

Supplementary Information

Whole-genome sequencing reveals that mutations in myosin-5 confer resistance to the fungicide phenamacril in *Fusarium graminearum*

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Fig. S1. Frequency of changes in bases in a phenamacril-resistant strain (YP-1) of *Fusarium graminearum* as indicated by whole-genome sequencing. Changes are relative to reference strain PH-1.

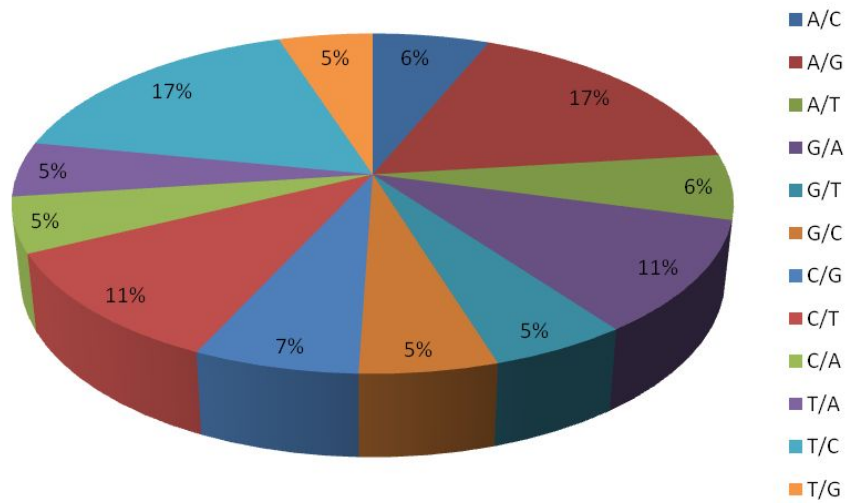


Fig. S2. The alignment of the amino acid sequences of myosin-5 motor domain with those from *F. oxysporum*, *B. cinerea*, *M. oryzae* and *B. graminis*. ATP-binding site and SH1 helix were blast by NCBI database (<http://www.ncbi.nlm.nih.gov/>). Codon 216 and 420 are conserved in all the five myosin motor.

ATP-binding site

FgMyo5 39 **CVSDLTLLSKVSNEAINENLKKRFEGREIYTYIGHVIVSVNPPFRDLGIYTDVLCQSYL** 98
 FoMyo1 39 **CVSDLTLLSKVSNEAINENLKKRFEGREIYTYIGHVIVSVNPPFRDLGIYTDVLESY** 98
 BcMyo1 40 **CVSDLTLLSKVSNEAINENLKKRFENREIYTYIGHVIVSVNPPFRDLGIYTDVLD** 99
 MgMyo1 42 **CVSDLTLLSKVSNEAINENLQKRFEGREIYTYIGHVIVSVNPPFRDLGIYTDVLD** 101
 BgMyo

ATP-binding site

FgMyo5 99 **NRLEMPPHVFAIAEASYYNMKAYS**DNQCVIISGESGAGKTEAAKRIMQYIASVSGGESGD 158
 FoMyo1 99 **NRLEMPPHVFAIAEASYYNMKAYS**DNQCVIISGESGAGKTEAAKRIMQYIASVSGGESGD 158
 BcMyo1 100 **NRLEMPPHVFAIAEASYYNMKAYKDNQCVIISGESGAGKTEAAKRIMQYIANVSGGS** 159
 MgMyo1 102 **NRLEMPPHVFAIAEASYYNMKAYKDNQCVIISGESGAGKTEAAKRIMQYIASVSGG** 161
 BgMyo 1 -----**MKAYKENQCVIISGESGAGKTEAAKRIMQYIASVSGGSNTS** 41

216 217

FgMyo5 **IQIKDMVLATNPLESFCNAKTLRNNNSSRFGKYLQIFNFNQGEFVGADITNYLLEKSR** 218
 FoMyo1 **IQIKDMVLATNPLESFCNAKTLRNNNSSRFGKYLQIFNFNQGEFVGADITNYLLEKSR** 218
 BcMyo1 **IQIKDMVLATNPLESFCNAKTLRNNNSSRFGKYLQIFNFNQGEFVGADITNYLLEKSR** 219
 MgMyo1 **IQIKDMVLATNPLESFCNAKTLRNNNSSRFGKYLQIFNFNSVGEFVGADITNYLLEKSR** 221
 BgMyo **IQIKDMVLATNPLESFCNAKTLRNNNSSRFGKYLQIFNFNSQGEFVGADITNYLLEKSR** 101

FgMyo5 **VVGQITNERNFHIFYQFAKGSACQYRETFVQKPEYVYTSRSKCLDVDGIDDLAEFQDT** 278
 FoMyo1 **VVGQITNERNFHIFYQFAKGSACQYRETFVQKPEYVYTSRSKCLDVDGIDDLAEFQDT** 278
 BcMyo1 **VVGQITNERNFHIFYQFAKGSACQYRETFVQKPEYVYTSRSKCLDVDGIDDLAEFQDT** 279
 MgMyo1 **VVGQITNERNFHIFYQFAKGSACQYRETFVQKPEYVYTSRSKCLDVDGIDDLAEFQDT** 281
 BgMyo **VVGQITNERNFHIFYQFAKGSACQYRETFVQKPEYVYTSRSKCLDVDGIDDLAEFQDT** 161

FgMyo5 **INAMKVIIGLSQEQDEIFRMLSAIIWIGNIQFCEDCGYAEVVDERSVVDFAAYLLEVD** 338
 FoMyo1 **INAMKVIIGLSQEQDEIFRMLSAIIWIGNIQFCEDCGYAEVVDERSVVDFAAYLLEVD** 338
 BcMyo1 **INAMKVIIGLSQEQDEIFRMLSAIIWIGNIQFCEDCGYAEVVDERSVVDFAAYLLEVD** 339
 MgMyo1 **INAMKVIIGLSQEQDEIFRMLSAIIWIGNIQFCEDCGYAEVVDERSVVDFAAYLLEVD** 341
 BgMyo **INAMKVIIGLSQEQDEIFRMLSAIIWIGNIQFCEDCGYAEVVDERSVVDFAAYLLEVD** 221

FgMyo5 **QLIKGITIRILTPRNGEVIESEANFAQACATRDALAMAIYSNLFWDWIVERINKSLKARQP** 398
 FoMyo1 **QLIKGITIRILTPRNGEVIESEANFAQACATRDALAMAIYSNLFWDWIVERINKSLKARQP** 398
 BcMyo1 **QLIKGITIRILTPRNGEVIESEANFAQACATRDALAKAIYNNLFDWIVERINKSLKARQP** 399
 MgMyo1 **QLIKGITIRILTPRNGEVIESEANFAQACATRDALAKAIYNNLFDWIVERINKSLKARQP** 401
 BgMyo **QLIKGITIRILTPRNGEVIESEANFAQACATRDALAKAIYNNLFDWIVERINKSLKARQP** 281

418 420

FgMyo5 **TINTIGILDYGFIEFEKNSFEQLCINYNVEKLOQIFQLTLKAEQEEYAREQIQWTPPIK** 458
 FoMyo1 **TINTIGILDYGFIEFEKNSFEQLCINYNVEKLOQIFQLTLKAEQEEYAREQIQWTPPIK** 458
 BcMyo1 **TINTIGILDYGFIEFEKNSFEQLCINYNVEKLOQIFQLTLKAEQEEYAREQIQWTPPIK** 459
 MgMyo1 **TINTIGILDYGFIEFEKNSFEQLCINYNVEKLOQIFQLTLKAEQEEYAREQIQWTPPIK** 461
 BgMyo **TINTIGILDYGFIEFEKNSFEQLCINYNVEKLOQIFQLTLKAEQEEYAREQIQWTPPIK** 341

FgMyo5 **YFDNKVVDLIEQIREVGFISAMKDATKTAHADEAACDRTFMQSINGMSIAHLTPRQGNF** 518
 FoMyo1 **YFDNKVVDLIEQIREVGFISAMKDATKTAHADEAACDRTFMQSINGMSIAHLTPRQGNF** 518
 BcMyo1 **YFDNKVVDLIEQIREVGFISAMKDATKTAHADEAACDRTFMQSINGMSIAHLTPRQGNF** 519
 MgMyo1 **YFDNKVVDLIEQIREVGFISAMKDATKTAHADEAACDRTFMQSINGMSIAHLTPRQGNF** 521
 BgMyo **YFDNKVVDLIEQIREVGFISAMKDATKTAHADEAACDRTFMQSINGMSIAHLTPRQGNF** 401

FgMyo5 **IIKHYAGDVYTYTVEGITDKNKDQLKGLLALFQHSQNFVHTLFRFVDQDNRKQPPSAG** 578
 FoMyo1 **IIKHYAGDVYTYTVEGITDKNKDQLKGLLALFQHSQNFVHTLFRFVDQDNRKQPPSAG** 578
 BcMyo1 **IIKHYAGDVYTYTVEGITDKNKDQLKGLLALFQHSQNFVHTLFRFVDQDNRKQPPSAG** 579
 MgMyo1 **IIKHYAGDVYTYTVEGITDKNKDQLKGLLALFQHSQNFVHTLFRFVDQDNRKQPPSAG** 581
 BgMyo **IIKHYAGDVYTYTVEGITDKNKDQLKGLLALFQHSQNFVHTLFRFVDQDNRKQPPSAG** 461

SH1 helix

FgMyo5 **DRIRASANALVDTIMKQPSYIRTIKENENKSPTEYN**GENVLHQIKYLGLENVRRIRAG 638
 FoMyo1 **DRIRASANALVDTIMKQPSYIRTIKENENKSPTEYN**GENVLHQIKYLGLENVRRIRAG 638
 BcMyo1 **DRIRASANALVDTIMKQPSYIRTIKENENKSPTEYN**GENVLHQIKYLGLENVRRIRAG 639
 MgMyo1 **DRIRASANALVDTIMKQPSYIRTIKENENKSPTEYN**GENVLHQIKYLGLENVRRIRAG 641
 BgMyo **DRIRASANALVDTIMKQPSYIRTIKENENKSPTEYN**GENVLHQIKYLGLENVRRIRAG 521

FgMyo5 **FAYRQDFDKFVDRFFLLSEATSYAGEFTWECTTEAAVKQILKDTSIPEEWCQMGVTKAFI** 698
 FoMyo1 **FAYRQDFDKFVDRFFLLSEATSYAGEFTWECTTEAAVKQILKDTSIPEEWCQMGVTKAFI** 698
 BcMyo1 **FAYRQDFDKFVDRFFLLSEATSYAGEFTWECTTEAAVKQILKDTSIPEEWCQMGVTKAFI** 699
 MgMyo1 **FAYRQDFDKFVDRFFLLSEATSYAGEFTWECTTEAAVKQILKDTSIPEEWCQMGVTKAFI** 701
 BgMyo **FAYRQDFDKFVDRFFLLSEATSYAGEFTWECTTEAAVKQILKDTSIPEEWCQMGVTKAFI** 581

FgMyo5 **KAPETLFALEHMR** 711
 FoMyo1 **KAPETLFALEHMR** 711
 BcMyo1 **KAPETLFALEHMR** 712
 MgMyo1 **KAPETLFALEHMR** 714
 BgMyo **KAPETLFALEHMR** 594

Fig. S3. Relative expression of 10 differentially expressed genes as indicated by RNA-Seq and qRT-PCR. Values are means (\pm SE for qRT-PCR) of three repeated experiments.

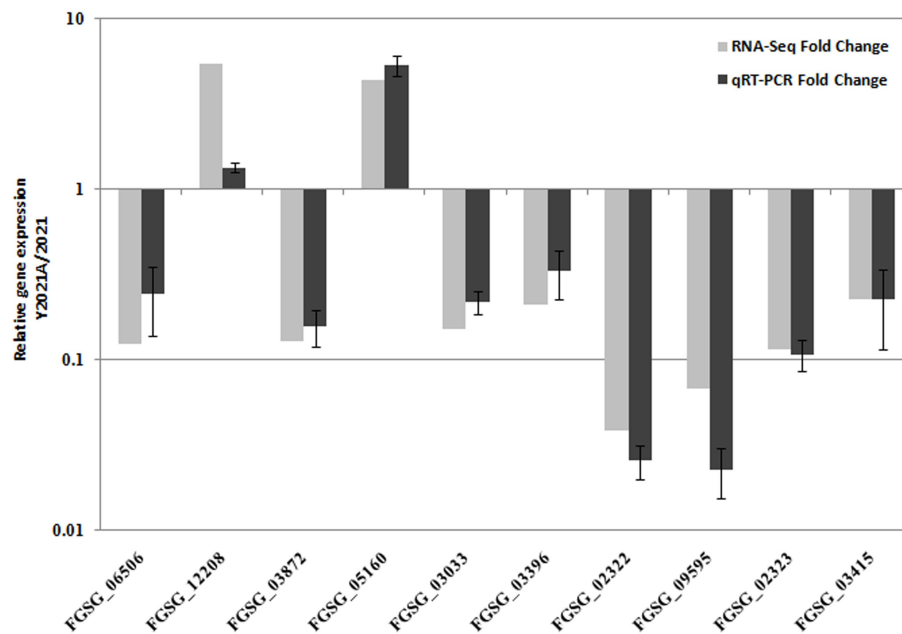


Fig. S4. Functional categorization of differentially expressed genes based on known genes in the Uniprot database. Gene Ontology (GO) terms at the 2nd level were plotted here, and in this ontology, “Cellular component”, “Molecular function”, and “Biological process” are categorized independently.

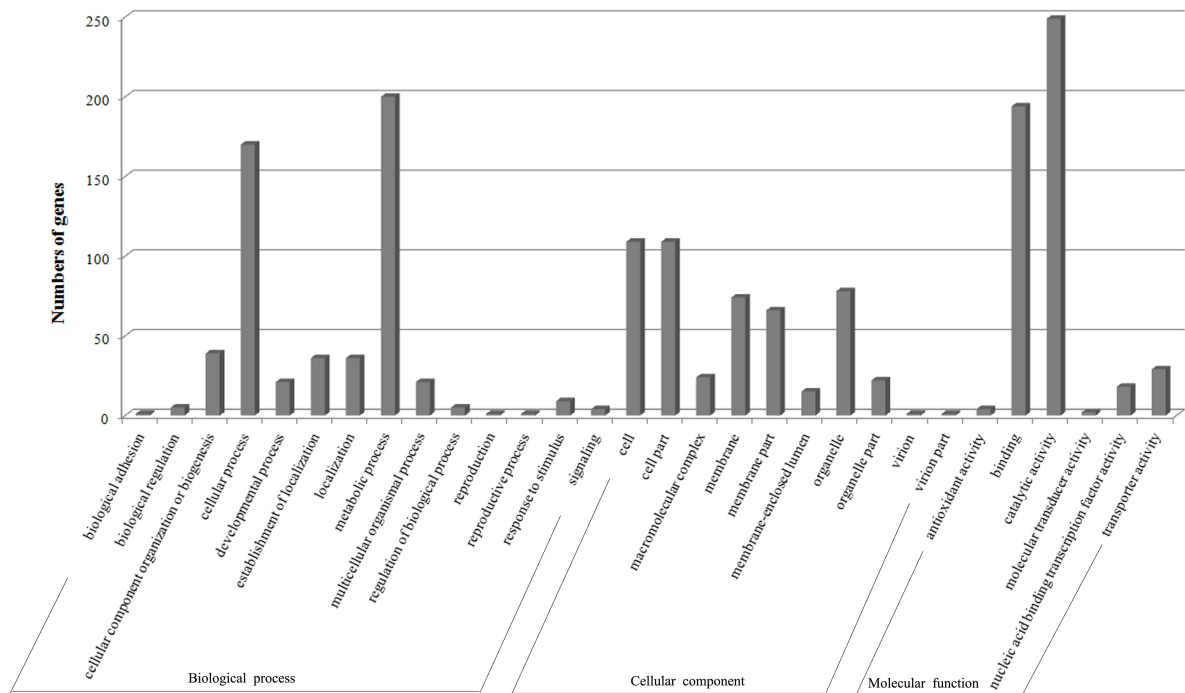


Fig. S5. Schematic representation of homology modelling of the *Fusarium graminearum* myosin-5 motor domain (A), general chemical structure of phenamacril (B), model of phenamacril (C), and ribbon models of myosin in *Dictyostelium discoideum* 4A7F (D). In (A) amino acids Lys216, Ser217, Ser418, and Glu420 of the myosin-5 motor domain are shown in yellow. Amino acids at these sites formed a similar ‘pocket’. In (C), blue and red represent nitrogen and oxygen atoms, respectively. In (D) Ribbon models of myosin in the crystal structure of the actin-tropomyosin-myosin complex in *Dictyostelium discoideum*, amino acids at sites 186, 187, 397 and 399 are conserved with that of the myosin-5 motor domain. These sites also formed a similar ‘pocket’.

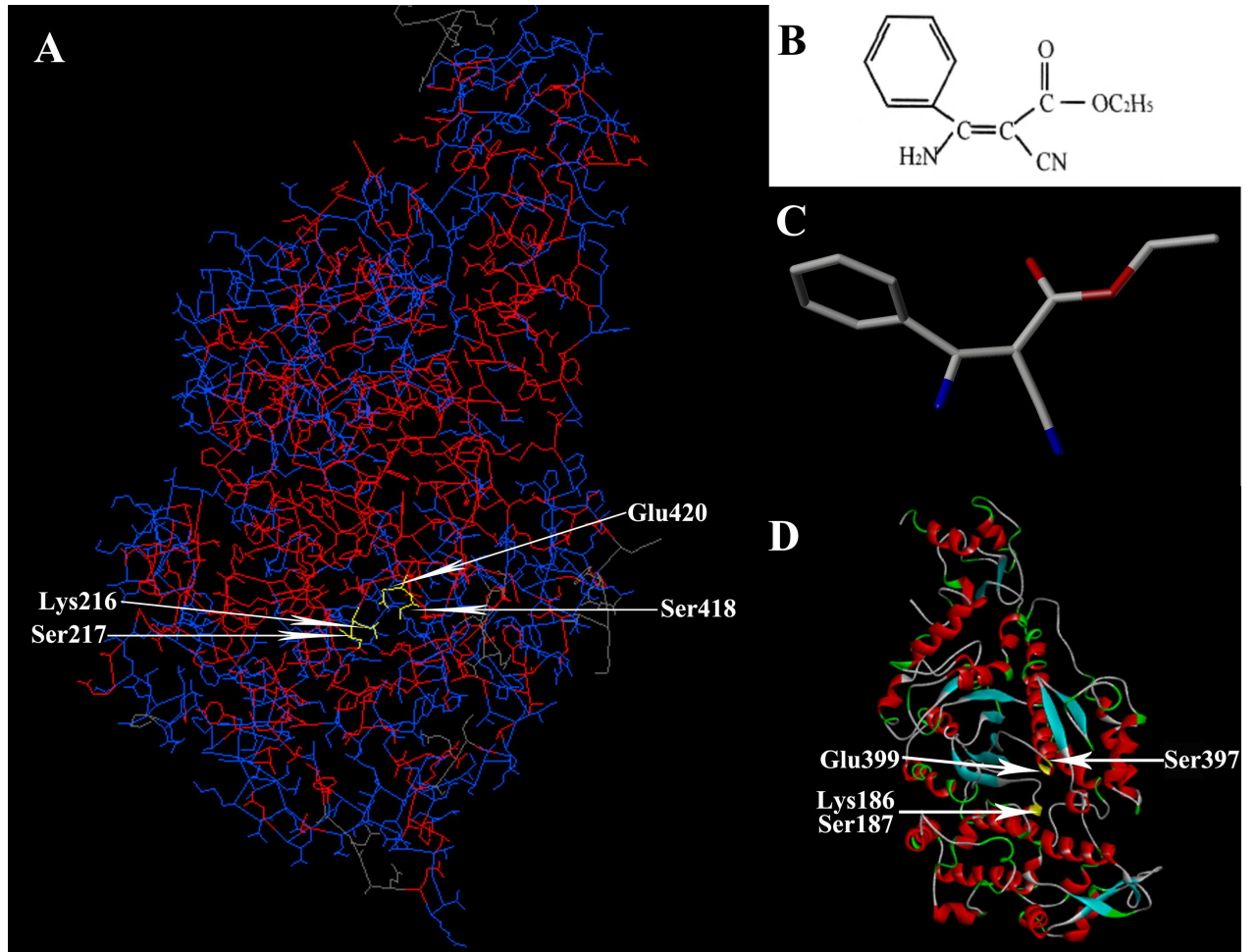


Table S1. Oligonucleotide primers used in this study.

Primer code	Primer	Sequence (5'-3')	Relevant characteristics
1	A1	TTGCGGTAGGAGAAGATG	PCR primers for amplification of the upstream <i>myosin-5</i> fragment
2	A2	AGCCAGCCAACAGCTCCCAAGGGAAACTCTGGAAGC	
3	A3	TACGCAAACCGCCTCTCCCCTCCTCCGTGCCGTGATA	PCR primers for amplification of the <i>myosin-5</i> mutant zone fragment
4	A4	TCGGCTGGGACTTGTGAA	A pair of PCR primers for identification of replacement integration at the left junction.
5	A5	CAGGCTTGGTCTGGTTGG	
6	A6	CCGTGGTTGGCTTGTATG	

7	A7	ATTTGGATGCTTGGGTAG	A pair of PCR primers for identification of replacement integration at the right junction.
8	A8	ATAGAAGCAGCGGCAGAG	
9	hphF	GGGAGCTGTTGGCTGGCTGGTGG	PCR primers for amplification of the 1.7-kb <i>hph</i> resistance gene containing the <i>Aspergillus nidulans</i> trpC promoter
10	hphR	GGGGAGAGGCGTTTGCCTATTG	
11	myo5-prob eF	GTTTACCGACATTACCGC	PCR primers to amplify the 622-bp myosin-5 upstream fragment used as probe for Southern blot
12	myo5-prob eR	TTCTAGTGGTGCCCTGACG	
13	Myo5-F	TCACAAGTCCCAGCCGATTA	Quantitative real-time PCR primers for analysis of <i>myosin-5</i> expression
14	Myo5-R	GGTTGCCTTGAATGCGAGA	
15	Myo2B-F	GCAGTCCCTCGGTTCTATGA	Quantitative real-time PCR primers for analysis of <i>myosin-2B</i> expression
16	Myo2B-R	CTGGTTGGCTTCCGTCTGT	
17	08719cF	GCCTTGGACAGGGTTTCATT	Quantitative real-time PCR primers for analysis of <i>type II myosin</i> expression
18	08719cR	TCCTCCTCAGATTCGGCAC	
19	GAPDH-F	CTTACTGCCTCCACCAACTG	Quantitative real-time PCR primers for analysis of the elongation factor 1-alpha gene expression
20	GAPDH-R	TGACGTTGGAAGGAGCGAAG	
21	01099F	GACCGCTCGCTCAACAGA	PCR primers for amplification of the FGSG_01099 fragment
22	01099R	CCATAATCACGCTGCTGT	
23	01234F	CGCTCATCACTCGGCTAC	PCR primers for amplification of the FGSG_01234 fragment
24	01234R	AGTCTGGCAACAGCATCG	
25	01410F	TCCCGTCAGGCACCACTA	PCR primers for amplification of the FGSG_01410 fragment
26	01410R	CGGTTGCCAGTTCAGTC	
27	01675F	CACTGGTTACGCTTCTCG	PCR primers for amplification of the FGSG_01675 fragment
28	01675R	GTCAAGGCGATGAGGATA	
29	02501F	TCCCTCCTCATCTCAACG	PCR primers for amplification of the FGSG_02501 fragment
30	02501R	TCAAGTGTAAGGGCGGTA	
31	03065F	CCAAGACCTGGGAACATC	PCR primers for amplification of the FGSG_03065 fragment
32	03065R	GTACTTGGAGTGCTGGGT	
33	03772F	ATGCCTCAGACGACAACA	PCR primers for amplification of the FGSG_03772 fragment
34	03772R	GCAAATCTGCCTGGGACT	
35	05615F	TCTGCTCCCCGCCACAAA	PCR primers for amplification of the FGSG_05615 fragment
36	05615R	TGGCATCGTTGGGCATAG	
37	05894F	GCCGAGACAATGCCACA	PCR primers for amplification of the FGSG_05894 fragment
38	05894R	AGAGCAATGGTGGTGTCC	
39	06998F	ACACTCGTTGGCTCTTGG	PCR primers for amplification of the FGSG_06998 fragment
40	06998R	TGCAACCCTAACTATCCC	
41	09397R	AGGCTTTGACAGGCTCCA	PCR primers for amplification of the FGSG_09397 fragment
42	09397R	GCTCAGTTCTCCTCGTC	
43	09895F	GTCCGTGCCCTATCCTGT	PCR primers for amplification of the FGSG_09895 fragment
44	09895R	TTCGCTCCGTCACAAACA	
45	10358F	GCCAGTTGGTGTCTTCT	PCR primers for amplification of the FGSG_10358 fragment
46	10358R	TTCGAAACAAGGAATGA	

47	11973F	CCTCGTTCCTTGCTGTA	PCR primers for amplification of the
48	11973R	CGACCATACGCCAGTTGA	FGSG_11973 fragment
49	12039F	TACCTGCGTTGAGGAAGA	PCR primers for amplification of the
50	12039R	CTTTCGCCAGCAAACCTCT	FGSG_12039 fragment
51	12129F	CTGTTGCGGTGGATGTCA	PCR primers for amplification of the
52	12129R	CCAAACGCCAACTGACTG	FGSG_12129 fragment
53	12369F	GTTCTCGCCACAGTTCAA	PCR primers for amplification of the
54	12369R	TGGTAGAACGGTCGGTGG	FGSG_12369 fragment
55	12745F	GGACCACCTTATTACCGC	PCR primers for amplification of the
56	12745R	GCGGATGTTGTTGCGTAG	FGSG_12745 fragment
57	13343F	GCACTGGTTCGTCGGTAG	PCR primers for amplification of the
58	13343R	GCAGAGGTCCGAGTTAGA	FGSG_13343 fragment
59	13620F	AGTTTCGTTCTTTCCCTC	PCR primers for amplification of the
60	13620R	AATGGAGACAATCTACCG	FGSG_13620 fragment
61	13823F	GATGGTGGACAAGATGAC	PCR primers for amplification of the
62	13823R	TCCAGCCTTGAAATACTC	FGSG_13823 fragment
63	13897F	CGCAAGGACACTACCAAC	PCR primers for amplification of the
64	13897R	AGAGCAAGGAGCCAATCA	FGSG_13897 fragment
65	02322rtF	TCTGGTGGTGCATCAACGG	Quantitative real-time PCR primers for
66	02322rtR	ATAAGACCGCCACCGACTGA	analysis of FGSG_02322 expression
67	02323rtF	CAGCAAGCACAATACCGCAG	Quantitative real-time PCR primers for
68	02323rtR	TGGGATGGTAAACCGAGAGC	analysis of FGSG_02323 expression
69	03033rtF	GCAATGTGCTTCAGTTTCGG	Quantitative real-time PCR primers for
70	03033rtR	AACAACAGCCATCGCATACC	analysis of FGSG_03033 expression
71	03396rtF	CGTCGGTGTATCAGTGCCA	Quantitative real-time PCR primers for
72	03396rtR	CAAATCGTCCTGCCAATGCT	analysis of FGSG_03396 expression
73	03415rtF	GCATCGCAAATGGCAGTG	Quantitative real-time PCR primers for
74	03415rtR	AGCATTGCGGAGCGACATAG	analysis of FGSG_03415 expression
75	03872rtF	ATCGCTGGTATCGGTTTCGTG	Quantitative real-time PCR primers for
76	03872rtR	CGGAGTGCTGAGGATGAATG	analysis of FGSG_03872 expression
77	05160rtF	CTTTGCTGGCTGCTGTGTTG	Quantitative real-time PCR primers for
78	05160rtR	ATAGCGTTGAAGATGCCCCA	analysis of FGSG_05160 expression
79	06506rtF	TGGTTACGCCAATGTTTCCG	Quantitative real-time PCR primers for
80	06506rtR	CAGCGTTGGCAGCATAGTTC	analysis of FGSG_06506 expression
81	09595rtF	GCAACTGGCGACTCATTCTG	Quantitative real-time PCR primers for
82	09595rtR	TGAGCACGAGGGGAATGGT	analysis of FGSG_09595 expression
83	12208rtF	GGACACTCCCGACAACACA	Quantitative real-time PCR primers for
84	12208rtR	AAGCCCACGATTTCTTGTTG	analysis of FGSG_12208 expression

Table S2. Statistics of 132 genes with amino acid mutations.

Gene	Description	Synonymous SNPs	Non-synonymous SNPs
FGSG_00100	hypothetical protein	1	0
FGSG_00180	hypothetical protein	1	3
FGSG_00410	hypothetical protein	0	1
FGSG_00554	hypothetical protein	0	1
FGSG_00614	hypothetical protein	2	2
FGSG_00727	hypothetical protein	2	11
FGSG_00924	hypothetical protein	0	1
FGSG_00925	hypothetical protein	0	2
FGSG_01099	GTP-binding nuclear protein GSP1/Ran	0	2
FGSG_01116	hypothetical protein	0	1
FGSG_01156	hypothetical protein	0	1
FGSG_01163	hypothetical protein	2	5
FGSG_01234	adenylate cyclase	0	2
FGSG_01410	myosin-5	0	1
FGSG_01434	hypothetical protein	0	2
FGSG_01501	hypothetical protein	1	2
FGSG_01545	hypothetical protein	0	1
FGSG_01641	hypothetical protein	0	1
FGSG_01675	dicarboxylic amino acid permease	0	2
FGSG_01899	hypothetical protein	0	1
FGSG_01927	hypothetical protein	0	1
FGSG_01981	hypothetical protein	0	2
FGSG_02229	hypothetical protein	1	2
FGSG_02501	transcriptional repressor rco-1	1	3
FGSG_02594	hypothetical protein	0	1
FGSG_02679	hypothetical protein	0	1
FGSG_02799	hypothetical protein	0	1
FGSG_02896	hypothetical protein	0	1
FGSG_03065	phytoene dehydrogenase	0	6
FGSG_03191	hypothetical protein	0	2
FGSG_03550	hypothetical protein	2	4
FGSG_03772	drug resistance protein	0	1
FGSG_04091	hypothetical protein	0	1
FGSG_04189	hypothetical protein	3	4
FGSG_04370	hypothetical protein	0	2
FGSG_04373	hypothetical protein	0	1
FGSG_04439	hypothetical protein	0	2
FGSG_04871	hypothetical protein	1	2
FGSG_04890	hypothetical protein	0	3
FGSG_04987	hypothetical protein	0	2
FGSG_05347	hypothetical protein	5	5

FGSG_05615	adenosylhomocysteinase	0	1
FGSG_05794	hypothetical protein	2	4
FGSG_05883	hypothetical protein	0	2
FGSG_05894	serine/threonine protein phosphatase 2A	1	2
FGSG_06175	hypothetical protein	0	1
FGSG_06302	hypothetical protein	0	1
FGSG_06431	hypothetical protein	0	2
FGSG_06445	hypothetical protein	0	1
FGSG_06998	glucan synthesis regulatory protein	4	12
FGSG_07013	hypothetical protein	0	1
FGSG_07078	hypothetical protein	0	1
FGSG_07133	hypothetical protein	0	1
FGSG_07525	hypothetical protein	2	5
FGSG_07526	hypothetical protein	0	1
FGSG_07562	hypothetical protein	0	2
FGSG_07807	hypothetical protein	0	1
FGSG_08249	hypothetical protein	0	1
FGSG_08608	hypothetical protein	0	1
FGSG_08701	hypothetical protein	0	1
FGSG_08759	hypothetical protein	0	1
FGSG_08842	hypothetical protein	0	5
FGSG_08955	hypothetical protein	2	3
FGSG_09281	hypothetical protein	2	4
FGSG_09318	hypothetical protein	0	3
FGSG_09329	hypothetical protein	0	2
FGSG_09397	glycylpeptide N-tetradecanoyltransferase	0	2
FGSG_09478	hypothetical protein	1	1
FGSG_09605	hypothetical protein	0	2
FGSG_09686	hypothetical protein	2	1
FGSG_09762	hypothetical protein	2	1
FGSG_09876	hypothetical protein	0	1
FGSG_09889	hypothetical protein	0	2
FGSG_09895	neutral trehalase	0	1
FGSG_09950	hypothetical protein	0	2
FGSG_10075	hypothetical protein	2	2
FGSG_10333	hypothetical protein	1	1
FGSG_10351	hypothetical protein	0	1
FGSG_10357	hypothetical protein	0	4
FGSG_10358	GMP synthase	5	3
FGSG_10488	hypothetical protein	0	1
FGSG_10577	hypothetical protein	1	1
FGSG_10620	hypothetical protein	3	3

FGSG_10807	hypothetical protein	0	2
FGSG_10812	hypothetical protein	0	2
FGSG_10932	hypothetical protein	0	1
FGSG_11097	hypothetical protein	0	1
FGSG_11615	hypothetical protein	1	2
FGSG_11638	hypothetical protein	0	1
FGSG_11673	hypothetical protein	0	1
FGSG_11710	hypothetical protein	0	1
FGSG_11862	hypothetical protein	1	2
FGSG_11884	hypothetical protein	0	1
FGSG_11912	hypothetical protein	2	3
FGSG_11936	hypothetical protein	1	3
FGSG_11973	phosphate-repressible phosphate permease	1	1
FGSG_11981	hypothetical protein	0	2
FGSG_12017	hypothetical protein	0	1
FGSG_12039	chitin synthase 6	0	1
FGSG_12040	hypothetical protein	1	1
FGSG_12086	hypothetical protein	1	1
FGSG_12129	peroxisomal copper amine oxidase	0	4
FGSG_12178	hypothetical protein	0	2
FGSG_12270	hypothetical protein	0	2
FGSG_12323	hypothetical protein	2	1
FGSG_12369	peroxidase/catalase 2	0	1
FGSG_12439	hypothetical protein	3	3
FGSG_12492	hypothetical protein	0	4
FGSG_12595	hypothetical protein	0	2
FGSG_12745	heat shock protein HSP98	0	1
FGSG_12781	hypothetical protein	0	2
FGSG_12827	hypothetical protein	0	1
FGSG_12946	hypothetical protein	0	1
FGSG_12980	hypothetical protein	0	1
FGSG_12985	hypothetical protein	0	1
FGSG_13148	hypothetical protein	0	1
FGSG_13181	hypothetical protein	3	1
FGSG_13292	hypothetical protein	0	1
FGSG_13342	hypothetical protein	1	1
FGSG_13343	glycerol kinase	0	1
FGSG_13561	hypothetical protein	0	2
FGSG_13581	hypothetical protein	0	1
FGSG_13620	alpha-1,2 mannosyltransferase KTR1	0	1
FGSG_13634	hypothetical protein	0	2
FGSG_13710	hypothetical protein	0	1

FGSG_13715	hypothetical protein	0	4
FGSG_13823	aconitate hydratase	1	1
FGSG_13897	amino-acid permease inda1	0	1
FGSG_13946	hypothetical protein	0	3
FGSG_13966	hypothetical protein	1	2
FGSG_13987	hypothetical protein	0	5
FGSG_14031	hypothetical protein	0	3

Table S3. Statistics of base variable frequency in *Fusarium graminearum* sequencing resistant strain YP-1.

Base changes	Numbers of site
A/C	123
A/G	336
A/T	123
G/A	213
G/T	105
G/C	105
C/G	126
C/T	215
C/A	104
T/A	104
T/C	336
T/G	99

Table S4. *Fusarium graminearum* strains used in this study.

Strain or mutant	Genotype	Reference
2021	Wild-type, phenamacril-sensitive strain	47
PH-1	Reference strain	41
Y2021A-10 ² -myo5	Myosin-5 replaced by 2021's myosin-5 fragment mutant in Y2021A genetic background	This study
Y2021A-24 ^F -myo5	Myosin-5 replaced by Y2021F's myosin-5 fragment mutant in Y2021A genetic background	This study
YP-1	Phenamacril-resistant strain generated from the reference strain PH-1 by fungicide treatment	This study
Y2021A		47
Y2021B		This study
Y2021C	Phenamacril-resistant strains generated from the wild-type strain 2021 by fungicide treatment	This study
Y2021D		This study
Y2021F		This study
2021-12 ^A -myo5	Myosin-5 replaced by Y2021A's myosin-5 fragment mutant in 2021 genetic background	This study
2021-22 ^B -myo5	Myosin-5 replaced by Y2021B's myosin-5 fragment mutant in 2021 genetic background	This study
2021-9 ^C -myo5	Myosin-5 replaced by Y2021C's myosin-5 fragment mutant in 2021 genetic background	This study
2021-19 ^D -myo5	Myosin-5 replaced by Y2021D's myosin-5 fragment mutant in 2021 genetic background	This study
2021-13 ^F -myo5	Myosin-5 replaced by Y2021F's myosin-5 fragment mutant in 2021 genetic background	This study

Table S5. Pathway of the more differentially expressed genes based on the KEGG database.

Pathway	Up-regulated genes	Down-regulated genes
Drug metabolism cytochrome P450	FGSG_07685	FGSG_02291, FGSG_04214, FGSG_03915, FGSG_10200, FGSG_05382, FGSG_11097, FGSG_04194
Microbial metabolism in diverse environments	FGSG_13963, FGSG_07685, FGSG_12344, FGSG_11542, FGSG_02217, FGSG_13962, FGSG_07080, FGSG_02906, FGSG_07838, FGSG_00489, FGSG_01692, FGSG_03996,	FGSG_02291, FGSG_10198, FGSG_01581, FGSG_05623, FGSG_09572, FGSG_04214, FGSG_08875, FGSG_00321, FGSG_13444, FGSG_08463, FGSG_10120, FGSG_08443, FGSG_01433, FGSG_00810, FGSG_09280, FGSG_05048, FGSG_02063, FGSG_00175, FGSG_09913, FGSG_03402, FGSG_02482, FGSG_03915, FGSG_01698, FGSG_10200, FGSG_06681, FGSG_05382, FGSG_04196, FGSG_05130, FGSG_06290, FGSG_02974, FGSG_08941, FGSG_11332, FGSG_04194, FGSG_10893, FGSG_03590, FGSG_13714
Cyanoamino acid metabolism	FGSG_00596, FGSG_10659,	FGSG_12231, FGSG_10250, FGSG_06480, FGSG_00313, FGSG_03387, FGSG_01685, FGSG_10859, FGSG_01698, FGSG_09141, FGSG_06290
Butanoate metabolism	FGSG_13963, FGSG_13962,	FGSG_03936, FGSG_05554, FGSG_09494, FGSG_01086, FGSG_06342, FGSG_05128, FGSG_01581, FGSG_00321, FGSG_04103, FGSG_06282, FGSG_00600, FGSG_05048, FGSG_02063, FGSG_04196, FGSG_08941
Aminobenzoate degradation	FGSG_07080, FGSG_07838, FGSG_00489, FGSG_03996,	FGSG_02824, FGSG_04302, FGSG_04736, FGSG_07678, FGSG_03199, FGSG_13176, FGSG_10250, FGSG_13444, FGSG_10120, FGSG_09913, FGSG_03402, FGSG_01698
Bisphenol degradation	FGSG_13962, FGSG_07080,	FGSG_02824, FGSG_04302,

		FGSG_00489,		FGSG_09494, FGSG_04736, FGSG_05128, FGSG_00321, FGSG_10120, FGSG_05048, FGSG_02063, FGSG_09913, FGSG_08941
Ribosome eukaryotes	biogenesis in	FGSG_00270, FGSG_02511, FGSG_04424, FGSG_01386, FGSG_00892, FGSG_07262, FGSG_01185, FGSG_07055, FGSG_07258, FGSG_00485, FGSG_06340, FGSG_00732, FGSG_09795, FGSG_05081, FGSG_06070, FGSG_10033, FGSG_06788, FGSG_07206, FGSG_01102, FGSG_10908, FGSG_07379, FGSG_08759, FGSG_10940, FGSG_10219, FGSG_06165, FGSG_01870,		FGSG_03933, FGSG_02500
Methane metabolism		FGSG_07685, FGSG_02217, FGSG_01692,		FGSG_07277, FGSG_00330, FGSG_07127, FGSG_06127, FGSG_02427, FGSG_00475, FGSG_09572, FGSG_00810, FGSG_09280, FGSG_03915, FGSG_10200, FGSG_06681, FGSG_06290, FGSG_02974, FGSG_10893
Glycine, serine and threonine metabolism		FGSG_12344, FGSG_03626, FGSG_07266, FGSG_04468, FGSG_06544, FGSG_01692, FGSG_08407,		FGSG_02357, FGSG_12373, FGSG_08055, FGSG_03638, FGSG_00118, FGSG_02427, FGSG_05623, FGSG_00810, FGSG_10743, FGSG_06290, FGSG_10893
Peroxisome		FGSG_11413, FGSG_02217, FGSG_04333,		FGSG_11069, FGSG_04217, FGSG_05011, FGSG_02287, FGSG_10672, FGSG_07173, FGSG_07104, FGSG_05596, FGSG_06012, FGSG_09424, FGSG_09263, FGSG_10893, FGSG_00840, FGSG_05379, FGSG_09756

Table S6. Transcription data for myosin-5, myosin-2B, and type II myosin for strains 2021 and Y2021A of *F. graminearum* in RNA-seq.

Gene	Description	Length^a	Fold-change Y2021A/2021^b	Fold-change 2021+/2021^c	Fold-change Y2021A+/Y2021A^d
FGSG_01410	Myosin-5	3645	-1.18	1.07	1.49
FGSG_07469	Myosin-2B	4752	1.11	1.27	1.51
FGSG_08719	Type II myosin	7029	-1.29	1.15	1.72

^a mRNA length in bp.

^b The fold-change is the ratio of RPKM Y2021A to RPKM 2021.

^c The fold-change is the ratio of RPKM 2021+ to RPKM 2021. 2021+ represents sensitive strain 2021 treated with 0.21 µg/ml JS399-19 (EC₅₀).

^d The fold-change is the ratio of RPKM Y2021A+ to RPKM Y2021A. Y2021A+ represents resistant strain Y2021A treated with 204 µg/ml JS399-19 (EC₅₀).