



S6 Fig. Targeted deletions of *VdSep3* and *VdSep5* genes in *V. dahliae* strain V592.

(A) Physical maps of the *VdSep3* locus and of the homologous recombination constructs obtained by fusion of the *VdSep3* 5'flack, hygromycin B resistance gene cassette and *VdSep3* 3'flack. Probes and relative positions of the primers used for PCR are indicated. *hph*, hygromycin resistance gene. (B) Southern blot analysis of targeted gene deletion mutants. BamHI digested genomic DNA from the V592 strain and two putative *VdΔSep3* transformants were blotted with the probe indicated in the schematic diagram. KpnI and HindIII digested genomic DNA from V592 wild type strain and two putative *VdΔSep5* transformants were analyzed as described above. (C)

PCR amplification of genomic DNA from the complemented transformants using the primer pair in-F and in-R produced a banding pattern consistent with the integration of an intact *VdSep3* and *VdSep5*. Lanes 1-4 were used for the verification of *VdΔSep3* complementation and lanes 5-8 were for the verification of *VdΔSep5* complementation. (D) Colony morphology of V592, *VdΔsep3* and *VdΔSep5* mutants and the complementary strains on PDA plates incubated for 2 weeks. (E) Penetration ability analysis of *VdΔSep3* and *VdΔSep5*. Colonies of V592, *VdΔSep3* and *VdΔSep5* on M0 medium overlaid with cellophane (Before) and removal of the cellophane membrane (After). Images in the first row were obtained at 6 dpi, and the colonies below the cellophane were obtained at 9 dpi. (F) Hyphopodium morphology analysis of V592, *VdΔSep3* and *VdΔSep5*. Photographs were taken at 6 dpi. (G) Deficient development of the hyphopodium in *VdΔSep3* and *VdΔSep5*. Fungi incubated on cellophane at 5 dpi were used for the observation. The numbers of hyphopodia were counted in three fields of the culture under a light microscope at x1000 magnification with three replicates. The mean and SD for (G) were calculated from three clones for each mutant (* $P < 0.05$; t-test). (H, I) Disease symptoms (H) and disease grades (I) of cotton plants infected with wild-type V592, *VdΔSep3* and *VdΔSep5* mutants and the complementary strains at 21 dpi. Three replicates of 36 plants were used for each inoculum. The asterisks indicate significant differences compared with V592 infection (* $P < 0.05$; t-test).