Journal of Fungi

MDPI

# Supplementary Materials

# A Kinesin Vdkin2 Required for Vacuole Formation, Mycelium Growth, and Penetration Structure Formation of *Verticillium dahliae*

**Xing Yang [1,2], Cuimei Guo [1,2], Chi Chen [1,2], Zhijuan Hu [1,2], Xinyao Zheng [1,2], Shan Xu [1,2], Xingyong Yang [3] and Chengjian Xie [1,2,*]**

[1] The Chongqing Key Laboratory of Molecular Biology of Plant Environmental Adaptations, Chongqing Normal University, Chongqing 401331, China; 2019110513033@stu.cqnu.edu.cn (X.Y.); 2019110513007@stu.cqnu.edu.cn (C.G.); 2020110513007@stu.cqnu.edu.cn (C.C.); 2021110513003@stu.cqnu.edu.cn (Z.H.); 2021210513054@stu.cqnu.edu.cn (X.Z.); 20200017@cqnu.edu.cn (S.X.)
[2] Chongqing Engineering Research Center of Specialty Crop Resources, The College of Life Science, Chongqing Normal University, Chongqing 401331, China
[3] College of Pharmacy, Chengdu University, Chengdu 610106, China; yangxingyong@cdu.edu.cn
* Correspondence: xcj614@163.com or 20131913@stu.cqnu.edu.cn; Tel.: +86-13594334787

ASSOCIATED CONTENT

Supplementary Figures and Tables

**Figure S1.** *VdKin2* knockout and complementation in *Verticillium dahliae*.

**Figure S2**. The results of the pathogenic analysis.

**Figure S3**. Number of conidia calculated in potato dextrose broth medium.

**Figure S4**. Strains were cultured on solid microsclerotia-inducing medium to induce microsclerotial formation.

**Figure S5**. Strains were cultured on solid microsclerotia-inducing medium with or without cellophane membrane.

**Table S1.** Primers used in this study.

**Table S2.** *Verticillium dahliae* strains used in this study.

**Supporting Information Methods**

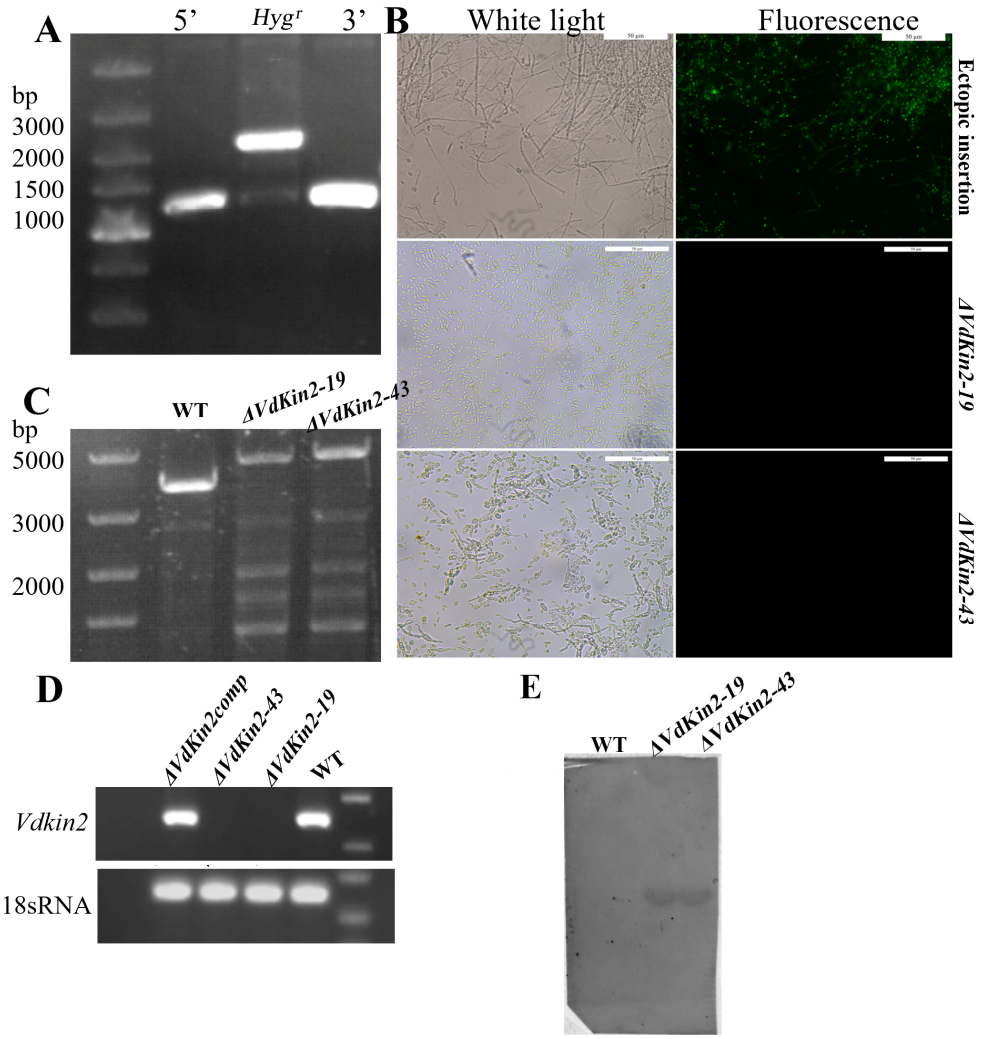



**Figure S1.** *VdKin2* knockout and complementation in *Verticillium dahliae*. (A) 5′- and 3′-flanking region of *VdKin2* and hygromycin resistance gene (*Hygr*). (B) GFP was not observed in null mutants but was present in ectopic insertion transformants or null mutants with ectopic insertions. (C) Gene-deletion event was verified using a pair of primers complementary to the region outside of the two homologous arms of the *VdAA9*-deletion cassettes. (D) RT-PCR revealed the elimination of *Vdkin2* in gene-deletion mutants. 18S rRNA served as an internal control. (E) Southern blotting indicates a single-copy insertion of the *Hygr* gene in *VdKin2* mutants.


**Figure S2.** The results of the pathogenic analysis. Four-week-old cotton seedlings were inoculated with wild-type (WT), *ΔVdKin2-19*, *ΔVdKin2-43*, or *ΔVdKin2comp* or sterile water (mock) at $10^7$ spores/mL and cultured in a greenhouse. Symptoms of *Verticillium* wilt were photographed at 40 days after inoculation.

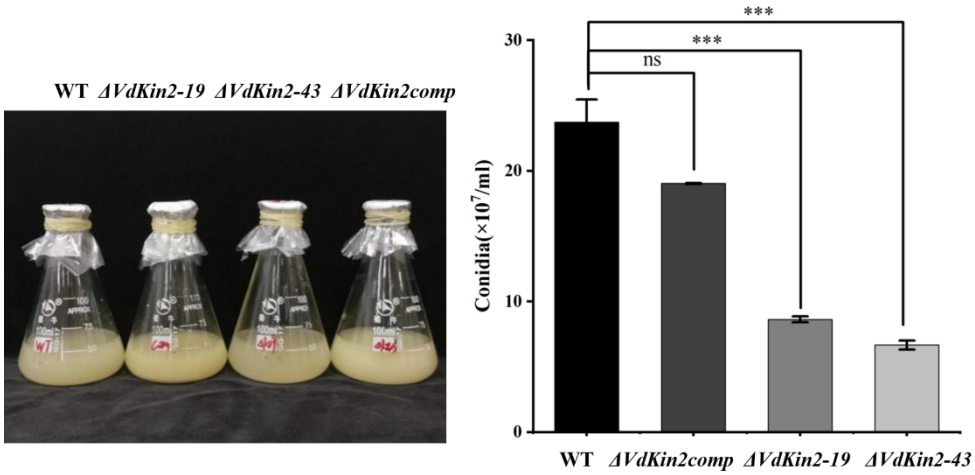



**Figure S3**. Number of conidia calculated in potato dextrose broth medium. Values are the means plus standard deviations (error bars) from three replicates. ***significant differences at $P < 0.001$, based on Student's *t*-test.

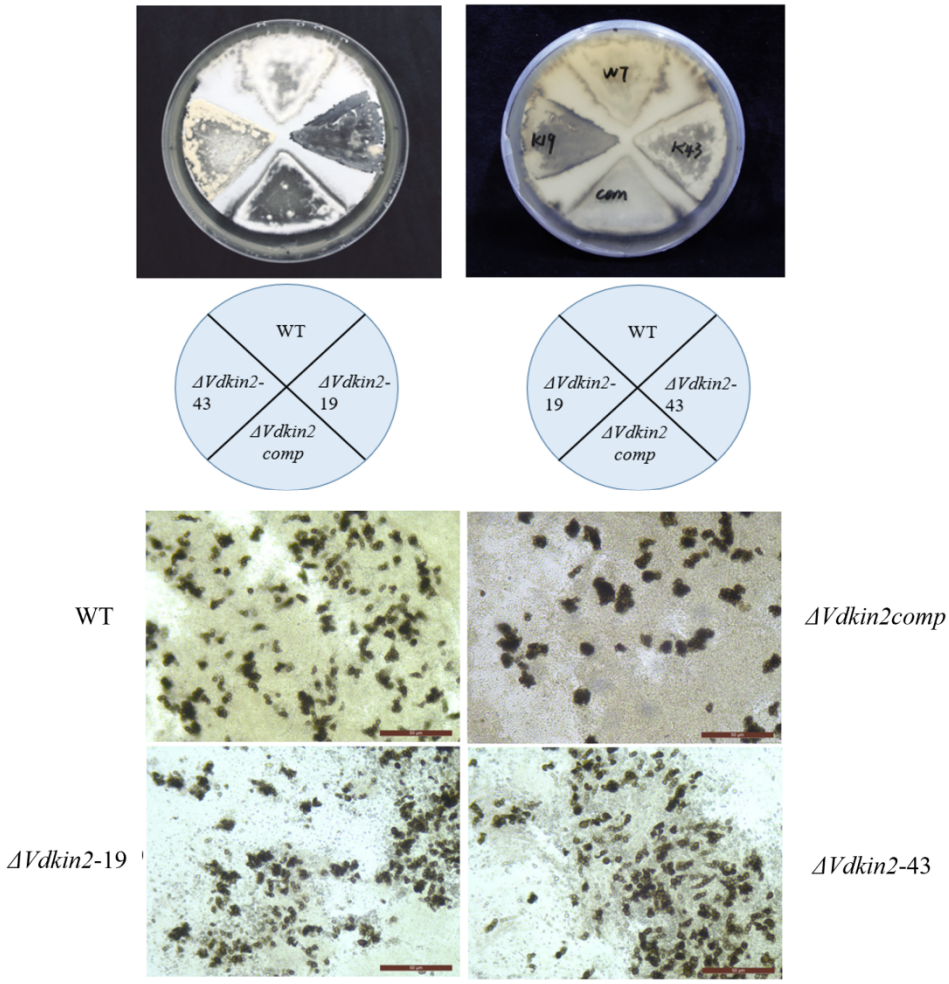



**Figure S4**. Strains were cultured on solid microsclerotia-inducing medium to induce microsclerotial formation. Mycelia and microsclerotia were scraped off the membrane for examination and counting under a microscope. Photos of the same Petri dishes were taken at 15 days post inoculation.

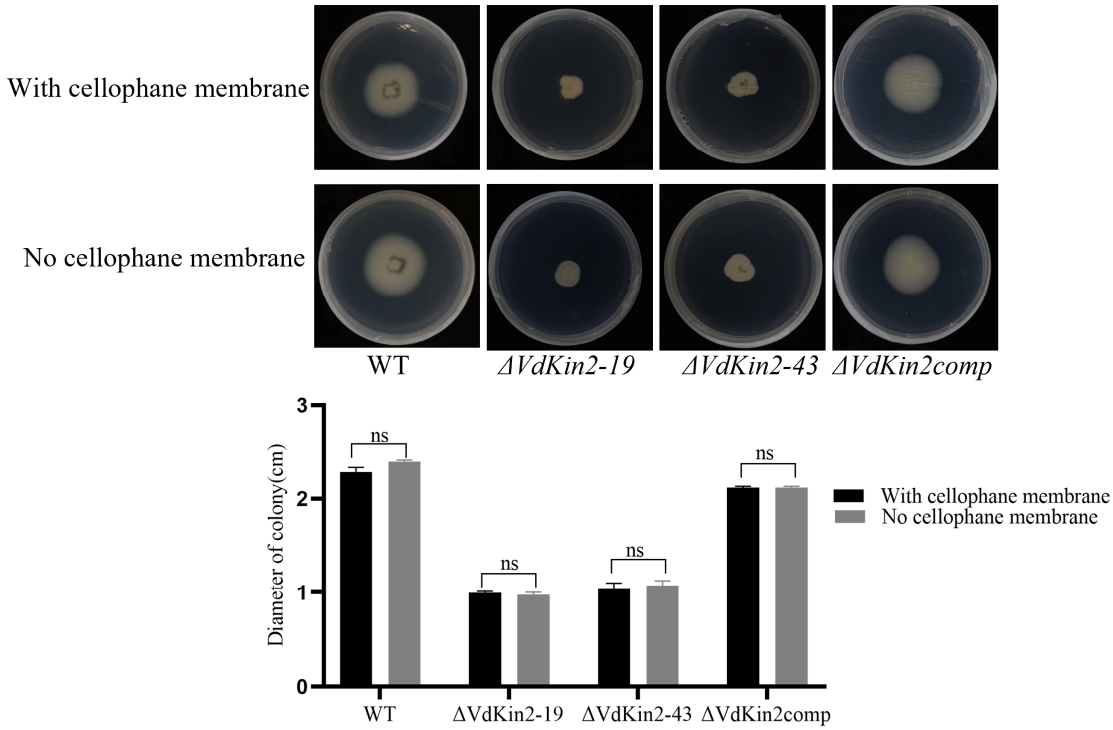



**Figure S5**. Strains were cultured on solid microsclerotia-inducing medium with or without cellophane membrane. The colonies were examined, and the diameter of each colony was measured at the 6th day.

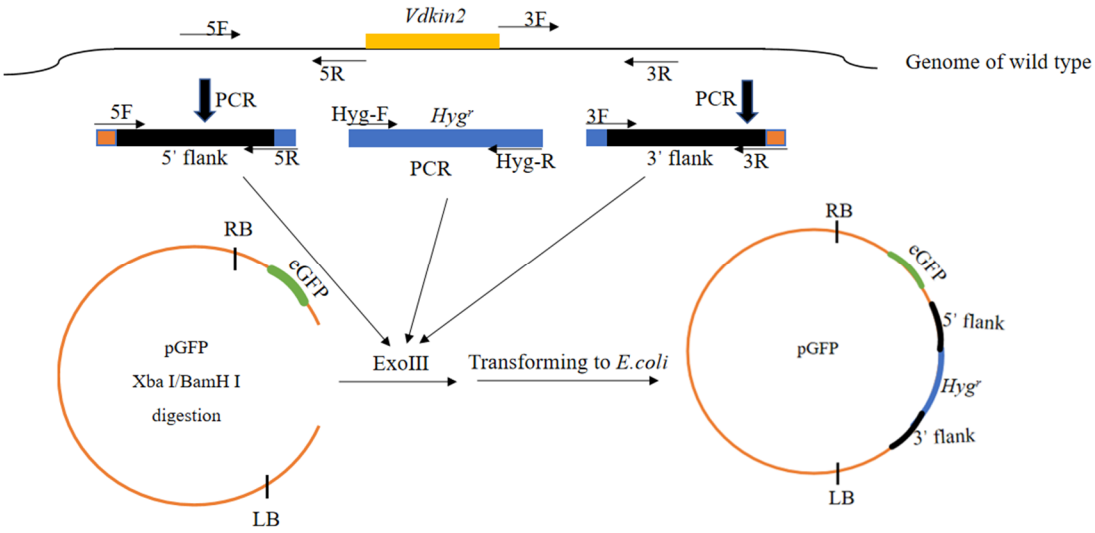
**Table S1.** Primers used in this study.

| Primer name | 5′-3′ | Use |
|---|---|---|
| 5F | AGAAGAGTAATCTAGCAAACGTCGAAGCCTCGAAC | Amplifying 5' homologous arms |
| 5R | TTAGTGAGGGTTAATTGCGCGCGCTAGACATGATGACGAC | Amplifying 5' homologous arms |
| 3F | AGTTGTTCCCAGTGATCTTCGTGGAAGGCGAGATTCAGATG | Amplifying 3' homologous arms |
| 3R | ATTACGAATTGGATCACGCGCTCGCAGCGGTTCTG | Amplifying 3' homologous arms |
| Hyg-F | GCGCAATTAACCCTCACTAAA | Amplifying Hygr cassette |
| Hyg-R | CGAAGATCACTGGGAACAACT | Amplifying Hygr cassette |
| 5OUT | GACATACGACGTGGCAGCTG | Identify mutant |
| 3OUT | CTGCTGAGCCCGGTTGTTCG | Identify mutant |
| Vdkin2comp f | CGGCCAGTGCCAAGCTTCAGTACCCGATAACGTCATG | Amplifying Vdkin2 complement |
| Vdkin2comp r | CAGTTAACGTCGAATTCCTCGCTATTGCCCGATGCTG | Amplifying Vdkin2 complement |
| 18sRNAf | CCGCCGGTCCATCAGTCTCTCTGTTTATA | Identify mutant |
| 18sRNAr | CGCCTGCGGGACTCCGATGCGAGCTGTAAC | Identify mutant |
| Probe F | CCGCGCTCCCGATTCCGGAAGTG | Southern blot probe |
| Probe R | GCTTCTGCGGGCGATTTGTGTAC | Southern blot probe |
| Vdkin2 exf | CGCCAACAGCATTAAGGTTG | Identify mutant |
| Vdkin2 exr | CTCGTCGTCGATGCTGGAAC | Identify mutant |
| G418Sep5F | CAATCTTCAAATCTAGAATGTCGTCTTCAGCTACTTT | Expressing *Sep5* |
| G418Sep5R | CCACCGCCTCCACTAGTTTTGCTGTCATTCTCGCCGT | Expressing *Sep5* |
| GFPF | TCTAGAGGATCCTTAATTAAATGGTGAGCAAGGGCGAGG | Colonization |
| GFPR | AGTTAACGTCGAATTCCTACTTGTACAGCTCGTCCATGCC | Colonization |

Table S2. *Verticillium dahliae* strains used in this study.

| Strain | Genotype description | Reference |
|---|---|---|
| V991 | Wild type | Xie et al., 2017 |
| *ΔVdkin2* | *Vdkin2* deletion mutant | This study |
| *Vdkin2comp* | *Vdkin2* complementary strain | This study |
| WT/GFP | Transformant of V991 expressing *GFP* | This study |
| *ΔVdkin2*/GFP | Transformant of ΔVdkin2 expressing *GFP* | This study |
| WT/VdSep5-GFP | Transformant of V991 expressing VdSep5-GFP | This study |
| *ΔVdKin2*/VdSep5-GFP | Transformant of ΔVdkin2 expressing VdSep5-GFP | This study |

**Supporting Information Methods**




**Construction of gene knock-out vectors.**

1, The 5′ (5F/5R) and 3′ (3F/3R) flanking sequence and *Hyg*$^r$ cassette (Hyg-f/Hyg-r) were amplified using high-fidelity enzymes.

2, The pGFP vector was digested using *Xba* I and *Bam*H I.

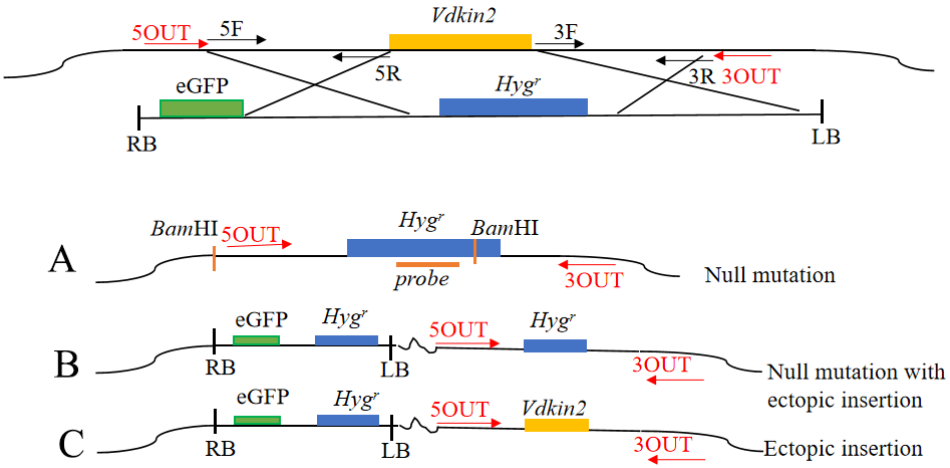
3, The digested pGFP vector, the 5′ (5F/5R) and 3′ (3F/3R) flanking sequence, *Hyg*$^r$ cassette were mixed with ExoIII (TaKaRa Dalian Biotechnology) or Gibson Assembly Cloning Kit (NEB). *5F and 3R contain overhangs homologous to the pGFP vector, and 5R and 3F have overhangs homologous to the *Hyg*$^r$ cassette , which allow for annealing.

4, The ligation mixture was transformed into *Escherichia coli* to generate gene knock-out vector.

5, The gene knock-out vector were introduced into *Agrobacterium tumefaciens* by electroporation.

6, ATMT of *V. dahliae* was performed as described previously (Xie et al. 2017 doi:10.1094/mpmi-01-17-0007-r).

7, The transformants were selected on PDA supplemented with cefotaxime at 500 μg/ml and hygromycin B at 50 μg/ml.




**Identification of null mutant transformants.**

1, Transformants are screened for GFP fluorescence under a microscope. The strains without GFP fluorescence were retained for further analyses. *Homologous recombination creates three types of transformants: **A:** null mutants, **B:** null mutants with ectopic insertions and **C:** ectopic insertion transformants. **B** and **C** have GFP fluorescence because the GFP gene was included in the T-DNA fragment (RB and LB). Null mutants (**A**) do have not GFP fluorescence because the GFP gene was eliminated in the null mutants.

2, Primers 5OUT and 3OUT are used for amplification gDNA from transformants. This length of null mutants should be longer than of WT or ectopic insertion transformants.

3, Reverse-transcription (RT)-PCR was performed to confirm the absence of *Vdkin2* mRNA in null mutants.

4, The gDNA were extracted with CTAB method (Kuhad, Kapoor et al. 2004 doi: 10.1007/BF02931383) and digested with *Bam*H I.

4, A *Hyg*$^r$ probe is used for southern blotting according to the protocol of the DIG High Prime DNA Labelling and Detection Starter Kit I (Roche, Mannheim, Germany)