**Title:** Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice

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## **Supplementary Data**

**Supplementary Fig. S1.** Differences of *G5* between susceptible-parent TN1 and resistant-parent RBPH54.

Supplementary Fig. S2. Complementation test for G4.

Supplementary Fig. S3. Expression of G5 alleles in transgenic lines.

Supplementary Fig. S4. BPH29-GFP fusion protein activity test.

**Supplementary Table S1.** Transcript levels of *BPH29* and *OsActin1* at different rice developmental stages

Supplementary Table S2. Primers used in this work

## **Supplemental Figures:**

Α			
TN1	1	ATGGCCACCATTGTTGCATGGGAGAGAGTAGAAACCTGCAGCTGCAAGGAGGAGGAGGTGGCCATGGCGGTGGCGGCGGCGGTG	82
RBPH54	1	ATGECCACCATTETTECATEGEAGAETAEAAACCTECAECTECAAGEAEGAEGAEGCECATEGCCETEGCGECEGCGC	82
TN1	83	GCGGCGGCGAGCGGCGGGAGTACATGTTCGAGAAGGTGGTGACGCCGAGCGACGTGGGGAAGCTGAACAGGCTGGTGGTGCC	164
RBPH54	83	GCGGCGGCGAGCGGGGAGTACATGTTCGAGAAGGTGGTGACGCCGAGCGACGTGGGGAAGCTGAACAGGCTGGTGGTGCC	164
TN1	165	GAAGCACTACGCCGAGAAGTACTTCCCGCTGGGGCCGGCGGGGACCAGCCCGGCGGGCACCGTGCTGTGCTTCGAGGAC	246
RBPH54	165	GAAGCACTACGCCGAGAAGTACTTCCCGCTGGGGCCGGTGGCGAGGACCAGCCCGGCGGGCACCGTGCTGTGCTTCGAGGAC	246
TN1	247	GCGCGGGGCGGCGACAGCACGTGGCGCTTCCGCTACTCCTACTGGAGCAGCCAGAGCTACGTCATCA	316
RBPH54	247	SCGCGGGGCGGCGGCGGCGGCGACAGCACGTGCGCGCTTCCGCTACTCCTACTGGAGCAGCAGCAGCGGCTACGTCATCA	328
TN1	317	CCAAGGGATGGAGCCGCTACGTCCGCGACAAGCGCCTCGCCGCCGGCGACACCGTCTCCTTCTGCCGCGCCGGTGCCCGCCT	398
RBPH54	329	CCAAGGGATGGAGCCGATACGTCCGCGACAAGCGCCTCGCCGGCGACACCGTCTCCTTCTGCCGCGCGGGGCCCGCCT	410
TN1	399	CTTCATCGACTGCCGGAAGCGCGCCGCCTCCGTGTCGTCGCTGGTGCCACCGGCCTTGATCAAGGTGCAGCTGCCG	480
RBPH54	411	CTTCATCGACTGCCGGAAGCGCGCCGCCTCCGTGTCGTCGTCGCTGGTGCCACCGGCCTTGATCAAGGTGCAGCTGCCG	492
TN1	481	CCGTCGCGGCCTGTCGTCGACGAGGAGGAGGGCGCGTGCGT	562
RBPH54	493	CCGTCGCGGCCTGTCGTCGACGAGGAGGAGGCTGCGTGTGGGCGGCGGTGCCTCCGGCTGTTCGGCGTGGACCTCCAGCTCC	574
TN1	563	GCGCCGATGCCTCGCCGGCGCTGGATTTGCAGCTTTGA 600	
RBPH54	575	GCGCCGATGCCTCGCCGGCGCTGGATTTGCAGCTTTGA 612	
TN1	1	MAT I VAWE SRNLOLOGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	82
RBPH54	1	MATIVAWESRNLQLQGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	82
TN1	83	ARGGDSTWRFRYSYWSSSQSYVITKGWSRYVRDKRLAAGDTVSFCRAGARLFIDCRKRAASVSSSSLVPPALIKVQLP	160
RBPH54	83	ARGGGGGG <mark>DSTWRFRYSYWSSSQSYVITKGWSRYVRDKRLAAGDTVSFCRAGARLFIDCRKRAASVSSSSLVPPALIKVQLP</mark>	164
TN1	161	PSRPVVDEEEAACGRRCLRLFGVDLQLRADASPALDLQL 199	
RBPH54	165	PSRPVVDEEEAACGRRCLRLFGVDLQLRADASPALDLQL 203	

**Supplementary Fig. S1.** Differences of *G5* between susceptible-parent TN1 and resistant-parent RBPH54.

Alignments of *G5* coding regions cDNA sequences (A) and amino acids sequences (B). The results showed differences included three single nucleotide polymorphism, resulting in one single amino acid mutation from alanine to valine, and a 12-bp insertion corresponding to four glycines in RBPH54. Identical amino acids are in pink, similar amino acids are marked with green.



Supplementary Fig. S2. Complementation test for G4.

(A) Gene and vector structure of G4. The eight exons of G4 are boxed, with the ORF indicated by a grey box. In the linear map of pCAMBIA1304, G4 is assembled downstream of the 35S promoter in the antisense form.

(B) BPH-resistance bioassay of G4-transgenic lines at the seedling stage. G4-1 to G4-6, 35S::anti-G4 transgenic T<sub>1</sub> lines; TN1 susceptible-parent control; RBPH54, resistant-parent control.

(C) qRT-PCR analysis of *G4* expression levels in transgenic lines. Data are means  $\pm$  SD (n = 3 individuals) and expression levels are shown relative to *OsActin1*.

(**D**) BPH resistance scores of the *G4* transgenic lines. Their lower scores indicate their high resistance to BPH. Data are means  $\pm$  SD (n = 20 plants). (B) and (D) show that *G4* transgenic lines retained their resistance from RBPH54 after transformation.



	TN1+/RBPH54+	TN1-/RBPH54+	Independent lines
G5-a	8	7	15
G5-b	6	6	12

Supplementary Fig. S3. Expression of G5 alleles in transgenic lines.

(A) Gene structure of G5. The ORF is indicated by a grey box, and the region with sequence differences between G5 dominant and recessive alleles is delineated between the black lines.

(**B**) The alleles expressed in the *G5* transgenic lines. Both G5-a and G5-b transgenic lines were performed in resistant-parent RBPH54, and the recessive *G5* allele of RBPH54 were consequently detected in all the transgenic lines (RBPH54+). The transgenic lines that dominant *G5* allele of TN1 were also detected (TN1+/RBPH54+) were confirmed as positive transformants and selected to evaluate BPH resistance.



Supplementary Fig. S4. BPH29-GFP fusion protein activity test.

(A) BPH-resistance bioassay of BPH29-GFP fusion protein transgenic lines at the seedling stage. BPH29-GFP fusion protein was assembled into the vector pCAMBIA1304. 1304-1 to 1304-4, 35S::GFP transgenic T<sub>1</sub> lines; G5gfp-1 to G5gfp-7, 35S::BPH29::GFP transgenic T<sub>1</sub> lines; TN1 susceptible-parent control; RBPH54, resistant-parent control.

(**B**) The alleles expression in the 35S::*BPH29*::*GFP* transgenic lines. As described in Supplementary Fig. S3 legend, the transgenic lines that *G5* allele of both TN1 and RBPH54 were detected (TN1+/RBPH54+) were confirmed as positive transformants. The results corresponded with BPH-resistance bioassay.

(C) BPH resistance scores of BPH29-GFP fusion protein transgenic lines. G5gfp positive transformants lines showed higher scores, indicating their high susceptibility to BPH and the activity of BPH29-GFP fusion protein. The lower scores of 1304 control transgenic lines indicate their high resistance to BPH. Data are means  $\pm$  SD (*n* = 20 plants).

## Supplemental Tables:

Supplemental Table S1. Transcript levels of *BPH29* and *OsActin1* at different rice developmental stages<sup>a</sup>

		Signal value <sup>b</sup>			
Number	Sample Name	BPH29 (Os06g01860)		OsActin1 (Os03g0718100)	
		Minghui 63	Zhenshan 97	Minghui 63	Zhenshan 97
1	calli, T2, 15 days after induction	9	5	4580	4593
2	calli, T3, 15 days after induction	25	25	6284	4618
3	calli, 15 days after subculture	14	38	4989	3361
4	calli, screening stage	36	29	6657	3912
5	root, seedling with 2 tillers	1574	3242	6759	4570
6	shoot, seedling with 2 tillers	21	24	5915	4254
7	leaf and root at three-leaf stage	102	129	11293	12509
8	flag leaf, 14 days after heading	17	3	2313	2215
9	stem, 5 days before heading	10	19	4992	4614
10	flag leaf, 5 days before heading	26	22	1978	2278
11	stem, heading stage	10	0	7341	4668
12	panicle, heading stage	7	24	10015	6358
13	leaf, 4-5cm young panicle	26	19	4760	2898
14	sheath, 4-5cm young panicle	3	16	3866	3918

15	panicle, 4-5cm young panicle	9	11	5528	4525
16	hull, one day before flowering	16	10	5114	4691
17	stamen, one day before flowering	12	14	21412	8720
18	spikelet, 3 days after pollination	8	10	6496	5176
19	endosperm, 7 days after pollination	19	14	8798	6619

a. Informations obtained from CREP (http://crep.ncpgr.cn).

b. Signal values represent average hybridization signal values. Minghui 63 and Zhenshan 97 are *indica* rice variety.

Purpose	Primer Name	Accession No.	Sequence $(5^{\circ} - 3^{\circ})$	Source
	DMOOO		CTTAAATGGGCCACATGCG	Gramene
	RIVI222		CAAAGCTTCCGGCCAAAAG	(http://www.gramene.org/)
	DN/244		CCGACTGTTCGTCCTTATCA	Gramene
	K1V1244		CTGCTCTCGGGTGAACGT	(http://www.gramene.org/)
			ATTACGTGCATGTCTGGCTG	Gramene
	KW1455		CGTACCTGACCATGCATCTG	(http://www.gramene.org/)
	<b>DN45</b> 40		GCCTTCTGGCTCATTTATGC	Gramene
	KM540		CTAGGCCTGCCAGATTGAAC	(http://www.gramene.org/)
Fine Mapping	g RM586		ACCTCGCGTTATTAGGTACCC	Gramene
			GAGATACGCCAACGAGATACC	(http://www.gramene.org/)
	DM500		GTTGCTCTGCCTCACTCTTG	Gramene
	KM388		AACGAGCCAACGAAGCAG	(http://www.gramene.org/)
	<b>D1</b> (500)		ATCATGGTCGGTGGCTTAAC	Gramene
	KW1389		CAGGTTCCAACCAGACACTG	(http://www.gramene.org/)
	BYL7		AAGCTAGGGAATCAGCGGTTA	Van a. et al. 2011
			TGTGGCATGTCACTCACTCAC	1 ang <i>et al.</i> , 2011
	BYL8		CCCACTTCCACAACCACA	Yang <i>et al.</i> , 2011

## Supplemental Table S2. Primers used in this work

			ATGCTCCTAGCTTCCTATTCC		
	DIDA		CACGTAATTATCGCGGTTAC		
	BID2		TACTAGGCAGGGTTCCTCTC		
	BID3		AAAATTTGAAACACGGGAAT	This study	
			GAATATGCTTTGCATCAGTCA		
			ATAATGCTTGCGACCGGTAC	This study.	
$C_{1}$	G4 qR1-PCK	– Os06g01850	TGTCAAACTCCTCCTTGTAG	This study	
64	G4 Vector		GCACTAGTTTAGTAGACTTCCACGTT	This study.	
			GCGGTCACCATGGCCGCCGTCACGGCT		
	G5 Sequencing	- Os06g01860 -	ATGGCCACCATTGTTGCATG	This study.	
			TCAAAGCTGCAAATCCAGCG	- This study	
	G5 qRT-PCR		CAAGGGATGGAGCCGATACG	This study	
			AAAGCTGCAAATCCAGCGCC		
<i>C</i> 5	G5 Vector		GCACTAGTATGGCCACCATTGTTGCA	This study.	
65			GCGGTCACCTCAAAGCTGCAAATCCAG		
	G5 Promoter		GCGGATCCGCTGGTTCCGAGCTAAGCC	This study	
			GCGGTCACCGCTAGATCAAAGCTG		
	Hygromycin test		ATTTGTGTACGCCCGACAGT	This study.	
			CTCTCGGAGGGCGAAGAATC		

	OsActin1	Os03g0718100	TCTGGCATCACACCTTCTACA	This study
			GGAAGGCTGGAAGAGGAC	<sup>-</sup> This study
	PR10	D38170	CCCTGCCGAATACGCCTAA	0: . 1 2007
			CTCAAACGCCACGAGAATTTG	- Qiù <i>et al.</i> , 2007
	DAI	X87946	GCACATCTTGGAGGGAAGCT	D 1. 2000
	PAL		GCGCGGATAACCTCAATTTG	– Du <i>et al.</i> , 2009
	CHS	X89859	CCGGCGAACTGCGTGTAC	01 1 2007
Defense			TTCCTGATCTGCGACTTGTCA	- Qiu <i>et al.</i> , 2007
related genes	NPR1	AY923983	TTTCCGATGGAGGCAAGAG	D I. 2000
			GCTGTCATCCGAGCTAAGTGTT	– Du <i>et al.</i> , 2009
	AOS2	AY062258	CTCGTCGGAAGGCTGTTGCT	D 1 2000
			ACGATTGACGGCGGAGGTT	– Du <i>et al.</i> , 2009
	LOX	D14000	GCATCCCCAACAGCACATC	01 1 2007
			AATAAAGATTTGGGAGTGACATATTGG	- Qiu <i>et al.</i> , 2007
	EIN2	11/20/5/0	CAAGGAACCAGTGACAACCA	D 1 2000
		AY 396568	GCAGTCGTCTCCGCAGTTAG	– Du <i>et al.</i> , 2009