCCGACAATTCATCT	
qRT-NbSOBIR1-F	CCAGCAAGTCACAGAAGGGA	
qRT-NbSOBIR1-R	CCAACACCACCAAAAGCTG	
NbActin-qPCR-F	TGGTCGTACCACCGGTATTGTGTT	qRT-PCR analysis in <i>N. benthamiana</i>
NbActin-qPCR-R	TCACTTGCCCATCAGGAAGCTCAT	

<i>Nb HIN1-q PCR-F</i>	CCAACTTGAACGGAGCCTATTA	
<i>Nb HIN1-q PCR-R</i>	AGGCATCCAAAGAGACAACTAC	
<i>NbPR1α-qPCR-F</i>	CCGCCTTCCCTCAACTCAAC	
<i>NbPR1α-qPCR-R</i>	GCACAACCAAGACGTACTGAG	
<i>NbPR2-qPCR-F</i>	AGGTGTTTGCTATGGAATGC	
<i>NbPR2-qPCR-R</i>	TCTGTACCCACCATCTTGC	
<i>NbLOX-qPCR-F</i>	AAAACCTATGCCTCAAGAAC	
<i>NbLOX-qPCR-R</i>	ACTGCTGCATAGGCTTTGG	
<i>NbPti5-qPCR-F</i>	CCTCCAAGTTTGAGCTCGGATAGT	
<i>NbPti5-qPCR-R</i>	CCAAGAAATTCTCCATGCACTCTGTC	
<i>NbAcre31-qPCR-F</i>	AATTCGGCCATCGTGATCTTGCTC	
<i>NbAcre31-qPCR-R</i>	GAGAACTGGGATTGCCTGAAGGA	
<i>NbWRKY7-qPCR-F</i>	CACAAGGGTACAAACAACACAG	
<i>NbWRKY7-qPCR-R</i>	GGTTGCATTTGGTTCATGTAAG	
<i>NbWRKY8-qPCR-F</i>	AACAATGGTGCCAATAATGC	
<i>NbWRKY8-qPCR-R</i>	TGCATATCCTGAGAAACCATT	
<i>Vdβt-F</i>	AACAACAGTCCGATGGATAATT C	
<i>Vdβt-R</i>	GTACCGGGCTCGAGATCG	
<i>VdM35-1-qPCR-F</i>	ACCAGATGCCTCCTACCGACAAG	Expression pattern analysis of <i>VdM35-1</i> and <i>VdASPF2</i>
<i>VdM35-1-qPCR-R</i>	TCCATAGCCTCCGTAGTCTTCAGTG	
<i>VdASPF2-qPCR-F</i>	GCTCTCTGCGGTGGTGGTTATAC	
<i>VdASPF2-qPCR-R</i>	CGGCGTAATCCTCAGCGTAGTG	
B303- <i>VdM35-1</i> -Up-Kp n	GGACCGGACGGGGCGGTACC	
1-F		Generation of <i>VdM35-1</i> and <i>VdASPF2</i> deletion and complementation mutant in <i>V.</i> <i>dahliae</i>
B303- <i>VdM35-1</i> -Up-R	CTTCAATATCAGTTAACGTCAACAAAATAGGAAACCCGCA	
B303- <i>VdM35-1</i> -HPH-F	TGCGGGTTTCTATTTGTTGACGTAACTGATATTGAAG	
B303- <i>VdM35-1</i> -HPH-R	TGCTCTGTTGCCGCTCCATCTATTCCTTGGCCCTCGGACG	

B303- <i>VdM35-1</i> -Down-F	CGTCCGAGGGCAAAGGAATAGATGGAGCGGGCAACAGAGCA
B303- <i>VdM35-1</i> -Down-Pac1-R	TAGTCCCGGGTCTTAATTAACCGAGTCTGAGTCGGTGTTC
B303- <i>VdASPF2</i> -Up-Kp n	GGACCGGACGGGGCGGTACCGCTATTCATTGTGATGAAA
1-F	
B303- <i>VdASPF2</i> -Up-R	CTTCAATATCAGTTAACGTCTCACGATGAACCAGGTAGGA
B303- <i>VdASPF2</i> -HPH-F	TCCTACCTGGTTCATCGTGAGACGTTAACTGATATTGAAG
B303- <i>VdASPF2</i> -HPH-R	AAAGGGAGTGCCCACTCACTCTATTCCTTTGCCCTCGGACG
B303- <i>VdASPF2</i> -Down-F	CGTCCGAGGGCAAAGGAATAGAGTGAGTGGGCACTCCCTTT
B303- <i>VdASPF2</i> -Down-Pac1-R	TAGTCCCGGGTCTTAATTAACCGTGAGTGGCTGTGAATGG
1302- <i>VdM35-1</i> -Xba1-F	GACAGCCCGCAAACCTAGACTAGCTCTATTATAGAGCTA
1302- <i>VdM35-1</i> -Xho1-R	ATTATTATGGAGAACTCGAGCATAGACTTTGACGACTCTCCG
1302- <i>VdASPF2</i> -Xba1-F	GACAGCCCGCAAACCTAGACGCTATTCTTGTGATGA
1302- <i>VdASPF2</i> -Xho1-R	ATTATTATGGAGAACTCGAGTCTTCAGCGAGTCGTGA
<i>VdM35-1</i> -F	ATGAAGTTTCTCGTGGCC
<i>VdM35-1</i> -R	GCAACGGGCGAAGATGGA
<i>VdASPF2</i> -F	ATGCTTTCTCTCCAGACC
<i>VdASPF2</i> -R	CGAGCAGTGAACAAAGCCATC
<i>HPH</i> -F	ATTTGTGTACGCCCAGAGT
<i>HPH</i> -R	GTGCTTGACATTGGGGAATT
<i>HPH</i> -southern-F	ATTTGTGTACGCCCAGAGT
<i>HPH</i> -southern-R	GTGCTTGACATTGGGGAATT

For southern blotting

Note: Underline for restriction site , and italics for genes or species.

