Supplemental material

Characterization of a Novel Bipartite Double-stranded RNA Mycovirus Conferring Hypovirulence in the Phytopathogenic Fungus *Botrytis porri*

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TABLE S1. Oligonucleotide primers/adaptor used in this study.

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TABLE S5. Comparison of volumes for the spherical viral particle of dsRNA viruses.

FIG. S1. A schematic diagram showing the strategy used for full-length cDNA cloning of dsRNA-1 and dsRNA-2 of Botrytis porri RNA virus 1 in strain GarlicBc-72 of *Botrytis porri*. The PCR primers and the 3’-adaptor used in the cDNA cloning are indicated on the diagram. The oligonucleotide sequences of the primers and the 3’-adaptor were listed in Table S1.

FIG. S2. Proline frequency profiles in the polypeptides encoded by ORF I and ORF II of BPRV1. Note the high proline frequency occurring in the region (221 to 264) on the ORF I-encoded polypeptide. Dotted line indicates the threshold value (0.33).

FIG. S3. The lesion length on detached garlic leaves (20 ºC, 72 h) (**A**) and the mycelial growth rates on potato dextrose agar (20 ºC) (**B)** for strains GarlicBc-38, OnionBc-95 and GP72SC35 of *Botrytis porri,* and the derivative isolates of strains GarlicBc-38 (72-38-1, 72-38-2, 72-38-3), OnionBc-95 (72-95-1, 72-95-2, 72-95-3) and GP72SC35 (72-35-1, 72-35-2, 72-35-3) in pair cultures of GarlicBc-38/GarlicBc-72, OnionBc-95/GarlicBc-72 and GP72SC35/GarlicBc-72 in the horizontal transmission experiment. Each parameter for each strain/isolate was expressed as the arithmetic mean ± S.E.M. Bars in graphs A or B labeled with the same letters are not significantly different ($P > 0.05$) according to Least Significant Difference Test. The symbols "+" and "–" indicate the presence and the absence of dsRNA-1 or dsRNA-2, respectively, according to the results of dsRNA extraction from mycelia and detection by agarose gel electrophoresis.

FIG. S4. A schematic graph showing the results of peptide mass fingerprinting (PMF) analysis for capsid proteins p85, p80 and p70 of Botrytis porri RNA virus 1. The colored areas on each gray bar represent amino acid sequences detected by PMF matched the deduced amino acid sequences. Ion scores higher and lower than 46 indicate good match and bad match, respectively.

TABLE S1. Oligonucleotide primers/adaptor used in this study.

Name	Sequence (5'→ 3')	Position [1]	Polarity [2]
to cDNA clone			
BP5-4 (primer)	CGTTCAGCGGGCACAATG	dsRNA-1: 2162–2145	–
BP3 (primer)	GCGGTGGCTTAGGTTTGATGC	dsRNA-1: 5382–5402	+
BPM3 (primer)	AGCGCTACGAGGGCACTGGA	dsRNA-1: 1396–1415	+
BPM4 (primer)	GTCGTCATCGCTATCTCGCTCTTC	dsRNA-1: 2200–2177	–
BPM5 (primer)	CCGTTGTAGAGGCAGGTATTGAGT	dsRNA-1: 2520–2543	+
BPM6 (primer)	CATCTTCCCGTTTCTTGCTATTTT	dsRNA-1: 4144–4121	–
BG1 (primer)	CACTGCTGCTCGCTCTG	dsRNA-2: 1695–1679	–
BG2 (primer)	GCATCGGCAAGAGCACAGG	dsRNA-2: 3324–3342	+
BPA (3'-adaptor)	TTCAAATCCTACTGGCGCCGTG-(NH2)	to 3'-end of dsRNA	+/–
RCBPA (primer)	CACGGCGCCAGTAGGATTTGAA	complementary to the adaptor BPA	–/+
to probe dsRNA-1 or dsRNA-2			
BVP1F (primer)	AAGAGCTGCCTATTTGAA	dsRNA-1: 8–25	+
BVP1R (primer)	TGTGTTGTAAGGCATTTTG	dsRNA-1: 430–412	–
BVP2F (primer)	AGATGGTGCCGGCAGACGAGA	dsRNA-1: 4140–4160	+
BVP2R (primer)	CCACCGACCCGACCGACAC	dsRNA-1: 4645–4627	–
BVP3F (primer)	GCGGTGCCAGGTGCTTATTCTT	dsRNA-2: 3921–3942	+
BVP3R (primer)	TCCGCTCAGTGGTTAGGCTCAG	dsRNA-2: 4371–4350	–
To test cDNA inserts in the pMD18-T vector			
M13F-47	CGCCAGGGTTTTCCCAGTCACGAC	pMD18-T vector	
M13R-48	AGCGGATAACAATTTCACACAGGA	pMD18-T vector	

[1] Positions for the PCR primers or the adaptor in the cDNA of dsRNA-1 or dsRNA-2 were labeled in FIG. S1.

[2] Polarity refers to the positive strand (+) and the negative strand (–) of dsRNA-1 or dsRNA-2.

TABLE S2. Summary of the peptide mass fingerprinting analysis of p85.

Amino acid position	Calculated Mass	Observed Mass	± delta	Amino acid sequence	Ion Score
407–422	1846.8793	1846.8551	-0.0242	DTIAYNVPFGMSVYNR	137
462–475	1653.7295	1653.7417	0.0122	GNNHIALGCTMYMR	<46
501–514	1556.7955	1556.7692	-0.0263	FLLPVEDGDDAPIR	<46
501–515	1684.8905	1684.8650	-0.0255	FLLPVEDGDDAPIRK	71
552–579	2984.5364	2984.5945	0.0581	ITTYVTAGPGAPDLAVHSTIDLYVPEQR	183
580–594	1777.8789	1777.8539	-0.025	FTLCPLNGSELIEER	94
595–608	1573.6765	1573.6554	-0.0211	DSDDDGAFKVDAYR	<46
616–630	1903.9636	1903.9301	-0.0335	YLTAHNLWQQFPVMR	58
672–693	2317.2371	2317.2307	-0.0064	GVILGERDHQTSLHASVVTAAR	<46
679–693	1592.8140	1592.7916	-0.0224	DHQTSLHASVVTAAR	<46
781–804	2778.2021	2778.2322	0.0301	ASSYLCMEETPEGEGWQVVFSESR	<46
806–817	1595.7847	1595.7623	-0.0224	KQILQQEYCTER	<46
807–817	1467.6897	1467.6700	-0.0197	QILQQEYCTER	78
818–828	1419.7301	1419.7113	-0.0188	EQLTCLWLTQK	<46
829–857	3187.5906	3187.6785	0.0879	VAQENVGAVTYYQHEILPSESVSDTPVVR	83
870–885	1635.8741	1635.8542	-0.0199	GAPILWGTSTTAVAYK	<46
989–1007	1999.1084	1998.9700	-0.1384	EGFLPVKGGARPTSVQVTR	<46
1016–1026	1159.6140	1159.6176	0.0036	SPMKVANSTPK	<46
1095–1105	1318.6096	1318.6554	0.0458	YYDRGMDAALK	<46
1491–1509	2225.2112	2225.1196	-0.0916	QTTLINTILNLTYHHIAMK	<46
1549–1558	1095.5173	1095.6099	0.0926	MGGMEINAIK	<46
1586–1598	1401.7122	1401.6222	-0.0900	SLASFVHGNVENK	<46

Note: The overall protein score is 791.

TABLE S3. Summary of the peptide mass fingerprinting analysis of p80.

Amino acid position	Calculated Mass	Observed Mass	± delta	Amino acid sequence	Ion Score
289–315	1419.7301	3160.4287	0.0698	SGQPVEEPEHAGWLSWLNDEDDTMFDR	<46
382–398	1467.6897	2032.9447	0.0054	AGYIDSYEVVQCTTDRR	<46
407–422	1573.6765	1846.8710	-0.0083	DTIAYNVPFGMSVYNR	137
423–445	1595.7847	2390.2266	0.0181	AQPFNSTASVTPLSDILSAVEDK	<46
462–475	1637.7345	1653.7310	0.0015	GNNHIALGCTMYMR	<46
476–500	1653.7295	2744.2922	-0.0347	LMALEMLAEQGAATYIDLTAEGFNR	<46
501–514	1556.7955	1556.7827	-0.0128	FLLPVEDGDDAPIR	<46
501–515	1777.8789	1684.8794	-0.0111	FLLPVEDGDDAPIRK	57
519–551	1777.8789	3640.8281	0.0871	SVVAHQPYNMITLPDQSADSDAILMYYLAGNSR	<46
552–579	1846.8793	2984.5830	0.0466	ITTYVTAGPGAPDLAVHSTIDLYVPEQR	190
580–594	1903.9636	1777.8689	-0.0100	FTLCPLNGSELIEER	94
595–608	1903.9636	1573.6680	-0.0085	DSDDDGAFKVDAYR	<46
616–630	2032.9393	1903.9479	-0.0157	YLTAHNLWQQFPVMR	67
672–693	2317.2371	2317.2339	-0.0032	GVILGERDHQTSLHASVVTAAR	<46
679–693	2390.2085	1592.8114	-0.0026	DHQTSLHASVVTAAR	<46
781–804	2744.3269	2778.2292	0.02710	ASSYLCMEETPEGEGWQVVFSESR	<46
806–817	2794.1970	1595.7775	-0.0072	KQILQQEYCTER	<46
807–817	2984.5364	1467.6798	-0.0099	QILQQEYCTER	77
818–828	3160.3589	1419.7208	-0.0093	EQLTCLWLTQK	<46
829–857	3187.5906	3187.6575	0.0669	VAQENVGAVTYYQHEILPSESVSDTPVVR	90
870–885	3187.5906	1635.8646	-0.0095	GAPILWGTSTTAVAYK	<46
1095–1105	3640.7410	1318.6613	0.0517	YYDRGMDAALK	<46
1491–1509	1318.6096	2225.1206	-0.0906	QTTLINTILNLTYHHIAMK	<46

Note: The overall protein score is 817.

TABLE S4. Summary of the peptide mass fingerprinting analysis of p70.

Amino acid position	Calculated Mass	Observed Mass	± delta	Amino acid sequence	Ion Score
263–276	1467.7732	1467.704	-0.0692	LISMTGALMAKMGK	<46
311–334	2724.3489	2724.3506	0.0017	VGTAYTDKHSVNLWSYRPTSAQSR	<46
319–334	1888.9413	1888.9532	0.0119	HSVNLWSYRPTSAQSR	47
337–348	1235.6379	1235.6412	0.0033	AVNTGIGYTSPR	64
370–378	1081.5347	1081.5372	0.0025	DYIVLGDMR	<46
380–387	1036.5463	1036.5413	-0.005	KVPSEWYK	<46
453–466	1654.8411	1654.8546	0.0135	IMEAVAHAWEWAIK	<46
505–521	2049.0176	2049.0256	0.008	TDLSIVPTYYTYQTDIR	98
657–667	1143.6190	1143.6218	0.0028	GIPMISIAGER	<46
714–723	1216.5845	1216.5865	0.002	YLEETFTTGR	84
753–765	1404.7845	1404.7924	0.0079	EATSVFVTKPVAR	<46
1424–1436	1481.7053	1481.7192	0.0139	MGEFSDPNAITRK	<46
1778–1786	1095.5615	1095.6187	0.0572	SWTRAMLSK	<46

Note: The overall protein score is 322.

TABLE S5. Comparison of volumes for the spherical viral particle of dsRNA viruses.

Virus genus	Largest size of dsRNA in one viral particle (bp)	Diameter of viral particle (nm)	Volume of viral particle (nm^3)[a]	Length of dsRNA per unit volume (bp/nm^3)
Birnaviridae				
Aquabirnavirus	6×10^3	70	1.80×10^5	0.03
Avibirnavirus	6×10^3	70	1.80×10^5	0.03
Blosnavirus	6×10^3	70	1.80×10^5	0.03
Entomobirnavirus	6.5×10^3	70	1.80×10^5	0.03
Cystoviridae				
Cystovirus	13.3×10^3	85	3.22×10^5	0.04
Chrysoviridae				
Chrysovirus	3.5×10^3	35 - 40	2.24×10^4 - 3.35×10^4	0.10 - 0.16
Partitiviridae				
Alphacryptovirus	2×10^3	30	1.41×10^4	0.14
Betacryptovirus	2.25×10^3	40	3.35×10^4	0.07
Cryspovirus	2.1×10^3	30 - 35	1.41×10^4 - 2.24×10^4	0.09 - 0.15
Partitivirus	2.1×10^3	30 - 35	1.41×10^4 - 2.24×10^4	0.09 - 0.15
Picobirnaviridae				
Picobirnavirus	4×10^3	35	2.24×10^4 - 3.35×10^4	0.12 - 0.18
Reoviridae[b]				
Aquareovirus	1.8×10^4 - 3.1×10^4	75	2.21×10^5	0.08 - 0.14
Coltivirus	2.9×10^4	60 - 80	1.13×10^5 - 2.68×10^5	0.11 - 0.26
Cypovirus	2.5×10^4	65	1.44×10^5	0.17
Dinovernavirus	2.3×10^4	50	6.54×10^4	0.35
Fijivirus	2.7×10^4	70	1.80×10^5	0.15
mycoreovirus	2.3×10^4	80	2.68×10^5	0.09
Idnoreovirus	2.7×10^4 - 3.1×10^4	70	1.80×10^5	0.15 - 0.17
Orthoreovirus	2.4×10^4	80	2.68×10^5	0.09
Oryzavirus	2.6×10^4	70	1.80×10^5	0.14
Cardoreovirus	1.9×10^4	55	8.71×10^4	0.22

Orbivirus	1.9×10^{4}	80	2.68×10^{5}	0.07
Mimoreovirus	2.5×10^{4}	90 - 95	3.81×10^{5} - 4.49×10^{5}	0.06 - 0.07
Phytoreovirus	2.6×10^{4}	70	1.80×10^{5}	0.14
Rotavirus	1.9×10^{4}	80	2.68×10^{5}	0.07
Seadornavirus	2.1×10^{4}	60	1.13×10^{5}	0.19
Totiviridae				
Giardiavirus	6.3×10^{3}	36	2.40×10^{4}	0.25
Leishmaniavirus	5.3×10^{3}	33	1.90×10^{4}	0.28
Totivirus	4.6×10^{3} - 6.7×10^{3}	40	3.35×10^{4}	0.14 - 0.20
Victorivirus	4.6×10^{3} - 6.7×10^{3}	40	3.35×10^{4}	0.14 - 0.20
Megabirnaviridae				
Megabirnavirus	8.9×10^{3}	50	6.54×10^{4}	0.14
Unassigned				
BpRV1[c]	$6.2\times10^{3}/1.2\times10^{4}$	35	3.35×10^{4}	0.28/0.53

* All viral information was obtained from ViralZone (http://viralzone.expasy.org/).

[a] The volumes of different viral particles were calculated with the diameter of corresponding viral particle by using the volume formula of a sphere.

[b] To simplify the calculation, the genome for all viruses in Reoviridae were temporarily considered to be encapsidated in one viral particle.

[c] the data for BpRV1 were calculated twice as two dsRNAs were encapsidated separately or together.

FIG. S1

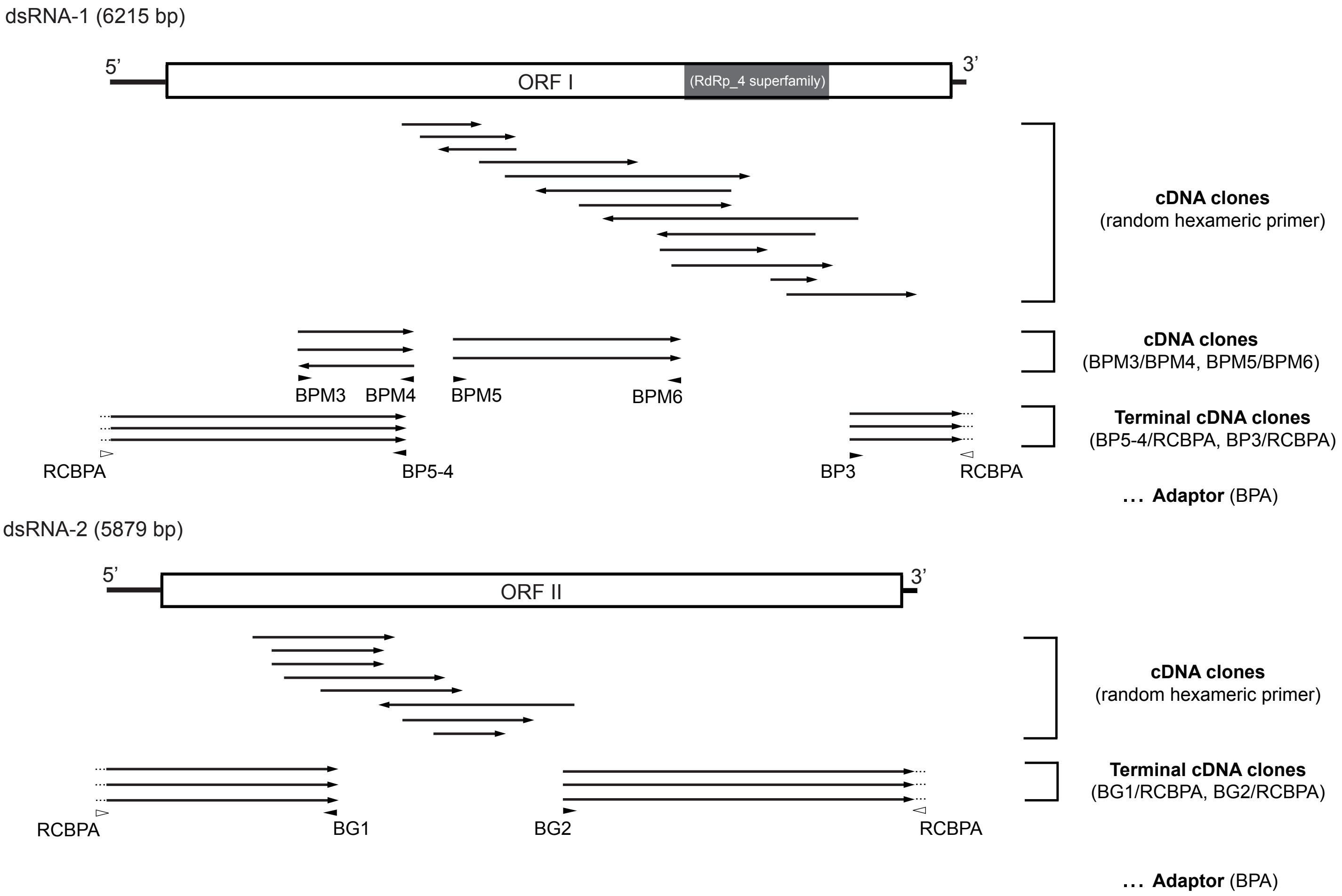
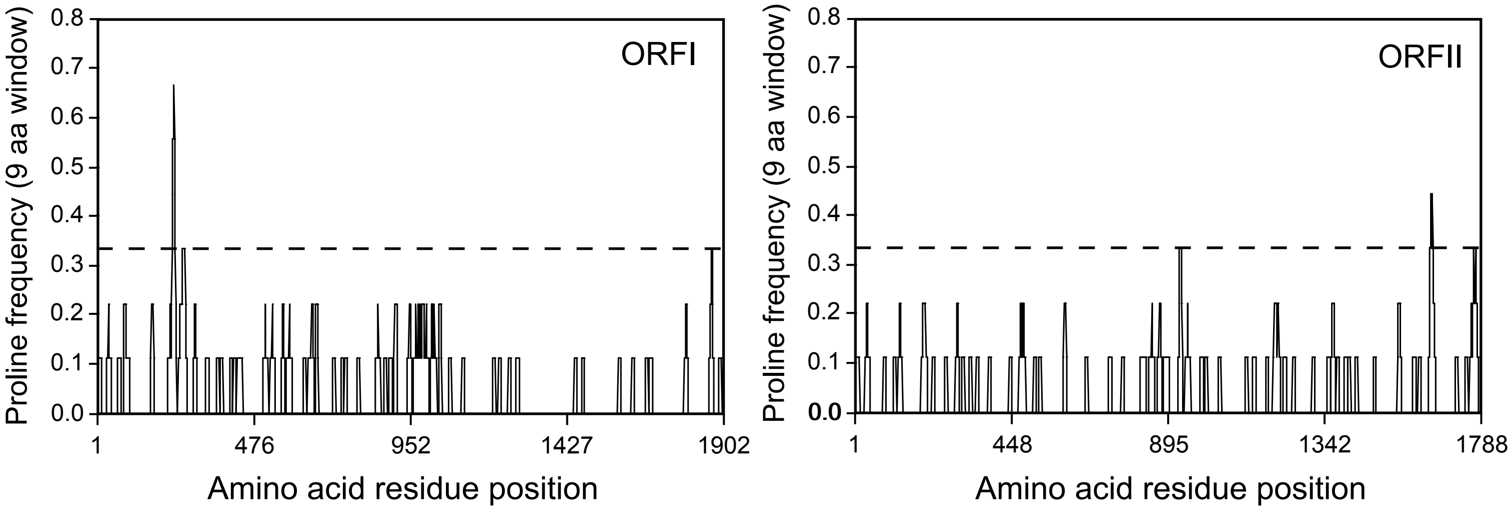

dsRNA-1 (6215 bp)
5'
ORF I
(RdRp_4 superfamily)
3'
cDNA clones
(random hexameric primer)
cDNA clones
(BPM3/BPM4, BPM5/BPM6)
BPM3
BPM4
BPM5
BPM6
Terminal cDNA clones
(BP5-4/RCBPA, BP3/RCBPA)
RCBPA
BP5-4
BP3
RCBPA
... Adaptor (BPA)
dsRNA-2 (5879 bp)
5'
ORF II
3'
cDNA clones
(random hexameric primer)
Terminal cDNA clones
(BG1/RCBPA, BG2/RCBPA)
RCBPA
BG1
BG2
RCBPA
... Adaptor (BPA)

FIG. S2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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
476
952
1427
1902
Amino acid residue position
ORFII
Proline frequency (9 aa window)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
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895
1342
1788
Amino acid residue position

Fig. S3

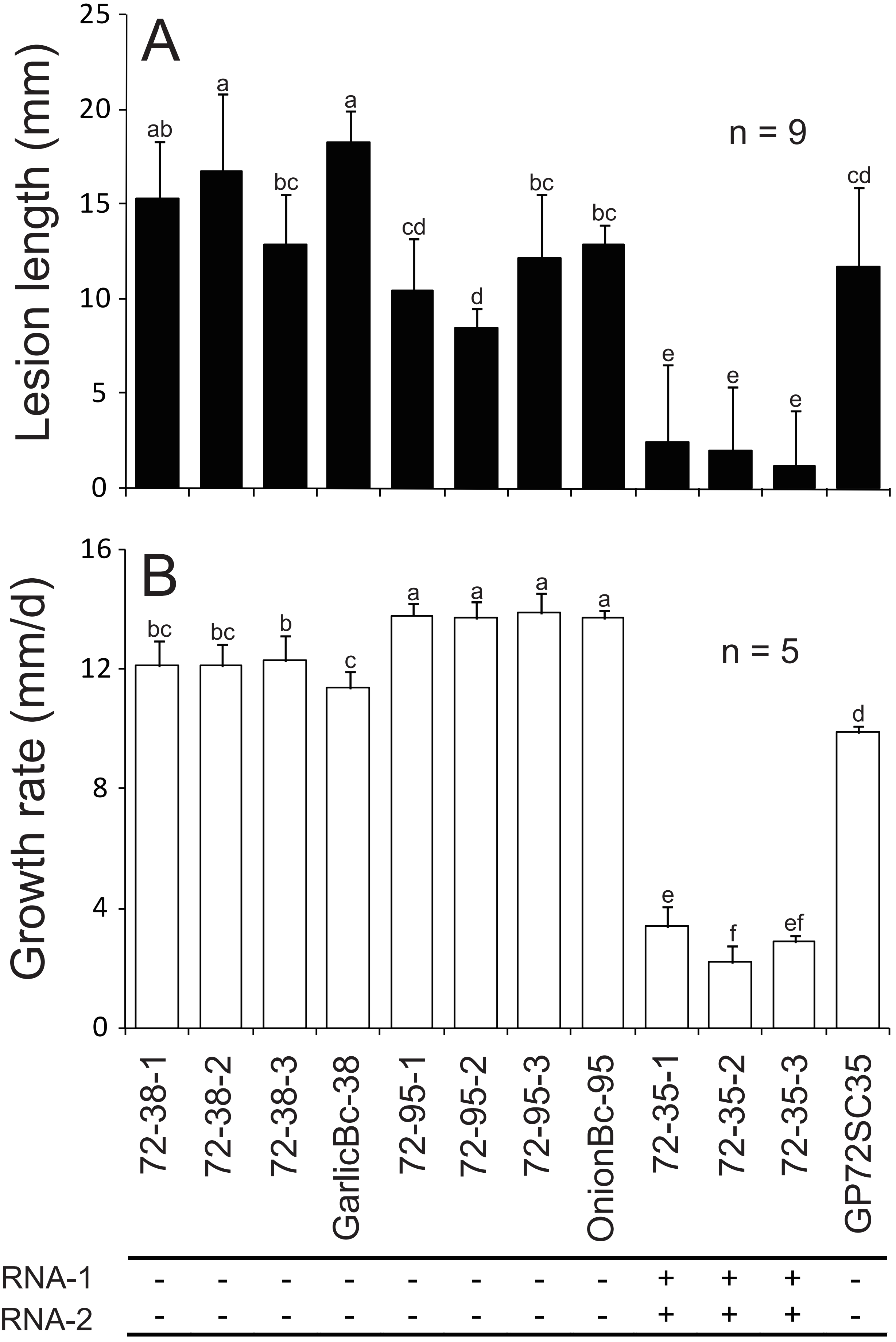
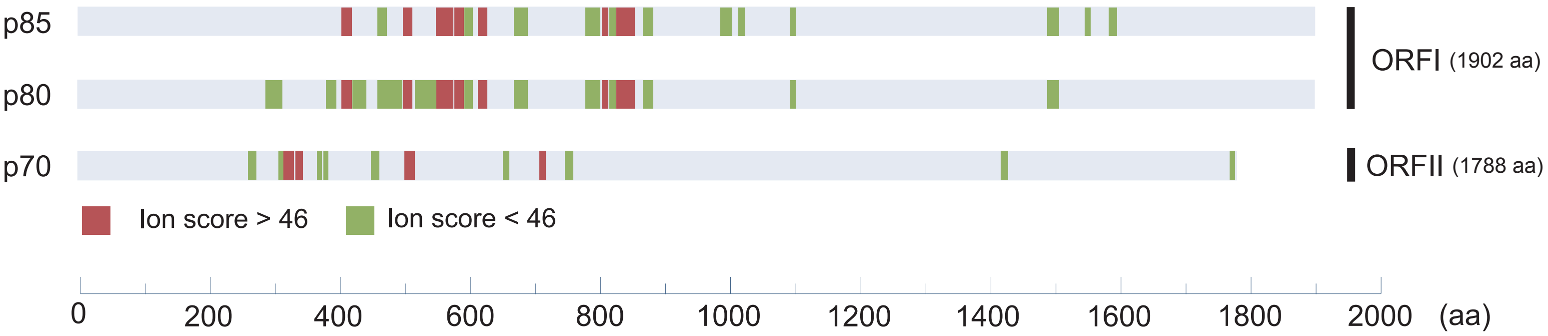

A
Lesion length (mm)
25
20
15
10
5
0
n = 9
ab
a
bc
a
cd
d
bc
bc
e
e
e
cd
B
Growth rate (mm/d)
16
12
8
4
0
n = 5
bc
bc
b
c
a
a
a
a
e
f
ef
d
72-38-1
72-38-2
72-38-3
GarlicBc-38
72-95-1
72-95-2
72-95-3
OnionBc-95
72-35-1
72-35-2
72-35-3
GP72SC35
dsRNA-1 - - - - - - - - + + + -
dsRNA-2 - - - - - - - - + + + -

Fig. S4


p85
p80
p70
ORFI (1902 aa)
ORFII (1788 aa)
🟥 Ion score > 46
🟩 Ion score < 46
0
200
400
600
800
1000
1200
1400
1600
1800
2000
(aa)