nature microbiology

SUPPLEMENTARY INFORMATION

ARTICLE NUMBER: 16167 | DOI: 10.1038/NMICROBIOL.2016.167

Root-hair endophyte stacking in finger millet creates a physicochemical barrier to trap the fungal pathogen *Fusarium graminearum*

Walaa K. Mousa[1,2], Charles Shearer[1], Victor Limay-Rios[3], Cassie L. Ettinger[4], Jonathan A. Eisen[4], and Manish N. Raizada[1*]

[1]Department of Plant Agriculture, University of Guelph, Guelph, ON Canada N1G 2W1

[2]Department of Pharmacognosy, Mansoura University, Mansoura, Egypt

[3]Department of Plant Agriculture, University of Guelph, Ridgetown Campus, Ridgetown, ON, Canada, N0P 2C0

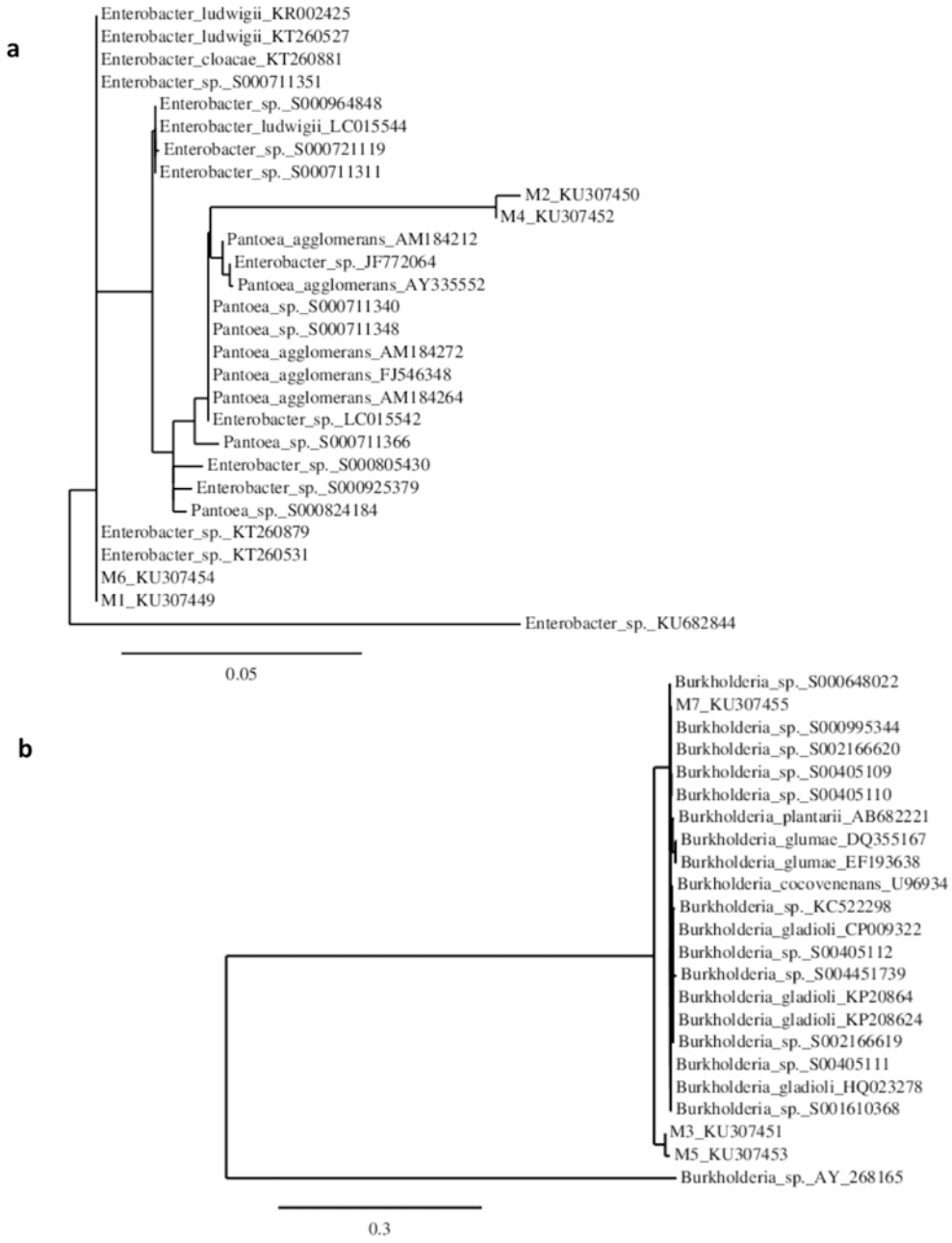
[4]University of California Davis Genome Center, Davis, California, USA 95616

*Author for correspondence and request for materials:

Manish N. Raizada

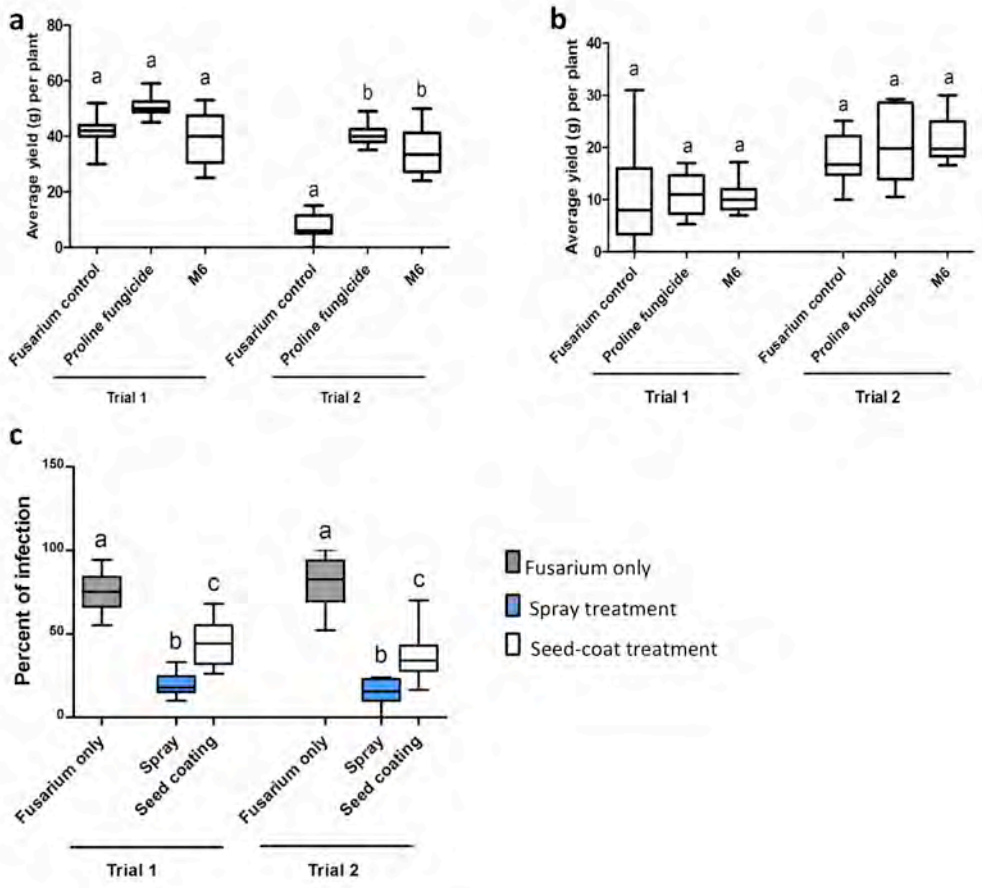
Email: raizada@uoguelph.ca; Phone 1-519-824-4120 x53396; FAX 1-519-763-8933

(1) Supplementary Figures

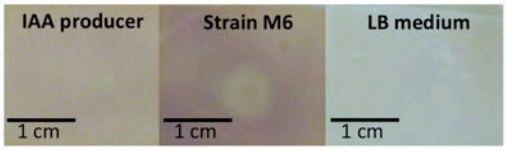


Supplementary Figure 1 | 16S rDNA based phylogenetic tree of finger millet endophytes based on Phylogeny.fr software. **a**, tree with endophytes M1, M2, M4, M6. **b**, tree with endophytes M3, M5, M7.

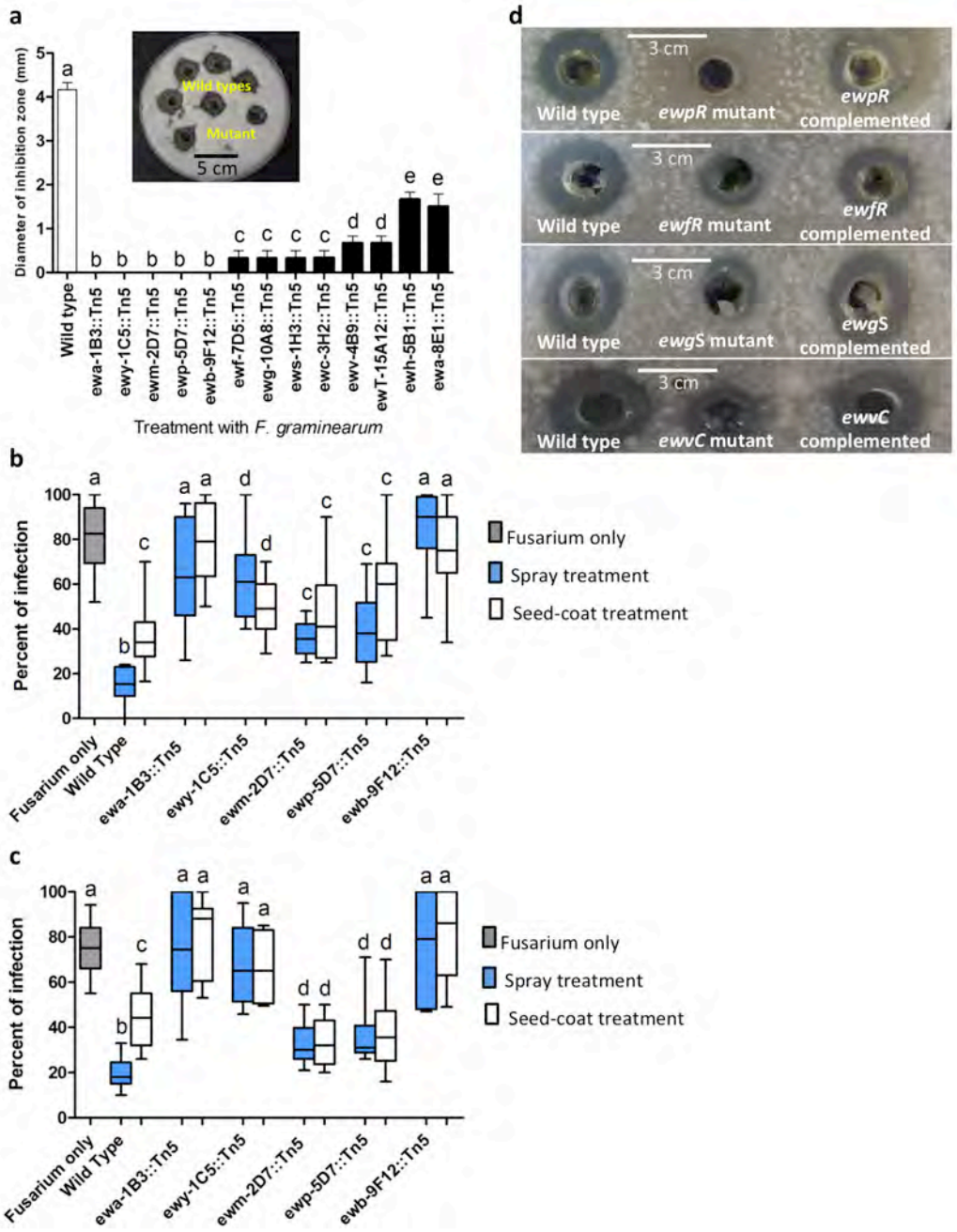
Supplementary Figure 2 | Image of finger millet seedlings previously seed-coated with GFP-tagged M6 showing no pathogenic symptoms, consistent with the strain behaving as an endophyte.



Supplementary Figure 3 | Suppression of *F. graminearum* (Fg) by M6 and its effect on grain yield in greenhouse trials. a-b, Effect of endophyte M6 on grain yield per plant in two greenhouse trials for (**a**) maize and (**b**) wheat. **c**, Effect of treatment with endophyte M6 on Fg disease symptoms in maize ears when the endophyte was applied as a seed coat or foliar spray compared to a *Fusarium* only control treatment. For greenhouse disease trials, n=20 biological replicates for M6 and 10 biological replicates for controls. Letters that are different from one another within a trial indicate that their means are statistically different ($P \leq 0.05$). The whiskers indicate the range of data.

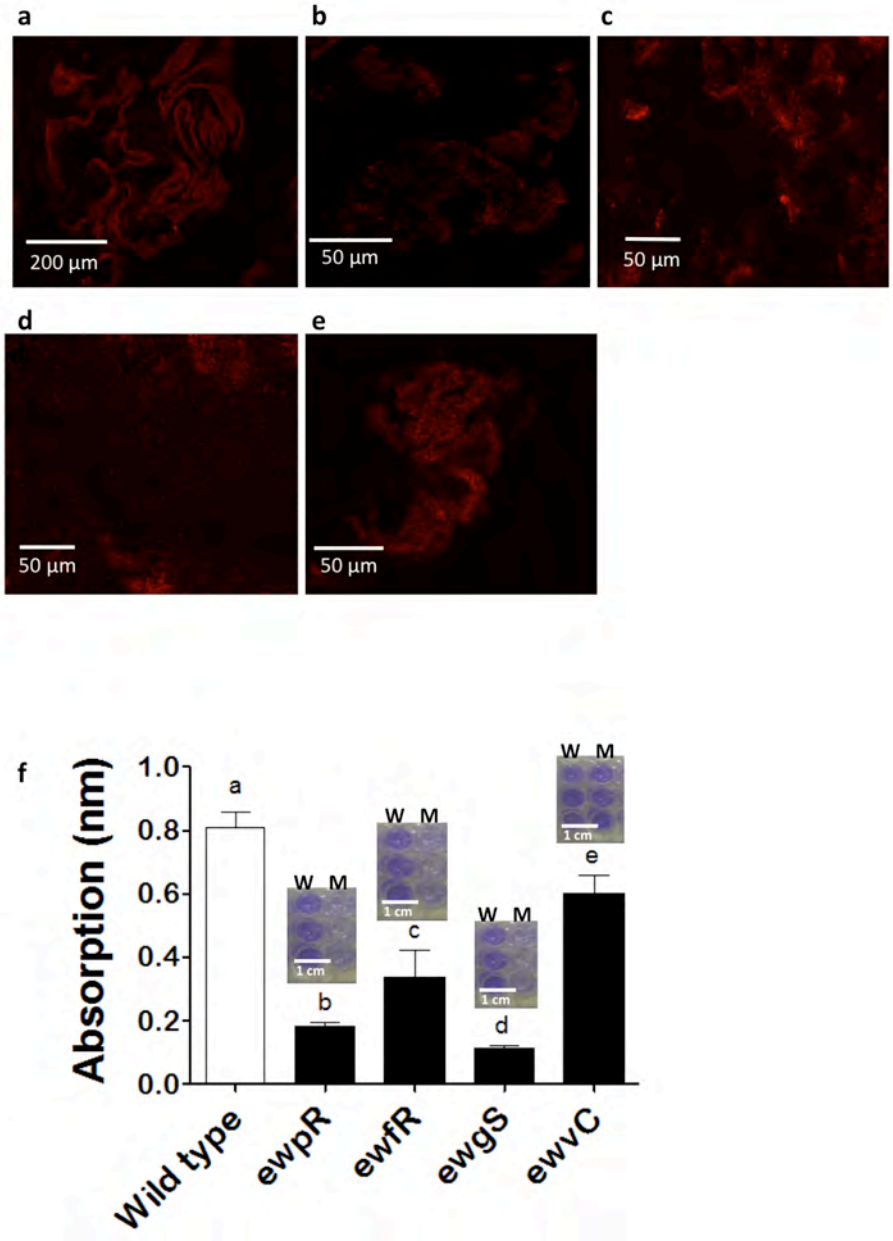


Supplementary Figure 4 | Assay for production of indole-3-acetic acid (IAA, auxin) by wild type strain M6. Production of indole-3-acetic acid (IAA) *in vitro* by wild type strain M6 compared to a positive control (bacterial endophyte strain 3E10) using the Salkowski reagent colorimetric assay.

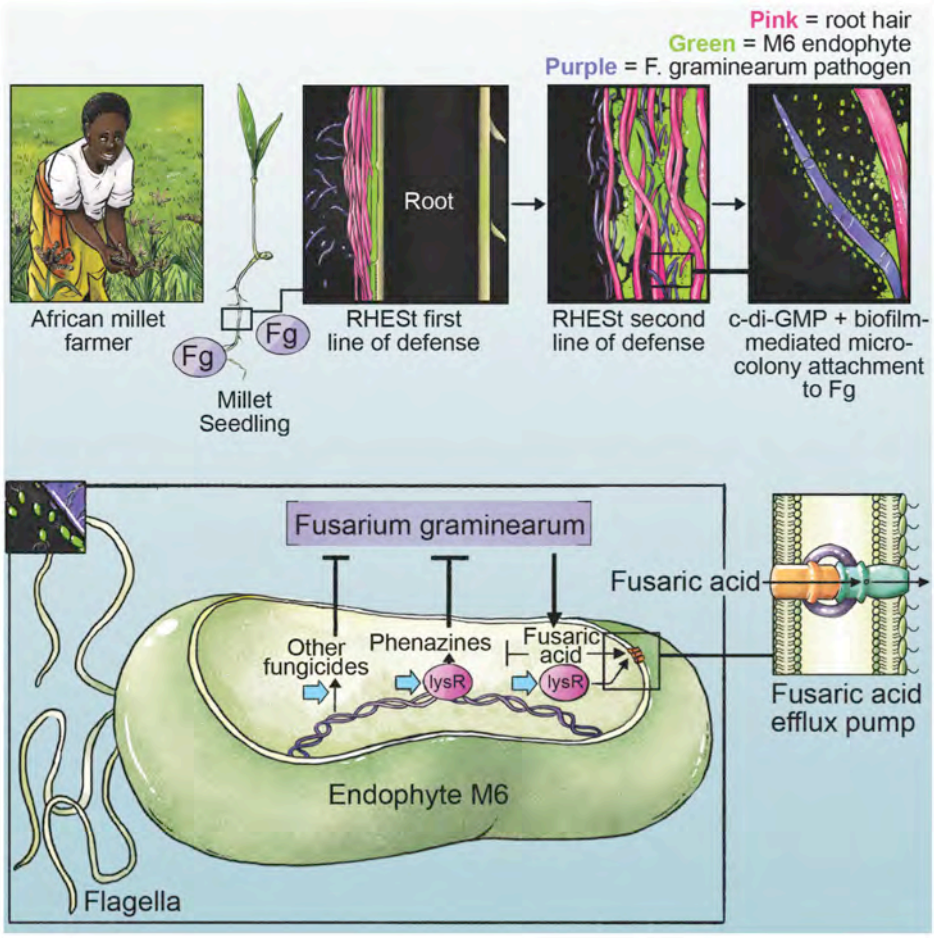


Supplementary Figure 5 | Tn5 mutagenesis-mediated discovery, validation and complementation of genes required for the anti-*Fusarium* activity of strain M6. **a**, Loss of anti-*F. graminearum* activity associated with each Tn5 insertion mutant using the *in vitro* dual culture diffusion assay, along with a representative image (inset) of the mutant screen. **b-c**, *In planta* validation of loss of anti-fungal activity of M6 mutant strains based on quantification of *F. graminearum* disease symptoms on maize ears, in (**b**) greenhouse trial 1, and (**c**) greenhouse trial 2. Only mutant strains that completely lost anti-fungal activity *in vitro* were selected for *in planta* validation. The whiskers indicate the range of data points. Letters that are different from one another indicate that their means are statistically different ($P \leq 0.05$). **d**, Genetic complementation of Tn5 mutants

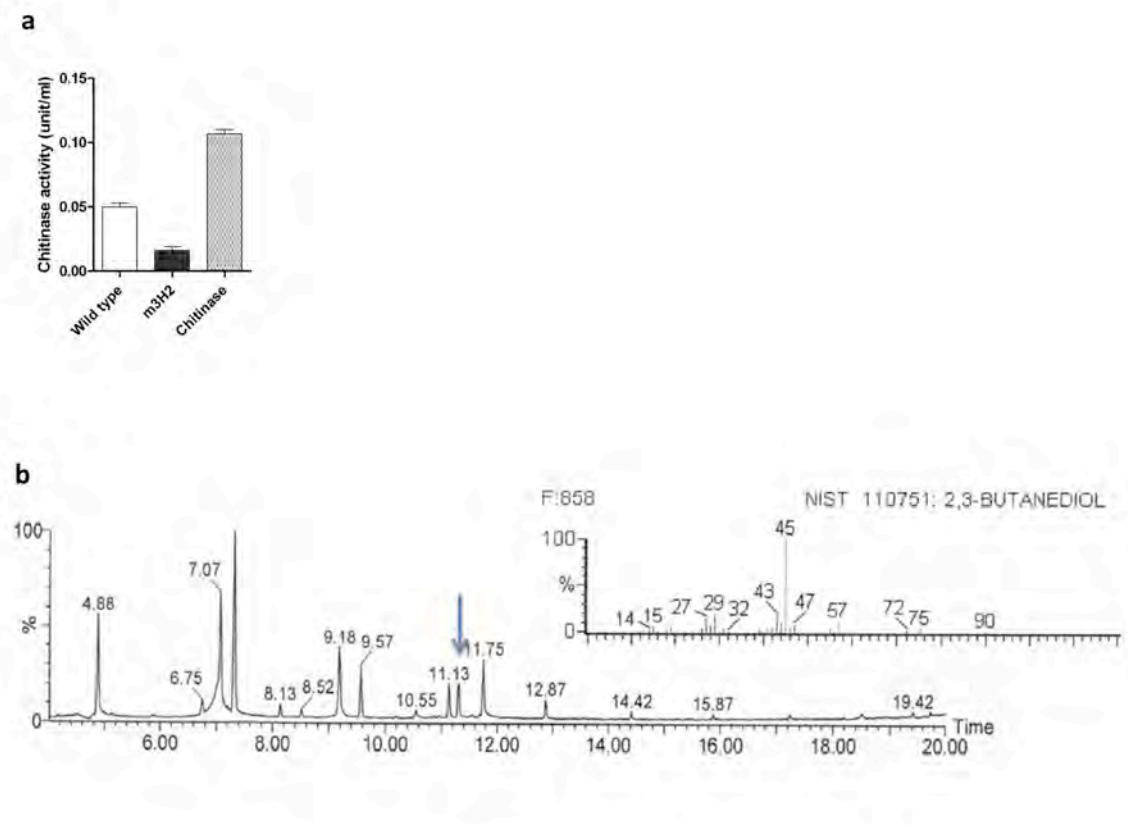
with the predicted, corresponding wild type coding sequences. Shown are representative images.



Supplementary Figure 6 | Supporting data on biofilm formation. **a-e**, Confocal microscopy images showing the proteinaceous biofilm matrix stained with Ruby film tracer (red) associated with: **a**, the wild type M6 strain, and **b-e**, mutant strains *in vitro*. Shown are mutant strains, **(b)** *ewpR*-5D7::Tn5C (LysR-phenazine biosynthesis), **(c)** *ewfR*-7D5::Tn5 (LysR-fusaric acid resistance), **(d)** *ewvC*-4B9::Tn5 (c-di-GMP biosynthesis), and **(e)** *ewvC*-4B9::Tn5 (colicin V production). **f**, Quantification of the average light absorption (n=3) of crystal violet indicator in wild type M6 and each mutant, along with corresponding images from the colorimetric assay (W denotes wild type and M denotes mutant). Letters that are different from one another indicate that their means are statistically different ($P \leq 0.05$).



Supplementary Figure 7 | Model to illustrate the interaction between strain M6, the host plant and *F. graminearum* pathogen. Following pathogen sensing, M6 swarm towards invading *Fusarium* hyphae. It then creates two lines of defence. First M6 forms a dense layer of microcolonies on the root surface (rhizoplane). Second, M6 is associated with induced root hair growth, then forms microcolony stacks between the elongated and bent root hairs resulting in root hair-endophyte stack (RHESt) formation, likely mediated by biofilm. The RHEST-associated barriers prevent entry and/or trap *F. graminearum* for subsequent killing. M6 killing requires diverse antimicrobial compounds including phenazines. *Fusarium* will produce fusaric acid toxin which interferes with phenazine biosynthesis. However, M6 has a specialized fusaric acid-resistance pump system which is predicted to pump the mycotoxin outside the endophyte cell.



Supplementary Figure 8 | Assays for production of chitinase and butanediol by strain M6. **a**, Quantification of chitinase production by an M6 mutant strain carrying a Tn5 insertion in a chitinase encoding gene (*ewc-3H2*::Tn5, Supplementary Table 5) compared to wild type M6. **b**, Entire GC chromatogram showing an arrow pointing to a peak with RT 11.13 with a molecular weight and fragmentation pattern (inset) that matches 2, 3 butanediol when searched against the NIST 2008 database.

(2) Supplementary Tables

Supplementary Table 1. Taxonomic classification of finger millet bacterial endophytes based on 16S rDNA sequences and BLAST analysis

ID (Genbank Accession)	Tissue source	BLAST analysis	% of max. identity	Length of aligned sequence
M1(KU307449)	Roots	*Enterobacter* sp. (CP015227)	99	646
M2 (KU307450)	Seeds	*Pantoea* sp. (FN796868)	99	701
M3(KU307451)	Seeds	*Burkholderia* sp. (KC522298)	99	587
M4 (KU307452)	Shoots	*Pantoea* sp. (KT075171)	95	751
M5 (KU307453)	Shoots	*Burkholderia* sp. (KP455296)	99	586
M6 KU307454	Roots	*Enterobacter* sp. (KU935452)	99	586
M7 (KU307455)	Shoots	*Burkholderia* sp. (HQ023278)	100	644

Supplementary Table 2. Effect of endophyte strain M6 isolated from finger millet on the growth of diverse fungal pathogens *in vitro.*

Target fungal species	Diameter of growth inhibition zone in mm		
	Nystatin (10.0 U/ml)	**Amphotericin (250 µg/ml)**	**M6**
Alternaria alternata	0.0±0.0	0.0±0.0	0.0±0.0
Alternaria arborescens	0.0±0.0	0.0±0.0	0.0±0.0
Aspergillus flavus	2.0±0.2	0.0±0.0	3.6±0.2
Aspergillus niger	0.0±0.0	2.0±0.0	0.0±0.0
Bionectria ochroleuca	2.0±0.2	0.5±0.2	0.0±0.0
Davidiella tassiana	1.5±0.2	0.5±0.3	0.0±0.0
Diplodia pinea	2.5±0.2	3.0±0.2	0.0±0.0
Diplodia seriata	3.0±0.2	2.0±0.2	0.0±0.0
Epicoccum nigrum	0.0±0.0	0.0±0.0	0.0±0.0
Fusarium avenaceum (isolate 1)	2.5±0.3	3.0±0.6	1.8±0.2
Fusarium graminearum	1.5±1.6	0.0±0.0	5.0±0.3
Fusarium lateritium	0.0±0.0	1.0±0.2	0.0±0.0
Fusarium sporotrichioides	1.0±0.2	1.0±0.2	2.8±0.2
Fusarium avenaceum (isolate 2)	0.0±0.0	0.0±0.0	0.0±0.0
Nigrospora oryzae	0.0±0.0	0.0±0.0	1.83±0.2
Nigrospora sphaerica	0.0±0.0	0.0±0.0	0.0±0.0
Paraconiothyrium brasiliense	0.0±0.0	0.0±0.0	0.0±0.0
Penicillium afellutanum	3.0±0.2	3.0±0.2	0.0±0.0
Penicillium expansum	2.0±0.2	5.0±0.2	4.9±0.1
Penicillium olsonii	1.5±0.3	3.5±0.3	0.0±0.0
Rosellinia corticium	2.0±0.2	4.5±0.3	0.0±0.0

Supplementary Table 3. Suppression of *F. graminearum* disease symptoms in maize and wheat by endophyte M6 in replicated greenhouse trials.

Treatment	% Infection (mean ± SEM)*	% Disease reduction relative to *Fusarium* only treatment	Average yield per plant*	% Yield increase relative to *Fusarium* only treatment
Greenhouse trial 1 in maize				
Fusarium only	33.69±2.3	0.0	41.77±1.4	0.0
Proline fungicide	23.10±2.0	31.4	50.9±1.2	21.85
M6	10.13±0.9	69.93	39.58±2.6	-5.2
Greenhouse trial 2 in maize				
Fusarium only	85.11±4.5	0.0	7.8±1.1	0.0
Proline fungicide	41.42±4.5	51.33	40.8±1.2	423
M6	6.9±0.9	91.89	34.70±2.7	344
Greenhouse trial 1 in wheat				
Fusarium only	51.8±6.3	0.0	10.5±2.6	0.0
Proline fungicide	31.70±2.6	38.8	10.9±1.3	3.8
M6	41.1±2.6	20.6	10.6±0.8	0.95
Greenhouse trial 2 in wheat				
Fusarium only	54.0±2.6	0.0	17.7±1.5	0.0
Proline fungicide	31.5±4.1	41.6	20.1±2.1	13.5
M6	30.7±3.6	43.1	21.1±1.3	19.2%

***SEM is the standard error of the mean.**

Supplementary Table 4. Reduction of DON mycotoxin accumulation during prolonged seed storage following treatment with endophyte M6.

Treatment	DON content (ppm) (mean ± SEM)*	% DON reduction relative to Fusarium only treatment*
Greenhouse trial 1 in maize		
Fusarium only	3.4±0.4	0.0
Proline fungicide	0.7±0.4	79.4
M6	0.1±0.0	97
Greenhouse trial 2 in maize		
Fusarium only	3.5±0.3	0.0
Proline fungicide	0.1±0.0	97.1
M6	0.1±0.0	97.1
Green house trial 1 in wheat		
Fusarium only	7.6±0.3	0.0
Proline fungicide	0.5±0.1	94.6
M6	1.5±0.6	81.33
Green house trial 2 in wheat		
Fusarium only	5.5±0.7	0.0
Proline fungicide	1.3±0.9	76.3
M6	2.0±0.4	63.6

***SEM is the standard error of the mean.**

Supplementary Table 5. Complete list of strain M6 Tn5 insertion mutants showing loss of antifungal activity against *F. graminearum in vitro*.

ID	Gene prediction	Swarming assay	Motility assay (mean ± SEM)*	Presence of flagella (%)	Biofilm (mean ± SEM)*
Wild type		+++	4.2±0.3	70%	0.8
ewa-1B3::Tn5 *ewa*-4B8::Tn5	Transcription regulator, AraC	---	0.6±0.02	50%	0.11±0.00
ewy-1C5::Tn5 *ewy-5D2::Tn5*	YjbH outer-membrane protein	++	0.5±0.03	10%	0.3±0.08
ewm-2D7::Tn5	4-hydroxyphenyl acetate 3-monoxygenase May catalyze production of phenylacetic acid (PAA)	---	0.8±0.05	20%	0.07±0.00
ewpR-5D7::Tn5	Transcription regulator, LysR	---	0.5±0.03	30%	0.18±0.01
ewb-9F12::Tn5 *ewb-7C5::Tn5*	Fatty acid biosynthesis	---	0.6±0.05	30%	0.3±0.11
***ewvC**-4B9::Tn5*	Colicin V production	+	1.8±0.30	60%	0.6±0.05
ewT-15A12::Tn5	Transport permease protein Within operon for biosynthesis of P-amino-phenyl-alanine antibiotics (PAPA).	+	1.5±0.00	30%	0.6±0.04

ews-1H3::Tn5	Sensor histidine kinase	+	0.8±0.16	50%	0.8±0.2
ewc-3H2::Tn5	Chitinase	---	1.0±0.00	20%	0.4±0.15
ewfR-7D5::Tn5	Transcription regulator, LysR	+	1.5±0.20	50%	0.3±0.08
ewgS-10A8::Tn5	Di-guanylate cyclase	---	0.8±0.30	40%	0.1±0.01
ewh-5B1::Tn5	Hig A protein	+++	1.6±0.30	30%	0.6±0.10
ewa-8E1::Tn5	Hypothetical protein	++	2.3±0.40	20%	0.5±0.09

*SEM is the standard error of the mean.

Supplementary Table 6. M6 wild type nucleotide coding sequences corresponding to Tn5 insertion mutants that showed loss of antifungal activity against *F. graminearum in vitro*

ID	Gene sequence
ewa-1B3::Tn5	gtgaataccattggcataaacagcgagcccatcctgacgcacagtggctttagcattaccgccgatacc actcttgccgcagacaggcactatgacgttatctatcttcctgccctgtggcgcaatcctcgtgcagtggtc agacaacagcctgaactcctggcatggcttagcgaacaggcggcgcgagggacccgcatcgcggcc gtcggaacgggctgctgtttcctggcggaatcgggattgctcaacgggaaacccgccaccacccactg gcactacttcaaacagttctcgcgtgactaccccaacgtaaaattacaaaccaaacattttctcacgcag gccgataatatttactgtgccgccagcgtcaaagccctctcagatctgaccatccatttcatcgaaacgat atacgggaaacgtgtagccacacatacccaacggacctttttccatgaaattcggagtcagtttgatcgc cagtgttacagtgaagaaaacaaaccccatccggatgaagatattgttcaaattcaaatctggataaaa gccaactgcgcctcggatatatccatgcaaaatcttgccgatatggctggcatgagtttgcgcaactttaat cgccgctttaaaaatgccaccgatatatccccccctgcagtatttattaaccgccagaattgaatccgccat gacaatgctgcaatccaccaatctgagcattcaggagattgcgaatgcggtgggatatcaggatattgc gcactttaatcgccagtttaagcataaaacaacggtttcaccgggggattaccgtaagaccgtccgggc aaagatgtttagtgcataa
ewy-1C5::Tn5	atgaaaagaacctatctctacagcatgctggcgctctgcgtgagtgccgcgtgccatgcagaaacgtat ccggcacccattggcccgtctcagtcagacttcggcggcgtcggtttgctgcaaacgcccaccgcgcgg atggcgcgcgaaggggaaattagccttaactaccgtgataacgatcagtatcgttactactcggcgtcgg tgcagctgttcccgtggcttgaaaccacgctgcgctacaccgacgtgcgtacgaaacagtacagcagc gttgatgcgttctccggcgaccagacctacaaagataaagccttcgacgtcaagctgcgcctgtgggaa gagagctactggatgccgcaggtgtccgtgggcgccaaagatatcggtggtaccggtctgtttgatgctg aatacatcgtggccagtaaagcctgggggccgttcgacttctcgctcggcctgggatggggctacctgg gcactggcggtaacgtgaaaaatccgttttgctcctacagcgataaatactgctaccgcgataacagcta taagaaagcgggttccatcaacggtgaccagatgttccacggtccggcatcgctgtttggcggcgtgga gtatcaaacgccctggcagccattacgcctgaagctggaatatgaagggaatgactactcgcaggactt cgccgggaagattgagcagaagagcaagtttaacgtcggcgccatttatcgcgtcaccgactgggccg acgttaacctcagctacgagcgcggcaacaccgtgatgtttggcttcacgctgcgcaccaactttaacga tatgcggccacactacaatgataacgcgcgccctgcataccagccggagccgcaggatgcgattctgc agcactccgtggtggcaaaccagctgacgctgctgaaatacaatgccggcctggcggatccgaaaatt caggtgaaaggcgatacgctgtacgtgaccggcgagcaggtgaaataccgcgactcgcgcgaaggg atcgaacgcgctaaccggatcgtaatgaacgatctgccggaggggatccgcacgatccgcgtgacgg aaaaccgccttaacctgccgcaggtgacgacggaaacggacgttgccagcctcaagcgccatctgga aggtgaaccgctcgggcatgaaaccgagctggtgcaaaaacgcgtagaaccgatcgtgccggagac caccgagcagggctggtatatcgacaaatcgcgcttcgatttccatatcgatccggtgctgaaccagtcc gtcggcgggccggaaaacttctacatgtatcagctgggcgtcatggcgacggcggatctgtggcttacc gaccacctgctgaccaccggtagcctgttcggcaacatcgctaataactacgacaagttcaactacacc aacccgccaaaagactcacagctgccgcgcgtgcgtactcgcgtgcgtgaatacgtgcagaacgatg cttacgtgaataacctgcaggccaactatttccagtacttcggcaatggcttctacggccaggtgtacggc gggtatctggaaaccatgtacggcggcgcgggggcggaagtgctttatcgtcctgtcgacagcaactgg gcgttcggggttgatgccaactacgtcaagcagcgtgactggcgcagcgcgcaggacatgatgaagtt caccgactacagcgtcaaaacgggccatctgaccgcctactggacgccgtcgttcgcgcctgacgtgct ggtgaaagccagcgttggtcagtacctggcgggcgataagggcggtacgctggatatctctaaacactt cgacagcggcgtcgtggtgggcggctatgccaccatcaccaacgtttcgccggacgaatacggggaa ggggacttcaccaaaggggtctacgtgtcgattccgctggatctgttctcgtcaggcccaacccgcagcc

	gtgcggcagtaggctggacgccgctgacgcgtgacgggggtcaacagcttggacgtaagttccagctg tatgacatgacgagcgataagaacattaacttccgctga
ewm-2D7::Tn5	atgaagcctgaagagttccgcgctgatgccaaacgcccgttaaccggcgaagagtatttaaaaagcct gcaggacggtcgtgagatttatatctacggcgagcgcgtcaaagacgtcaccacccatccggcatttcg caacgcggcggcctccatcgcgcagatgtacgacgcgctgcacaagcctgacatgcaggacgcgctc tgctggggcaccgacaccggcagcggcggctatacccacaagtttttccgcgtggcgaaaagtgccga cgacctgcgccagcagcgcgacgccatcgccgagtggtcgcgcctgagctacggctggatgggccgc acgccggactacaaagccgcgttcggctgcgcgctcggcgccaacccggccttctacggccagttcga acagaacgcccgtaactggtacacgcgcattcaggaaaccggcctgtactttaaccacgccatcgttaa cccgccgatcgaccgtcacaagccggcggacgaggtgaaagacgtctacatcaagctggagaaag agaccgacgccgggattatcgtcagcggcgcgaaagtggtagccaccaactccgccctgacccacta caacatgatcggcttcggctccgcgcaggtgatgggcgaaaacccggacttcgcgctgatgttcgtcgc gccgatggatgccgaaggcgtgaagctgatctcccgcgcctcttacgaaatggtggcaggcgcaaccg gatccccgtacgactacccgctctccagccgctttgatgagaacgacgcgatcctggtgatggatcacgt gctgatcccgtgggaaaatgtgctgatctaccgcgatttcgaccgctgccgtcgctggacgatggaagg cggtttcgcgcggatgtacccgctgcaggcctgcgtgcgtctggcggtgaagctcgacttcatcaccgcc ctgctgaagaaatccctggagtgcaccggcaccctggagttccgcggcgtacaggcggatctcggcga agtggtggcctggcgtaacatgttctgggcgctgagcgactccatgtgttccgaagccaccccgtgggtc aacggcgcgtatctgccggatcacgccgcgctgcaaacctaccgcgtgatggcgccgatggcctacgc caagatcaagaacatcatcgagcgtaacgtgacgtccggtctgatctacctgccgtccagcgcccggg atctgaacaacccgcagatcgaccagtatctggcgaaatacgtgcgcggctccaacgggatggatcac gtcgaacgcatcaagattttgaagctgatgtgggatgccatcggcagcgagtttggcggtcgtcacgagc tatacgaaatcaactactccggcagccaggatgagatccgcctgcagtgtctgcgccaggcgcagagc tccggcaacatggacaaaatgatggcgatggtcgaccgctgcatgtccgaatacgaccagcacggct ggaccgtaccgcacctgcacaacaacagcgatatcaacatgctcgacaagctgctgaaatag
ewp-5D7::Tn5	atggacttaacccagcttgaaatgtttaacgccgtcgcgctgacgggcagcatcacccaggcggcgca gaaggtgcatcgcgtgccgtccaacctgacgacccgcatccgccagctggaagccgatcttggcgttg agctgtttattcgtgagaaccagcgtttgcgcttatctcccgccgggcataacttcctgcgctacagcaggc agatcctcgccctggtggatgaagcgcgcatggtcgtcgcgggtgatgagccgcaggggttatttgccct cggcgcgctggaaagcaccgccgcggtgcgcattcccgaaacgctggcgcagtttaaccagcgctat ccgcgcattcagtttgccctttctaccgggccttccgggacgatgattgatggcgtactggagggcaccttа agcgccgcctttgtcgacgggccgctgtcgcacccggagctggagggcatgccggtctaccgggaag agatgatgctagtcacgcctgccgggcacgccgaggttgcacgcgccacgcaggttagcggcagcga cgtttacgcatttcgcgcgaactgctcgtaccgtcgacatctggaaagctggtttcatgcggacagagcca cgcctggccgcattcatgagatggagtcctaccacggcatgctcgcctgcgtcattgcgggcgcgggca tcgcgctgatgccgcgctcgatgctggagagtatgccgggacatcatcaggttgaagcctggccgctgg cggaaaactggcgctggcttacaacctggctggtgtggcggcgcggggcgatgacccgccagctgga agcttttatagcgctgctaaacgaacgtcttcaaccagcgccttctccataa
ewb-9F12::Tn5	atgcccgcaaaatcatcaggaagcgcgtgggagcgttttgccggagtattacgtaatgcgcaaacgga atgtattgttacgacagcaaagggagcagaaacgctaggccagctatcacttccgttatccccgcttatttt taccttcgacaaaccagacacagctgcgctccctgcgggctatcgtctgcatcctcttgatcgcacgttctc cggggcatttcaccccgttccggtagcggaaaacgatctggcttttttacagtacacctcgggttcgacgg gctctccgaagggcgtgatggtgacccatggcaacctgtgggccaactcgcatgccattcaccgcttttc ggccatcacagcgaaagccggggcacgatctggctgccgcattttcatgatatggggctgattggcggg ctactgcagcccgtgtttggcgcattcccctgtcgggtgatgtcgcccatgatgttaatgaaaaatcccctta actggctcaaacatatttctgactatcaggcgacgacctccggcggccctaatttcgcctacgatctgtgc gtgcgcaagattggcagagagcaagttgaggcattagatctttctcgctgggatgtggctttttgcggcgc

	agagccgattcggcccgccacgctacagcagtttagcgaacactttgcgcccgcagggtttcggcccg gcgcgttcctgccctgctacggcatggcggaaaccacgctcatcgtcaccgggatggagaaaggaca agggctccgcgtttccgacgaggccggtacggtgagctgcgggcaggctctgccggataccgaggtgc gtatcgtcgatcccgatcgccatcagccgcttgctgatggtgagagcggggaaatatggctgcgtggtcc gagcgttgccgcaggatactgggacaatgacgccgccacacgcgaaaccttccaggcgtctctggctg gccatccgcacccgtggctgcgcagcggcgatatgggttttctccagtccggccatttgtacgtcaccgg acgactcaaagagctgctgattatcaacggtcagaaccactacccgacggatattgaagagacgatcc gccaggccgatcccgcgctggcggaagcgacggtttgcgtgtttgccagtgaagacgagcgcccggtt gcgctgcttgagctgatgacgcgccataaaaacgatctcgatatggcgacgctggcaccgtccgtgacc gcggcggtggcggagcggcacggcatcacgctggatgaactgctccttgtcgggcgaagggcaattc cccgcaccaccagcgggaaactacagcgcacccgcgcgaaagcgatgcaccagcagggaaccct ggaagtagcctggcgcagctgccaggacgcgtcgaaacctgttgaactcgcgggggaaaccccacc cgcgctggcggcgctgatagccgggataatcagcagcgcgatgaacacgacgatcggcgaatccca gtgggacgaggcgtttaccggctttggcatgagctctctgcaggcggtgggcgtgattggcgagcttgaa cagcggctgggccgcgagctctctcccgcgctgatttatgactaccccaccatcaatcggctggcggcc gcgctggggcaacccgctgcggcccggccggtcagctcagccgtcgcggagagcgccattgcggtg attggcatcggcgtggagctgccgggacatagcggcgtggaggcgctgtggtcgctgctgcagcaggg ccacagcacgaccggcgagatcccggcgcaccgctggcgtacctcgtcccttgacggttttaaccgtaa aggcagtttcttcgacgaggtcgacgcgttcgacgcaggctacttcggcatctctccccgtgaggccgtct atatcgatccgcagcatcgtctgctgttagaaaccgttcaacaggcgctaaccgatgccggccttaaggc gtcctccctgcgcggtagcgatacggcggtctttgttgggatcagcgccagcgactacgcgctggcctgc ggcgataacgtctcggcctacagcggcttaggcaacgcgcacagtatcgcggccaaccgaatttcttat ctttatgatttaaaaggtccaagcgtcgccgtcgacacggcctgttcttcctcgctggtggcgatagaggg ggcaatgcagagcctgcgggccggacgttgcgctctggccattgccggaggcgttaatctggcgctgac gccacatttgcaaaaagtcttcaccgaagcccagatgctggcccccgacggccggtgtaaaaccttcg acgcccgcgcggatggctatgttcgtggcgaagggtgtggcgtcgtggtgcttaagccgctttcacaggc gctggcggatggcgatcgggtttatgccacgctggtggcgagcgccgtgaatcaggacggccgcagc aacggcattaccgcgccaaatggcccatcgcagcaggcggtcatcctgcaggcgatggcggacgcc gggttggacagcgacagcattgactatatcgaagcgcacggtacgggaaccgcgcttggcgatctgatt gaatatcaggcgctggaagcggtgtttgcggaccggaaaaagaccgcacctgtccaggtgggttcgat caaaaccaacattggccaccttgaggcggcggcgggcgtgctgggcgtggtgaaaacgtctctgatgc tgcacttccggcaatacgtacctcacctcaattttcagcagaaaaacccgcatattgcggcgattagccgt catgttgaggtgagcggcgcgcagcctgcctcatggcatgccgatggcgaagcgcgctatgcgggcgt aagcagctttggcttcggcggtaccaacggtcatgtgattttgcgcagcgcgccagcggtggaaaaacg ccaggagcccgctgcgccgcacggcctgcttctggtcggttcacatgataaaggggcgtttaccttcag cgggaggcggtcaaaaaagggttatcgacgtgccaggagagcgatattgccacctggtgtcggctggt gaacacccgctacgacgcggcccgctatcgcggcgtggcgtatggcgcggatcgctcccagctggcg gaaagccttgcgcagctcaccgtctgcaaggtgggtaaagcccagccccaggtctggctcttcccggg gcagggcacccagcaaatcggcatgggtgccgagctgtatcaccatctgccgcactatcgcacccagt ttgacgcgctggcgacgactattcagcagcgctatcagattgatattacgcaggcgctgtttgcccgtgac gacagctggcagcgctgcgccagaacgtgtcagctctcattatttgcctgtagctacgcgcttgctcagag cgtgatgcagttcggcccgcgtccggctgccgtaatggggcacagcctgggagagtactgcgcggcgg ttatcgctggctatctctcgctggacgacgggctggcaatggttcatcagcgcgcgctgttgatgtcagccc tgacgcaggaaggggcgatggctgtcgtcttcagcggcgaagccgacgtccgtcagatgatttccccct ggacgggcgacattgatattgccgcattcaacacgccgacattgaccaccatcgcaggcagtcgggcg gcgattgacgcctgccttcaggccatatcttcaaaaggcggtcacgccagaaaaattaaaactgccagc gcatttcactcctcgatgatggatccgatcctcggcgcctggcgcgagtggctggtcaacaacgtcacctt cacccgcgggacgatcccgttttacagcaacctgaacggtgaggcgtgcgaccgcaccgacgccgac tactggacccggcaaattcgccagcccgtgagtttccttcagggcgtgcaaaacgtgctggcacagggt gagttcacctttatcgatctgagcgcggacggttcgctgggcaaatttgtgaccgcaactgaccgccgtca ccgggtgctggccgcaggcgaccggcgacatgagtacaaatcactgctgacgctgctgggtacgctgt ggcagcaagggcacgacatcaactggagcgggctgtaccacgcgaccacgcgggaggcgctaacc ctgcccgccatccagttctgccgcaaacgttactggctggcgggtgagacgccagcgcagaccccatct gcaaaagaggacgctatgtcaaatcaacaccatttagccgctgaaataaaagcgattattgccggttttct tgaggcggatcccgccgcgcttgacgactctctgccgttcctggaaatgggggcggactcgctggtgctg ctggatgccatcaataccattaaagaccgctttggcgtagccatcccggtgcgggcgctgtttgaagagc

	tcaatacgctggacgcggtgatcggatatgtggtggagcacgcgcagccggcggcttcgctcaccacc ccggaaaccgccggcctggcggcacagcctgtcgcggcaccgcagggtaccagcaggccggtgcct gatacggttcaggatctgattgcccgccagctggagctcatgtcccagcagctaaatttgcttaacggcac ggcgcaggctctcccgatgccagccgcacccgcgacgccggacgttatcgcgcctgcgcccgtcgtg gccccgaccgcaccggtgaaggccagcgcgcacaacagctggtttaaaaaagagaccaaaaaggt ctccctcggcgctgagcgcgaccagcatctggcgcaactcaccgaacggtttgtcgataaaacgggcg ggtcaaaacgcaatgcccagcaataccgcgccgtgctcgcggataaccgggcctctgcgggctttcgtt tatccaccaaagagatgctttaccccttagtgggagagcgctctcagggttcgcgcatctgggatgtcgat ggcaacgagtatattgattttacgatggggtttggcgctaacctgctgggacatgcgccggactgcgtgca gcaggccgttgccgaccagctggcgcggggcatgcagattggtccgcaaagcgccctggcgggcga ggtggcaacgcttatcagcgagctgaccggccagcagcgcgtggcattttgtaactccggctccgaagc ggtaatgagcgccgtgcgcctggcgcgggccgtgacggggaaaaataaagtcgcgctgtttagcggct cgtatcatggcgtgtttgacggcattctggggcgacagcagggggggagaaacccccgagcgcgcgac gccgattgccgcaggcacgccgccatcgctggtggacgacctgctggtgctcgactacggcagcgaa gagagcctggcgcttatcgcccgctatgccgccgagttggcggtggtgatcgtcgaaccggtgcagagc cgttatcccgatcatcagccacgcgactatctgcataccctgcgtgaactgacgacaacccacaacattg cgctgatgtttgacgaggtgatcaccggtttccgtctggctgccggcggcgcgcaggcgtattacggcgtt caggcggatatcgcctcctacggaaagattgtcggcggcggtatgccgatcggcgtgattgcaggctct gcgcgctttatggacagtatcgacggcggattctggcagtatggcgatgactcctggccgcaggctgaa ctgatcttctttgccggtacgttctccaagcatccgctgaccatggcggcaagcaaagccgtgctggagta catcaaaacgcatccggcgctgtatgacgacatcaaccaaaaaaccgcgcgtctggcgatgtcgctaa atacctggttctcagcgaccggcacgccgattgagatcgtctcagccggcagcctgttccgtttcaaattc aacggtaactacgacatcctcttccaccacctgatgctgcgcggcattttcatctgggaagggcgtaactg ttttgtctcggtcgcgcacgcggatgaggatatcgatcggtttattgcggcggtgaaagagagcgttaacg ccatgcgcgtggacggcttctttggggaggcgggattgcgccccgacacgcgttatgccgttgcagaca gccagcagcgttttctgcagctggccgcgcaggatgaaagcgggctgttggccgggacgatcggcgg cgtgatcgaaacgccgcgcaatgtagatggcgacatcatgcgcgccgcctggcaactgctgtgcgagc gccatgacgcgctgcgtatgcaattcaccgacgcgggtgaccttcaggtggccctggcgcccatcgtcg atatctgcgaggaaaacgctgcgcctgccgcctgtctcgctgaatttgctgcgcgtccttttgatcttaccgc cacgccgctggctcgcctgctgctggtccgccacgaggggaaaaccacgctcgccatctcggcacatc atacggtggcggacggctggtcgttcatggtgatgctgcgcgagctgttgcatctttacgatgccctggcgt cgggtaaacgtcctgacctggcggcagcagcaagctatctgcaggctatccgcgcgcagaacatcgtc agcgaggctgagctacctgcgcgtcttgcggcgcttccagcgcgccgggtaacgccggaagttctgag cgtgcaagatccctccctgacgtatcaggggcagcggctggtacagcgcctgtcgtatccgggcctgac cgggcagcttcgcaaagcctccgccgaactgcgggtgacccgcttcgcgatgctgaatgcgctctttacc ctgacgcttgagaaggcgttcggccattccccggtgccggtcggcgtgccggatgccgggcgggacttc gggcaggggacgcgcttgtcgggcagtgcgtcaggctgttgccgctatgtatcgacagcgctgcctgc gcgtcggtgtccggggtcgccagggcaatccatgacggtatcctcgcgcagcgcggcgagcccgcac tgccgtcacgctgcttccacggtcaggatgcgccgcttccgctgctggcgacctttaacgtcgagccgcat gctccgctggctgagatgcgtcagtgggaagccagcctgtcgctgctcccgatcggcgccgtcgaattc ccgctgatggttaacattctcgagacgaaagaggggctgtccgtcgagctggattaccagatgcgctattt caccgaggaacgagcccgcgcgctactggagcacttcctgaaggcgataaccgtgctggccgagca gggagaagaggctgttgaagcattattctcgtcgtcagaggcgctggccgcatcatga
ewv-4B9::Tn5	atggtctggattgattacgccatcattgcggtgattggtttttcctgtctggttagcctgatccgtggctttgttcgt gaagcgttatcgctggtgacttggggttgtgctttctttgtcgccagtcattactacacttacctgtctgtctggtt cacgggctttgaagatgaactggtccgaaatggaatcgctatcgcggtgctgtttatcgcaacgctgattgt cggcgctatcgtgaattacgtgataggtcagctggtcgagaaaaccggtctgtcaggaacggacaggg tactcgggatctgtttcggggcgttgcgaggcgtgctgattgtggccgcgatcctgttcttcctggataccttt accgggttctccaaaagcgaagactggcagaaatcgcagctcattccagagttcagcttcatcatcaga tggttctttgactatctgcaaagctcgtcgagttttttgcccagggcataa

ewT-15A12::Tn5	atgtcatggcaatacttcaaacagacttacctggttaagttctggtcacctgtcccggccgttatcgcggca ggcattctctctacctactatttcggcatcaccggcaccttctgggccgtgaccggcgagttcacccgctgg ggtggtcaattgctacagctcgcaggcgtacatgccgaggagtggggttactttaaactcatccatctgga cggcacaccgctcacccgcatcgacgggatgatgatcgtcggcatgttcggcggctgtttcgcggcggc gctgtgggcaaacaacgttaagttgcggatgcccaaaagccgcattcgtataatgcaggcggtgggcg gcggtatcattgccgggtttggcgcccgtctggcgatgggctgtaacctggccgctttctttacggggattcc tcagttttcgcttcacgcctggttctttgctgtcgccacggccatcggctcttacttcggtgcaaaattcaccct gctaccgctgttccgcattccggtgaaaatgacaaaagtcagcgcagcgtctccgttaacccagaagcc ggaccaggcgcgtcgtcgtttccgcctcggtatgctggtctttttggctatggtcgcctgggcgctttgcactg cgatgaatcagcccaaactcggcctggcgatgctgttcggcgtgggttttggtctgctcattgaacgcgcg cagatctgctttacctccgcgtttcgcgatatgtggatcaccggccgtacgatgatggcaaaggcgattat cgccgggatggcggtcagcgccatcggcatcttcagctatgttcaactgggcgtcgaaccgaaaatcat gggctggccctaacgccgtgatcggcggcctgctgtttggcttcgggatcgtgctggctggcgggtgtga aaccggctggatgtaccgcgccgtcgaaggccaggtacactactggtgggtgggtctgggaaatgttat cggctcgacgatcctggcgtacttctgggacgatctctccccggcgctggccacgagctgggataaggt caacctgctgagcaccttcggccccctcggcggcctgctggtcacctacgccctgctgctggtggctttttt actggttgtcgcacaggagaaacgcttcttccgccgcgcaagtgttaaaacagaaacccaggagaatg ctgcatga
ews-1H3::Tn5	atgacgagccgtaaacctgcccatcttttactggtggatgacgatcccgggctgttaaagctgctggggat gcgtctggtgagtgaaggctacagcgtcgtgaccgccgaaagcgggctggaggggctgaagatcctc acccgcgagaaaatcgatctggtgataagcgacctgcggatggacgaaatggatggcctgcagctgtt cgcggagatccagaggcagcagccgggtatgcccgtgatcattctgacggcgcacgggtcgatcccg gatgcggttgccgcgacgcagcaggggtcttcagcttcctgaccaagccggtggacaaagacgcgct gtataaggctatcgacagcgcgctggaacatgccgccccggcgggggatgaagcgtggcgggagtc catcgtcacccgcagccccattatgctgcgtctccttgaacaggcccggatggtggcgcagtccgacgtc agcgtgctcatcaacggccagagcggaaccgggaaagagatcctggcccaggcgatccataacgcc agcccgcgcagtaaaaatgcctttatcgccattaactgcggcgcgcttccggaacagcttctcgaatctg aactgtttggtcatgcccgcggcgcattcaccggcgcggtgagcagtcgggaagggctgttccaggcg gcggaaggcggcacgctgttcctggacgagattggcgacatgcccgcgccgctgcaggtcaaactgct gcgcgtattgcaggagcgaaaagttcgcccgctgggcagcaaccgcgatatcgatattaacgtgcgca ttatttccgccacccaccgcgacttgccaaaagtgatggcccgcaacgagtttcgcgaagatctctacta ccgtctgaacgtggtgaatctgaagatccctgcgctggcggagcgcgcggaagacattccgctgctggc gaatcatcttctgcgccaggcggccgatcgtcataaaccgtttgtgcgcgcgttttccaccgacgcgatga agcggctgatggccgcaggctggccgggtaacgtgcgccagctggtgaacgtgattgagcagtgcgtg gcgctgacctcctcaccggtaatcagcgatgcgcttgtagagcaggcgctggaaggagaaaacacgg cgctgccgacgtttgcggaagcgcggaatcagttcgagctgaactatctgcgcaagctattgcagatca ccaaaggcaacgtgacccacgcggcgcgcatggccggacgcaaccgcaccgagttctacaagctgc tgtcgcgccacgagctggaagcaaacgattttaaagagtaa
ewc-3H2::Tn5	atgaacaaaagaacattactcagtgttcttattgcgggcgcatgtgttgcaccgtttatggctcaggcaacc ctgctgcaggccagcagcgaaccttacacccttaaggccagcgatctgcagaagaaagagcaggag ttaaccaacttcccgctgatggcttcggtgaagtcaaccatccgcacgctggacaacagcctggtggaa caaattgagcctggtaaatcgacaaacccggaaaacgttaagcgcgtggaagggattatcaaggcca gcgactgggattatctcttcccactgcgtgcgccggaatatacgtacagcaacttcctgaaagcggtcggt aaattcccggcactgtgccagacctataccgatggtcgtaacagcgatgccatctgccgtaagtctctgg caaccatgttcgcgcacttcgcgcaggaaactggcggccacgagtcctggcgtccggaagccgagtg gcgtcaggcgctggtttacgtgcgtgagatgggctggagcgaaggtcagaagggcggatataacggc gaatgtaacccggatgtctggcaggggcagacctggccgtgcggtaaagacaaagatggcgatttcgt tagctattttggccgcggtgcgaaacagctctcctataactacaactacggcccgttctctgaagcgatgta cggcgacgtccgcgtactgctggacaaacctgagctggtggcggacacctggctgaaccttgcttccgc gatcttcttcttcgcctacccgcagccgccaaaaccaagcatgctgcaggttatcgacggcacatggca

	gccgaacgatcacgataaggcgaatggtctggtaccgggcttcggagtgaccacgcagatcatcaac ggcggcgtggagtgtggcggcccgactgagatcgcccagtctcaaaaccgtatcaaatactacaaag agttcgccaactacctgaaagtgcctgttccgtctaacgaagtgctgggctgcgccaacatgaagcagtt cgacgaaggcggtgctggcgcgctgaagatctactgggaacaggactggggatggagcgcggatac cccgtcaggccagacctactcttgccagctggtgggttaccagacgccattcagcgcctttaaagagggt gactacaccaaatgcgtgaagcatttcttcaatgtgaacgtggtgggtgaagacgggacttccgacggc ggtagcgtcacgccagccccgacgccgacccctgtcgacccaacggacgaaggcaacaccacgcc ggtgccagacgataacaccccggctccggacgataatacgccagcgccggtaaaccatgcgccggt agcaaaaatcgccggtccggtgggtgcggttgaagcgggtaaatcagtttctctgaacgcgtctggctcg accgacgaagacggtaaccacctgacttatacctggacggctccgaacggccagaccgtaagcggc gaagataaagcgattattaccttcaatgcgccggaagtggcagcggcgacgcagtacccgatcaacct taccgtcagtgacggcgagctgagcagcaccaccagctatacgctgaacgtacaggctaagcagacc aacggcggccagaccgggacttacccgacctggacctccaaaaccaaatggaaagcaggcgatatc gttaacaaccgcggccagctgttccagtgcaaaccttatccgtacagcggctggtgcaataacgcgcca tcctactacgagccaggtaaagggattgcatggcaggacgcgtggactgcgctgtaa
ewf-7D5::Tn5	atgatgaatcttttacactggcgcttgctggttgccgtggccgatgccagtaatatttcccgtgccgctgaag aggtcggtatgacgcagtcaggtgccagccaggcgatcgctcagttagaggcctcattaggatttgcggt attcacgcgagagcgacgccacatcggcgtgactgccctgggcgagcgggttgtcaaagaagcaag gagtatgctggcgcggctggatgccatccgtgcactggcggatgagagccgggggctgagcggcgga cgcattcgtcttgccagttttccttcggtgacatccacgtttttgcccgggctgctgcgcgacttaaagcgcct gcatcctgaaatcgaggtggtcgtcctcgaaggcacggaccaggaggtcgaagagtggctggcggca gatacggttgatctgggcgtggtcatgaaccctgttcccgggcgtgctgaagcgatcttagggcaggatg catgggtcgtggtgttgccagccagtcaccgtcaggctcgttatacacgtacgcgcggcatcacccttga ggaactggcagaccagcccttcgtgctcgcaaccggcggttgcgccgtcaatggaaaaagcctgatgg aacaggctggcctgcagctgcctgacgtgcgggtgaccgtccgcgactggatcagcgcctgcctgctg gtgcgcgaaggcatgggcgtcgcgctgatacctgaatccgccctgccggatgaattacgcggtttgtgc gtcgtacccgtgacgccttcgctgtgccgggaatttgggctggtatgttcgcaggtgggcaaatattcccg cgcgacgcaggcgttattagaagggttgctcaaaatgaaaaggtctgcgtga
ewg-10A8::Tn5	atgacccacaacatttctctcagaaatagggtgtgcgatgaacaaaagtttgtcatctcagcctgcggtttt acgttccagggatatcgtctgttccttaaccagtacggaatacaggcttcgcatattcattttgatggggatg aggcatcgcaacaggatatgaaaaatatccttataaatcagaatgctcatgttgtggtgtttctcggaaaa ggtatcttaagtctcctggagagcctgaagcgactggcgtccgtgctcaatgcgttgcccgttattcgacgc gtcacgctgtatggcgacataccggatggctggctatatcgcaccctgggcagtcttttaaataatagttat caattatcattgattcgactagcccgcgtttcggatgtcgtcactggttctcatacgcaccatcatgtatttaa ggaacgttcgtacttattacgcgatcgctacagggataattcttcgcaagacaacgtcaagtggctaaca aaaagagagattgacgttttattaaatttctaccgcggcatgtccgtaaaagaaatgtgcgatgaaatgg gactatctaataaaacggtttatacccaccgtaaggaaggcgtgctgaaattacgcttaattaagcggtg gctacacgattcgcacaatatcaatgcggaaagaagtatcaagcggcggagtcaaaacacggaatttt acggataaggaagcagagatttttaatgcattatataaaaaagagatcttccctgcttatcagatcattacc gatcgtgacaaaaaaggcgtaggctttgagatactgatccgctggaacaaaaacggtaaaattgtcaa gccgaccagttttctcacggatatttcaaatcatgagatatggttgaaaattaccgcgctagttattcatgcc gccgtgtcgggaattaataagtataatggtaaatactatttttctgtgaatattccacctcgcctggcctccgg aaatgcattgcctgatatggcaaggaaagctatcgacatgctgctcaaaccgcagtgggccgagaagc tggtctttgaattcgcagaagatattgacgtgacgaaggacaaagggatcccagaaaccatgcggcatt tgcgtaatacagggtgtcgattgtttctggatgactgcttctctaatcaccaaaccatgttcccggtgcggca ggtgcattttgatggactcaagctggatcgggatatcgttgagcattttgtggcaaacgacaatgactataa cctgatcaaagcgatacagatttatagcgacatgaccggaacggactgtattgcagagggagtagaca gtgaggaaaaatttgaaaaattagtcgcgctgggcgtcaaaaactttcagggatattatttatcgcgagcc gtgaaagaggacgagttggatcgcatggtcagaatttttagttaa

ewh-5B1::Tn5	Atgacacttcaacaggcactccgcaaacccaccacgccgggcgacgtgttgcagtatgagtatcttgaacc gctcaatctgaaaatcagcgatctggcggagatgctcaatgttcaccgcaacaccatcagcgcgctggtca ataacaatcgcaaacttactgccgatatggcgatcaaactggcaaaagcctttgataccacgattgaattttgg ctgaacttacagctgaacgtcgatatctgggaagcgcaatctaactccaggactcaggaggagctaagccg tataaagaccgttgcggaagttatggctaagcgaaaatctggccagccggatgttgcctga
ewa-8E1::Tn5	gtgatccgcaaagagaaagggcgcgtggatgtctatggcgaccgctttcgcgcacgtgcgcatcagct gacgccgcgtctgctgcaggttgcacgctatatccatgacaaccgcgaagcggtgatggaacagaccg caatggagattgcgacgctgttgaaaacgtcggatgccacggtggtccgcgccattcaggcgctgggttt cgccgggctgcgcgatcttaagcaaacgctggagcagtggtttggtccggtggtcacctccagcgaaa agatgtccactacggtgaatacgctcgccagcgacgtcaacgcgagcattgatttcgtgctggaggggc atcagcacacctgcgaggtgttatccgagccccacaaccgctacgcaatggcccaggcggtctctctgc tggcgcaggcgaggcaggtcgccatatttggcattggcgcctccggcattctggccgagtataccgcca ggctgttcagccgtatggggttacccgccacgccgcttaaccgtaccggaatagggcttgccgagcagc tgattgcgcttcagcgcggcgacgtgctggtgatgatggcgcagaaatccgcgcaccgggaggggca aaccacgctgcgtgaagcaaaacggctgggcattcccaccatcctgctgaccaacgccctcgattcgc gtttcagcaaggaggccagcgtggtgatccacgttccgcgcggcggcgaaaaaggcaaaattccgct ccacggcacggtgctgctgtgtctggagatgatcattttatccgtcgcctccaccgagccgcagcgcacc atcaagtcgatgaagcgcatcaacgagttacaccgtgggttaaagccggggaagaaaagtacctaa

Supplementary Table 7. Gene-specific primers used in quantitative real-time PCR analysis.

Gene ID	PCR primers
ewpR	5D7F: 5′- GGCATAACTTCCTGCGCTAC - 3′ 5D7R: 5′: CAGTACGCCATCAATCATCG - 3′
ewpF1	PhzF1F: 5′- TTTTCTCACCGGGCGTTTC- 3′ PhzF1R: 5′- GTATGTGCGGAGCCGGTAA- 3′
ewpF2	PhzF2F: 5′- AAATGGCGCAGCAGCATAA- 3′ PhzF2R: 5′- GTCGGTGCGCACGAAAA- 3′
ewfR	D5F : 5′- GGGGACAGTAACGACGAAAC - 3′ D5R : 5′- CGGCAATCTGTCGATATGAA - 3′
ewfD	FusE/MFPF: 5′- TGGCCGTGCGGGATAAT- 3′ FusE/MFPR: 5′- GGATCGATGGTGTAAAGCACATC- 3′
ewfE	FusEF: 5′- CGTCGAGCCCACCTTTAGC- 3′ FusER: 5′- TCCGGCAATCTGTCGATATG- 3′
ewgS	A8F 5: GGAGTCAAAACACGGAATTTACG - 3′ A8R 5: ATCTGATAAGCAGGGAAGATCTCTTT - 3′
ewvC	B9F 5: TGTTTTATGCTTAAACTGGCGATT - 3′ B9R 5: CGAATGCGGTGGGATATCA - 3′
16SrDNA (housekeeping gene)	799F: 5′- AACMGGATTAGATACCCKG- 3′ 1492R: 5′- GGTTACCTTGTTACGACTT- 3′

(3) Supplementary video legends

Supplementary video 1 | 3D video showing RHESt. A 62 µm confocal stack with 46 sections was imaged from a 12 day old finger millet root previously seed-coated with GFP-tagged endophyte M6 (green colour) then inoculated with *F. graminearum* (*Fg*) at a distance of 0.5 cm to the left-hand side of the image followed by a 72 h incubation. On the right side of the image is the root (purple red) oriented downward. M6 cells (green, right of the root) stack to form a deep physical barrier on the rhizoplane on the same side as *Fg* inoculation. Root hairs (purple) unusually elongate on the same side (left) as *Fg* inoculation, bend parallel to the rhizoplane and become intercalated with M6 cells, in contrast to the side of the root that is distal to *Fg* (right). In this video, few *Fg* mycelia (purple threads) are observed, perhaps because most mycelia had not yet reached the root system (mycelia were clearly visible ~1 mm away), were obscured or due to earlier death by the RHESt complex.

Supplementary video 2 | 3D imaging of a biofilm associated with endophyte M6 *in vitro*. Shown is a 45 µm confocal stack rendered as a 3D video. The biofilm was grown on a microscopic slide immersed in LB liquid medium inoculated with M6 at 37 °C and 50 rpm for 5 days, then stained with Ruby Film Tracer.