RESEARCH ARTICLE

SCREENING OF NATIVE BACILLUS THURINGIENSIS (BT) ISOLATES FOR THE PRESENCE OF CRY 1 AB & *VIP 3A*

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Abstract: Insecticidal *cry* and *vip* genes from *Bacillus thuringiensis* (Bt) have been used for control of lepidopteran insects in transgenic crops. However, novel genes are required for gene pyramiding to delay evolution of resistance to the currently deployed genes.PCR-based techniques were employed for screening of *cry1Ab* type genes in 96 Bt isolates from diverse habitats in India and 8 known Bt strains. 96 native Bt isolates, recovered from different locations in India and 8 known Bt strains were screened for the presence of cry 1 Ab, cry 1Ac, Cry 3A & vip 3A for Isolation of plasmid DNA from native Bt isolates of Bacillus thuringiensis, Screening for the presence of cry 1 Ab, cry 1Ac, cry 3A & vip 3A gene using PCR amplification and Cloning of partial cry 1 Ab & vip 3A gene using different sets of primers. *Cry1Ab* type genes were more prevalent than *cry1Aa*- and *cry1Ac* type genes. Correlation between source of isolates and abundance of *cry1*-type genes was not observed..

Keywords: *Bacillus thuringiensis*, *Cry1Ab* genes, *Cry1Ac*, *Cry3A*, Vip*3A*, *Helicoverpa armigera*, Insecticidal genes

INTRODUCTION

Bacillus thuringiensis (Bt) is an aerobic, Gram-positive, spore-forming, facultative bacterial pathogen that produces parasporal crystals containing 1 or more insecticidal crystal proteins (Cry), which are selectively toxic to insects (Schnepf et al. 1998; van Frankenhuyzen 2013; Adang et al. 2014). Bt has been used as a biopesticide in agriculture, forestry, and mosquito control (Kaur 2000). Synthetic *cry* genes, modified for plant-preferred codon usage, have been transferred into crop plants for insect resistance (Koziel et al. 1993). Bt transgenic corn, cotton, tomato, potato, and soybean have been developed (James 2013). The search for novel cry and *vip* (vegetative insec- ticidal proteins) genes from native Bt isolates is an on- going effortworldwide (Kaur 2006). *Helicoverpa armigera* is a polyphagous pest of several crops, such as cotton, pigeon pea, chickpea, sorghum and tomato worldwide, which has developed resistance to chemical pesticides (Subramanian and Mohankumar 2006). Of the different types of *cry* genes, *cry1Ac* has been found to be most toxic towards *H. armigera* (Liao et al.2002). However, development of resistance in *H. armigera*, exposed to cry1Ac gene upon large-scale cultivation of first-generation transgenic crops, is a disconcerting possibility (Kaur 2012; Pardo López et al. 2013; Fabrick *et al.*2014). Gene pyramiding, deploying more than 1 type of insecticidal genes, having different receptors in the in- sect midgut, is the best strategy to delay the onset of resistance (Tabashnik et al. 2013; Carriere *et al.* 2015). *cry2*-type genes are promising candidates for gene pyra- miding owing to a difference in structure and mode of action In heliothine-type insects such as *H. armigera*, Cry2-type proteins have been shown to have binding sites different from those of other Cry proteins deployed in transgenic crops such as Cry1Ac, Cry1F, and Vip3A proteins (Gouffon et al. 2011). *cry1Ac*-resistant *H. armigera* did not show cross resistance to *cry2Aa* (Akhurst et al. 2003). Furthermore, a synergistic effect from the combination of Cry1Ac and Cry2Ab proteins has been observed towards *H. armigera* (Ibargutxi et al. 2008).

Novel *cry2*-type genes have been identified by PCR and cloned in many laboratories in India and elsewhere (Misra et al. 2002; Sauka et al. 2005; Beard et al. 2008; Lin et al. 2008; Shu et al 2013; Somwatcharajit et al. 2014). PCR- based identification of *cry* genes was first developed by Carozzi et al. (1991) and has remained the method of choice because of its sensitivity, rapidity, and ease of performance (Porcar and Juarez-Perez 2003). In this study, 96 native Bt isolates, recovered from different locations in India (Table 1), and 8 known Bt strains (Table 2) were screened for the presence of cry 1 Ab, cry 1Ac, Cry 3A & vip 3A with the objectives of .Isolation of plasmid DNA from native Bt isolates of Bacillus thuringiensis, Screening for the presence of cry 1 Ab, cry 1Ac, cry 3A & vip 3A gene using PCR amplification and Cloning of partial cry 1 Ab & vip 3A gene using different sets of primers.

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MATERIAL AND METHOD

Bacterial strain: 98 Native Bt isolates recovered from diverse agricultural & Non agricultural location of India. These strain were provided by Dr. Sarvjeet Kaur Laboratory NRCPB. IARI New Delhi

The objective of the study was screening of native Bt isolates for the presence of cry 1Ab & vip 3A gene. This could be used as a promising tool for the effective control and resistant management of agricultural important species of insect pests. The methodology includes:

- Overnight grown Culture of *Bacillus thuringiensis* was prepared using LB containing appropriate amount of penicillin at 28^0c- 30^0c.
- The bacterial plasmid DNA from overnight grown culture of bacillus thuringiensis was isolated by alkali lyses method.
- The plasmid was observed as a discrete band of size ~23kb on agarose gel electrophoresis.
- Since cry gene is plasmid encoded, therefore the isolated plasmids were further screened for the presence of cry gene.
- The most common method utilized for screening was polymerase chain reaction (PCR). Set of gene specific primers were used to detect and amplify the cry 1Ab & *vip* 3A gene.
- The PCR product was observed on 0.8% Agarose gel electrophoresis as discrete band.
- The band with desired gene size was finally cut under UV transilluminator and kept at-200C.
- Competent cells of Ecoli DH5a were prepared.
- The band was further eluted out and ligated in a pDrive vector.
- Transformation was carried out by incubating competent cells with ligation mixture.
- Transformed cells were then plated onto LA+ ampicillin + X-gal- IPTG plates.
- Plates were screened for white colonies.
- Plasmid was isolated from these transformed colonies and screened for the presence of cry gene.

RESULT AND DISCUSSION

Molecular characterization: plasmid DNA was isolated from 88 native Bt isolates (Table 1) & 8 Bt reference strain (Table 2). The extracted DNA was analyzed by the PCR with specifically designed primer.

Isolation of Plasmid DNA: plasmid DNA of native strain showed a band of ~23kb in size.

PCR screening of natives Bt isolates for presence of full length cry 1Ab gene.

PCR amplification was carried out with primer 3 & 4 corresponding to full length cry 1Ab gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	2.5ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	1ul				
D/H_2o	-	9.5ul				

The Full length of cry 1 Ab gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates. (Table 3).

PCR screening of natives Bt isolates for presence of full length cry 1Ab gene.

PCR amplification was carried out with primer 110&111 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of vip 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates.(Table 4)

PCR screening of natives Bt isolates for presence of full length cry 3A gene.

PCR amplification was carried out with primer 63&64 corresponding to full length cry 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	

PCR buffer	-	2.5ul
Taq. Polymerase	-	0.5ul
D/H_2o	-	9.5ul

The Full length of cry 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates.(Table 5)

PCR screening of natives Bt isolates for presence of full length cry 3A gene.

PCR amplification was carried out with primer 114&115 corresponding to full length cry 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of cry 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates. (Table 6)

PCR screening of natives Bt isolates for presence of full length cry 1 Ac gene.

PCR amplification was carried out with primer 112&113 corresponding to full length cry 1 Ac gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	2.5ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	1.0ul				
D/H_2o	-	9.5ul				

The Full length of cry 1 Ac gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates (Table 7).

PCR screening of natives Bt isolates for presence of full length vip 3A gene.

PCR amplification was carried out with primer 1&2 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of vip 3A gene was observed in 4N1, T-55, 986, 4J2 isolates. (Table 8)

PCR screening of natives Bt isolates for presence of full length vip 3A gene.

PCR amplification was carried out with primer 1&3 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The expected size of 2.37 kb was observed in 4A6 reference of native Bt isolates. (Table 9)

Table 1. List of native Bt strain isolates from different location of India.

S.No.	Strain no	Plasmid DNA		S. No.	Strain No.	Plasmid DNA		S. No.	Strain No.	Plasmid DNA		S.No.	Strain No.	Plasmid DNA	
		23kb	48kb			23kb	48kb			23kb	48kb			23kb	48kb
1	1	+	-	23	220	+	-	45	784	+	-	67	922	+	-
2	3	+	-	24	222	+	-	46	791	+	-	68	935	+	-

3	4	+	-	25	223	+	-	47	792	+	-	69	980	+	-
4	5	+	-	26	229	+	-	48	700	+	-	70	942	+	-
5	9	+	-	27	232	+	-	49	701	+	-	71	945	+	-
6	13	+	-	28	301	+	-	50	707	+	-	72	948	+	-
7	20	+	-	29	302	+	-	51	721	+	-	73	949	+	-
8	28	+	-	30	303	+	-	52	722	+	-	74	950	+	-
9	48	+	-	31	304	+	-	53	727	+	-	75	951	+	-
10	51	+	-	32	305	+	-	54	729	+	-	76	952	+	-
11	63	+	-	33	307	+	-	55	739	+	-	77	954	+	-
12	82	+	-	34	611	+	-	56	741	+	-	78	955	+	-
13	84	+	-	35	608	+	-	57	758	+	-	79	956	+	-
14	88	+	-	36	629	+	-	58	754	+	-	80	957	+	-
15	94	+	-	37	668	+	-	59	753	+	-	81	958	+	-
16	110	+	-	38	669	+	-	60	783	+	-	82	959	+	-
17	113	+	-	39	678	+	-	61	793	+	-	83	960	+	-
18	208	+	-	40	680	+	-	62	794	+	-	84	961	+	-
19	213	+	-	41	751	+	-	63	807	+	-	85	977	+	-
20	214	+	-	42	763	+	-	64	911	+	-	86	980	+	-
21	217	+	-	43	767	+	-	65	918	+	-	87	986	+	-
22	219	+	-	44	772	+	-	66	918	+	-	88	995	+	-

Table 2. List of Reference Strain Bt strain isolates from different location of India.

S.No.	Strain no. (88 Samples)	Plasmid DNA	
		23 kb	48 kb
1	4A6	+	-
2	4M2	+	-
3	4K1	+	-
4	4F1	+	-
5	4J2	+	-
6	4K1	+	-
7	4R1	+	-
8	4S2	+	-
Total 8			

Table 3. List of PCR amplification for full length cry 1 Ab gene with primer 3&4

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		23	302	-		45	772	-		67	957	-	
2	3	-		24	303	-		46	783	-		68	958	-	
3	4	-		25	304	-		47	671	-		69	959	-	
4	9	-		26	305	-		48	792	-		70	960	-	
5	13	-		27	307	-		49	793	-		71	961	-	
6	20	-		28	611	-		50	807	-		72	977	-	
7	28	-		29	618	-		51	851	-		73	986	-	
8	32	-		30	629	-		52	911	-		74	995	-	
9	51	-		31	668	-		53	916	-		75	996	-	
10	63	-		32	678	-		54	918	+(2kb)		76	4M2	-	
11	82	-		33	680	-		55	922	-		77	4A6	-	
12	84	-		34	700	+ (1kb)		56	935	-		78	4W1	+(500bp)	
13	88	-		35	701	-		57	980	-		79	4M2	+(>1kb)	
14	94	-		36	721	-		58	944	+(2kb)		80	4R1	-	
15	110	-		37	792	-		59	945	-		81	4K1	+(2.0kb)	
16	113	-		38	729	+(1.5 kb)		60	948	-		82	4F1	-	
17	217	-		39	739	-		61	949	+(250bp)		83	4S2	-	
18	219	-		40	750	-		62	950	+(2kb)		84	T-55	-	
19	220	-		41	751	-		63	951	-		85	4C3	-	
20	222	-		42	753	-		64	952	-		86	4L3	-	
21	223	-		43	754	-		65	954	+(2kb)		87	T-94	+(250)bp	
22	301	-		44	758	-		66	955	-					

Table 4. List of PCR amplification for full length vip 3A gene with primer 110 & 111

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		23	302	-		45	758	-		67	957	-	
2	3	-		24	303	-		46	772	-		68	958	-	
3	4	-		25	304	-		47	783	-		69	959	-	
4	9	-		26	305	-		48	671	-		70	960	-	
5	13	-		27	306	-		49	792	-		71	961	-	
6	20	-		28	307	-		50	793	-		72	977	-	
7	28	-		29	611	-		51	807	-		73	986	-	
8	32	-		30	618	-		52	911	-		74	995	-	
9	51	-		31	629	-		53	916	-		75	996	-	
10	63	-		32	668	-		54	918	-		76	4M2	+(1.2kb)	
11	82	-		33	678	-		55	922	-		77	4A6	-	
12	84	-		34	680	-		56	935	-		78	4W1	-	

13	88	-		35	700	-		57	980	-		79	4K1	-	
14	94	-		36	701	-		58	944	-		80	4C3	-	
15	110	-		37	721	-		59	945	-		81	4L3	-	
16	113	-		38	792	-		60	948	-		82	4F1	-	
17	217	-		39	729	-		61	949	-		83	4R1	-	
18	219	-		40	739	-		62	950	-		84	T-55	-	
19	220	-		41	750	-		63	951	-		85	T-94	-	
20	222	-		42	751	-		64	952	-		86	T-15	-	
21	223	-		43	753	-		65	954	-					
22	301	-		44	754	-		66	955	-					

Table 5. List of PCR amplification for full length cry 3A gene with primer 63& 64

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	1	-	25	304	-		49	918	-		73	995	-	
2	2	3	-	26	305	-		50	922	-		74	996	-	
3	3	4	-	27	307	-		51	935	-		75	4M2	-	
4	4	9	-	28	611	-		52	955	-		76	4A6	+(1.2kb)	
5	5	13	-	29	618	-		53	936	-		77	4W1	-	
6	6	20	-	30	629	-		54	951	-		78	4R1	-	
7	7	28	-	31	668	-		55	950	-		79	4K1	-	
8	8	32	-	32	678	-		56	957	-		80	4F1	-	
9	9	51	-	33	680	-		57	960	-		81	4S2	-	
10	10	63	-	34	700	-		58	700	-		82	4J2	-	
11	11	82	-	35	701	-		59	749	-		83	4N1	-	
12	12	84	-	36	721	-		60	T-94	-		84	T-55	-	
13	13	88	-	37	722	-		61	4M2	-		85	4C3	-	
14	14	94	-	38	792	-		62	942	-		86	4L3	-	
15	15	110	-	39	729	-		63	952	-		87	T-94	-	
16	16	113	-	40	739	-		64	954	-		88	T-27	-	
17	17	217	-	41	750	-		65	955	-		89	942	-	
18	18	219	-	42	751	-		66	956	-		90	4K1	-	
19	19	220	-	43	753	-		67	977	-		91	4L3	-	
20	20	222	-	44	783	-		68	958	-		92	956	-	
21	21	223	-	45	792	-		69	959	-		93	4J2	-	
22	22	301	-	46	772	-		70	960	-		94	4K1	-	
23	23	302	-	47	793	-		71	977	-		95	4S2	-	
24	24	303	-	48	851	-		72	986	-		96	4L3	-	

Table 6. List of PCR amplification for full length cry 3A gene with primer 114& 115

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		26	305	-		51	851	-		76	948	-	
2	3	-		27	307	-		52	911	-		77	4M2	-	
3	4	-		28	611	-		53	916	-		78	4A6	-	
4	9	-		29	618	-		54	918	-		79	4W1	-	
5	13	-		30	629	-		55	922	+(750bp)		80	4W1	-	
6	20	-		31	668	-		56	935	+(750bp)		81	4R1	-	
7	28	-		32	678	-		57	980	+(300bp)		82	4M2	-	
8	32	-		33	680	-		58	944	+(250bp)		83	4W1	-	
9	51	-		34	700	-		59	945	-		84	942	-	
10	63	-		35	701	-		60	948	-		85	4K1	-	
11	82	-		36	721	-		61	949	-		86	4F1	-	
12	84	-		37	792	-		62	950	-		87	4S2	-	
13	88	-		38	729	+(1.2kb)		63	951	-		88	4J2	-	
14	94	-		39	739	-		64	952	-		89	4N1	-	
15	110	-		40	750	-		65	954	-		90	T-55	-	
16	113	-		41	751	-		66	955	-		91	4C3	-	
17	217	-		42	753	-		67	957	-		92	4L3	-	
18	219	-		43	754	-		68	958	-		93	T-94	-	
19	220	-		44	758	-		69	959	-		94	4S2	-	
20	222	-		45	772	-		70	960	-		95	4K1	-	
21	223	-		46	793	-		71	961	-		96	4C3	-	
22	301	-		47	671	-		72	977	-		97	4L3	-	
23	302	-		48	792	-		73	977	-		98	T-94	-	
24	303	-		49	793	-		74	986	-		99	4J2	-	
25	304	-		50	807	-		75	995	-		100	4K1	-	

Table 7. List of PCR amplification for full length cry 1 Ac gene with primer 112&113

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		25	304	-		49	807	-		73	4J3	-	
2	3	-		26	305	-		50	669	-		74	986	+(1.7kb)	
3	4	-		27	405	-		51	911	-		75	995	+(1.1kb)	
4	9	-		28	611	-		52	916	-		76	948	-	
5	680	-		29	618	-		53	918	-		77	4M2	-	

6	20	-		30	729	-		54	922	-		78	4A6	-	
7	20	-		31	668	-		55	935	-		79	4M2	-	
8	918	-		32	669	-		56	980	-		80	T-27	-	
9	51	-		33	678	-		57	942	+(500bp)		81	4K1	-	
10	63	-		34	680	-		58	945	+(250bp)		82	4M2	-	
11	82	-		35	700	-		59	948	-		83	4W1	-	
12	84	-		36	701	-		60	949	+(500bp)		84	942	-	
13	88	-		37	721	-		61	950	-		85	4K1	-	
14	94	-		38	792	+(1.2kb)		62	951	+(1kb)		86	4F1	-	
15	110	-		39	729	-		63	952	-		87	952	-	
16	113	-		40	739	-		64	954	-		88	4J2	-	
17	217	-		41	750	-		65	955	+(1kb)		89	4N1	-	
18	219	-		42	751	-		66	956	-		90	T-55	-	
19	T-94	-		43	753	-		67	957	+(1.7,1.1,.8kb)		91	4L3	-	
20	222	-		44	754	-		68	958	+(1.7,1.1,.8kb)		92	4R1	-	
21	223	-		45	758	-		69	959	-		93	4J2	-	
22	301	-		46	671	-		70	960	-		94	4K1	-	
23	302	-		47	792	-		71	961	-		95	4L3	-	
24	303	-		48	793	-		72	977	-		96	4C3	-	

Table 8. List of PCR amplification for full length vip 3A gene with primer 1&2

S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		26	305	-		51	1			76	986	+(400bp)	
2	3	-		27	405	-		52	669			77	995		
3	4	-		28	954	-		53	911			78	110		
4	9	-		29	618			54	916			79	4M2		
5	680	-		30	792			55	918			80	4A6	+(400bp)	
6	20	-		31	668			56	918			81	729		
7	20	-		32	669			57	303			82	T-27		
8	110	-		33	678			58	935			83	110		
9	51	-		34	680			59	980			84	950	+(400bp)	
10	63	-		35	700			60	942			85	4W1		
11	82	-		36	701			61	959			86	739	+(400bp)	
12	84	-		37	721			62	948			87	4K1		
13	88	-		38	792			63	949			88	4F1		
14	94	-		39	729			64	950			89	952	+(500bp)	
15	110	-		40	739			65	950			90	492	+(400bp)	
16	113	-		41	750			66	952			91	4J2	+(400bp)	
17	217	-		42	751			67	954			92	4M1	+410bp	
18	219	-		43	753			68	955			93	T-55	+410bp	
19	T-94	-		44	754			69	962			94	4L3		
20	680	-		45	758			70	945			95	4R1		
21	223	-		46	671			71	958			96	4A6		
22	301	-		47	783			72	977			97	4J2		
23	302	-		48	671			73	960			98	4K1		
24	303	-		49	792			74	977						
25	304	-		50	793			75	452	+(400bp)					

Table 9. List of PCR amplification for full length vip 3A gene with primer 1&3

S.No.	Bt Isolates	PCR Products	
		(+)	(-)
1	4A6	+(2.37kb)	
1	4A6	+(2.37kb)	
2	4J2	-	
3	4J2	-	
4	986	-	
5	995	-	
6	4M2	-	
7	4A6	+(2.37kb)	
8	950	-	
9	739	-	
10	952	-	
11	4N1	-	
12	J-55	-	
13	4L3	-	
14	20	-	
15	20	-	
16	110	-	
17	63	-	
18	T-94	-	
19	301	-	
20	680	-	

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