The chitin synthase FgChs2 and other FgChss co-regulate vegetative development and virulence in *F. graminearum*

Zunyong Liu[1], Xiaoping Zhang[1], Xin Liu[2], Chaoyu Fu[1], Xinyue Han[1], Yanni Yin [1]& Zhonghua Ma[1,3]

[1]Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China. [2]Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, 210014, China. [3]State Key Laboratory of Rice Biology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China. Correspondence and requests for materials should be addressed to Yanni Yin (email: ynyin@zju.edu.cn) or Zhonghua Ma (zhma@zju.edu.cn).

SUPPORTING INFORMATION

Figure S1. Generation and identification of the *FgChs* single and double deletion mutants, and the complemented strain. **(a)** Gene deletion strategy for *FgChs2*. The hygromycin resistance cassette (*HPH*) is denoted by the large black arrow. Primer binding sites are indicated by arrows (see Table S1 for the primer sequences). **(b)** Southern blot hybridization analysis of *FgChs2* locus in the wild-type PH-1, *FgChs2* deletion mutant ΔFgChs2, and the complemented strain ΔFgChs2-C, using the 1.1-kb upstream fragment of *FgChs2* as a probe. Genomic DNA preparation of each strain was digested with *Pvu* I. **(c)** Southern blot hybridization analysis of *NEO* locus in PH-1 and ΔFgChs2-C using the 1.1-kb *NEO* fragment as a probe. Genomic DNA preparation of each strain was digested with *Stu* I. **(d)** Gene deletion strategy for *FgChs1*, *FgChs3a*, *FgChs4*, *FgChs7*, *FgChs5* and *FgChs6*. Genomic DNA preparations from deletion mutants ΔFgChs1, ΔFgChs3a, ΔFgChs4, ΔFgChs7, ΔFgChs5 and ΔFgChs6 were digested with *Stu* I, *Hind* III, *Xho* I, *Stu* I, *Nru* I and *EcoR* V respectively. Genomic DNA preparations from double deletion mutants ΔFgChs2/1, ΔFgChs2/3a, ΔFgChs2/4, ΔFgChs2/7, ΔFgChs2/5 and ΔFgChs2/6 were digested with *Stu* I, *Hind* III, *Xho* I, *Xho* I, *Bgl* II and *Nru* I respectively. **(e)** The 1.3-kb *HPH* fragment was used as a probe in the Southern blot hybridization analyses of the single deletion mutants ΔFgChs1, ΔFgChs4, ΔFgChs7, ΔFgChs5 and ΔFgChs6. **(f)** The 1.1-kb *NEO* fragment was used as a probe in the Southern blot hybridization analyses of the double deletion mutants ΔFgChs2/1, ΔFgChs2/3a, ΔFgChs2/4, ΔFgChs2/7, ΔFgChs2/5 and ΔFgChs2/6.

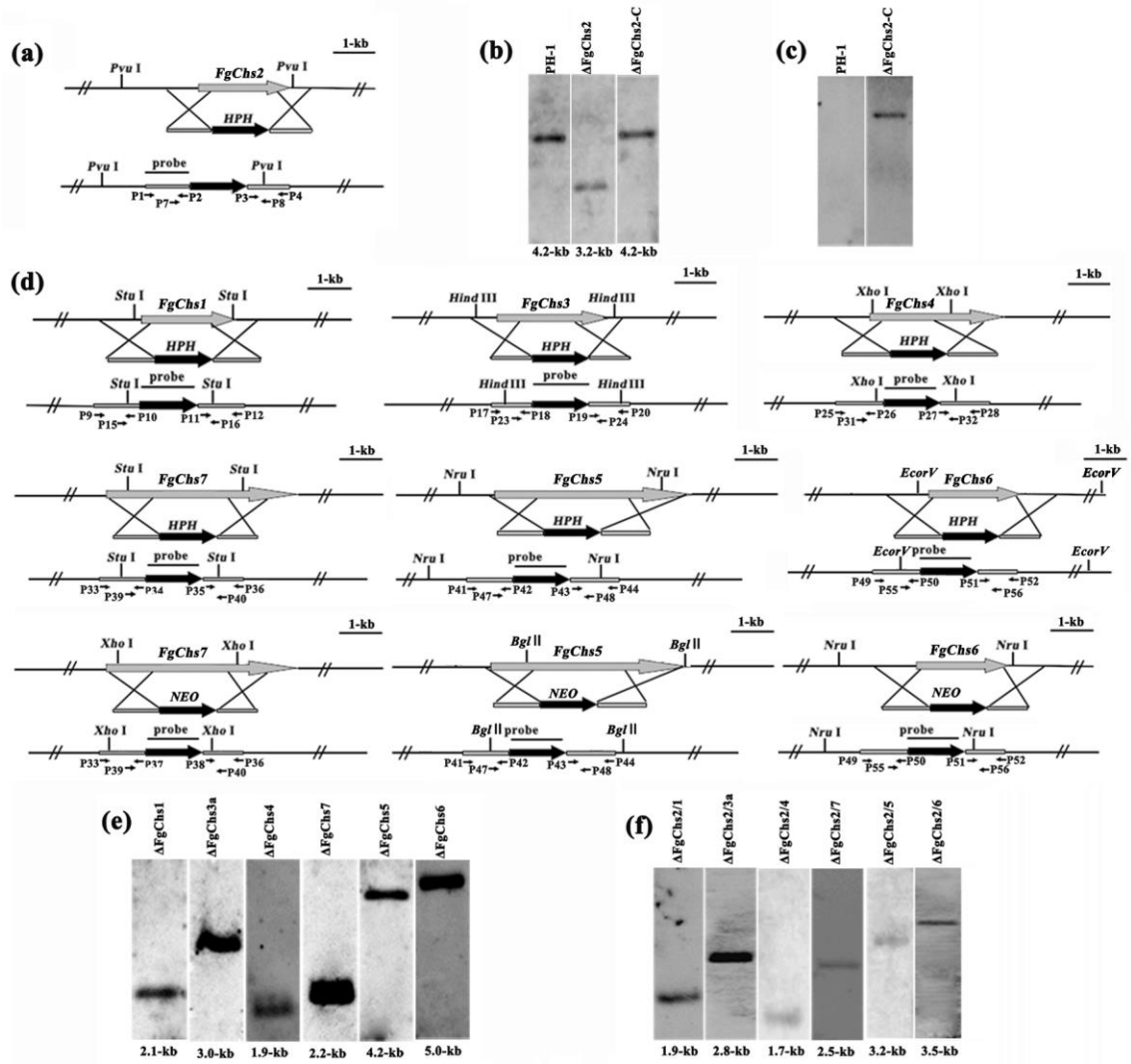
(a)
1-kb
Pvu I
FgChs2
Pvu I
HPH
Pvu I
probe
Pvu I
P1
P7
P2
P3
P8
P4
(b)
PH-1
ΔFgChs2
ΔFgChs2-C
4.2-kb
3.2-kb
4.2-kb
(c)
PH-1
ΔFgChs2-C
(d)
1-kb
Stu I
FgChs1
Stu I
HPH
Stu I
probe
Stu I
P9
P15
P10
P11
P16
P12
Hind III
FgChs3
Hind III
HPH
Hind III
probe
Hind III
P17
P23
P18
P19
P24
P20
Xho I
FgChs4
Xho I
HPH
Xho I
probe
Xho I
P25
P31
P26
P27
P32
P28
Stu I
FgChs7
Stu I
HPH
Stu I
probe
Stu I
P33
P39
P34
P35
P40
P36
Nru I
FgChs5
Nru I
HPH
Nru I
probe
Nru I
P41
P47
P42
P43
P48
P44
EcorV
FgChs6
EcorV
HPH
EcorV
probe
EcorV
P49
P55
P50
P51
P56
P52
Xho I
FgChs7
Xho I
NEO
Xho I
probe
Xho I
P33
P39
P37
P38
P40
P36
Bgl II
FgChs5
Bgl II
NEO
Bgl II
probe
Bgl II
P41
P47
P42
P43
P48
P44
Nru I
FgChs6
Nru I
NEO
Nru I
probe
Nru I
P49
P55
P50
P51
P56
P52
(e)
ΔFgChs1
ΔFgChs3a
ΔFgChs4
ΔFgChs7
ΔFgChs5
ΔFgChs6
2.1-kb
3.0-kb
1.9-kb
2.2-kb
4.2-kb
5.0-kb
(f)
ΔFgChs2/1
ΔFgChs2/3a
ΔFgChs2/4
ΔFgChs2/7
ΔFgChs2/5
ΔFgChs2/6
1.9-kb
2.8-kb
1.7-kb
2.5-kb
3.2-kb
3.5-kb

Figure S2. Effect of *FgChs* deletion on conidial germination in *F. graminearum*. Comparisons in conidial germination among the wild-type PH-1, *FgChs* deletion mutants (ΔFgChs1-7), double deletion mutants of *FgChs2* and other *FgChss* (ΔFgChs2/1-7), and the complemented strain ΔFgChs2-C. After incubation in 2% (w/v) sucrose for 4 hrs, conidial germination of 150 conidia was examined for each strain.

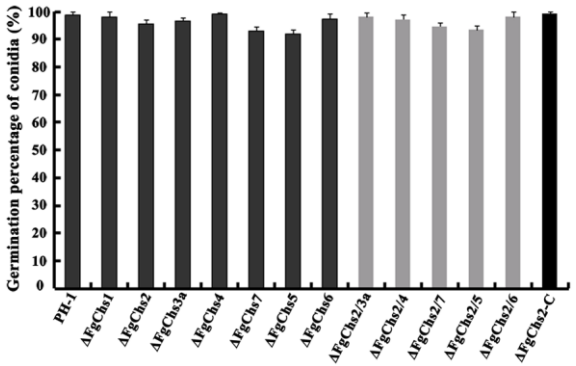

Figure S3. Penetration assays of *FgChs7*, *FgChs5*, *FgChs2/1*, *FgChs2/7* and *FgChs2/5* deletion mutants into cellophane membrane. Fungal colonies of each strain were grown at 25 ℃ for 3 days on top of cellophane membranes placed on minimal medium (Before). And then, the cellophane membranes with the fungal colonies were removed, and the plates were incubated at 25 ℃ for two additional days to examine the presence of mycelial growth on the plate, indicating penetration of the cellophane (After).

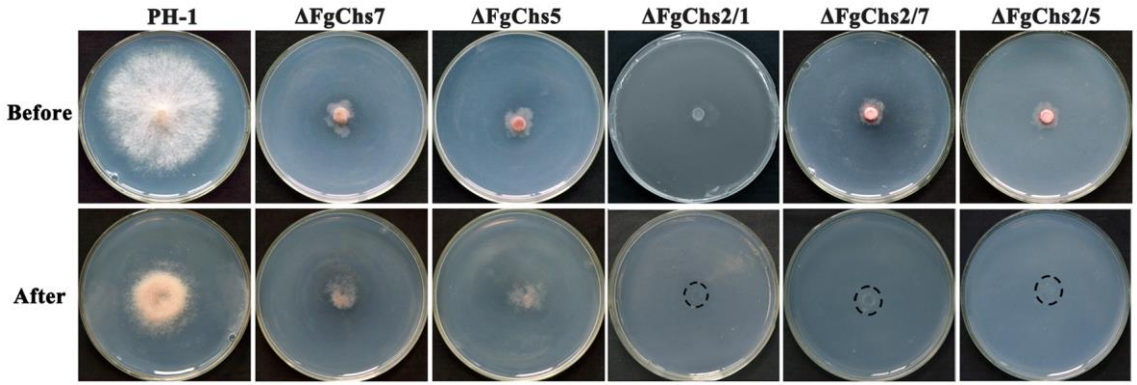

Table S1 PCR primers used in this study

Primer	Sequence (5′-3′)	Relevant characteristics
P1	CAACACCTTCTCCTCTCAACC	PCR primers to amplify *FgChs2* upstream fragment for the construction of *FgChs2* deletion mutants
P2	CAAAATAGGCATTGATGTGTTGACCTCCTTGTCGGGATCTTACACTCCA	
P3	CTCGTCCGAGGGCAAAGGAATAGAGTAGTATTCTCGGCATGGCTGTGT	PCR primers to amplify *FgChs2* downstream fragment for the construction of *FgChs2* deletion mutants
P4	TGAGCGCTTCGGTGACTTCT	
P5	AGACACACTAAACGAAGGCGA	PCR primers for identification of *FgChs2* deletion transformants
P6	TTGATCACATTAAGGATGGCA	
P7	ACACTCAAGCGAACATTCACA	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs2* deletion mutants
P8	TGGTTATAGCATGGAACTGGG	
P9	TTACGCATCTCTTCTCGCTGA	PCR primers to amplify *FgChs1* upstream fragment for the construction of *FgChs1* deletion mutants
P10	CAAAATAGGCATTGATGTGTTGACCTCCTACAGCTGCGTTGAGGCTCA	
P11	CTCGTCCGAGGGCAAAGGAATAGAGTAGGACTTGGATGCGCAATATCA	PCR primers to amplify *FgChs1* downstream fragment for the construction of *FgChs1* deletion mutants
P12	CTTTGCAAACTCCTTCACCTC	
P13	TTCAATTGCCTTCAGACGAC	PCR primers for identification of *FgChs1* deletion transformants
P14	ATCCAAAGAAGGACAACACCG	
P15	TCTGTGCATTCATCGTACCA	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs1* deletion mutants
P16	TTTTGCGGGATGTTATGTGG	
P17	GGATGGAGGGATGGAAATGTA	PCR primers to amplify *FgChs3a* upstream fragment for the construction of *FgChs3a* deletion mutants
P18	CAAAATAGGCATTGATGTGTTGACCTCCTGTACGGTGCATGACGGAAA	
P19	CTCGTCCGAGGGCAAAGGAATAGAGTAGCCCATGTATTTCCGTTTCCT	PCR primers to amplify *FgChs3a* downstream fragment for the construction of *FgChs3a* deletion mutants
P20	TCGGAAGTTTCACATTCAAAG	
P21	TCATTATCCTCCTGACCTCCA	PCR primers for identification of *FgChs3a* deletion transformants
P22	AAGACATTCATCCCCAAGAAG	
P23	AAAGAACGAGGTTTCTTGCCA	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs3a* deletion mutants
P24	TTCGAGTGGTTTCGGGAGTTT	
P25	TTCAATCGCCATCTCTCGTT	PCR primers to amplify *FgChs4* upstream fragment for the construction of *FgChs4* deletion mutants
P26	CAAAATAGGCATTGATGTGTTGACCTCCTGTTTCTCTCAGGACGAATCA	
P27	CTCGTCCGAGGGCAAAGGAATAGAGTAGACCGATTATCCCAACAGTCAC	PCR primers to amplify *FgChs4* downstream fragment for the construction of

P28	TTCCAGATTGGCAATGCAAG	*FgChs4* deletion mutants
P29	ACAATCAGGCTTCACACCAA	PCR primers for identification of *FgChs4* deletion transformants
P30	TTCAGCATACCCAAGCAGACA	
P31	TTCGCTCTCGTCATTGATCAC	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs4* deletion mutants
P32	AAAGATCACGGACCAGAACCA	
P33	TGTCCATGTATTCGATGGCCT	PCR primers to amplify *FgChs7* upstream fragment for the construction of *FgChs7* deletion mutants
P34	CAAAATAGGCATTGATGTGTTGACCTCCTCTCAGCGTTAATGCCCAAA	
P35	CTCGTCCGAGGGCAAAGGAATAGAGTAGGCAACGTTGTTCCATATATCG	PCR primers to amplify *FgChs7* downstream fragment for the construction of *FgChs7* deletion mutants
P36	AAGTTGTCCTGGTTCCAGAAG	
P37	CTCCAAACGAAAACGAGGAT	PCR primers for identification of *FgChs7* deletion transformants
P38	CACGGTTCAGGAAGCTCATAA	
P39	TGCTGAAGATGGATGTGTTGT	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs7* deletion mutants
P40	GGTAACCAATGTAGACGGTGG	
P41	TACCATAACCCACCGAATCGT	PCR primers to amplify *FgChs5* upstream fragment for the construction of *FgChs5* deletion mutants
P42	CAAAATAGGCATTGATGTGTTGACCTCCTCATCTTGGTCTTGTAACCCA	
P43	CTCGTCCGAGGGCAAAGGAATAGAGTAGTTCTCACAACTTGTCCAAGGC	PCR primers to amplify *FgChs5* downstream fragment for the construction of *FgChs5* deletion mutants
P44	TTCAGTTGCTGGAAGGAATG	
P45	TTGAGACAACTAGCCAGACCA	PCR primers for identification of *FgChs5* deletion transformants
P46	TTGCTGACGAACAAAGGCTT	
P47	TTGCCAGTCGTTTCCATGTC	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs5* deletion mutants
P48	AAGAATTGTGAGCCACCGAA	
P49	ATGCAAGACCACAGCGAAT	PCR primers to amplify *FgChs6* upstream fragment for the construction of *FgChs6* deletion mutants
P50	CAAAATAGGCATTGATGTGTTGACCTCCATGGACCAGTGTTCCGACTTT	
P51	CTCGTCCGAGGGCAAAGGAATAGAGTAGATGGACGATCGAGAAACGTA	PCR primers to amplify *FgChs6* downstream fragment for the construction of *FgChs6* deletion mutants
P52	TAAGAGAAGAATTGCCCCTCC	
P53	GAGTCGTTATAGGCACTGGCA	PCR primers for identification of *FgChs6* deletion transformants
P54	TGTCTACTTTCGCAACCAGCA	
P55	TGCTTATCAATTGTAACGCGA	PCR primers to amplify upstream-HPH-downstream fragment for the construction of *FgChs6* deletion mutants
P56	TGCTGCAACTGCGTTACATT	

P57	TTACGCATCTCTTCTCGCTGA	PCR primers to amplify *FgChs1* upstream fragment for the construction of *FgChs2* and *FgChs1* double genes deletion mutants
P58	CCAAAATAGCATTGATGTGTTGACCTCCTACAGCTGCGTTGAGGCTCA	
P59	CTATCGCCTTCTTGACGAGTTCTTCTGAGACTTGGATGCGCAATATCA	PCR primers to amplify *FgChs1* downstream fragment for the construction of *FgChs2* and *FgChs1* double genes deletion mutants
P60	CTTTGCAAACTCCTTCACCTC	
P61	TCTGTGCATTCATCGTACCA	PCR primers to amplify *FgChs1* up-*NEO*-down fragment for the construction of *FgChs2* and *FgChs1* double genes deletion mutants
P62	TTTTGCGGGATGTTATGTGG	
P63	TTCAATTGCCTTCAGACGAC	PCR primers for identification *FgChs2* and *FgChs1* double genes deletion transformants
P64	ATCCAAAGAAGGACAACACCG	
P65	GGATGGAGGGATGGAAATGTA	PCR primers to amplify *FgChs3a* upstream fragment for the construction of *FgChs2* and *FgChs3a* double genes deletion mutants
P66	CCAAAATAGCATTGATGTGTTGACCTCCTGTACGGTGCATGACGGAAA	
P67	CTATCGCCTTCTTGACGAGTTCTTCTGACCCATGTATTTCCGTTTCCT	PCR primers to amplify *FgChs3a* downstream fragment for the construction of *FgChs2* and *FgChs3a* double genes deletion mutants
P68	TCGGAAGTTTCACATTCAAAG	
P69	AAAGAACGAGGTTTCTTGCCA	PCR primers to amplify *FgChs3a* up-*NEO*-down fragment for the construction of *FgChs2* and *FgChs3a* double genes deletion mutants
P70	TTCGAGTGGTTTCGGGAGTTT	
P71	TCATTATCCTCCTGACCTCCA	PCR primers for identification *FgChs2* and *FgChs3a* double genes deletion transformants
P72	AAGACATTCATCCCCAAGAAG	
P73	TTCAATCGCCATCTCTCGTT	PCR primers to amplify *FgChs4* upstream fragment for the construction of *FgChs2* and *FgChs4* double genes deletion mutants
P74	CCAAAATAGCATTGATGTGTTGACCTCCTGTTTCTCTCAGGACGAATCA	
P75	CTATCGCCTTCTTGACGAGTTCTTCTGAACCGATTATCCCAACAGTCAC	PCR primers to amplify *FgChs4* downstream fragment for the construction of *FgChs2* and *FgChs4* double genes deletion mutants
P76	TTCCAGATTGGCAATGCAAG	
P77	TTCGCTCTCGTCATTGATCAC	PCR primers to amplify *FgChs4* up-*NEO*-down fragment for the construction of *FgChs2* and *FgChs4* double genes deletion mutants
P78	AAAGATCACGGACCAGAACCA	
P79	ACAATCAGGCTTCACACCAA	PCR primers for identification *FgChs2* and *FgChs4* double genes deletion transformants
P80	TTCAGCATACCCAAGCAGACA	
P81	TGTCCATGTATTCGATGGCCT	PCR primers to amplify *FgChs7* upstream fragment for the construction of *FgCHS2* and *FgChs7* double genes deletion mutants
P82	CCAAAATAGCATTGATGTGTTGACCTCCTCTCAGCGTTAATGCCCAAA	
P83	CTATCGCCTTCTTGACGAGTTCTTCTGAGCAACGTTGTTCCATATATCG	PCR primers to amplify *FgChs7* downstream fragment for the construction of *FgChs2* and *FgChs7* double genes deletion mutants
P84	AAGTTGTCCTGGTTCCAGAAG	
P85	TGCTGAAGATGGATGTGTTGT	PCR primers to amplify *FgChs7* up-*NEO*-down fragment for the construction of

P86	GGTAACCAATGTAGACGGTGG	*FgChs2* and *FgChs7* double genes deletion mutants
P87	CTCCAAACGAAAACGAGGAT	PCR primers for identification *FgChs2* and *FgChs7* double genes deletion transformants
P88	CACGGTTCAGGAAGCTCATAA	
P89	TACCATAACCCACCGAATCGT	PCR primers to amplify *FgChs5* upstream fragment for the construction of *FgChs2* and *FgChs5* double genes deletion mutants
P90	CCAAAATAGCATTGATGTGTTGACCTCCTCATCTTGGTCTTGTAACCCA	
P91	CTATCGCCTTCTTGACGAGTTCTTCTGATTCTCACAACTTGTCCAAGGC	PCR primers to amplify *FgChs5* downstream fragment for the construction of *FgChs2* and *FgChs5* double genes deletion mutants
P92	TTCAGTTGCTGGAAGGAATG	
P93	TTGCCAGTCGTTTCCATGTC	PCR primers to amplify *FgChs5* up-*NEO*-down fragment for the construction of *FgChs2* and *FgChs5* double genes deletion mutants
P94	AAGAATTGTGAGCCACCGAA	
P95	TTGAGACAACTAGCCAGACCA	PCR primers for identification *FgChs2* and *FgChs5* double genes deletion transformants
P96	TTGCTGACGAACAAAGGCTT	
P97	ATGCAAGACCACAGCGAAT	PCR primers to amplify*FgChs6* upstream fragment for the construction of *FgChs2* and *FgChs6* double genes deletion mutants
P98	CCAAAATAGCATTGATGTGTTGACCTCCATGGACCAGTGTTCCGACTTT	
P99	CTATCGCCTTCTTGACGAGTTCTTCTGAATGGACGATCGAGAAACGTA	PCR primers to amplify *FgChs6* downstream fragment for the construction of *FgChs2* and *FgChs6* double genes deletion mutants
P100	TAAGAGAAGAATTGCCCCTCC	
P101	TGCTTATCAATTGTAACGCGA	PCR primers to amplify *FgCHS6* up-*NEO*-down fragment for the construction of *FgChs2* and *FgChs6* double genes deletion mutants
P102	TGCTGCAACTGCGTTACATT	
P103	GAGTCGTTATAGGCACTGGCA	PCR primers for identification *FgChs2* and *FgChs6* double genes deletion transformants
P104	TGTCTACTTTCGCAACCAGCA	
P105	TATCGACGATCGGCATGTAT	PCR primers for amplification of the partial *FgChs2* gene in quantitative real-time PCR assays
P106	AGCTCGACAGTCTCACCCTT	
P107	ATGTGCTGGTTCTGGGCTAT	PCR primers for amplification of the partial *FgChs1* gene in quantitative real-time PCR assays
P108	ATACTGGGCGAATGATGTGA	
P109	CCAACATTGTCAAGGATGAGA	PCR primers for amplification of the partial *FgChs3a* gene in quantitative real-time PCR assays
P110	CACCGAGAGAGCTAAAGTCGT	
P111	TCTACCTGCTTGCATTGCC	PCR primers for amplification of the partial *FgChs4* gene in quantitative real-time PCR assays
P112	ACTTCTTGACCGCTTGTCG	
P113	TCGTTATCTCACCACCCTGA	PCR primers for amplification of the partial *FgChs7* gene in quantitative real-time PCR assays
P114	AAACACAACGAAGCGCATAC	

P115	AGGCTCAAGGACCCTTCTCT	PCR primers for amplification of the partial *FgChs5* gene in quantitative real-time PCR assays
P116	AACTCTTGCTTGATGCCCTT	
P117	GATGGACATAAGCGCCAAC	PCR primers for amplification of the partial *FgChs6* gene in quantitative real-time PCR assays
P118	CTGAATGTGCATGAGGGAAG	
P119	AAGTCGAGAAGGAGCAGGAG	PCR primers for amplification of the partial *FgChs3b* gene in quantitative real-time PCR assays
P120	CTTTCGTTCTGTGGCAGGT	
P121	ACTCACTATAGGGCGAATTGGGTACTCAAATTGGTTAGAGGAACACTACGACCCTCA	PCR primers to amplify the entire *FgChs2* gene for the construction of complemented plasmid
P122	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACATTGCGTAGGTTGCTTGACA	
P123	TGCGCTTCCTTCTCTGGT	PCR primers for identification of the complementation of *FgChs2* deletion mutants
P124	TCAGCTTGCCGTAGGTGGCA	
P125	CAACACCTTCTCCTCTCAACC	PCR primers for amplification of *FgChs2* upstream fragment for Southern hybridization assay
P126	TTGTCGGGATCTTACACTCCA	
hph-F	GGAGGTCAACACATCAATGCCTATT	PCR primers for amplification of hygromycin resistance gene (*HPH*) and *HPH* probe
hph-R	CTACTCTATTCCTTTGCCCT	
neo-F	GGAGGTCAACACATCAATGCT	PCR primers for amplification of neomycin resistance gene (*NEO*) and *NEO* probe
neo-R	TCAGAAGAACTCGTCAAGAAG	
Actin-F	ATCCACGTCACCACTTTCAA	PCR primers for amplification of the reference *ACTIN* gene in quantitative real-time PCR assays
Actin-R	TGCTTGGAGATCCACTTTG	
Tri4-F	ACGTGTGGCTACTCAGGAGAA	PCR primers for amplification of the *TRI4* gene in quantitative real-time PCR assays
Tri4-R	TGGAATTGCCTTGGGGTA	
Tri5-F	AGCAGATGGTTGCTGTCTTCT	PCR primers for amplification of the *TRI5* gene in quantitative real-time PCR assays
Tri5-R	TTCTGAGCCTCCTTCACATCG	
Tri6-F	AAATGCCCATTCCCTAGTTG	PCR primers for amplification of the *TRI6* gene in quantitative real-time PCR assays
Tri6-R	ATCTCGCATGTTATCCACCCT	