A CCCH-Type Zinc Finger Nucleic Acid-Binding Protein Quantitatively Confers Resistance against Rice Bacterial Blight Disease

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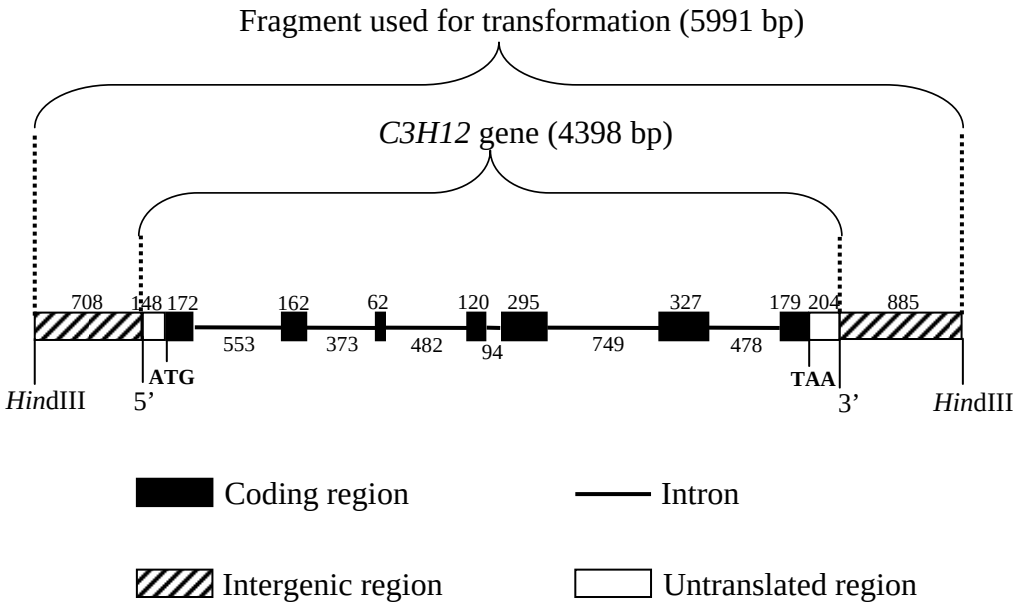
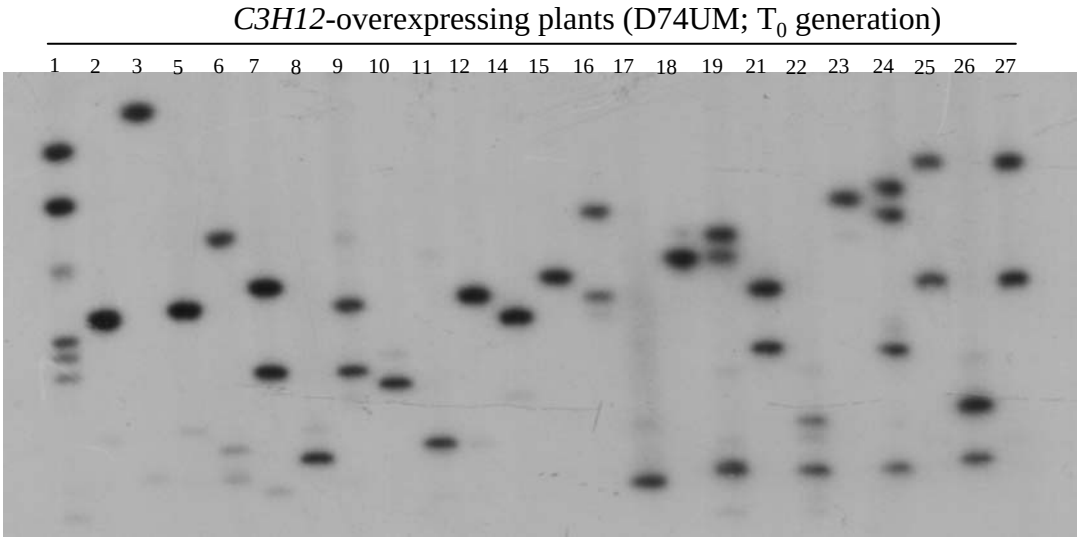
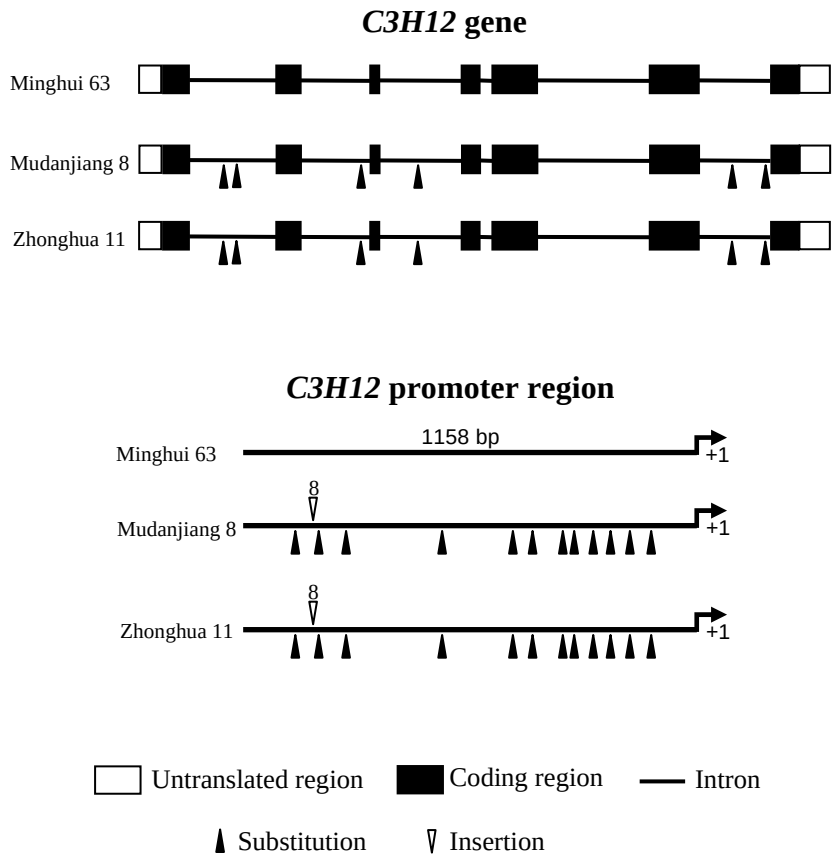
Supplemental Data



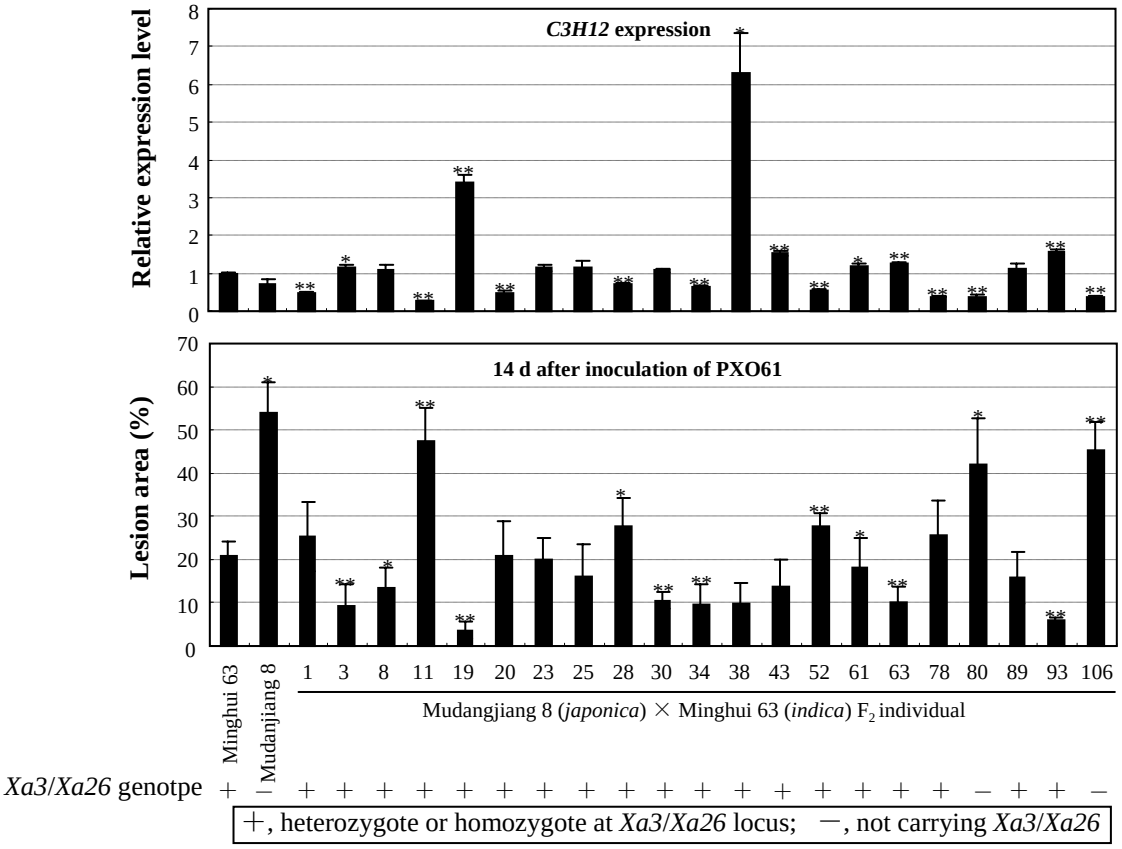
Supplemental Figure S1. The structures of *C3H12* gene and rice DNA fragment for transformation.



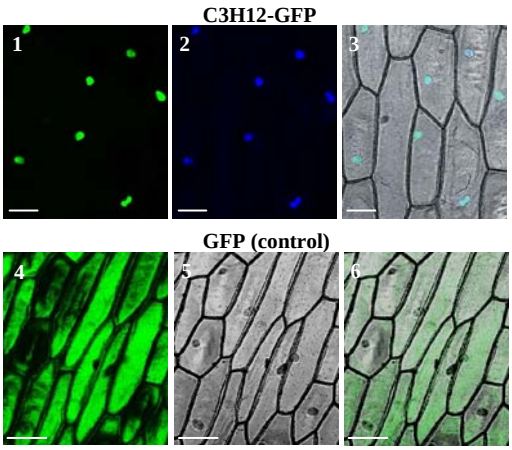
Supplemental Figure S2. Southern blot analysis of the copy numbers of transgene *C3H12*.



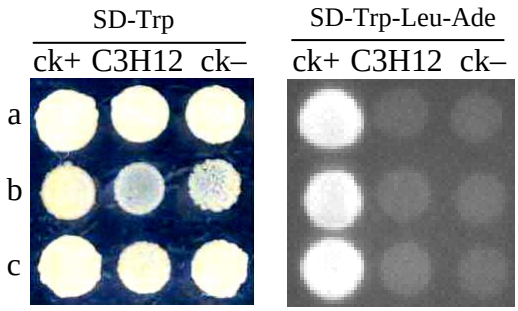
Supplemental Figure S3. Sequence comparison of the *C3H12* gene and its promoter region from rice varieties Minghui 63, Mudanjiang 8, and Zhonghua 11. Nucleotide substitution or insertion are identified in different rice varieties with reference to the sequence of Minghui 63. The figure above a triangle indicates the numbers of nucleotides inserted.



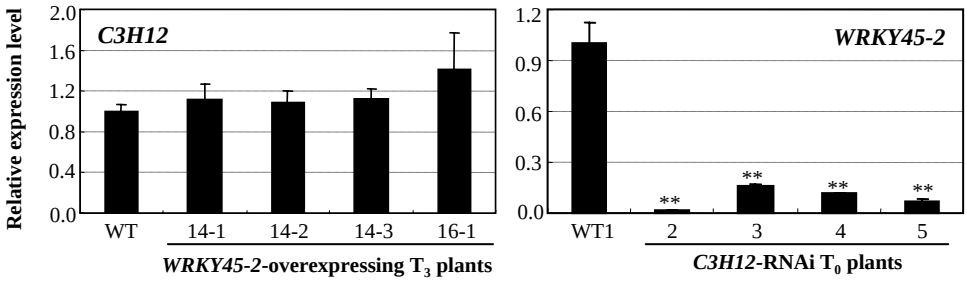
Supplemental Figure S4. Relationship of *C3H12* expression level and the resistance level in F_2 plants. Each expression datum represents mean (3 replicates) ± standard deviation. The lesion area and genotype at *Xa3/Xa26* locus were from our previous report (Zhou Y et al. Theor. Appl. Genet. 120:127-138, 2009). Two or one asterisks indicate that a significant difference between an F_2 individual and its resistant parent Minghui 63 was detected at $P < 0.01$ or $P < 0.05$, respectively.



Supplemental Figure S5. C3H12 localized in the nuclei of onion epidermal cells. 1, C3H12-GFP expression; 2, staining of nuclei using DAPI as control; 3, transmission image overlain with 1 and 2; 4, GFP expression; 5, transmission image; 6, overlay of 4 and 5. Scale bars: 100 μm.



Supplemental Figure S6. C3H12 displayed no transactivation activity as compared to the positive control. The transactivation activity was analyzed by growing yeast cells carrying transgene on plates lacking tryptophan (Trp), leucine (Leu), and adenine (Ade). ck+: positive control (rice transcription factor OsbZIP23); ck-: negative control (empty pGBKT7 vector); a, b and c, three replicates (independent clones) for ck+, C3H12, and ck–.



Supplemental Figure S7. *C3H12* influenced the expression of defense-responsive gene *WRKY45-2*. Bars represent mean (3 replicates) ± standard deviation. Two asterisks indicate that a significant difference between transgenic and wild-type plants was detected at $P < 0.01$. WT, wild-type Mudanjiang 8; WT1, wild-type Minghui 63.

Supplemental Table S1. Resistance of T_0 *C3H12*-overexpressing plants (D74UM) to *Xoo* strain PXO61 at booting stage[a]

Rice material	Lesion area (%)	*P*	Copy number[b]
Mudanjiang 8 (wild type)	64.9.0 ± 8.6		
D74UM1	54.8 ± 12.9	0.3143	6
D74UM2	61.0 ± 12.8	0.5858	1
D74UM3	43.8 ± 7.6	0.0034	1
D74UM4	45.1 ± 12.4	0.0215	–
D74UM5	48.6 ± 7.5	0.0127	1
D74UM6	50.5 ± 7.8	0.0240	3
D74UM7	24.0 ± 10.3	0.0045	2
D74UM8	47.1 ± 7.2	0.0074	1
D74UM9	39.5 ± 12.9	0.0196	2
D74UM10	57.0 ± 8.6	0.1827	1
D74UM11	53.4 ± 12.0	0.1243	1
D74UM12	49.7 ± 5.7	0.0132	1
D74UM13	65.7 ± 10.7	0.9068	–
D74UM14	46.9 ± 7.4	0.0075	1
D74UM15	51.5 ± 12.9	0.0952	1
D74UM16	38.1 ± 12.5	0.0055	2
D74UM17	45.6 ± 11.3	0.0184	1
D74UM18	42.1 ± 10.0	0.0047	1
D74UM19	36.5 ± 8.3	0.0007	3
D74UM20	2.8 ± 2.1	0.0000	–
D74UM21	46.4 ± 6.8	0.0055	2
D74UM22	56.9 ± 5.6	0.1241	2
D74UM23	39.9 ± 8.9	0.0020	1
D74UM24	51.9 ± 12.3	0.0927	4
D74UM25	41.4 ± 5.8	0.0014	2
D74UM26	35.7 ± 8.2	0.0006	2
D74UM27	51.5 ± 8.1	0.0343	2

[a]Three to five uppermost fully expanded leaves of each plant were inoculated. Lesion length and leaf length were measured two weeks after inoculation.

[b]The "–" indicates that the copy number of transgene was not analyzed.

Supplemental Table S2. PCR primers used for construction of vectors, gene structure analysis, gene mapping, and transgene copy number analysis

Gene (GenBank accession number)	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (nt)	Use
C3H12 (JF799943)	38D7RIF/38D7RIR	GCCACTAGTGGTACCAAGCCCTAGATGGCAGAATC[a]	GCCGAGCTCGGATCCTAGTTGAAGCAGAGCCGTAG[b]	538	Amplifying cDNA fragment for constructing RNAi construct
	38D7CF1/38D7CR	GAGGATCCAT*GGTACC*GATGGACGACGCCGGAAG[c]	GCTGAGCTCGGATCCGGAAGTGTACGCGGATGCGG[d]	1349	Amplifying coding region for constructing *C3H12-GFP* or trans-activation activity assay
	38D7CF2/38D7CR2	AAGGATCCATGGACGACGCCGGAAG[d]	TTAAGCTTAAGGAAGTGTACGCGGATGCGG[e]	1335	Amplifying coding region for expressing C3H12 protein in *Escherichia coli*
	MF/GSP1	GGGTATGTGGCAGCAGATGA	ATTTGAGGGATTCTGC	1507	Analyzing *C3H12*-knockout mutant
	38D7GSP2		CTCGGTCGGAGTGGAT	666	5'-RACE analysis
	38D75UF5/38D7RT5UR	GCCACCGCGCGAGTGCACGT	AGGATATGGTCCAGGTTG	1050	Amplifying genomic DNA for sequencing
	38D7MF/38D75UR	GGGTATGTGGCAGCAGATGA	CTCAGGGTATTCTCCTTTCA	572	Amplifying genomic DNA for sequencing
	38D7G1/38D7GSP1	ATTGCCTCTGCCAGAATGAA	ATTTGAGGGATTCTGC	990	Amplifying genomic DNA for sequencing

	38D7NPPL/38D7G3	TTATTCCAAGCCCTAGATGG	GTAGGGTGACAACATTCCCT	967	Amplifying genomic DNA for sequencing
	38D7G2/38D7stop	GCCTGTTTCATCTTCTGAGA	GTGGTTTGGGTCACAACT	1008	Amplifying genomic DNA for sequencing
	38D7dCAPSF/R	ATCCACTCTTACACCAATTGCTCGA	AGAAGTTCCATAGGTTTGTTGAGCC	130	A dCAPS (*Xho*I) for *C3H12* mapping
WRKY45-1 (GQ331930) *WRKY45-2* (GQ331927)	w45F6/w45R6	ATCACAAAGCATAGCATCATCT	CTCAGCACCTCCTCCTGGTCGG		Identifying different alleles of *WRKY45* in F_2 plants
OsbZIP23 (AK072062)	OsbZIP23F/OsbZIP23R	TAA<u>CCATGG</u>AGATGGATTTTCCGGGAGGGA[f]	TAA<u>GGATCC</u>TGGACCCGTCAGAGTCCT[d]	1087	Amplifying coding region for trans-activation activity assay
Agrobacterium T-DNA	LBT1		CTCGTCCGAGGGCAAAGAAATAGAGTAGA		Analyzing *C3H12*-knockout mutant
Hpt[g] (V01499)	hptF/htpR	CGTCTGCTGCTCCATACAAG	GAGCCTGACCTATTGCATCTC	583	Amplifying *Hpt* fragment for DNA gel blot analysis

[a]The underlined nucleotides are the digestion site of *Spe*I and the double underlined nucleotides are the digestion site of *Kpn*I.

[b]The underlined nucleotides are the digestion site of *Sac*I and the double underlined nucleotides are the digestion site of *Bam*HI.

[c]The underlined nucleotides are the digestion site of *Nco*I and the nucleotides showing in italic are the digestion site of *Kpn*I.

[d]The underlined nucleotides are the digestion site of *Bam*HI.

[e]The underlined nucleotides are the digestion site of *Hin*dIII.

[f]The underlined nucleotides are the digestion site of *Nco*I.

[g]*Hpt*, hygromycin phosphotransferase gene.

Supplemental Table S3. Primers used for quantitative PCR in gene expression analysis

Gene (GenBank accession number)	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (nt)
C3H12 (JF799943)	38D7RT1F/ 38D7RT1R	ACCGAGTGAAAAGGAGTGTGCATAT	AAGCCATAGCATTGAAAAGTTCTGG	100
AOS2 (AY062258)[d]	AOS2-F/R	CAATACGTGTACTGGTCGAATGG	AAGGTGTCGTACCGGAGGAA	120
LOX (D14000)	LOX-F/R	GCATCCCCAACAGCACATC	AATAAAGATTTGGGAGTGACATATTGG	110
PR5 (X68197)	PR5-F/R	CAACAGCAACTACCAAGTCGTCTT	CAAGGTGTCGTTTTATTCATCAAC	130
PR10 (D38170)	PR10-F/R	CCCTGCCGAATACGCCTAA	CTCAAACGCCACGAGAATTTG	120
Cht1 (D16221)	Cht1-F/R	CGTGGTGACCAACATCATCA	GAGTTGAAAGGCCTCTGGTTGT	120
PAL1 (X16099)	PAL-F/R	AGCACATCTTGGAGGGAAGCT	GCGCGGATAACCTCAATTTG	120
ICS1 (AK120689)	ICS1-F/R	TATGGTGCTATCCGCTTCGAT	CGAGAACCGAGCTCTCTTCAA	120
PAD4 (CX118864)	PAD4-F/R	GCCAGCTCCCCTACGACTTC	CGTGTGCGGTGTAGGTTGTT	120
PR1a (AJ278436)	PR1a-F/R	CGTCTTCATCACCTGCAACTACTC	CATGCATAAACACGTAGCATAGC	130
NH1 (AY923983)	NH1-F/R	CACGCCTAAGCCTCGGATTA	TCAGTGAGCAGCATCCTGACTAG	120
OsDR10 (CX109127)	OsDR10-F/R	TCATCAAGCTGATTCATCAGACA	CGTACTTGTAGAACGCCATGGA	120
WRKY62 (DQ298182)	OsWRKY62-F/R	ATGGACGACGACGGCGACGGCT	GCGGTCGGCGGCTGCTGTCTC	476

NRR (AY846391)	NRR-F/R	CGGGTGCTCACGGATTACAA	AGCGATTGATTAACCAGGTCTCAC	120
TGA2.1 (AB051295)	TGA2.1-F/R	TAGCTGCAAAGGCCGATGT	AAGCTCAGATGGACGGAAACC	120
PLDβ1 (AJ419630)	OsPLD β 1-F/R	TCTTTTGTCTTGGCAATCGTG	CTGGATAGTTGAAGCCTTCCT	342
Actin (X15865)	Actin-F/R	TGTATGCCAGTGGTCGTACCA	CCAGCAAGGTCGAGACGAA	121