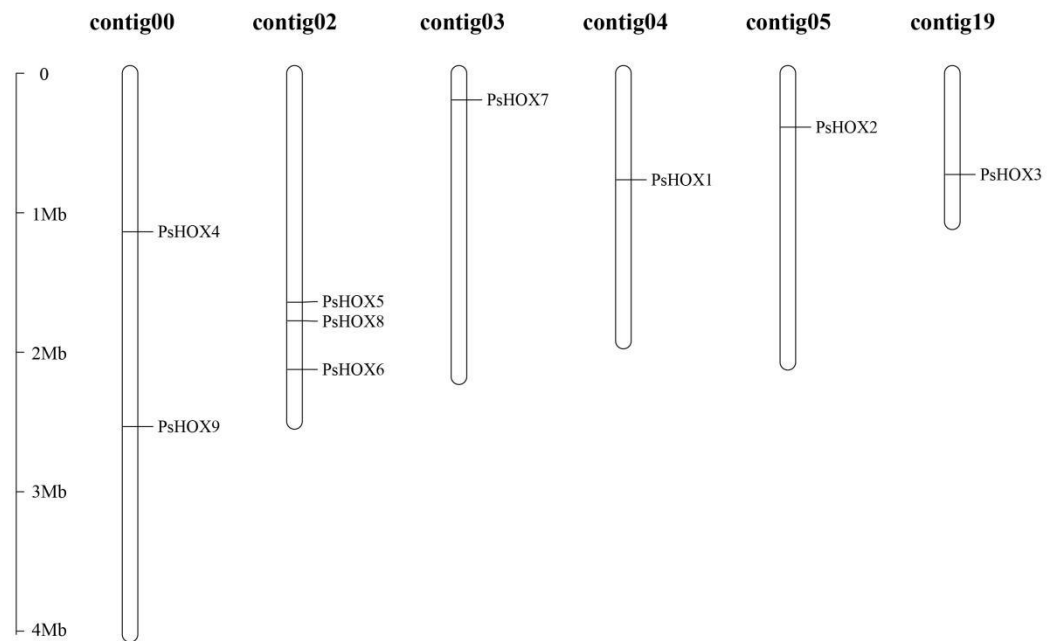
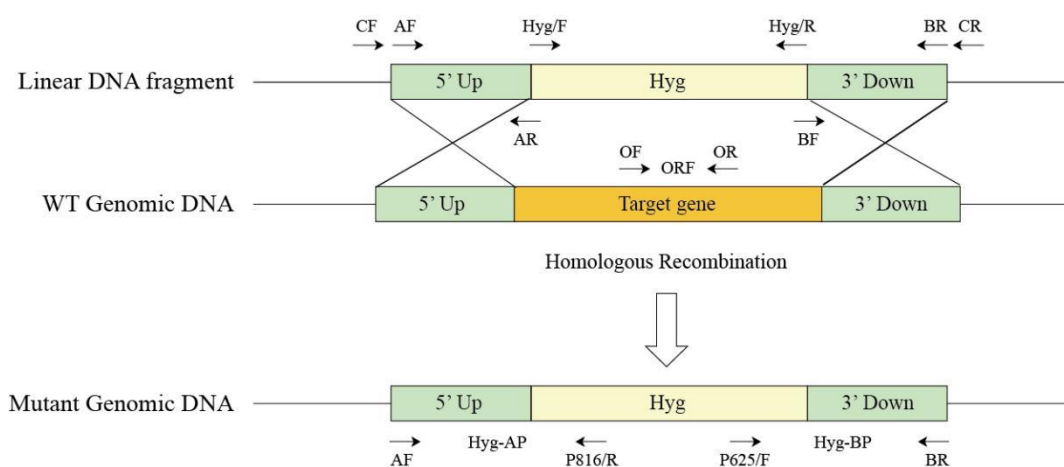


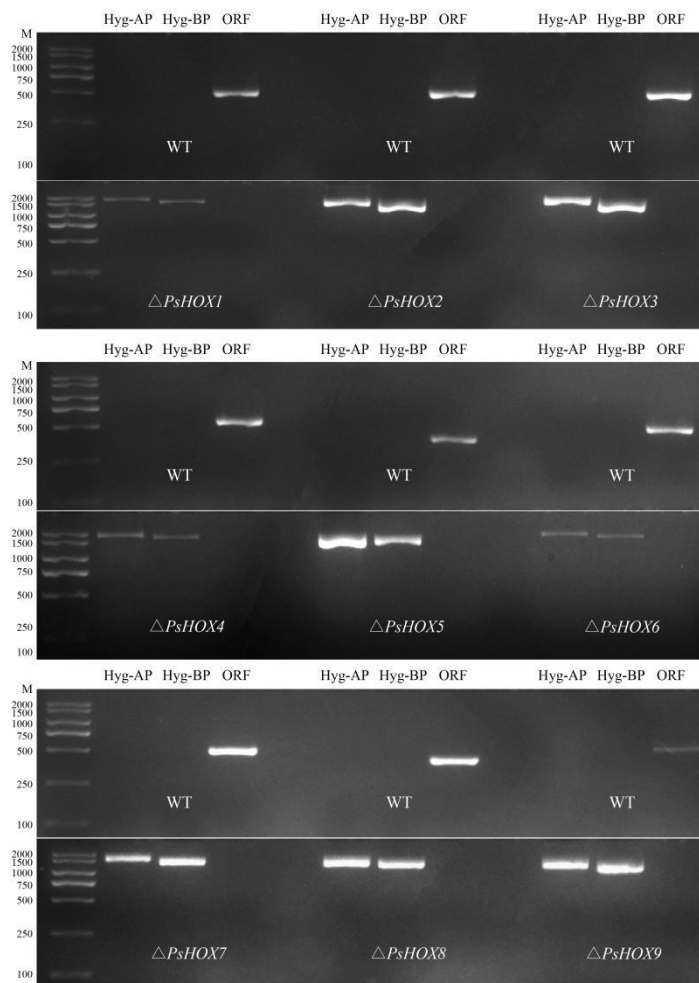
**Supplementary Figures:**



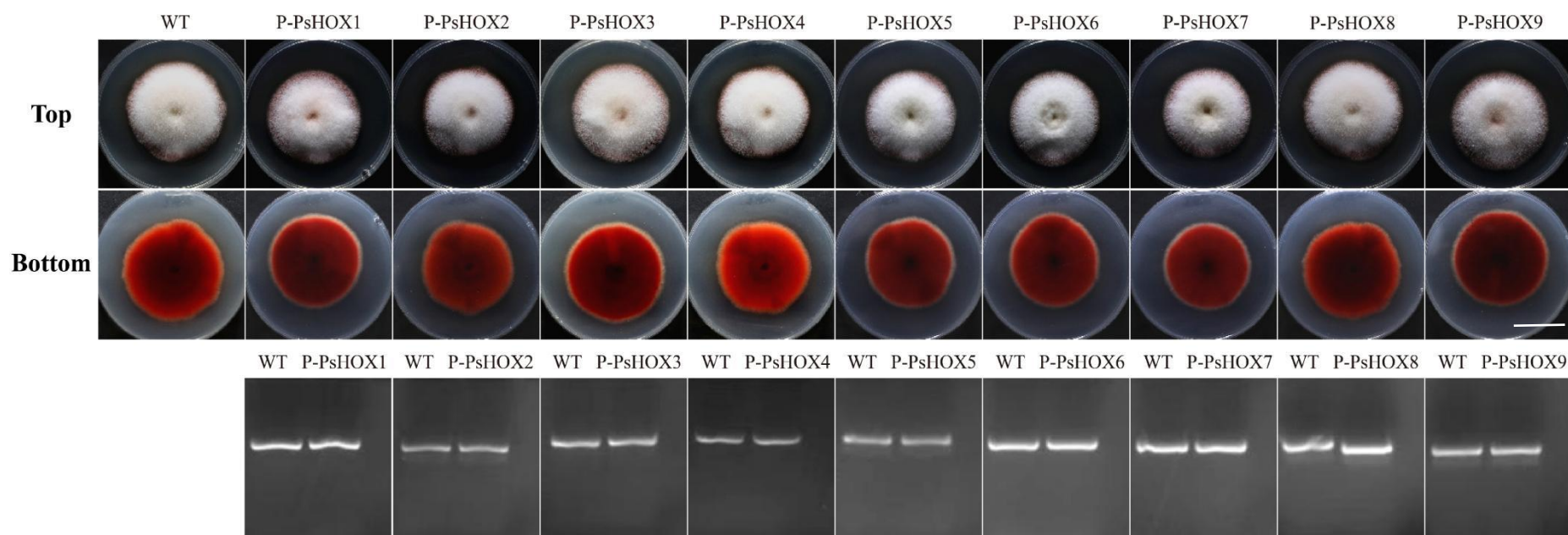
**Figure S1.** Chromosomal locations of *HOX* genes are shown in *Phoma sorghina* var. *saccharum* BS2-1 genome.



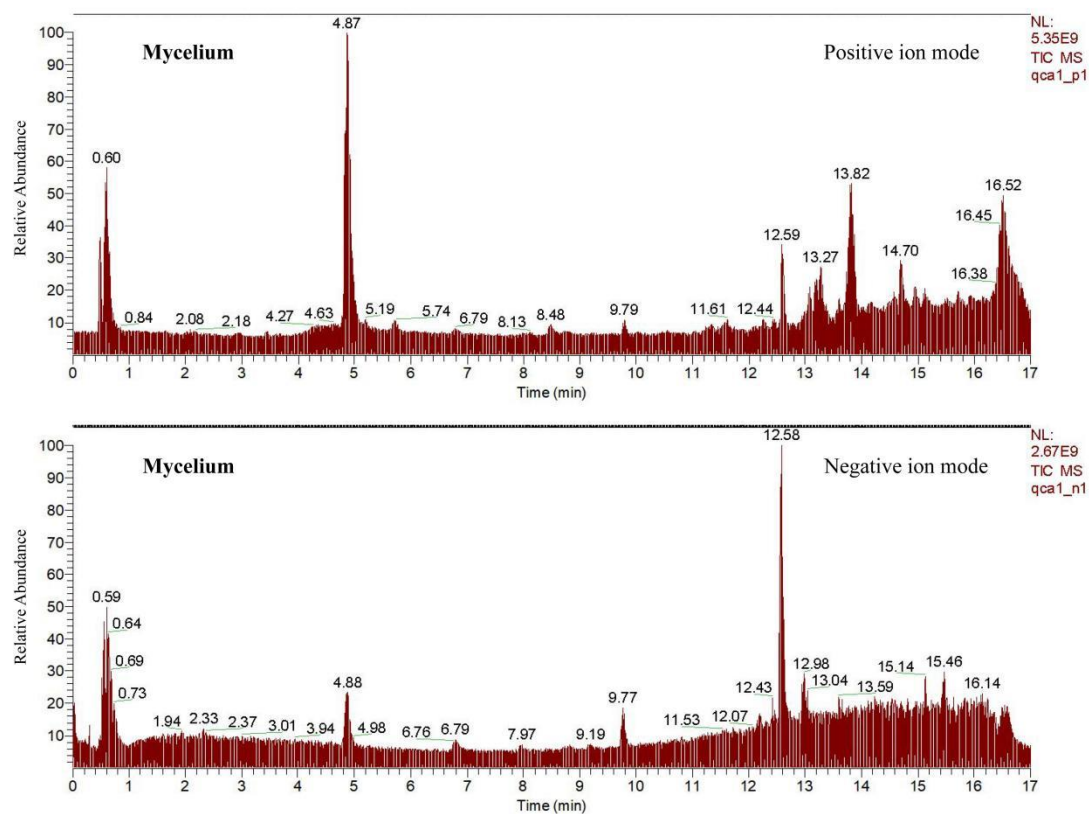
**Figure S2.** The gene replacement strategy of *HOX*.



**Figure S3.** Confirmation of HOX genes deletion in BS2-1 using diagnosed PCR. Hyg-AP, Hyg-BP, and ORF, represent the indicated DNA fragments shown in Figure S5.



**Figure S4.** Confirmation of *HOX* genes complement in *Phoma sorghina* var. *saccharum* BS2-1 using phenotype observed (up) and diagnosed PCR (down). Bars=3 cm.



**Figure S5.** Total ion chromatogram (TIC) of *P. sorghina* BS2-1 and four mutants ( $\Delta PsHOX1$ ,  $\Delta PsHOX3$ ,  $\Delta PsHOX6$ ,  $\Delta PsHOX9$ ) from mycelium.



**Figure S6.** Typical twisting and curling of crown leaves symptoms in field sugarcane.

**Supplementary Tables:**

**Table S1. Global statistics for the *P. sorghina* BS2-1 genome assembly, BUSCO analysis and the length of chromosomes.**

	telomeric repeat 3'	telomeric repeat 5'	Length (bp)
Contig 00	+	+	4,023,921
Contig 01	+	+	2,588,534
Contig 02	+	—	2,497,106
Contig 03	+	—	2,176,165
Contig 04	+	—	1,920,476
Contig 05	+	—	2,072,022
Contig 07	+	—	1,973,798
Contig 08	+	+	1,858,818
Contig 10	+	+	1,705,504
Contig 13	+	+	1,602,205
Contig 14	+	—	1,419,212
Contig 16	+	+	1,386,965
Contig 17	+	+	1,316,475
Contig 18	+	—	693,251
Contig 19	+	+	1,064,691
Contig 20	+	—	473,809
Contig 21	+	+	1,085,172
Contig 22	+	—	1,084,869
Contig 23	+	+	1,108,709
Contig 25	+	+	977,488
Contig 29	—	—	28,739
Mitochondrion			66,703
Total			33,124,632
<b>Summary</b>			
Genome size (bp)			33,124,632
Depth of coverage			97 ×
Contig number			22
Contig N50 (bp)			1,920,476
Contig N90 (bp)			1,084,869
Contig max (bp)			4,023,921
GC content			52.12 %
Complete BUSCOs (C)			286 (98.6 %)
Complete and single-copy BUSCOs (S)			286 (98.6 %)
Complete and duplicated BUSCOs (D)			0
Fragmented BUSCOs (F)			1 (0.3 %)
Missing BUSCOs (M)			3 (1.1 %)
Total BUSCO groups searched			290

Note: + shows that chromosomes with telomeric repeats sequences (TTAGGG) on the ends; — shows that chromosomes can not found the complete telomeric repeats sequences (TTAGGG) on the ends.

**Table S2. Functional annotation of predicted protein-coding genes in *P. sorghina* BS2-1.**

Method	Software		<i>P. sorghina</i> BS2-1
<i>Ab initio</i>	Augustus	-	13,349
	Glimmer HMM	-	7,788
	SNAP	-	10,808
Homolog-based	GeMoMa	<i>Ascochyta rabiei</i>	9,952
Integration	EVM	-	7,973
Gene length (bp)			17,689,450
Average gene length (bp)			2,219
Exon length (bp)			13,034,658
Average exon length (bp)			595.3
Intron length (bp)			21,895
Average Intron length (bp)			212.6
<b>Database</b>			
GO (Gene Ontology)			4,415 (55.4 %)
COG (Cluster of Orthologous Groups of proteins)			3,556 (44.6 %)
KEGG (Kyoto Encyclopedia of Genes and Genomes)			2,767 (34.7 %)
Pfam			6,169 (77.4 %)
Swiss-Prot			5,239 (65.7 %)
TrEMBL			7,854 (98.5 %)
Nr (non-redundant proteins)			7,851 (98.5 %)
CAZyme (Carbohydrate-Active EnZymes Database)			515 (6.5 %)
PHI (Pathogen–Host Interactions Database)			2,440 (30.6 %)
TCDB (Transporter Classification Database)			115 (1.4 %)
SP (Secreted protein)			583 (7.3 %)
tRNA			168 (2.1 %)
rRNA			113 (1.4 %)
Pseudogene			76 (1.0 %)



**Table S3. Repeat sequences identification and classification in *P. sorghina* BS2-1 genome.**

<b>Type</b>	<b>Number</b>	<b>Length (bp)</b>	<b>Rate (%)</b>
ClassI/?/?	25	30,265	0.09
ClassI/LINE/I	8	731	0
ClassI/LINE/Jockey	1	57	0
ClassI/LINE/L1	7	490	0
ClassI/LTR/?	7	1,066	0
ClassI/LTR/Copia	34	4,968	0.01
ClassI/LTR/Gypsy	158	107,912	0.33
ClassI/PLE/Penelope	32	4,302	0.01
ClassI/SINE/?	50	3,266	0.01
ClassII/?/?	1	170	0
ClassII/?/Kolobok	3	120	0
ClassII/Helitron/Helitron	3	199	0
ClassII/TIR/CACTA	4	151	0
ClassII/TIR/P	9	511	0
ClassII/TIR/PIF-Harbinger	2	140	0
ClassII/TIR/Tc1-Mariner	57	52,395	0.16
ClassII/TIR/hAT	10	835	0
<b>Total</b>	<b>411</b>	<b>207,578</b>	<b>0.63</b>

**Table S4. The statistical results of transcription factors identified in *P. sorghina* BS2-1.**

<b>Families</b>	<b>Number</b>	<b>Families</b>	<b>Number</b>
AF-4	3	Others	3
ARID	2	RFX	1
bHLH	8	SRF	2
Fork head	3	TEA	1
HMG	9	TSC22	1
HMGA	1	ZBTB	15
Homeobox	9	zf-BED	1
HSF	3	zf-C2H2	39
MYB	19	zf-CCCH	8
NDT80_PhoG	1	zf-GAGA	1
NF-YA	1	zf-GATA	6
NF-YB	3	zf-LITAF-like	1
NF-YC	3	zf-MIZ	3
<b>Total</b>			<b>147</b>

**Table S5. The Ct value of wild-type strain and mutants to sugarcane.**

<b>Sample</b>	<b>Ct value*</b>	<b>Status</b>	<b>Sample</b>	<b>Ct value*</b>	<b>Status</b>
WT_D1	17.94	Infected	$\Delta PsHOX5\_D1$	17.54	Infected
WT_D2	19.45	Infected	$\Delta PsHOX5\_D2$	19.09	Infected
WT_D3	17.34	Infected	$\Delta PsHOX5\_D3$	<b>35.21</b>	<b>Healthy</b>
WT_D4	18.99	Infected	$\Delta PsHOX5\_D4$	<b>36.94</b>	<b>Healthy</b>
WT_D5	17.22	Infected	$\Delta PsHOX5\_D5$	18.98	Infected
WT_D6	17.86	Infected	$\Delta PsHOX5\_D6$	19.35	Infected
WT_D7	18.09	Infected	$\Delta PsHOX5\_D7$	20.34	Infected
<b>WT_D8</b>	<b>36.51</b>	<b>Healthy</b>	$\Delta PsHOX5\_D8$	21.87	Infected
WT_D9	18.42	Infected	$\Delta PsHOX5\_D9$	20.88	Infected
WT_D10	17.02	Infected	$\Delta PsHOX5\_D10$	23.98	Infected
$\Delta PsHOX1\_D1$	25.32	Infected	$\Delta PsHOX6\_D1$	<b>35.65</b>	<b>Healthy</b>
$\Delta PsHOX1\_D2$	<b>37.22</b>	<b>Healthy</b>	$\Delta PsHOX6\_D2$	<b>36.22</b>	<b>Healthy</b>
$\Delta PsHOX1\_D3$	26.43	Infected	$\Delta PsHOX6\_D3$	<b>35.55</b>	<b>Healthy</b>
$\Delta PsHOX1\_D4$	25.33	Infected	$\Delta PsHOX6\_D4$	<b>35.71</b>	<b>Healthy</b>
$\Delta PsHOX1\_D5$	<b>37.53</b>	<b>Healthy</b>	$\Delta PsHOX6\_D5$	<b>36.20</b>	<b>Healthy</b>
$\Delta PsHOX1\_D6$	<b>37.98</b>	<b>Healthy</b>	$\Delta PsHOX6\_D6$	<b>36.09</b>	<b>Healthy</b>
$\Delta PsHOX1\_D7$	20.23	Infected	$\Delta PsHOX6\_D7$	25.09	Infected
$\Delta PsHOX1\_D8$	23.31	Infected	$\Delta PsHOX6\_D8$	<b>36.21</b>	<b>Healthy</b>
$\Delta PsHOX1\_D9$	20.15	Infected	$\Delta PsHOX6\_D9$	<b>35.15</b>	<b>Healthy</b>
$\Delta PsHOX1\_D10$	19.88	Infected	$\Delta PsHOX6\_D10$	<b>36.92</b>	<b>Healthy</b>
$\Delta PsHOX2\_D1$	20.43	Infected	$\Delta PsHOX7\_D1$	21.65	Infected
$\Delta PsHOX2\_D2$	19.22	Infected	$\Delta PsHOX7\_D2$	19.98	Infected
$\Delta PsHOX2\_D3$	26.65	Infected	$\Delta PsHOX7\_D3$	20.54	Infected
$\Delta PsHOX2\_D4$	24.55	Infected	$\Delta PsHOX7\_D4$	20.17	Infected
$\Delta PsHOX2\_D5$	<b>35.64</b>	<b>Healthy</b>	$\Delta PsHOX7\_D5$	<b>35.97</b>	<b>Healthy</b>
$\Delta PsHOX2\_D6$	<b>36.21</b>	<b>Healthy</b>	$\Delta PsHOX7\_D6$	23.53	Infected
$\Delta PsHOX2\_D7$	23.43	Infected	$\Delta PsHOX7\_D7$	<b>36.01</b>	<b>Healthy</b>
$\Delta PsHOX2\_D8$	<b>35.90</b>	<b>Healthy</b>	$\Delta PsHOX7\_D8$	20.44	Infected
$\Delta PsHOX2\_D9$	20.11	Infected	$\Delta PsHOX7\_D9$	21.32	Infected
$\Delta PsHOX2\_D10$	19.32	Infected	$\Delta PsHOX7\_D10$	19.68	Infected
$\Delta PsHOX3\_D1$	<b>35.43</b>	<b>Healthy</b>	$\Delta PsHOX8\_D1$	<b>35.98</b>	<b>Healthy</b>
$\Delta PsHOX3\_D2$	<b>36.01</b>	<b>Healthy</b>	$\Delta PsHOX8\_D2$	<b>36.02</b>	<b>Healthy</b>
$\Delta PsHOX3\_D3$	<b>36.99</b>	<b>Healthy</b>	$\Delta PsHOX8\_D3$	21.89	Infected
$\Delta PsHOX3\_D4$	<b>36.29</b>	<b>Healthy</b>	$\Delta PsHOX8\_D4$	20.47	Infected
$\Delta PsHOX3\_D5$	<b>35.56</b>	<b>Healthy</b>	$\Delta PsHOX8\_D5$	21.98	Infected
$\Delta PsHOX3\_D6$	27.91	Infected	$\Delta PsHOX8\_D6$	19.55	Infected
$\Delta PsHOX3\_D7$	28.99	Infected	$\Delta PsHOX8\_D7$	22.45	Infected
$\Delta PsHOX3\_D8$	<b>36.43</b>	<b>Healthy</b>	$\Delta PsHOX8\_D8$	<b>35.99</b>	<b>Healthy</b>
$\Delta PsHOX3\_D9$	<b>35.55</b>	<b>Healthy</b>	$\Delta PsHOX8\_D9$	23.01	Infected
$\Delta PsHOX3\_D10$	<b>35.98</b>	<b>Healthy</b>	$\Delta PsHOX8\_D10$	22.95	Infected
$\Delta PsHOX4\_D1$	26.54	Infected	$\Delta PsHOX9\_D1$	<b>35.99</b>	<b>Healthy</b>
$\Delta PsHOX4\_D2$	23.43	Infected	$\Delta PsHOX9\_D2$	<b>35.32</b>	<b>Healthy</b>

$\Delta PsHOX4\_D3$	27.09	Infected	$\Delta PsHOX9\_D3$	37.19	Healthy
$\Delta PsHOX4\_D4$	28.33	Infected	$\Delta PsHOX9\_D4$	36.96	Healthy
$\Delta PsHOX4\_D5$	35.06	Healthy	$\Delta PsHOX9\_D5$	36.01	Healthy
$\Delta PsHOX4\_D6$	36.37	Healthy	$\Delta PsHOX9\_D6$	24.15	Infected
$\Delta PsHOX4\_D7$	23.98	Infected	$\Delta PsHOX9\_D7$	35.82	Healthy
$\Delta PsHOX4\_D8$	24.09	Infected	$\Delta PsHOX9\_D8$	36.98	Healthy
$\Delta PsHOX4\_D9$	37.01	Healthy	$\Delta PsHOX9\_D9$	35.31	Healthy
$\Delta PsHOX4\_D10$	26.94	Infected	$\Delta PsHOX9\_D10$	36.79	Healthy
CK1	37.02	Healthy	CK6	35.64	Healthy
CK2	36.22	Healthy	CK7	36.90	Healthy
CK3	35.79	Healthy	CK8	36.43	Healthy
CK4	37.98	Healthy	CK9	36.32	Healthy
CK5	36.11	Healthy	CK10	35.97	Healthy

\* Ct value < 35 (infected)

**Table S6. The pathogenicity of wild-type strain and mutants to sugarcane.**

<b>Strains</b>	<b>Incidence rate (%)</b>
WT (BS2-1)	90.00
$\Delta PsHOX1$	70.00
$\Delta PsHOX2$	70.00
$\Delta PsHOX3$	20.00
$\Delta PsHOX4$	70.00
$\Delta PsHOX5$	80.00
$\Delta PsHOX6$	10.00
$\Delta PsHOX7$	80.00
$\Delta PsHOX8$	70.00
$\Delta PsHOX9$	10.00
CK	0.00

**Table S7. Gene-specific primers for gene knock-out.**

Primer name	Sequence (5'-3')	Target gene	Size (bp)
PHOM_00410/AF	CGATCACAGCTAGCCAGCTG	<i>PsHOX1</i> A fragment	1,000
PHOM_00410/AR	TCTTTCTAGAGGATCCCCGGGTACATCCCATGTTCTCAGGCAAGC		
PHOM_00410/BF	ATATCATCTTCTGTGCGACCTGCAGGCGTCGCCGCCAGGCTTC	<i>PsHOX1</i> B fragment	1,000
PHOM_00410/BR	GAACAGCAGCAACGACGACAACTG		
PHOM_00410/CF	CGATCACAGCTAGCCAGCTG	<i>PsHOX1</i> A-target gene- <i>PsHOX1</i> -B gene cassette	4,400
PHOM_00410/CR	GAACAGCAGCAACGACGACA		
PHOM_00410/OF	ATTGGCTCTGACTGAACCAGGC	<i>PsHOX1</i> ORF	544
PHOM_00410/OR	GCAGGACTTGACACGGAAACAC		
PHOM_01626/AF	GCTGGATGCAAATGAGAACAGGA	<i>PsHOX2</i> A fragment	1,000
PHOM_01626/AR	TCTTTCTAGAGGATCCCCGGGTACTCATGTTCTGGGTAGAGTAAGGA		
PHOM_01626/BF	ATATCATCTTCTGTGCGACCTGCAGGGTTATTCGCGACTGTATCTGGCA	<i>PsHOX2</i> B fragment	1,000
PHOM_01626/BR	CAGTTGGAAGAGAGCAAAAAGGA		
PHOM_01626/CF	GCTGGATGCAAATGAGAACAGGA	<i>PsHOX2</i> A-target gene- <i>PsHOX2</i> -B gene cassette	3,809
PHOM_01626/CR	CAGTTGGAAGAGAGCAAAAAGGA		
PHOM_01626/OF	TGTCGTGCCTGATTCTGCGC	<i>PsHOX2</i> ORF	553
PHOM_01626/OR	CGCGAACCTCATGGATCTCC		
PHOM_02470/AF	CCTGAAGCAGACCTGGCCG	<i>PsHOX3</i> A fragment	1,000
PHOM_02470/AR	TCTTTCTAGAGGATCCCCGGGTACGTTGGGGAGGTGGACACCTG		
PHOM_02470/BF	ATATCATCTTCTGTGCGACCTGCAGGTGGCATTCTGGTTTCGCGTT	<i>PsHOX3</i> B fragment	1,000
PHOM_02470/BR	GCGCGGAAAACAAACTAGAAAGT		
PHOM_02470/CF	CCTGAAGCAGACCTGGCCG	<i>PsHOX3</i> A-target gene- <i>PsHOX3</i> -B gene cassette	3,857
PHOM_02470/CR	GCGCGGAAAACAAACTAGAAAGT		
PHOM_02470/OF	TCAACTTCCGCCCTGCCTCA	<i>PsHOX3</i> ORF	479
PHOM_02470/OR	TGCCGAGTGCGGAGGAG		
PHOM_03333/AF	CCTTCAGGGACCAGTGAGAGG	<i>PsHOX4</i> A fragment	1,000
PHOM_03333/AR	TCTTTCTAGAGGATCCCCGGGTACTCTGTGAGAGTGTGCGGGAT		
PHOM_03333/BF	ATATCATCTTCTGTGCGACCTGCAGGTTTTGTCTCTGGTTGGACCAAATTTGT	<i>PsHOX4</i> B fragment	1,000
PHOM_03333/BR	CCCTGGCACATTCGGTTTCG		
PHOM_03333/CF	CCTTCAGGGACCAGTGAGAGG	<i>PsHOX4</i> A-target gene- <i>PsHOX4</i> -B gene cassette	3,454
PHOM_03333/CR	CCCTGGCACATTCGGTTTCG		
PHOM_03333/OF	TTCCTTTGCTCAGCCACCCC	<i>PsHOX4</i> ORF	366
PHOM_03333/OR	ACCTGGTTCAATGTCAAGTTTGTGTCAG		

PHOM_04409/AF	ATCGAAGGCTACTGGAACGGC	<i>PsHOX5</i> A fragment	1,000
PHOM_04409/AR	TCTTTCTAGAGGATCCCCGGGTACTTATCTCTCTTTTCCGGCGTTTCG		
PHOM_04409/BF	ATATCATCTTCTGTGCGACCTGCAGGGTGCTCGGCGCTCGACAA	<i>PsHOX5</i> B fragment	1,000
PHOM_04409/BR	AGCTACTTCGTGGTTCTCACTTGC		
PHOM_04409/CF	ATCGAAGGCTACTGGAACGGC	<i>PsHOX5</i> A-target gene- <i>PsHOX5</i> -B gene cassette	3,611
PHOM_04409/CR	AGCTACTTCGTGGTTCTCACTTGC		
PHOM_04409/OF	AGAGTGGTTGTTTCGCACTTATGAT	<i>PsHOX5</i> ORF	531
PHOM_04409/OR	TGACCACATGGTTCACCAACATTTCG		
PHOM_05072/AF	ACAGGCGCACAGTGCGG	<i>PsHOX6</i> A fragment	1,000
PHOM_05072/AR	TCTTTCTAGAGGATCCCCGGGTACGCTGGCGAACGGCCCCCTT		
PHOM_05072/BF	ATATCATCTTCTGTGCGACCTGCAGGAGCTACCTCTTGCAGGCCAC	<i>PsHOX6</i> B fragment	1,000
PHOM_05072/BR	AAGAGATGATATGCGTAGATGGTCCT		
PHOM_05072/CF	ACAGGCGCACAGTGCGG	<i>PsHOX6</i> A-target gene- <i>PsHOX6</i> -B gene cassette	4,686
PHOM_05072/CR	AAGAGATGATATGCGTAGATGGTCCTATTCTCGT		
PHOM_05072/OF	AGATGCATCCCGACGACG	<i>PsHOX6</i> ORF	508
PHOM_05072/OR	CGGCAACTCCATACACGGC		
PHOM_05197/AF	ATCTCGGGGCCACAAAAGAA	<i>PsHOX7</i> A fragment	1,000
PHOM_05197/AR	TCTTTCTAGAGGATCCCCGGGTACTGTAGTGTTTCATAGACACAGACAAGTT		
PHOM_05197/BF	ATATCATCTTCTGTGCGACCTGCAGGCCTCTAATGATGCAAGCAGACTGT	<i>PsHOX7</i> B fragment	1,000
PHOM_05197/BR	CAGCTGCCTTCCAGAGCTCG		
PHOM_05197/CF	ATCTCGGGGCCACAAAAGAAG	<i>PsHOX7</i> A-target gene- <i>PsHOX7</i> -B gene cassette	5,701
PHOM_05197/CR	CAGCTGCCTTCCAGAGCTCG		
PHOM_05197/OF	TCTGCAATGATTCTCTTCCTCTCCTCC	<i>PsHOX7</i> ORF	527
PHOM_05197/OR	CGACTTCGTGCTGAAGATCCAATTGA		
PHOM_06420/AF	ATTCGGCCTATGATCGTCGGT	<i>PsHOX8</i> A fragment	1,000
PHOM_06420/AR	TCTTTCTAGAGGATCCCCGGGTACGTGGGGTCTACGAAGAATGCA		
PHOM_06420/BF	ATATCATCTTCTGTGCGACCTGCAGGCGTGGTAGCCTTTCGGGACT	<i>PsHOX8</i> B fragment	1,000
PHOM_06420/BR	AGTCGATATGTTCAAGTTCAGTGCG		
PHOM_06420/CF	ATTCGGCCTATGATCGTCGGT	<i>PsHOX8</i> A-target gene- <i>PsHOX8</i> -B gene cassette	5,092
PHOM_06420/CR	AGTCGATATGTTCAAGTTCAGTGCG		
PHOM_06420/OF	TACTAGGTTCAAGTTCGCCGCAATAC	<i>PsHOX8</i> ORF	492
PHOM_06420/OR	CTCTTACAGCTATTGCCCAGGCC		
PHOM_07085/AF	GACACGTTTGAGGGAGAGCCA	<i>PsHOX9</i> A fragment	1,000
PHOM_07085/AR	TCTTTCTAGAGGATCCCCGGGTACGAGGGAGGGGGGTTCTGGC		

<i>PHOM_07085/BF</i>	ATATCATCTTCTGTGCGACCTGCAGGCTCATTAAACCGATTGTATGCGTGAAA	<i>PsHOX9</i> B fragment	1,000
<i>PHOM_07085/BR</i>	CCTAGCTTGGTCGCAAGTACT		
<i>PHOM_07085/CF</i>	GACACGTTTGAGGGAGAGCCA	<i>PsHOX9</i> A-target gene- <i>PsHOX9</i> -B gene cassette	6,059
<i>PHOM_07085/CR</i>	CCTAGCTTGGTCGCAAGTACT		
<i>PHOM_07085/OF</i>	ACGAGCCAGCACGTTCTTTGA	<i>PsHOX9</i> ORF	424
<i>PHOM_07085/OR</i>	AGCATGCAACTCGAAGCTTGTC		
<i>Hyg/F</i>	CGGTACCCGGGGATCCTCTA	Hygromycin gene	2,145
<i>Hyg/R</i>	GCCTGCAGGTCGACAGAAGA		
<i>P816/R</i>	ATGGCTGTGTAGAAAGTACTCGCC	Hyg-AP fragment (amplified with Hyg/AF)	A+816
<i>P625/F</i>	CTGAATTCCCAATGTCAAGCACT	Hyg-BP fragment (amplified with Hyg/BR)	B+625

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