

Supplementary tables

Supplementary Table 1. Single nucleotide polymorphism and amino acid polymorphism in HC-Pro gene/protein of PRSV-P in relation to Bangalore-1 isolate (MF481203)

Sr No.	PRSV-P strains	SNP (Single Nucleotide Polymorphism)			AAP (Amino Acid Polymorphism)		
		Accession no.	SNP	Percent SNP	Accession no.	AAP	Percent AAP
1.	Bengaluru-1*	MF481203.1	0	0	AWP45088.1	0	0
2.	Bengaluru-2	MN103525.1	32	2.33	QHW08431.1	7	1.96
3.	Delhi-1	KY448321.1	41	2.99	ATZ76589.1	6	1.68
4.	Delhi-2	KC149504.1	43	3.13	AGE32247.1	7	1.96
5.	Tirupati AP	MG383641.1	51	3.72	AYB35846.1	7	1.96
6.	Bengaluru-IIHR	MN103527.1	54	3.94	QHW08433.1	8	2.24
7.	Sikar Raj-1	KJ922614.1	73	5.32	AIN40015.1	13	3.64
8.	Pune-1	MF405299.1	77	5.62	ASV48703.1	16	4.48
9.	Gadag-W	KC136309.1	80	5.83	AGD79972.1	15	4.2
10.	Hyderabad	KP743981.1	80	5.83	AKQ98195.1	14	3.92
11.	Koppal	KC149506.1	80	5.83	AGE32249.1	14	3.92
12.	Pune -2	MF405297.1	80	5.83	ASV48701.1	14	3.92
13.	Gadag-P	JQ394692.1	81	5.91	AFI25156.1	15	4.2
14.	Sikar Raj -2	KJ922615.1	82	5.98	AIN40016.1	14	3.92
15.	Dharwad	KU196788.1	83	6.05	APM86411.1	12	3.36
16.	Indore MP	KC149503.1	83	6.05	AGE32246.1	15	4.2

Supplementary Table 2. List of effective siRNAs targeting to PRSV HC-Pro gene

Sr no	Target position	Target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5'→3') 21nt passenger (5'→3')	Functional siRNA selection: Ui-Tei	Seed-duplex stability (T _m)		
						Guide	Passenger
1.	62-84	GTCTGATATGGACGTTACAATGT	AUUGUAACGUCCAUAUCAGAC CUGAUAUGGACGUUACAAUGU	U		14.7 °C	14.9 °C
2.	64-86	CTGATATGGACGTTACAATGTGT	ACAUUGUAACGUCCAUAUCAG GAUAUGGACGUUACAAUGUGU	U		13.5 °C	21.2 °C
3.	127-149	GTCACAAAATAACTTGCAATACT	UAUUGCAAGUUAUUUUGUGAC CACAAAUAACUUGCAAUACU	U		20.0 °C	5.3 °C
4.	234-256	TCGTCATATGGAGGTTCAATTTGG	AAUGAACCUGCAUAUGACGA GUCAUAUGGAGGUUCAUUUGG	U		14.8 °C	14.9 °C
5.	308-330	CACGAATGATGGAGCTTTTAAAG	UUAAGAGCUCCAUCAUUCGUG CGAUGAUGGAGCUUUUAAAG	U		13.7 °C	16.2 °C
6.	309-331	ACGAATGATGGAGCTTTTAAAGA	UUUAAAAGCUCCAUCAUUCGU GAAUGAUGGAGCUUUUAAAGA	U		-3.8 °C	13.6 °C
7.	319-341	GAGCTTTTAAAGAAATTGCTAAG	UAGCAAUUUCUUUAAAAGCUC GCUUUUAAAGAAAUUGCUAAG	U		18.5 °C	-3.8 °C
8.	320-342	AGCTTTTAAAGAAATTGCTAAGA	UUAGCAAUUUCUUUAAAAGCU CUUUUAAAGAAAUUGCUAAGA	U		19.7 °C	-9.1 °C
9.	335-357	TGCTAAGAAAATTGATGAAAAGA	UUUUCAUCAUUUUCUUGCA CUAAGAAAAUUGAUGAAAAGA	U		16.3 °C	7.1 °C
10.	402-424	ATCAAAGGTTGCTAGCAATTGG	AAUUGCUAGCGAACCUUUGAU CAAAGGUUCGCUAGCAAUUGG	U		19.7 °C	17.3 °C
11.	412-434	CGCTAGCAATTGGCTACGAATTT	AUUCGUAGCCAAUUGCUAGCG CUAGCAAUUGGCUACGAAUUU	U		20.9 °C	19.7 °C
12.	477-499	CTCAAAGGACGGAGTCAATAAA	UAUUGACUCCGUCCUUUUGAG CAAAGGACGGAGUCAAUAAA	U		19.2 °C	18.7 °C
13.	506-528	CAGCGTTGAAAGCTTCAGAAATA	UUUCUGAAGCUUUCACGCUG	U		20.4 °C	21.1 °C

			GCGUUGAAAGCUUCAGAAUA				
14.	507-529	AGCGTTGAAAGCTTCAGAAATAA	AUUUCUGAAGCUUUCACGCU CGUUGAAAGCUUCAGAAUAA	U		20.4 °C	14.9 °C
15.	508-530	GCGTTGAAAGCTTCAGAAATAAG	UAUUUCUGAAGCUUUCACGC GUUGAAAGCUUCAGAAUAA	U		12.0 °C	12.2 °C
16.	533-555	TTCTGGAAAAGCTCATTTCAACC	UUGAAAUGAGCUUUUCCAGAA CUGGAAAAGCUCAUUUCAACC	U		13.8 °C	20.1 °C
17.	557-579	AGCTCTTACATGTGATAATCAAT	UGAUUAUCACAUGUAAGAGCU CUCUUACAUGUGAUAAUCAAU	U		11.6 °C	20.3 °C
18.	559-581	CTCTTACATGTGATAATCAATTG	AUUGAUUAUCACAUGUAAGAG CUUACAUGUGAUAAUCAAUUG	U		8.9 °C	13.5 °C
19.	563-585	TACATGTGATAATCAATTGGACA	UCCAAUUGAUUAUCACAUGUA CAUGUGAUAAUCAAUUGGACA	U		17.7 °C	20.5 °C
20.	612-634	AGGCAATACCACGCCAAAAGATT	UCUUUUGGCGUGGUUUUGCCU GCAAUACCACGCCAAAAGAUU	U		16.1 °C	20.0 °C
21.	613-635	GGCAATACCACGCCAAAAGATTC	AUCUUUUGGCGUGGUUUUGCC CAAUACCACGCCAAAAGAUUC	U		12.2 °C	20.0 °C
22.	629-651	AAGATTCTTTGCCAACTATTTTG	AAAUAGUUGGCAAAGAAUCUU GAUUCUUUGCCAAACUAUUUUG	U		4.6 °C	5.3 °C
23.	638-660	TGCCAACTATTTTGAGAAAATTG	AUUUUCUCAAAAUAGUUGGCA CCAACUAUUUUGAGAAAUUG	U		14.8 °C	11.6 °C
24.	639-661	GCCAACTATTTTGAGAAAATTGA	AAUUUUCUCAAAAUAGUUGGC CAACUAUUUUGAGAAAUUGA	U		5.3 °C	4.6 °C
25.	642-664	AACTATTTTGAGAAAATTGATCA	AUCAUUUUCUCAAAAUAGUU CUAUUUUGAGAAAUUGAUC	U		7.4 °C	-1.4 °C
26.	701-723	TCCAAATGGTGTGAGAAAAGTAG	ACUUUUCUGACACCAUUUGGA CAAUGGUGUCAGAAAAGUAG	U		10.3 °C	18.8 °C
27.	727-749	TTGGCAATTTGATATTTTCAACA	UUGAAAAUAUCAAUUGCCAA GGCAAUUUGAUUUUUAACA	U		7.4 °C	14.0 °C
28.	729-751	GGCAATTTGATATTTTCAACAAA	UGUUGAAAAUAUCAAUUGCC CAAUUUGAUUUUUAACAAA	U		14.9 °C	7.4 °C
29.	801-823	CCGATTACTCGCGAATGCATTGC	AAUGCAUUCGCGAGUAAUCGG	U		19.9 °C	14.5 °C

			GAUUACUCGCGAAUGCAUUGC				
30.	820-842	TTGCATTACGCAATAACAATTAT	AAUUGUUAUUGCGUAAUGCAA GCAUUACGCAAUAACAAUUAU	U		6.9 °C	13.7 °C
31.	827-849	ACGCAATAACAATTATGTTTCATG	UGAACAUAAUUGUUAUUGCGU GCAAUAACAAUUAUGUUCAUG	U		16.1 °C	6.9 °C
32.	828-850	CGCAATAACAATTATGTTTCATGT	AUGAACAUAAUUGUUAUUGCG CAAUAACAAUUAUGUUCAUGU	U		20.5 °C	6.9 °C
33.	834-856	AACAATTATGTTTCATGTATGTAG	ACAUACAUGAACAUAAUUGUU CAAUUAUGUUCAUGUAUGUAG	U		13.3 °C	-1.8 °C
34.	847-869	ATGTATGTAGCTGCGTAACTTTA	AAGUUACGCAGCUACAUACAU GUAUGUAGCUGCGUAAUUA	U		19.6 °C	13.1 °C
35.	883-905	CTGCAACAAGTGAGTTGAAGACT	UCUUCAACUCACUUGUUGCAG GCAACAAGUGAGUUGAAGACU	U		19.2 °C	16.7 °C
36.	905-927	TCCTACTAAGAACCATATCGTTC	ACGAUAUGGUUCUAGUAGGA CUACUAAGAACCAUAUCGUUC	U		15.3 °C	11.3 °C
37.	915-937	AACCATATCGTTCTTGGTAATTC	AUUACCAAGAACGAUAUGGUU CCAUAUCGUUCUUGGUAAUUC	U		20.0 °C	15.3 °C
38.	916-938	ACCATATCGTTCTTGGTAATTCT	AAUUACCAAGAACGAUAUGGU CAUAUCGUUCUUGGUAAUUCU	U		20.0 °C	16.4 °C
39.	1000-1022	AAGGTTACTGCTACAAGAACATC	UGUUCUUGUAGCAGUAACCUU GGUUCUGCUACAAGAACAUC	U		19.2 °C	19.0 °C
40.	1007-1029	CTGCTACAAGAACATCTTTTATAG	AAAAAGAUGUUCUUGUAGCAG GCUACAAGAACAUCUUUUUAG	U		5.3 °C	16.4 °C
41.	1008-1030	TGCTACAAGAACATCTTTTATAGC	UAAAAAGAUGUUCUUGUAGCA CUACAAGAACAUCUUUUUAGC	U		5.5 °C	20.3 °C
42.	1105-1127	TCGGAGAATGGCCAACGATGTTA	ACAUCGUUGGCCAUUCUCCGA GGAGAAUGGCCAACGAUGUUA	U		21.1 °C	20.4 °C
43.	1122-1144	ATGTTAGATGTCGCAACATGTGC	ACAUGUUGCGACAUCAAACAU GUUAGAUGUCGCAACAUGUGC	U		18.1 °C	13.4 °C
44.	1139-1161	ATGTGCAAATCAGCTAATAATTT	AUUAUUAGCUGAUUUUGCACAU GUGCAAUCAGCUAAUAAUUU	U		-2.3 °C	20.0 °C
45.	1141-1163	GTGCAAATCAGCTAATAATTTTT	AAAUUAUUAGCUGAUUUGCAC	U		-10.3 °C	13.8 °C

			GCAAAUCAGCUAAUAAUUUUU				
46.	1142-1164	TGCAAATCAGCTAATAATTTTTC	AAAAUUAUUAGCUGAUUUGCA CAAUCAGCUAAUAAUUUUUC	U		-10.3 °C	12.0 °C
47.	1180-1202	ATGCAGAATTGCCACGAATTTTA	AAAUUCGUGGCAAUUCUGCAU GCAGAAUUGCCACGAAUUUUA	U		15.2 °C	12.0 °C
48.	1181-1203	TGCAGAATTGCCACGAATTTTAG	AAAAUUCGUGGCAAUUCUGCA CAGAAUUGCCACGAAUUUAG	U		7.4 °C	12.0 °C
49.	1251-1273	GTGGATTCTGGGTACCATATACT	UAUAUGGUACCCAGAAUCCAC GGAUUCUGGGUACCAUAUACU	U		20.0 °C	20.4 °C

Supplementary Table 3. List of VIGS candidates targeting the PRSV-P HC-Pro gene based on potential siRNA sequence

Sl. No.	Range on target sequence	Length	Number of siRNAs	Number of off-target	Significant off-targets/Number of hits
1.	557-757	201	14	184	4
2.	1068-1267	200	14	216	10
3.	1081-1280	200	14	218	10
4.	188-387	200	13	199	2
5.	214-413	200	13	207	6
6.	27-226	200	13	223	4
7.	308-507	200	10	145	10
8.	370-569	200	12	160	9
9.	37-236	200	13	217	4
10.	411-610	200	11	158	5
11.	515-714	200	11	185	4
12.	696-895	200	14	207	8
13.	720-919	200	14	211	12
14.	763-962	200	15	226	8
15.	847-1046	200	12	204	6

16.	913-1112	200	12	197	8
17.	946-1145	200	13	208	10
18.	95-294	200	14	237	2
19.	997-1196	200	11	184	10
20.	1092-1290	199	14	226	10
21.	1102-1300	199	14	227	12
22.	264-462	199	11	160	8
23.	451-649	199	12	181	5
24.	53-251	199	12	205	2
25.	665-863	199	14	212	8
26.	814-1012	199	13	206	2
27.	827-1025	199	13	212	4
28.	1026-1223	198	12	201	10
29.	1113-1310	198	14	228	12
30.	179-376	198	13	196	2
31.	278-475	198	10	142	8
32.	293-490	198	10	144	10
33.	904-1101	198	12	199	6
34.	1125-1321	197	14	234	12
35.	530-726	197	11	189	4
36.	597-793	197	11	183	6
37.	607-803	197	11	184	6
38.	629-825	197	12	191	10
39.	678-874	197	14	210	8
40.	783-979	197	14	203	4
41.	794-990	197	14	207	4
42.	875-1071	197	12	211	4
43.	111-306	196	14	226	2
44.	121-316	196	14	217	2
45.	231-426	196	13	198	8
46.	487-682	196	11	177	7

47.	888-1083	196	12	214	4
48.	16-210	195	13	215	4
49.	356-550	195	12	173	13
50.	992-1186	195	11	187	12
51.	1051-1244	194	13	214	2
52.	206-399	194	13	200	4
53.	6-199	194	13	206	4
54.	621-814	194	11	181	10
55.	91-284	194	14	233	2
56.	940-1133	194	12	194	10
57.	1063-1255	193	13	210	10
58.	590-782	193	11	186	6
59.	393-583	191	11	153	9
60.	442-632	191	12	176	5
61.	718-908	191	13	190	8
62.	762-952	191	14	209	8
63.	932-1122	191	12	197	8
64.	139-328	190	14	217	2
65.	505-694	190	11	179	7
66.	549-738	190	11	184	6
67.	745-932	188	14	212	8
68.	662-845	184	13	204	10
69.	1166-1341	176	15	251	10

Supplementary Table 4. The 201 bp (557-757) sequence was used in a comparative analysis involving Ui-Tei, Amarzguioui, Hsieh, Takasaki, Biopreds, i-Score, Reynolds, Katoh, DSIR with the I-score analyzer online tool

A. List of ten best i-Score containing the siRNA rank as follows

Pos.	Sense	Seed G	5' end	3' end	Whole dG	GC stretch	% GC	Ui-Tei	Amarzguioui	Hsieh	Takasaki	s-Biopredsi		i-Score		Reynolds	Katoh	DSIR	
												Score	Rank	Score	Rank			Score	Rank
161	GGCCAACUAUUUUGAGAAA	1.0	-3.3	-0.9	-33.2	4	36.8	Ia	6	1	9.7	0.866	5	80.6	1	8	82.3	93.1	7
167	CUAUUUUGAGAAAUUGAA	1.6	-2.1	-0.9	-26.3	1	21.1	Ia	2	1	-2.2	0.874	2	78.3	2	8	94.2	90.8	12
254	GGCAAUUUGAUAUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.874	1	78.0	3	7	82.4	96.8	1
233	GGCAAUUUGAUAUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.858	8	78.0	4	7	82.4	93.8	5
194	GAGAAAAUUGAUCAUCCAA	1.1	-2.4	-0.9	-31.5	2	31.6	Ib	4	0	14.7	0.861	7	75.8	5	6	85.9	92.5	9
183	GAAACUAUUUUGAGAAAAU	1.1	-2.4	-1.1	-26.4	1	21.1	Ia	3	2	9.7	0.849	14	75.7	6	9	108.1	86.9	31
163	CCAACUAUUUUGAGAAAAU	1.0	-3.3	-1.1	-28.5	2	26.3	Ia	4	2	2.3	0.808	56	74.8	7	9	103.2	79.0	109
141	CCAACUAUUUUGAGAAAAU	1.0	-3.3	-1.1	-28.5	2	26.3	Ia	4	2	2.3	0.842	18	74.8	8	9	103.2	84.9	40
115	CAAGAUUCUUUGCCAACUA	1.1	-2.1	-1.3	-33.2	3	36.8	Ib	2	0	-0.3	0.858	9	74.6	9	9	81.4	93.9	4
196	GAAAAUUGAUAUCCAAAU	0.3	-2.4	-1.1	-29.0	2	26.3	Ia	3	2	2.5	0.831	31	74.4	10	8	86.7	83.5	60

B. List of ten best s-Biopredsi containing the siRNA rank as follows

Pos.	Sense	Seed G	5' end	3' end	Whole dG	GC stretch	% GC	U i- T ei	Amarzguioui	Hsieh	Takasaki	s-Biopredsi		i-Score		Reynolds	Kato	DSIR	
												Score	Rank	Score	Rank			Score	Rank
254	GGCAAUUUGAUAUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.874	1	78.0	3	7	82.4	96.8	1
167	CUAUUUUGAGAAAUUGAA	1.6	-2.1	-0.9	-26.3	1	21.1	Ia	2	1	-2.2	0.874	2	78.3	2	8	94.2	90.8	12
258	AUUUGAUAUUUCCAACAA	-1.1	-1.1	-0.9	-25.1	1	15.8	II	2	0	1.1	0.869	3	74.1	12	7	103.4	90.9	11
211	AAAUGGUGUCAGAAAAGUA	1.7	-0.9	-1.3	-32.1	2	31.6	II	0	1	-6.6	0.867	4	71.9	17	8	93.8	94.1	3
161	GGCCAACUAUUUUGAGAAA	1.0	-3.3	-0.9	-33.2	4	36.8	Ia	6	1	9.7	0.866	5	80.6	1	8	82.3	93.1	7
11	UGUGAUAUAUCAAUCUCUUA	0.4	-2.1	-1.3	-30.9	1	26.3	II	1	0	4.3	0.862	6	72.4	15	7	84.7	91.5	10
194	GAGAAAAUUGAUCAUCCAA	1.1	-2.4	-0.9	-31.5	2	31.6	Ib	4	0	14.7	0.861	7	75.8	5	6	85.9	92.5	9
233	GGCAAUUUGAUAUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.858	8	78.0	4	7	82.4	93.8	5
115	CAAGAUUCUUUGCCAACUA	1.1	-2.1	-1.3	-33.2	3	36.8	Ib	2	0	-0.3	0.858	9	74.6	9	9	81.4	93.9	4
226	AGUAGUUGGCAAUUGAUA	0.6	-2.1	-1.3	-32.4	3	31.6	II	2	1	-8.6	0.853	10	71.6	20	7	75.3	93.5	6

C. List of ten best DSIR containing the siRNA rank as follows

Pos.	Sense	Seed G	5' end	3' end	Whole dG	GC stretch	% GC	U i- T ei	Amarzguioui	Hsieh	Takasaki	s-Biopredict		i-Score		Reynolds	Kato	DSIR	
												Score	Rank	Score	Rank			Score	Rank
254	GGCAAUUUGAUAUUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.874	1	78.0	3	7	82.4	96.8	1
30	CAUGUGAUAUAUCAAUUGUA	0.9	-2.1	-1.3	-29.4	1	26.3	Ia	2	1	2.7	0.852	11	73.3	14	7	87.6	95.0	2
211	AAAUGGUGUCAGAAAAGUA	1.7	-0.9	-1.3	-32.1	2	31.6	II	0	1	-6.6	0.867	4	71.9	17	8	93.8	94.1	3
115	CAAGAUUCUUUGCCAACUA	1.1	-2.1	-1.3	-33.2	3	36.8	Ib	2	0	-0.3	0.858	9	74.6	9	9	81.4	93.9	4
233	GGCAAUUUGAUAUUUUCAA	-0.6	-3.3	-0.9	-28.7	3	26.3	Ia	4	0	5.2	0.858	8	78.0	4	7	82.4	93.8	5
226	AGUAGUUGGCAAUUUGAUA	0.6	-2.1	-1.3	-32.4	3	31.6	II	2	1	-8.6	0.853	10	71.6	20	7	75.3	93.5	6
161	GGCCAACUAUUUUGAGAAA	1.0	-3.3	-0.9	-33.2	4	36.8	Ia	6	1	9.7	0.866	5	80.6	1	8	82.3	93.1	7
20	CAAUCUCUUACAUGUGAUA	0.5	-2.1	-1.3	-31.9	1	31.6	Ia	3	2	5.0	0.851	12	71.5	21	8	79.1	92.8	8
194	GAGAAAAUUGAUAUCCAA	1.1	-2.4	-0.9	-31.5	2	31.6	Ib	4	0	14.7	0.861	7	75.8	5	6	85.9	92.5	9
11	UGUGAUAAUCAAUCUCUUA	0.4	-2.1	-1.3	-30.9	1	26.3	II	1	0	4.3	0.862	6	72.4	15	7	84.7	91.5	10

Supplementary Table 5. Potential siRNA for off targets prediction against the *N. benthamiana* using the NCBI BLAST tool

1. AUUGAUUAUCACAUGUAAGAG

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Target
1.	<i>N. benthamiana</i> expansin responded kinase 1 mRNA, complete cds	26.3	112	76 %	0.19	100.00 %	2496	MW841628.1
2.	<i>N. benthamiana</i> NLR-required for cell death 3 (NRC3) mRNA, complete cds	24.3	69.4	71 %	0.74	100.00 %	2667	MK692736.1
3.	<i>N. benthamiana</i> 5-epi-aristolochene synthase mRNA, complete cds	24.3	53.0	85 %	0.74	100.00 %	1647	MH939184.1
4.	<i>N. benthamiana</i> NbEAS1a mRNA for 5-epi-aristolochene synthase, complete cds	24.3	53.0	85 %	0.74	100.00 %	1647	LC015755.1
5.	<i>N. benthamiana</i> sesquiterpene synthase (TPS1) mRNA, complete cds	24.3	53.0	85 %	0.74	100.00 %	1647	KF990999.1

2. CACCCCAUAAGAAAUUGCCAU

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> clone (v3) GT011eq UDP-glycosyltransferase mRNA, complete cds	24.3	75.3	80 %	0.74	100.00 %	1749	MT945337.1
2.	<i>N. benthamiana</i> putative RNA-dependent RNA polymerase RdRP2 mRNA, complete cds	24.3	169	95 %	0.74	100.00 %	3527	AY722009.1
3.	<i>N. benthamiana</i> clone (v3) GT005eq UDP-glycosyltransferase mRNA, complete cds	24.3	67.4	66 %	0.74	100.00 %	1649	MT945332.1
4.	<i>N. benthamiana</i> SBT4 mRNA, complete cds	24.3	110	57 %	0.74	100.00 %	2298	MN534999.1
5.	<i>N. benthamiana</i> phytoalexin (DEK) mRNA, complete cds	24.3	301	100 %	0.74	100.00 %	6429	AY450851.1

3. UAUUGCCUCUCACCCCAUAAG

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> MAPKKKb mRNA for protein kinase, complete cds	24.3	38.7	57 %	0.74	100.00 %	2477	AB649283.1
2.	<i>N. benthamiana</i> casein kinase 1.4 mRNA, complete cds	22.3	83.7	61 %	2.9	100.00 %	1419	MT363964.1
3.	<i>N. benthamiana</i> NbEXA1 gene for essential for potexvirus accumulation 1, complete cds	22.3	220	100 %	2.9	100.00 %	9419	LC171418.1
4.	<i>N. benthamiana</i> clone 8-148 unknown mRNA	22.3	51.0	71 %	2.9	93.33 %	813	AY310773.1
5.	<i>N. benthamiana</i> promoter and 5' UTR-like (SOD) gene, promoter region	20.3	34.7	80 %	12	100.00 %	1147	KP747644.1

4. UUUGGCGUGGUAUUGCCUCUC

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> clone (v3) GT005eq UDP-glycosyltransferase mRNA, complete cds	22.3	38.7	52 %	2.9	100.00 %	1649	MT945332.1
2.	<i>N. benthamiana</i> cysteine proteinase aleuran type mRNA, complete cds	22.3	38.7	52 %	2.9	100.00 %	1326	DQ084022.1
3.	<i>N. benthamiana</i> NbEXA1 gene for essential for potexvirus accumulation 1, complete cds	20.3	128	100 %	12	100.00 %	9419	LC171418.1
4.	<i>N. benthamiana</i> VPS15 mRNA, complete cds	20.3	20.3	47 %	12	100.00 %	651	KU561371.1
5.	<i>N. benthamiana</i> NRC2a mRNA, complete cds	20.3	53.0	61 %	12	100.00 %	2661	KT936525.1

5. CAAAGAAUCUUUUGGCGUGGU

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> NbNup88 mRNA for nuclear pore complex protein Nup88b, complete cds	28.2	249	95 %	0.047	94.44 %	2445	AB897509.1
2.	<i>N. benthamiana</i> clone (v3) GT006c UDP-glycosyltransferase	26.3	155	85 %	0.19	100.00 %	2753	MT945333.1

	mRNA, complete cds							
3.	<i>N. benthamiana</i> NbNup88a mRNA for nuclear pore complex protein Nup88a, complete cds	24.3	230	95 %	0.74	100.00 %	2445	AB897508.1
4.	<i>N. benthamiana</i> sulfite reductase mRNA, partial cds	24.3	83.7	80 %	0.74	93.75 %	2079	FJ440631.1
5.	<i>N. benthamiana</i> sulfite reductase mRNA, complete cds	24.3	83.7	80 %	0.74	93.75 %	2082	FJ465091.1

6. AAUAGUUGGCAAAGAAUCUU

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> MAP kinase phosphatase mRNA, complete cds	24.3	136	85 %	0.74	100.00 %	2580	KX923320.1
2.	<i>N. benthamiana</i> NbPDR10 mRNA for PDR-type ACB transporter, complete cds	24.3	169	80 %	0.74	100.00 %	4293	LC015773.1
3.	<i>N. benthamiana</i> clone (v3) GT052ce UDP-glycosyltransferase mRNA, complete cds	24.3	251	95 %	0.74	100.00 %	5179	MT945367.1
4.	<i>N. benthamiana</i> clade XI lectin receptor kinase (LecRK) gene, complete cds	24.3	53.0	61 %	0.74	100.00 %	2242	KT225325.1
5.	<i>N. benthamiana</i> lectin receptor kinase (LecRK) gene, complete cds	24.3	38.7	61 %	0.74	100.00 %	1342	KT225324.1

7. ACUGUGAUCAAUUUUCUCAA

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> SBT10 mRNA, complete cds	26.3	100	90 %	0.19	100.00 %	2454	MN535005.1
2.	<i>N. benthamiana</i> GRETCHEN HAGEN 3.6 mRNA, complete cds	24.3	128	71 %	0.74	100.00 %	2123	KP941063.1
3.	<i>N. benthamiana</i> NbNup50c mRNA for nuclear pore complex protein Nup50c, complete cds	24.3	55.0	71 %	0.74	100.00 %	1332	AB897833.1
4.	<i>N. benthamiana</i> NbNup50b mRNA for nuclear pore complex protein Nup50b, complete cds	24.3	55.0	71 %	0.74	100.00 %	1332	AB897832.1
5.	<i>N. benthamiana</i> fructokinase-like protein 1 mRNA, complete cds	24.3	134	80 %	0.74	100.00 %	1461	HM211399.1

8. CAUAACCCUUACUGUGAUCAA

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> nine-cis-epoxycarotenoid dioxygenase-like protein mRNA, complete cds	26.3	40.6	90 %	0.19	100.00 %	1119	GQ477382.1
2.	<i>N. benthamiana</i> chromomethylase 3 (CMT3) mRNA, complete cds	24.3	59.0	100 %	0.74	100.00 %	2303	JQ965931.1
3.	<i>N. benthamiana</i> suppressor of gene silencing 3 (SGS3) mRNA, complete cds	22.3	22.3	52 %	2.9	100.00 %	1908	KJ190939.1
4.	<i>N. benthamiana</i> NbRibA mRNA for GTP cyclohydrolase II/3,4-dihydroxy-2-butanone 4-phosphate synthase, complete cds	22.3	22.3	52 %	2.9	100.00 %	1674	AB538870.1
5.	<i>N. benthamiana</i> NbSAHH1a mRNA for S-adenosylhomocysteine hydrolase, complete cds	22.3	36.7	76 %	2.9	100.00 %	1458	LC008356.1

9. CUGUAGUGCUCAUAACCCUUA

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> nucleotide binding leucine rich repeat protein (ZAR1) mRNA, complete cds	26.3	85.7	95 %	0.19	100.00 %	2538	MH532570.1
2.	<i>N. benthamiana</i> clone NbenPCNA8 Proliferating cell nuclear antigen protein (PCNA) mRNA, complete cds	24.3	24.3	76 %	0.74	93.75 %	795	MK424843.1
3.	<i>N. benthamiana</i> clone NbenPCNA14 Proliferating cell nuclear antigen protein (PCNA) mRNA, complete cds	24.3	24.3	76 %	0.74	93.75 %	795	MK424842.1
4.	<i>N. benthamiana</i> HSP90 mRNA for heat shock protein 90, complete cds, isolate: NbHSP90	24.3	53.0	66 %	0.74	100.00 %	2100	LC314272.1
5.	<i>N. benthamiana</i> myosin XI-F mRNA, complete cds	24.3	102	95 %	0.74	100.00 %	4710	DQ875136.1

10. AUUCUGGUGUUGACUGUAGUG

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> ER luminal-binding protein (BLP4) mRNA, complete cds	24.3	81.8	76 %	0.74	100.00 %	2004	FJ463755.1
2.	<i>N. benthamiana</i> NbNup155a mRNA for nuclear pore complex protein Nup155a, complete cds	24.3	112	85 %	0.74	100.00 %	4461	AB898778.1
3.	<i>N. benthamiana</i> neuroblastoma-amplified gene (NAG) mRNA, complete cds	24.3	284	95 %	0.74	100.00 %	7230	EU602317.1
4.	<i>N. benthamiana</i> putative RNA-dependent RNA polymerase SDE1 mRNA, complete cds	24.3	98.1	71 %	0.74	100.00 %	4098	AY722008.1
5.	<i>N. benthamiana</i> clade XVI lectin receptor kinase (LecRK) gene, complete cds	22.3	112	80 %	2.9	100.00 %	3568	KT225334.1

11. CACCAUUUGGAUUCUGGUGUU

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> NbTPRb mRNA for nuclear pore complex protein TPRb, complete cds	26.3	143	80 %	0.19	100.00 %	6126	AB898658.1
2.	<i>N. benthamiana</i> NbTPRa mRNA for nuclear pore complex protein TPRa, complete cds	26.3	128	80 %	0.19	100.00 %	6138	AB898657.1
3.	<i>N. benthamiana</i> NbNup136b mRNA for nuclear pore complex protein Nup136b, complete cds	24.3	304	100 %	0.74	100.00 %	3915	AB897836.1
4.	<i>N. benthamiana</i> NbWRKY13 mRNA for transcription factor, complete cds	22.3	98.1	80 %	2.9	100.00 %	2124	AB711134.1
5.	<i>N. benthamiana</i> voucher YU 270680 chloroplast ATP-dependent Clp protease chaperone protein (ClpC1B) mRNA, complete cds; nuclear gene for chloroplast product	22.3	96.1	85 %	2.9	100.00 %	3014	KJ406177.1

12. UACUUUUCUGACACCAUUUGG

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> external transcribed spacer, 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 25S ribosomal RNA gene, complete sequence; and external transcribed spacer, partial sequence	28.2	260	100 %	0.048	100.00 %	7903	KP824745.1
2.	<i>N. benthamiana</i> NOA1 mRNA for nitric oxide associated 1, complete cds	24.3	118	95 %	0.74	100.00 %	1707	AB303300.1
3.	<i>N. benthamiana</i> NbPLD_beta mRNA for phospholipase D beta, complete cds	22.3	83.7	90 %	2.9	100.00 %	2493	LC033852.1
4.	<i>N. benthamiana</i> clone (v3) GT052ce UDP-glycosyltransferase mRNA, complete cds	22.3	231	100 %	2.9	89.47 %	5179	MT945367.1
5.	<i>N. benthamiana</i> Nbgp210b mRNA for nuclear pore complex protein gp210b, complete cds	22.3	274	95 %	2.9	100.00 %	5826	AB898667.1

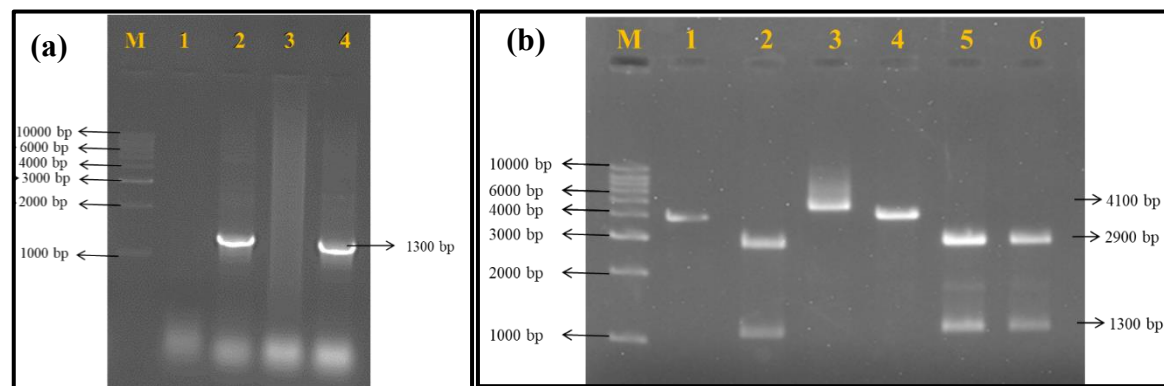
13. AAUUGCCAAUGGCUACUUUU

Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> TOM1-like protein 2 mRNA, partial cds	22.3	65.4	66 %	2.9	100.00 %	1566	KX272620.1
2.	<i>N. benthamiana</i> clade XII lectin receptor kinase (LecRK) gene, complete cds	22.3	53.0	90 %	2.9	100.00 %	1753	KT225330.1
3.	<i>N. benthamiana</i> clade XII lectin receptor kinase (LecRK) gene, complete cds	22.3	38.7	61 %	2.9	100.00 %	2040	KT225329.1
4.	<i>N. benthamiana</i> lectin receptor kinase (LecRK) gene, complete cds	22.3	55.0	71 %	2.9	100.00 %	1342	KT225324.1
5.	<i>N. benthamiana</i> clone (v3)GT088c UDP-glycosyltransferase mRNA, complete cds	22.3	83.7	90 %	2.9	100.00 %	1794	MT945389.1

14. UCCAAAUUUGUUGAAAAUAUC

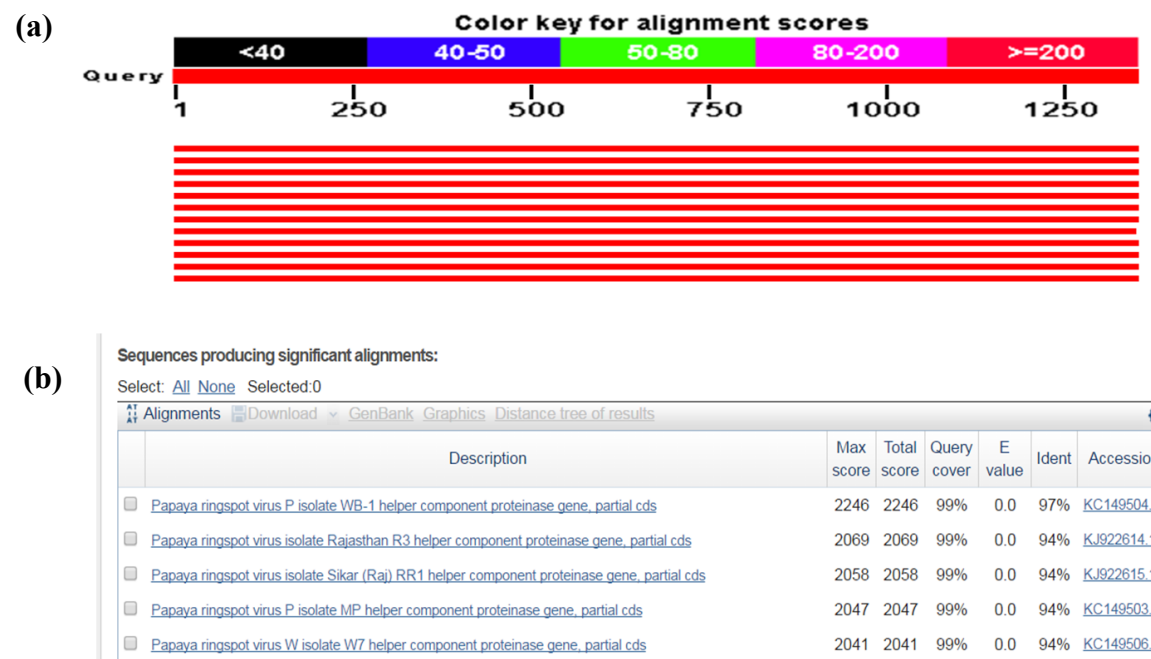
Sr No.	Description of off targeted genes	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1.	<i>N. benthamiana</i> NbHMGR1a mRNA for 3-hydroxy-3-methylglutaryl-CoA reductase, complete cds	24.3	53.0	57 %	0.74	100.00 %	1818	LC382274.1
2.	<i>N. benthamiana</i> NbPDR12 mRNA for PDR-type ACB transporter, complete cds	24.3	206	100 %	0.74	100.00 %	4422	LC015775.1
3.	<i>N. benthamiana</i> lanosterol synthase (LAS1) mRNA, partial cds	24.3	53.0	57 %	0.74	100.00 %	409	KM452915.1
4.	<i>N. benthamiana</i> chloroplast photosystem I reaction center subunit II-like protein mRNA, complete cds; nuclear gene for chloroplast product	24.3	24.3	57 %	0.74	100.00 %	624	MW458937.1
5.	<i>N. benthamiana</i> neuroblastoma-amplified gene (NAG) mRNA, complete cds	24.3	608	100 %	0.74	100.00 %	7230	EU602317.1

Supplementary figures



Supplementary Fig. 1. Cloning of HC-Pro gene into pTZ57R/T.

(a) Colony PCR of transformed colonies using gene-specific primers, lane M: 1kb DNA ladder, lane 1-4: transformed colonies; (b) Restriction profile of recombinant pTZ57R/T::HCPro plasmid, lane M : 1 kb ladder, lane 1: undigested pTZ57R/T::HCPro plasmid, lane 2, 5 and 6: double digested recombinant pTZ57R/T vector, lane 3-4: single digested recombinant pTZ57R/T vector



Supplementary Fig. 2. BLAST homology search based on HC-Pro gene sequences of PRSV strain utilizing NCBI database.

a) Graphical summary of BLAST search query with alignment scores; b) Significant alignments from BLAST algorithm for HC-Pro gene

	10	20	30	40	50	60
MF481203.1 Bgl-1-2017-P		C	TAGGCTCGGT	TTTAATAGGG	CGTTTCTGCG	GCACAGAAAA
MN103525.1 Bgl-2-2019-P	AATGACGTGG	CTGAAAAATT	CT...T			
KY44832.1.1 DH-1-2017-P	AATGACGTGG	CTGAAAAATT	CT		A	
KC149504.1 DH-2-2012-P	AATGACGTGG	CTGAAAAATT	CT			
MG383641.1 Trpt-2017-P	AATGACGTGG	CTGAAAAATT	CT			
MN103527.1 Blr-IIHR-2019	AATGACGTGG	CTGAAAAATT	CT			
KJ922614.1 Skr-2014-P	AATGACGTGG	CTGAAAAATT	CT	C	A	G
KJ922615.1 Skr-2-2014-P	AATGACGTGG	CTGAAAAATT	CT	C		
KU196788.1 Drd-2015-P	AATGACGTGG	CTGAAAAATT	CT			
KC149503.1 Idr-2012-P	AATGACGTGG	CTGAAAAATT	CT	C		
KC149506.1 Kpl-2012-P	AATGACGTGG	CTGAAAAATT	CT	C		
JQ394692.1 Gdg-2012-P	AATGACGTGG	CTGAAAAATT	CT	C		
KC136309.1 Gdg-2012-W	AATGACGTGG	CTGAAAAATT	CT	C		
MF405299.1 Pune-1-2017-P				C		
MF405297.1 Pune-2-2017-P				T		
KP743981.1 Hyd-2015-P				C		

	70	80	90	100	110	120
MF481203.1 Bgl-1-2017 P	C C A A C G G A T C	A C A C T T G C A C	G T C T G A T A T G	G A C G T T A C A A	T G T G T G G G G A	G G T G G C G G C T
MN103525.1 Bgl-2-2019 P	.	.	.	G T .	.	.
KY44832.1 L Dh-1-2017 P	G .	.	C	.	.	.
KC149504.1 Dh-2-2012 P	.	A	C	.	.	.
MG383641.1 Trpt-2017 P	.	.	.	.	.	.
MN103527.1 Bhr-IIHR-2019	.	A . G	C	C	.	A
KJ922614.1 Skr-2014 P	.	C	C	.	.	A . T
KJ922615.1 Skr-2-2014 P	.	C	C	.	.	A . T
KU196788.1 Drd-2015 P	.	T	T	T . A	A	.
KC149503.1 Idr-2012 P	.	C	.	.	.	A . T
KC149506.1 Kpl-2012 P	.	C	.	.	.	A . T
JQ394692.1 Gdg-2012 P	.	C	.	.	.	A . T
KC136309.1 Gdg-2012 W	.	C	.	.	.	A . T . A
MF405299.1 Pune-1-2017 P	.	.	A	C	.	.
MF405297.1 Pune-2-2017 P	.	.	C	.	.	A . T
KP743981.1 Hyd-2015 P	.	C	C	.	.	A . T

	130	140	150	160	170	180
MF481203.1 Bgl-1-2017-P	C T T G C A A C T A	T A A T C C T G T T	T C C G T G T C A C	A A A A T A A C T T	G C A A T A C T T G	C A T G A G C A A A
MN103825.1 Bgl-2-2019-P						
KY44832.1 L1 DH-1-2017-P	T					
KC149504.1 DH-2-2012-P					G	
MG383641.1 Trpt-2017-P			T		G	C
MN103827.1 Blr-IIHR-2019					G	
KJ922614.1 Skr-2014-P	C	A	T			
KJ922615.1 Skr-2-2014-P	C	A	T			
KU196788.1 Drd-2015-P			T			T
KC149503.1 Idr-2012-P	C	A	T			
KC149506.1 Kpl-2012-P	C	A	T			
JQ394692.1 Gdg-2012-P	C	A	T			
KC136309.1 Gdg-2012-W	C	A	T	G		
MF405299.1 Pune-1-2017-P	C	T	T			
MF405297.1 Pune-2-2017-P	C	A	T			
KP743981.1 Hyd-2015-P	C	A	T			

	190	200	210	220	230	240
MF481203.1 Bgl-1-2017-P	G T A A A G G G A A	G G G T T A T T G A	C G A A G T C G G T	G A G G A T T T G A	A T T G T G A A C T	C G A A C G C T T G
MN103525.1 Bgl-2-2019-P						
KY44832.1 L1 DH-1-2017-P	G		T			
KC149504.1 DH-2-2012-P						
MG383641.1 Trpt-2017-P						
MN103527.1 Blr-IIHR-2019				A		
KJ922614.1 Skr-2014-P	A			A		T
KJ922615.1 Skr-2-2014-P	A			A		T
KU196788.1 Drd-2015-P		T			C	T
KC149503.1 Idr-2012-P	A					T
KC149506.1 Kpl-2012-P	A			A		T
QJ394692.1 Gdg-2012-P	A			A		T
KC136309.1 Gdg-2012-W	A			A		T
MF405299.1 Pune-1-2017-P	A			G		T
MF405297.1 Pune-2-2017-P				G		T
KP743981.1 Hyd-2015-P	A			G		T

250 260 270 280 290 300
 MF481203.1 Bgl-1-2017-P C G C G A A A C T C T T T C G T C A T A T G G A G G T T C A T T T T G G G C A T G T C T C A A C A T T A C T T G A T C A A
 MN103525.1 Bgl-2-2019-P A G
 KY448321.1 DII-1-2017-P A C A T
 KC149504.1 DII-2-2012-P .
 MG383641.1 Trpt-2017-P G .

MF405299.1 Pune-1-2017-P	. T .	. A .	. C .	G .
MF405297.1 Pune-2-2017-P	. T .	. A .	. C .	G .
KP743981.1 Hyd-2015-P	. T .	. A .	. C .	G .

	310	320	330	340	350	360
MF481203.1 Bgl-1-2017 P	C T G A A C A G G G	T C T T G A A C G C	G C G G A A C A C G	A A T G A T G G A G	C T T T T A A A G A	A A T T G C T A A G
MN103525.1 Bgl-2-2019- P	T	T	T			
KY44832.1 Dh-1-2017- P	 A .			C	 C .	
KC149504.1 Dh-2-2012- P	 A .	 C .				
MG383641.1 Trpt-2017- P	. A A .			C	 C .	
MN103527.1 Blr-IIHR-2019	 A .			C	 C .	
KJ922614.1 Skr-2014- P	 A A .	 T . . T .	 T .	 A .		
KJ922615.1 Skr-2-2014- P	 A A .	 T . . T .	 T .	 A .		
KU196788.1 Drd-2015- P	 A A .		A	C		
KC149503.1 Idr-2012- P	 A A .	 T . . T .	 T .	 A .		
KC149506.1 Kpl-2012- P	 A A .	 T . . T .	 T .	 A .		
JQ394692.1 Gdg-2012- P	 A A .	 T . . T .	 T .	 A .		
KC136309.1 Gdg-2012- W	 A A .	 T . . T .	 T .	 A .		
MF405299.1 Pune-1-2017- P	 A A .	 T . . T .	 T .	C A .	 C .	
MF405297.1 Pune-2-2017- P	 A A .	 T . . T .	 T .	C A .	 C .	
KP743981.1 Hyd-2015- P	 A A .	 T . . T .	 T .	C A .	 C .	

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MF481203.1 Bgl-1-2017-P	AAAAATTGATG	AAAAGAAGGA	GAGTCCTTGG	ACCCACATGA	CAGTTATTAA	CAACACGCTC
MN103525.1 Bgl-2-2019-P		G		T		
KY44832.1.1 DH-1-2017-P		G		T		
KC149504.1 DH-2-2012-P				T		
MG383641.1 Trpt-2017-P			T	T		
MN103527.1 Blr-IIHR-2019				T		
KJ922614.1 Skr-2014-P		C	A			C
KJ922615.1 Skr-2-2014-P		C	A			C
KU196788.1 Drd-2015-P	G	G	A			C
KC149503.1 Idr-2012-P		C	A			C
KC149506.1 Kpl-2012-P		C	A			C
KC149506.1 Kpl-2012-P		C	A			C
JQ394692.1 Gdg-2012-P		C	A			C
KC136309.1 Gdg-2012-W		C	A			C
MF405299.1 Pune-1-2017-P		C	A	A		C
MF405297.1 Pune-2-2017-P		C	A			C
KP743981.1 Hyd-2015-P		C	A			C

	430	440	450	460	470	480
MF481203.1 Bgl-1-2017-P	A T C A A A G G T T	C G C T A G C A A T	T G G C T A C G A A	T T T G A A A G A G	C G T C T G A T A G	T C T T C G G G A G
MN103525.1 Bgl-2-2019-P						
KY44832.1.1 DH-1-2017-P						
KC149504.1 DH-2-2012-P			T			
MG383641.1 Trpt-2017-P					C	
MN103527.1 Blr-IIHR-2019					C	
KJ922614.1 Skr-2014-P		C	T		A	A
KJ922615.1 Skr-2-2014-P		C	T		A	A
KU196788.1 Drd-2015-P		C	T	A	A	A
KC149503.1 Idr-2012-P		C	T		A	A
KC149506.1 Kpl-2012-P		C	T		A	A
JQ394692.1 Gdg-2012-P		C	T		A	A
KC136309.1 Gdg-2012-W		C	T		A	A
MF405299.1 Pune-1-2017-P		C	T		A	A
MF405297.1 Pune-2-2017-P		C	T		A	A
KP743981.1 Hyd-2015-P		C	T		A	A

	490	500	510	520	530	540
MF481203.1 Bgl-1-2017-P	A T C G T G A G A T	G G C A T C T C A A	A A G G A C G G A G	T C A A T A A A A G	C T G G C A G C G T	T G A A A G C T T C
MN103525.1 Bgl-2-2019-P	.	.	A .	.	.	T
KY44832.1.1 Dh-1-2017-P	T .	.	A .	.	T .	.
KC149504.1 Dh-2-2012-P	T .	.	A .	.	.	.
MG383641.1 Trpt-2017-P	T .	.	A .	.	.	.
MN103527.1 Blr-IIIHR-2019	.	.	A .	.	.	C .
KJ922614.1 Skr-2014-P	T .	.	A .	.	T .	T .
KJ922615.1 Skr-2-2014-P	T .	.	A .	.	T .	T .
KU196788.1 Drd-2015-P	T .	.	A .	.	T .	T .
KC149503.1 Idr-2012-P	T .	.	A .	.	T .	T .
KC149506.1 Kpl-2012-P	T . A .	.	A .	.	T .	T .
JQ394692.1 Gdg-2012-P	T .	.	A .	.	T .	T .
KC136309.1 Gdg-2012-W	T .	.	A .	.	T .	T .
MF405299.1 Pune-1-2017-P	T .	.	A .	.	T .	T .
MF405297.1 Pune-2-2017-P	T .	.	A .	.	T .	T .
KP743981.1 Hyd-2015-P	T .	.	A .	.	T .	T .

550 560 570 580 590 600

KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

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 GACAGGAAATG GCAATTTCTT ATGGGGTGAG AGGCAATACC ACGCCAAAAG ATTCTTTGCC
 MN481203.1 Bgl-1-2017-P
 MN103525.1 Bgl-2-2019-P
 KY44832.1 DB-1-2017-P
 KC149504.1 DB-2-2012-P
 MG38364.1 Trp1-2017-P
 MN103527.1 Bhr-IJHR-2019
 KJ922614.1 Skr-2014-P
 KJ922615.1 Skr-2-2014-P
 KU196788.1 Drd-2015-P
 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

670 680 690 700 710 720
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 MN481203.1 Bgl-1-2017-P
 MN103525.1 Bgl-2-2019-P
 KY44832.1 DB-1-2017-P
 KC149504.1 DB-2-2012-P
 MG38364.1 Trp1-2017-P
 MN103527.1 Bhr-IJHR-2019
 KJ922614.1 Skr-2014-P
 KJ922615.1 Skr-2-2014-P
 KU196788.1 Drd-2015-P
 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

730 740 750 760 770 780
 CCAAATGGTG TCAGAAAAGT AGCCATTGGC AATTTGATAT TTTCAACAAA TTTGGAAAGG
 MN481203.1 Bgl-1-2017-P
 MN103525.1 Bgl-2-2019-P
 KY44832.1 DB-1-2017-P
 KC149504.1 DB-2-2012-P
 MG38364.1 Trp1-2017-P
 MN103527.1 Bhr-IJHR-2019
 KJ922614.1 Skr-2014-P
 KJ922615.1 Skr-2-2014-P
 KU196788.1 Drd-2015-P
 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

790 800 810 820 830 840
 TTTCGGCAGC AAATGGTTGA ACACCACATT GATCAAGGAC CGATTACTCG CGAATGCATT
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 MN103525.1 Bgl-2-2019-P
 KY44832.1 DB-1-2017-P
 KC149504.1 DB-2-2012-P
 MG38364.1 Trp1-2017-P
 MN103527.1 Bhr-IJHR-2019
 KJ922614.1 Skr-2014-P
 KJ922615.1 Skr-2-2014-P
 KU196788.1 Drd-2015-P
 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

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 MN481203.1 Bgl-1-2017-P
 MN103525.1 Bgl-2-2019-P
 KY44832.1 DB-1-2017-P
 KC149504.1 DB-2-2012-P
 MG38364.1 Trp1-2017-P

MN103525.1 Bgl-2-2019-P
 KY448321.1 DH-1-2017-P
 KC149504.1 DU-2-2012-P
 MG383641.1 Trpt-2017-P
 MN103527.1 Bhr-IHR-2019
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 KJ922615.1 Skr-2-2014-P
 KU196788.1 Drd-2015-P
 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
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 KP743981.1 Hyd-2015-P

MF481203.1 Bgl-1-2017-P
 MN103525.1 Bgl-2-2019-P
 KY448321.1 DH-1-2017-P
 KC149504.1 DU-2-2012-P
 MG383641.1 Trpt-2017-P
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 KJ922615.1 Skr-2-2014-P
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 KC149503.1 Idr-2012-P
 KC149506.1 Kpl-2012-P
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 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

MF481203.1 Bgl-1-2017-P
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 KP743981.1 Hyd-2015-P

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 KJ922615.1 Skr-2-2014-P
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 KC149506.1 Kpl-2012-P
 JQ394692.1 Gdg-2012-P
 KC136309.1 Gdg-2012-W
 MF405299.1 Pune-1-2017-P
 MF405297.1 Pune-2-2017-P
 KP743981.1 Hyd-2015-P

Supplementary Fig. 3. Nucleotide sequence alignment of PRSV-P helper-component proteinase of the Bangalore-1 isolate and fifteen similar isolates from Gene bank.

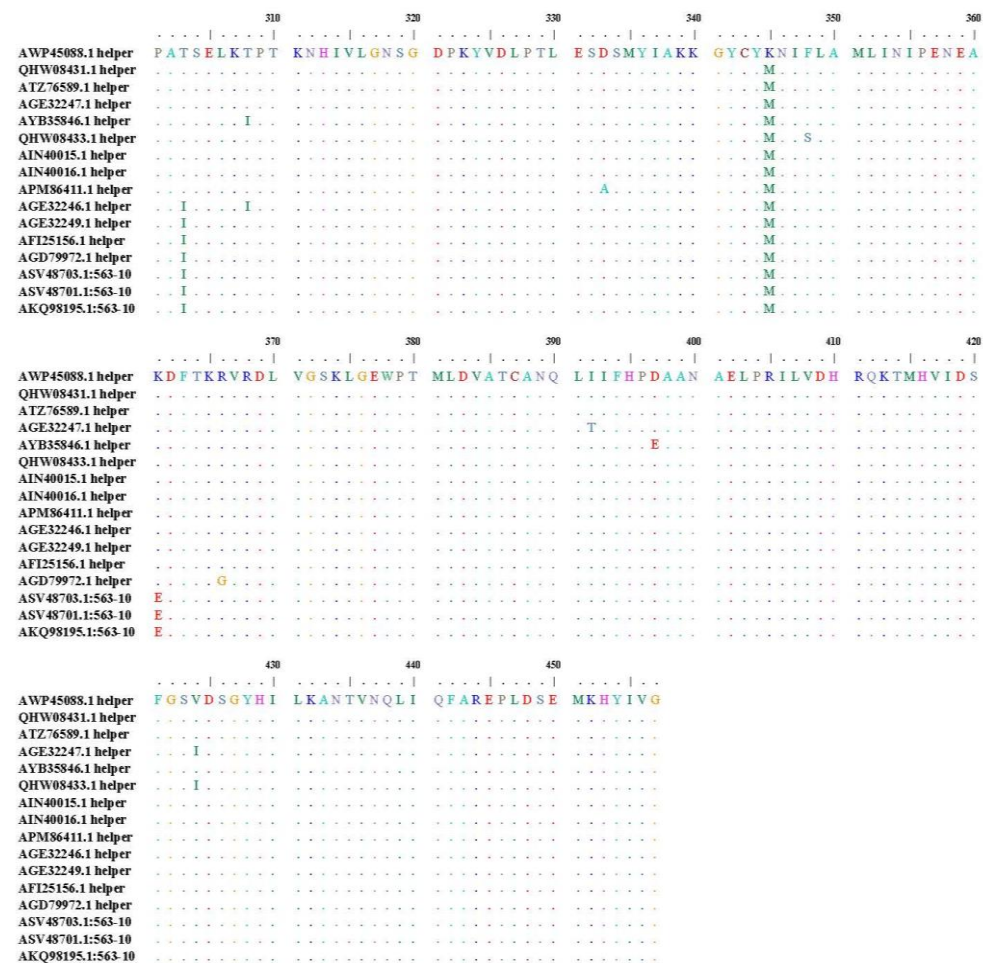
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QHW08431.1 helper	RLG	FNRAFLRHRK	PTDHTCTSDM	DVTMCGEVAA	LATIIILFPC	KITCNTCMSK
ATZ76589.1 helper	NDVAEKFW.			V.....	V.....	
AGE32247.1 helper	NDVAEKFW.					
AYB35846.1 helper	NDVAEKFW.					
QHW08433.1 helper	NDVAEKFW.	R.	G.....			
AIN40015.1 helper	NDVAEKFW.					
AIN40016.1 helper	NDVAEKFW.					
APM86411.1 helper	NDVAEKFW.		S.....			
AGE32246.1 helper	NDVAEKFW.					
AGE32249.1 helper	NDVAEKFW.					
AFI25156.1 helper	NDVAEKFW.				L.....	
AGD79972.1 helper	NDVAEKFW.					
ASV48703.1:563-10				S.....	L.....	
ASV48701.1:563-10						
AKQ98195.1:563-10						

	70	80	90	100	110	120
AWP45088.1 helper	VKGRVIDEVG	EDLNCELERL	RETLSSYGGG	FGHVSTLLDQ	LNRVLNARNT	NDGAFKEIAK
QHW08431.1 helper				F.....		
ATZ76589.1 helper						
AGE32247.1 helper						
AYB35846.1 helper						
QHW08433.1 helper						
AIN40015.1 helper	E				K.....M	E.....
AIN40016.1 helper	E				K.....M	E.....
APM86411.1 helper	E				K.....M	E.....
AGE32246.1 helper	E				K.....M	E.....
AGE32249.1 helper	E				K.....M	E.....
AFI25156.1 helper	E				K.....M	E.....
AGD79972.1 helper	E				K.....M	E.....
ASV48703.1:563-10	E				K.....M	E.....
ASV48701.1:563-10	E				K.....M	E.....
AKQ98195.1:563-10	E				K.....M	E.....

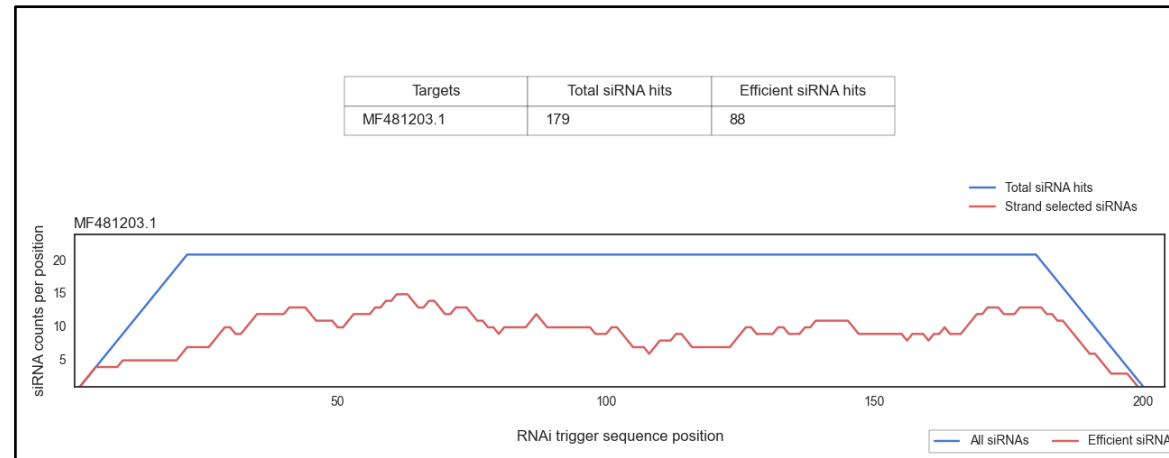
	130	140	150	160	170	180
AWP45088.1 helper	KID EK KES PW	THMTVINNTL	IKGSLAIGYE	FERASDSLRE	IVRWHLKRTE	SIKAGSVESF
QHW08431.1 helper	R.					
ATZ76589.1 helper	G.					
AGE32247.1 helper						
AYB35846.1 helper	D.					
QHW08433.1 helper						
AIN40015.1 helper	A.	A	T	N		
AIN40016.1 helper	A.	A	T	N		
APM86411.1 helper	A.	A	T	N		
AGE32246.1 helper	A.	A	T	N		
AGE32249.1 helper	A.	A	T	N		
AFI25156.1 helper	A.	A	T	N		
AGD79972.1 helper	A.	A	T	N		
ASV48703.1:563-10	A.	A	T	N		
ASV48701.1:563-10	A.	A	T	N		
AKQ98195.1:563-10	A.	A	T	N		

	190	200	210	220	230	240
AWP45088.1 helper	RNKRSGKAHF	NPALTCDNQL	DRNGNFWLGE	RQYHAKRFFA	NYFEKIDHSK	GYESHYSQHQN
QHW08431.1 helper				S.....		R.....
ATZ76589.1 helper			K.....			R.....
AGE32247.1 helper			K.....			R.....
AYB35846.1 helper			K.....			R.....
QHW08433.1 helper						
AIN40015.1 helper						Y.....R
AIN40016.1 helper	R.	A.				Y.....R
APM86411.1 helper						Y.....R
AGE32246.1 helper						Y.....R
AGE32249.1 helper						Y.....R
AFI25156.1 helper						Y.....R
AGD79972.1 helper						Y.....R
ASV48703.1:563-10				L.		Y.....R
ASV48701.1:563-10				L.		Y.....R
AKQ98195.1:563-10				L.		Y.....R

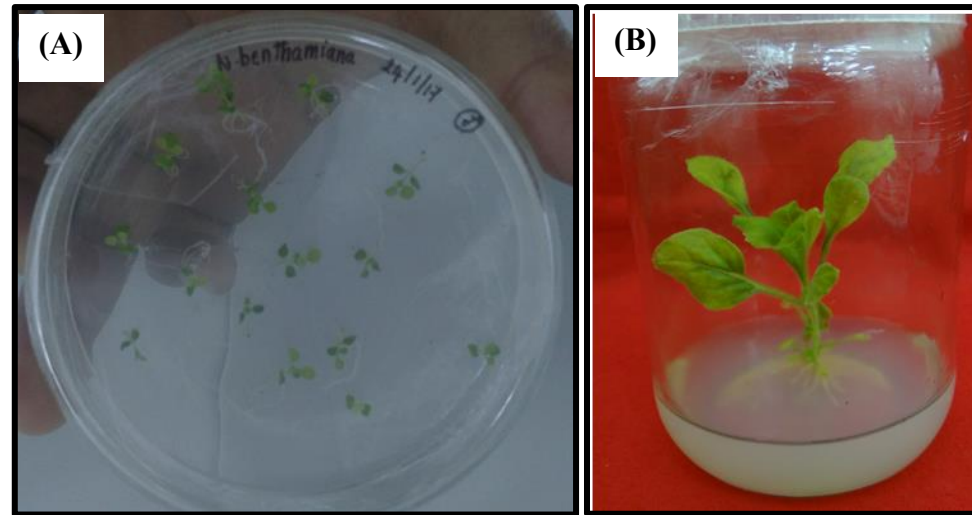
	250	260	270	280	290	300
AWP45088.1 helper	PNGVVKVAIG	NLIFSTNLER	FRQQMVEHHI	DQGPITRECI	ALRNNNNYVHV	CSCVTLLDDGT
QHW08431.1 helper	I.					
ATZ76589.1 helper			V.....			
AGE32247.1 helper						
AYB35846.1 helper						
QHW08433.1 helper						
AIN40015.1 helper	I					
AIN40016.1 helper	I					



Supplementary Fig. 4. Amino acid alignment of PRSV-P helper-component proteinase with the Bangalore-1 isolate and fifteen similar isolates from Gene Bank.

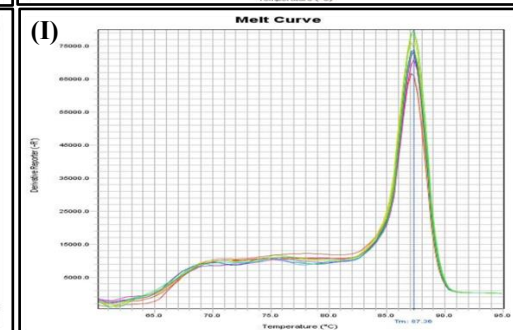
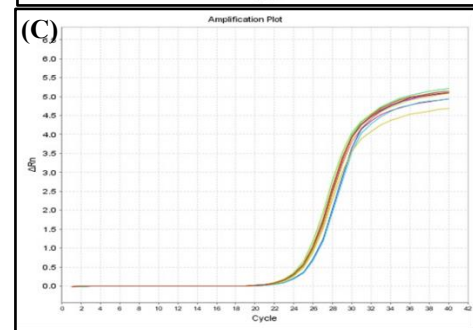
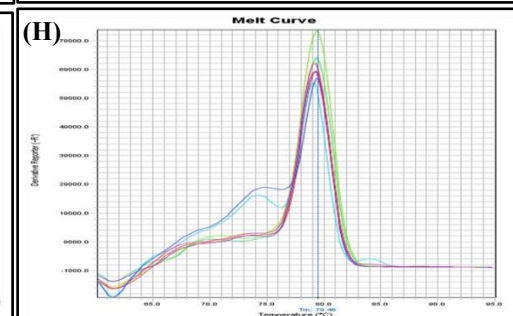
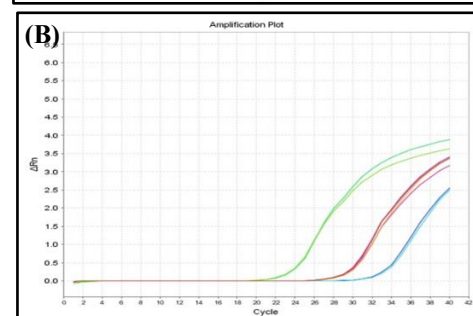
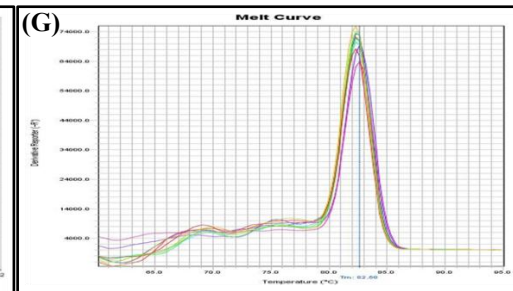
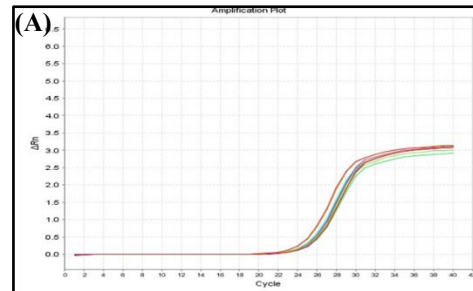


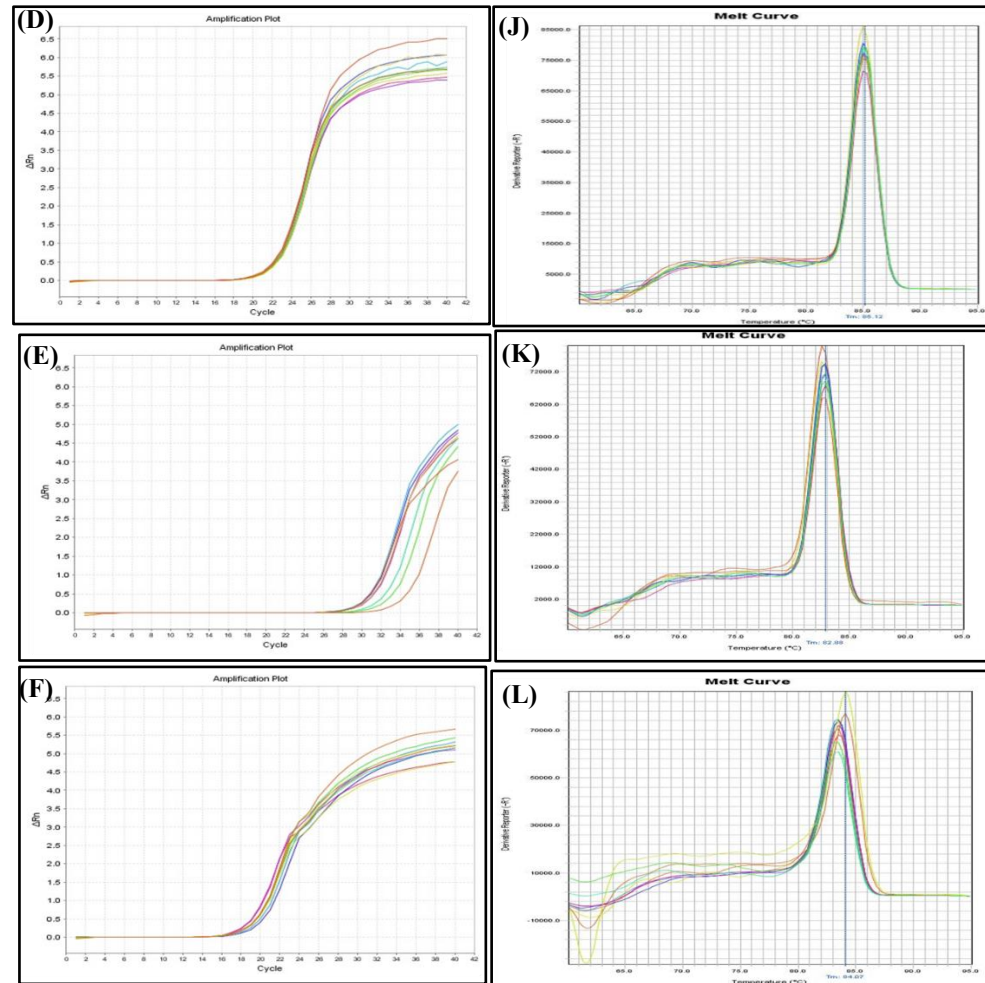
Supplementary Fig. 5. Off-target prediction analysis of the 201 bp HC-Pro gene construct sequence submitted against *N. benthamiana* Express Sequence Tag (EST) library with siFi21 software.



Supplementary Fig. 6. The *in vitro* germinated *N. benthamiana*, as the source of sterile explants for the transformation.

(A) Germinating seedlings (20 days after sowing) in MS media without growth hormones; (B) Source of leaf explant (45 days after sowing) with hormones (IAA 1 mg/L and BAP 0.5 mg/L)





Supplementary Fig. 7. Amplification plots and the dissociation curves of 6 genes in cDNA samples, the green horizontal line represents the threshold fluorescence at which the C T value was determined.

(A) to (F): Amplification plots of tubulin, HC-Pro, SOD, APX, POD and CAT genes respectively; (G) to (L): Dissociation curves of Tubulin, HC-Pro, SOD APX, POD and CAT genes respectively

Appendix 1

Ihp HC-Pro gene sequence

5'CTCTTACATGTGATAATCAATTGGACAGGAATGGCAATTTCTTATGGGGTGAGAGGCAATACCAACGCCAAAAGATTCTTT
GCCAACTATTTTGAGAAAATTGATCACAGTAAGGGTTATGAGCACTACAGTCAACACCAGAATCCAAATGGTGTCAGAAAA
GTAGCCATTGGCAATTTGATATTTTCAACAAATTTGGAGGTACCAGGTCCAAGGAAAATGGAGGCACACACATTATCATAAA
AATGACTTCTAAATATATTGTGACTTAATTAATAGAACAATAAAGAGCCAAATGGGGCAACACTCATATTTAATATCAAGGA
AACCCTGACAGGTTATTCATATCCAGAA GAATTCTCCAAATTTGTTGAAAATATCAAATTGCCAATGGCTACTTTTCTGACAC
CATTTGGATTCTGGTGTTGACTGTAGTGCTCATAACCCTTACTGTGATCAATTTTCTCAAAATAGTTGGCAAAGAATCTTTTGG
CGTGGTATTGCCTCTCACCCCATAAGAAATTGCCATTCTGTCCAATTGATTATCACATGTAAGAG3'

*The sequence indicated in yellow is the cowpea intronic sequence