

A transcription factor FgAtrR regulates the asexual and sexual development, virulence, DON production and contributes to the intrinsic resistance to azole fungicides in *Fusarium graminearum*

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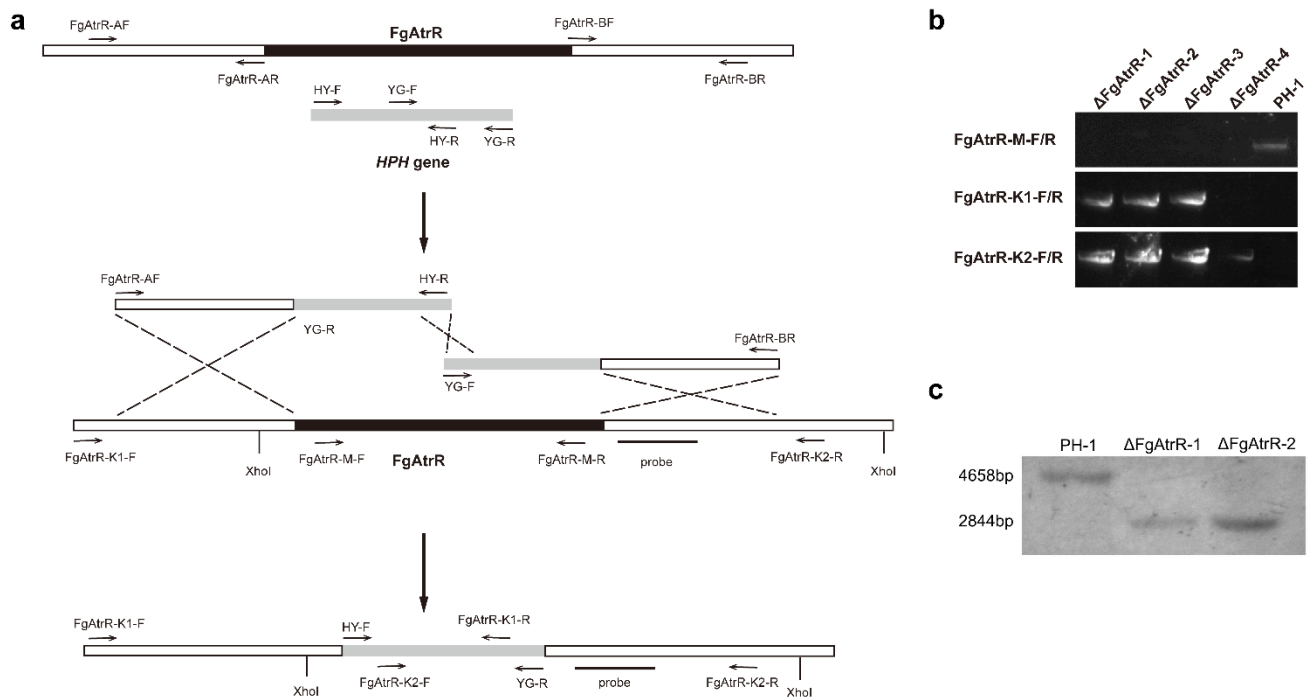


Figure S1 Generation and identification of *FgAtrR* deletion mutants. (a) Schematic representation of the gene deletion strategy. The *FgAtrR* gene was replaced by the hygromycin gene (*HPH*) through homologous recombination. Primer binding sites are labeled by arrows. (b) PCR analysis of transformants showed $\Delta FgAtrR$ -1, -2, and -3 might be the correct *FgAtrR* deletion mutants. (c) Southern blot analysis of *FgAtrR* deletion mutants and the wild-type strain PH-1 confirmed the $\Delta FgAtrR$ -1 and $\Delta FgAtrR$ -2 were corrected. A 524 bp downstream fragment was used as a probe. The genomic DNA sample of each strain was digested with *Xho*I.

1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
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FOXG_02014 .MDHM..G.GQ..V.PGMA..PPIMNHPP.QVFGSY..DGIPQ..LHPRIA.AQMFNDGAMM..LEDANDPKRRRIAR....ACDMCRKKKIKCDGKMPSCHICINMYKECVFTQVEKKRPFPGAKYIEGLENNRLRMHLLLSGLLDDDD...DLGALKRRIMRQHKES
FPRO_04832 .MDHM..V.PGMA..PPIMNHPP.QVFGSY..DGIPQ..LHPRIA.AQMFNDGAMM..LEDANDPKRRRIAR....ACDMCRKKKIKCDGKMPSCHICINMYKECVFTQVEKKRPFPGAKYIEGLENNRLRMHLLLSGLLDDDD...DLGALKRRIMRQHKES
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NCU01478 .MDHLLHGQ..MP.NA..P.LLP..P.OIFGGYEHGIPQQLPHDLAVAQMGHGL..LDDDEAKRRRIAR....ACDMCRKKKIKCDGKLPACHICINMYKDDCVFTQVEKKRPFPGAKYIEGLENNRLRMHLLLSGLLDDDDNGATDLGLTLEKRAKTAQS
MGG_07450 .MDRM..G.GQ..MPFMG..APPIMNHPP.QVIFGGY..DGMFMQ..LFFPMT..AHMPADHSTL..LDDANEAKRRRIAR....ACDMCRKKKIKCDGKLPACHICINMYKDDCVFTQVEKKRPFPGAKYIEGLENNRLRMHLLLSGLLDDDDGGTDLGLTLEKRAKRNRS
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500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660
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NCU01478 .LYPVSALVTLFNIQNDPDRASDTRLNMNVVFLSMGQABEAGGVHMDIGICAEFERAKAVLDIAEKEQSSRRKRKNQD.....TNKSSVNAITATARQSTAESATTSVSSSQRRS...SQAQLSPNGASAM.....GOFSM..GSPMNDPSPSAMSAG
MGG_07450 .LYPVSALVTLFNIQNDPDRASDTRLNMNVVFLSMGQABEAGGVHMDIGICAEFERAKAVLDIAEKEQSSRRKRKNQD.....TNKSSVNAITATARQSTAESATTSVSSSQRRS...SQAQLSPNGASAM.....GOFSM..GSPMNDPSPSAMSAG
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VDAG_08521 .LYPVSALVTLFNIQNDPDRASDTRLNMNVVFLSMGQABEAGGVHMDIGICAEFERAKAVLDIAEKEQSSRRKRKNQD.....TNKSSVNAITATARQSTAESATTSVSSSQRRS...SQAQLSPNGASAM.....GOFSM..GSPMNDPSPSAMSAG
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PDIP_71050 .LYPVSALVTLFNIQNDPDRASDTRLNMNVVFLSMGQABEAGGVHMDIGICAEFERAKAVLDIAEKEQSSRRKRKNQD.....TNKSSVNAITATARQSTAESATTSVSSSQRRS...SQAQLSPNGASAM.....GOFSM..GSPMNDPSPSAMSAG
AfU2g02690 .LYPVSALVTLFNIQNDPDRASDTRLNMNVVFLSMGQABEAGGVHMDIGICAEFERAKAVLDIAEKEQSSRRKRKNQD.....TNKSSVNAITATARQSTAESATTSVSSSQRRS...SQAQLSPNGASAM.....GOFSM..GSPMNDPSPSAMSAG
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Figure S2 Multiple sequence alignment of FgAtrR with homologs in other filamentous fungi. Sequences for analysis are as follows: FgAtrR from *Fusarium graminearum*, FOXG_02014 from *F. oxysporum* f. sp. *lycopersici*, FFUJ_03061 from *F. fujikuroi*, FPRO_04832 from *F. proliferatum*, NCU01478 from *Neurospora crassa*, MGG_07450 from *Pyricularia oryzae*, BCIN_03g02160 from *Botrytis cinerea*, PDIP_71050 from *Penicillium digitatum*, Afu2g02690 from *Aspergillus fumigatus*, AO090026000614 from *A. oryzae*, VDAG_08521 from *Verticillium dahliae*, GLRG_03129 from *Colletotrichum graminicola*.

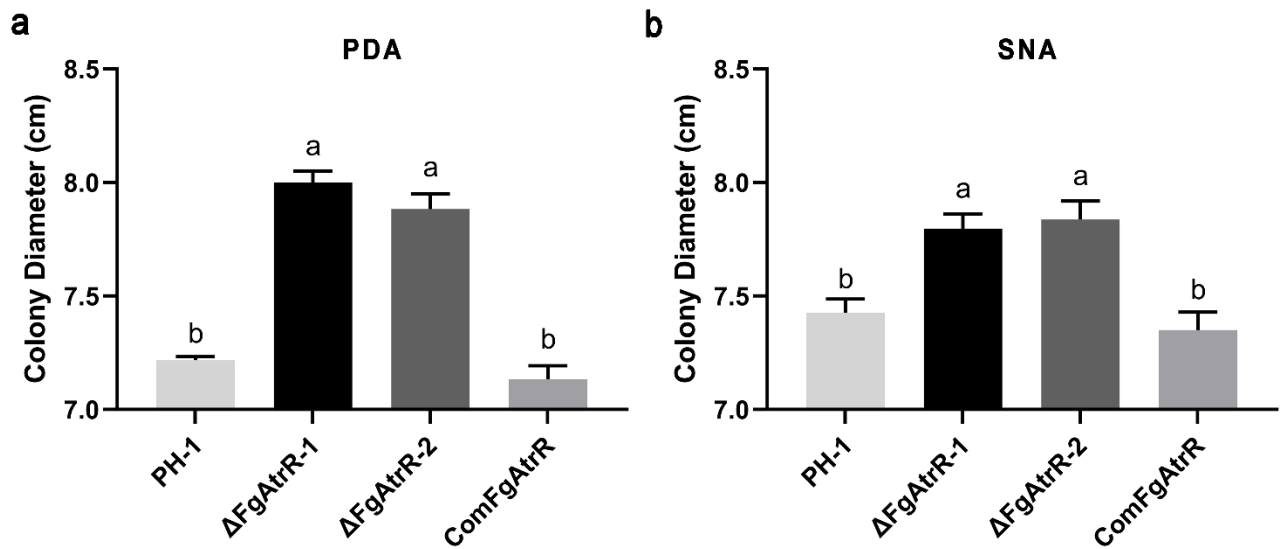


Figure S3 Deletion of FgAtrR promotes the radial growth of *Fusarium graminearum*. The colony diameter of each strain was measured after incubation for 96 h on PDA plates or 144 h on SNA plates. The experiment was repeated three times with three replicates each time. Linear bars denote standard errors of three experiments. The different letters on the bars indicate a significant difference at the $p < 0.05$ level.

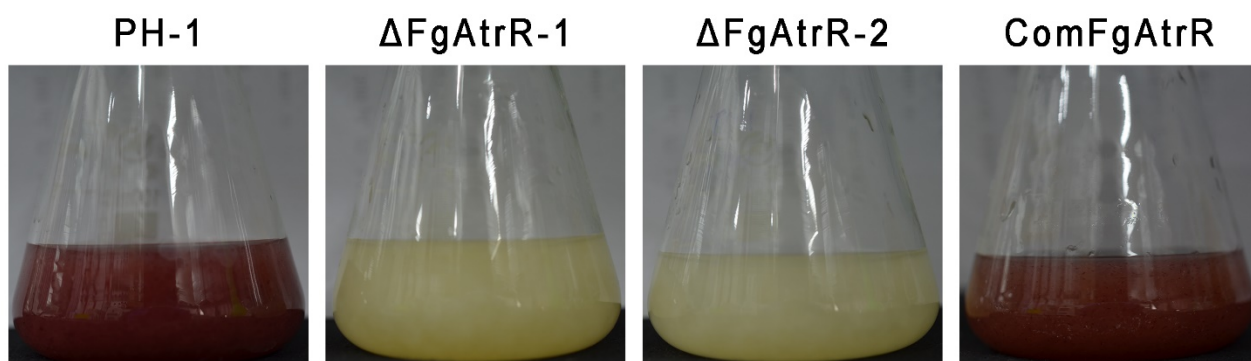


Figure S4 Deletion of FgAtrR blocks the aurofusarin biosynthesis of *Fusarium graminearum* in PDB medium. Each strain was cultured in flasks containing PDB medium at 25°C for 5 d.

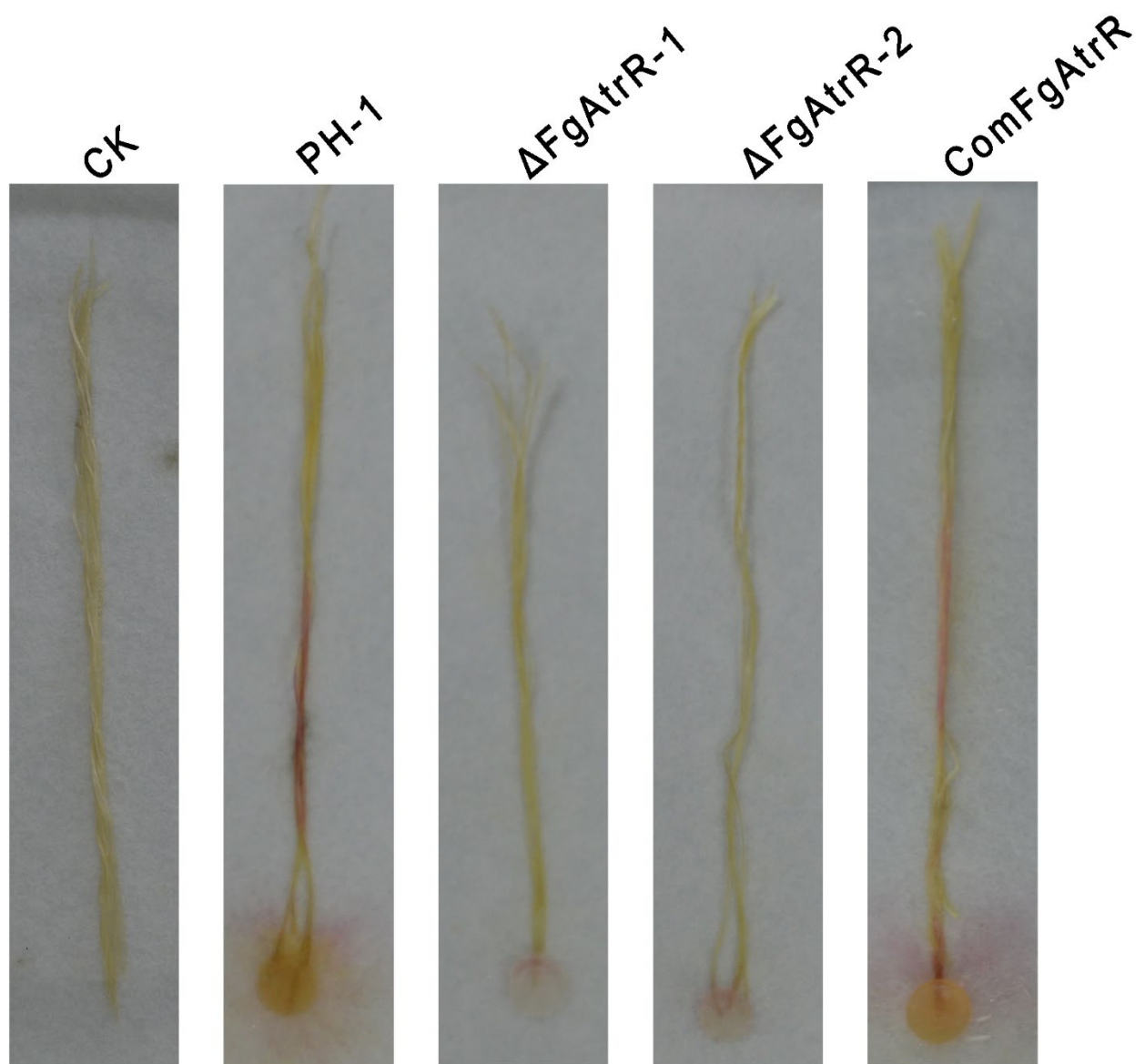


Figure S5 Deletion of FgAtrR impairs the pathogenicity of *Fusarium graminearum* on corn silks.

A mycelial plug was placed at one end of a bundle of silk corn. The photographs were taken after 7 days of incubation in a humid chamber at 25 °C.

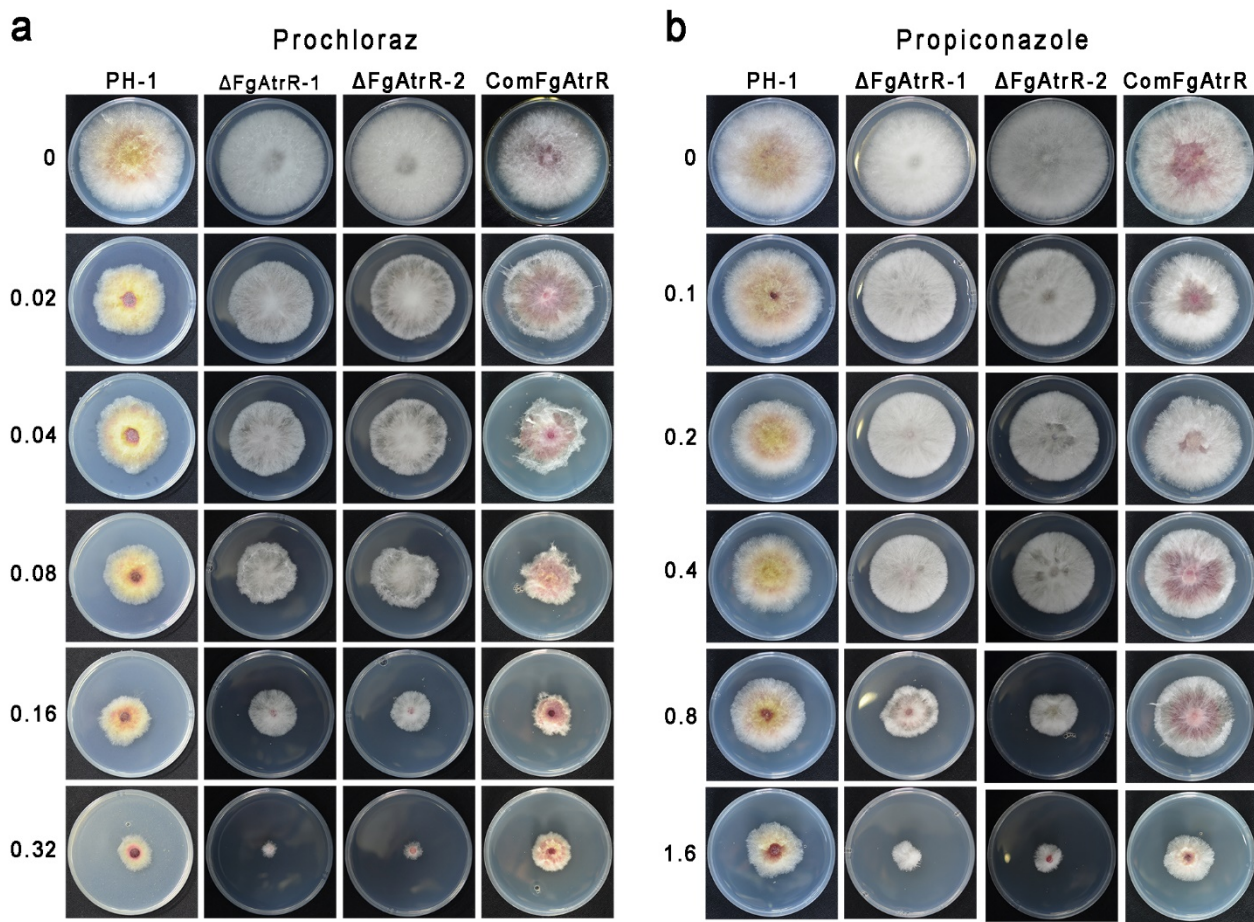


Figure S6 The growth inhibition effect of azole fungicide prochloraz (a) or propiconazole (b) on the *Fusarium graminearum* PH-1, FgAtrR deletion mutants, and FgAtrR-complemented strain at different concentrations. The concentration (mg/L) was labeled on the left side of each pane.