APPENDIX-I

CHEMICALS AND REAGENTS

A number of reagents and chemicals were used in the present study out of which the major chemicals are enlisted below. All other common salts, acids and solvents used were purchased from SRL Pvt. Ltd., Mumbai, India and Merck Specialities Pvt. Ltd., Mumbai, India. In addition to the common laboratory reagents, following chemicals were used during the work:

Chemicals	**Company**
Acetic acid	SRL Pvt. Ltd., Mumbai, India
Acetone	SRL Pvt. Ltd., Mumbai, India
Acrylamide	SRL Pvt. Ltd., Mumbai, India
Agarose	SRL Pvt. Ltd., Mumbai, India
2-aminobutyric acid	Fluka, Switzerland
3-aminobutyric acid	Fluka, Switzerland
Ammonium persulphate	SRL Pvt. Ltd., Mumbai, India
2,1,3-Benzothiadozole	Fluka, Switzerland
Bis-acrylamide	SRL Pvt. Ltd., Mumbai, India
Bovine serum albumin	HiMedia, Mumbai, India
Bromophenol blue	HiMedia, Mumbai, India
β-mercaptoethanol	SRL Pvt. Ltd., Mumbai, India
Casein	HiMedia, Mumbai, India
Coomassie brilliant blue R-250	HiMedia, Mumbai, India
Cetyltrimethylammonium bromide, Molecular Biology Grade	Calbiochem, EMD Biosciences, Inc. La Jolla, CA.
Chloroform	SRL Pvt. Ltd., Mumbai, India
Congo red	SRL Pvt. Ltd., Mumbai, India
Dextrose	SRL Pvt. Ltd., Mumbai, India
p-Dimethylaminobenzaldehyde	SRL Pvt. Ltd., Mumbai, India
Di-pottasium hydrogen phosphate	SRL Pvt. Ltd., Mumbai, India

dNTP mix (2.5mM each)	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
dinitrosalicylic acid	SRL Pvt. Ltd., Mumbai, India
di-Sodium tetraborate (Borax)	Merck, India
Ethanol	Bengal chemical, Kolkata India
Ethidium bromide	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Ethyl acetate	SRL Pvt. Ltd., Mumbai, India
Ethylenediaminetetra acetic acid disodium salt extrapure A.R.	SRL Pvt. Ltd., Mumbai, India
Ethylenediamine tetra acetic acid (EDTA)	SRL Pvt. Ltd., Mumbai, India
Ferric chloride	HiMedia, Mumbai, India
Folin ciocalteau	s.d. fine chem. Ltd., Mumbai, India
Gel loading buffer (6X)	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Glacial acetic acid	SRL Pvt. Ltd., Mumbai, India
Glucose	SRL Pvt. Ltd., Mumbai, India
Glutaraldehyde	Sigma Aldrich Chemicals, USA
Glycerol	SRL Pvt. Ltd., Mumbai, India
Glycine	SRL Pvt. Ltd., Mumbai, India
Guiacol	SRL Pvt. Ltd., Mumbai, India
Hydrochloric acid	E. Merck, Mumbai, India
Hydrogen peroxide	E. Merck, Mumbai, India
isoamyl alcohol	SRL Pvt. Ltd., Mumbai, India
isopropanol	SRL Pvt. Ltd., Mumbai, India
ITS1	Sigma Aldrich Chemicals Pvt. Ltd., India
ITS4	Sigma Aldrich Chemicals Pvt. Ltd., India
Laminarin	Sigma Aldrich Chemicals, USA
Lactophenol cotton blue	HiMedia Laboratories Ltd, India
L-phenylalanine	HiMedia Laboratories Ltd, India

Magnesium chloride	HiMedia Laboratories Ltd., Mumbai, India
Mercuric chloride ($HgCl_2$)	E. Merck, Mumbai, India
Methanol	SRL Pvt. Ltd., Mumbai, India
Nitric acid	E. Merck, Mumbai, India
One Step M-MuLV RT-PCR kit	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Ortho phosphoric acid	SRL Pvt. Ltd., Mumbai, India
PAL1-F	Sigma Aldrich Chemicals Pvt. Ltd., India
PAL1-R	Sigma Aldrich Chemicals Pvt. Ltd., India
Polyvinyl pyrrolidone	HiMedia, Mumbai, India
Potassium tetraborate	SRL Pvt. Ltd., Mumbai, India
Quick PCR Purification Kit	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Riboflavin	SRL Pvt. Ltd., Mumbai, India
RNase A	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Sodium azide	HiMedia, Mumbai, India
Sodium carbonate	E. Merck, Mumbai, India
Sodium chloride	SRL Pvt. Ltd., Mumbai, India
Sodium dodecyl sulphate (SDS)	HiMedia, Mumbai, India
Sodium hydroxide	SRL Pvt. Ltd., Mumbai, India
Sodium molybdate	SRL Pvt. Ltd., Mumbai, India
Sodium nitrate	HiMedia, Mumbai, India
Sodium thiosulphate	SRL Pvt. Ltd., Mumbai, India
Step Up 500bp DNA ladder	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Sucrose	SRL Pvt. Ltd., Mumbai, India
Tetramethyl ethylene diamine (TEMED)	SRL Pvt. Ltd., Mumbai, India
10 X Taq Polymerase buffer E with 15mM $MgCl_2$	Bangalore Genei (India) Pvt. Ltd., Bangalore, India

10X Taq buffer A (Tris with 15mM $MgCl_2$)	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Taq DNA polymerase (3U/µl)	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Thiobarbituric acid (TBA)	BDH chemicals limited, Poole, England
Toluene	SRL Pvt. Ltd., Mumbai, India
Trichloroacetic acid (TCA)	Universal laboratories Pvt. Ltd, Mumbai, India
Tris	E. Merck, Mumbai, India
Tris- saturated phenol	Bangalore Genei (India) Pvt. Ltd., Bangalore, India
Tween 20	HiMedia, Mumbai, India

APPENDIX II

BUFFERS AND SOLUTIONS

1. 0.2M Sodium phosphate buffer, pH 6.5

A. $Na_2HPO_4.7H_2O$, 0.2 M = 35.61 g in 1000 ml distilled water.

B. $NaH_2PO_4.2H_2O$ = 31.21 g in 1000 ml distilled water.

(32 ml of solution A and 68 ml of solution B were mixed and pH was adjusted to 6.5.)

2. Acetate buffer

Stock solution A:

Acetic acid	0.1 M
Distilled water	1000 ml

Stock solution B:

Sodium acetate (tri hydrate)	13.6 g
Distilled water	1000 ml

847 ml Stock solution A and stock solution B 153 ml were mixed to obtain a buffer of pH 5.

3. 0.2 M Borate buffer, pH 8.7

A. Boric acid, 1.24 g in 100 ml distilled water

B. Borax, 1.90g in 100 ml distilled water

(50 ml of solution A and 22.5 ml of solution B were mixed and pH was adjusted to 8.7)

4. 1X TAE buffer

50X TAE composition:

Tris base	242 g
Glacial acetic acid	57.1 ml
EDTA (0.5 M)	100 ml
Distilled water (final volume make up to)	1000 ml
Final pH	8.0

To make 1X TAE buffer, 1 ml 50X stock buffer was diluted in 49 ml distilled water to make final volume 50 ml.

5. TE buffer

Tris-HCl	10 mM
EDTA	1 mM
Final pH	8.0

6. DNA extraction buffer

Chemicals requirements	Concentration of stock solution	Working volume for 1g sample
Tris (pH 8.0)	1M	500µl
NaCl	5M	1.4ml
EDTA (pH 8.0)	0.5M	200µl
B-mercaptoethanol		10µl
Distilled water (sterile)		2.89ml
CTAB 2% (w/v)		100mg

7. Polyacrylamide gel electrophoresis: Stock solutions

Polyacrylamide gel electrophoresis (PAGE) was done following the method as described by Davis (1964) with some modifications. The compositions of the solutions used for preparing resolving and stacking gels are as follows:-

Preparation of stock solution

Solution A: Acrylamide stock solution (resolving gel)

Acrylamide stock solution was prepared for the resolving gel, by dissolving 30g of acrylamide and 0.8 gm of N N' methylene bisacrylamide dissolved in 100 ml of warm distilled water. The stock solution was filtered with Whatmann No. I filter paper in dark and stored in dark bottle in 4^0C.

Solution B: Tris-HCL (resolving gel)

Tris HCL buffer was prepared by dissolving 18.15 gm of Tris base in distilled water and 0.4 ml of TEMED was added. The pH of the solution was adjusted to 8.9 with 1N HCL. The final volume of the solution was made upto 100 ml with distilled water. The solution was then stored at 4^0C for further use.

Solution C: Ammonium per sulphate

Fresh solution of ammonium per sulphate was prepared by dissolving 60mg of APS in 100 ml of distilled water.

Solution D: Acrylamide stock solution (stacking gel)

For the preparation of acrylamide stock solution for the stacking gel, 5 gm of acrylamide and 1.25 gm of bisacrylamide was dissolved in 100 ml warm distilled water. The stock solution was then filtered with Whatmann No. I filter paper and stored at 4^0 C in dark bottle.

Solution E: Tris HCL (stacking gel)

2.1 gm of Tris base was mixed with distilled water and 0.2 ml of TEMED was added to it. Finally the pH of the solution was adjusted to 6.7 with 1N HCL. The final volume of the solution was made upto 100 ml with distilled water. The solution was then stored at 4^0C for further use.

Solution F: Riboflavin solution

Fresh riboflavin solution was prepared by dissolving 2 mg of riboflavin in 2M sucrose (100ml). The solution was kept in dark bottle to protect it from light.

Solution G: Electrode buffer (pH 8.4)

Fresh electrode buffer was prepared by dissolving 6 gm of Tris base and 28.8 gm of glycine in 1 litre distilled water.

8. Ethidium bromide solution (10 mg ml-1)

To prepare 10 ml of 10 mg/ml ethidium bromide, 100 mg of ethidium bromide powder was dissolved in 8 ml water and stirred on a magnetic stirrer for several hours to dissolve the dye completely. The volume was adjusted up to 10 ml and stored in dark brown bottle.

9. Agarose gel (1.0%)

Agarose powder (1.0 g) was taken in a conical flask and 1X TAE buffer was added to make the final volume to 100 ml. The mixture was then properly

boiled, added proper amount of EtBr solution when its temperature is about 55-60°C and poured on a gel casting plate.

10. Agarose gel (1.5%)

Agarose powder (1.5 g) was taken in a conical flask and 1X TAE buffer was added to make the final volume to 100 ml. The mixture was then properly boiled, added proper amount of EtBr solution when its temperature is about 55-60°C and poured on a gel casting plate.

11. 2% CTAB in 1M NaCl (100ml) for DNA isolation

CTAB	1.0g
1M NaCl	100ml

Measured amount of CTAB was added to sterile 1M NaCl and heated in water bath to dissolve completely at 60°C.

12. NaCl (5M) for DNA isolation

NaCl	29.2g
Distilled water	100ml

Measured amount of NaCl was added to distilled water, mixed properly till the salt dissolves and thereafter sterilized by autoclaving at 15 lbs p.s.i pressure for 15 min at 121°C.

13. EDTA (0.5 mM) (pH 8.0)

Disodium EDTA-dihydrate	18.6g
Distilled water	100ml

Measured amount of disodium EDTA-dihydrate was dissolved in distilled water and mixed vigorously on a magnetic stirrer. The pH was adjusted to 8.0 with NaOH (~ 10 g of NaOH pellets). Solution was sterilized by autoclaving at 15 lbs p.s.i pressure for 15 min at 121°C.

14. Tris-HCl (1M)

Tris base	12.32 g
Distilled water	100ml

Measured amount of tris base was dissolved in 100 ml of water and pH was adjusted the pH 8.0 with concentrated HCl, made up the final vol to 100 ml. Solution was sterilized by autoclaving at 15 lbs p.s.i pressure for 15 min at 121°C.

15. 70% ethanol for DNA isolation

Absolute ethanol	70 ml
Distilled water	30 ml

16. K_2HPO_4 (0.5 M)

Dipotassium hydrogen phosphate- 0.87gm

Dissolved in 100ml double distilled water

17. Guaiacol (0.05M) 20ml

Guiacol- 221.6µl

Dissolved in 19.77ml double distilled water

18. Dinitro salicylic acid (DNS) reagent

Dinitro salicylic acid	:	1.0 g
Crystal phenol	:	200mg
Sodium sulfate	:	50 mg
Sodium hydroxide	:	1.5
Distilled water	:	100 ml

By stirring dissolved all the constituents and stored the reagent in a stopper bottle at 4^0 C. The reagent deteriorates during storage due to atmospheric oxidation of the sulphite present. If required to be stored, the reagent was prepared without dilution and added just before use (Mahadevan and Sridhar, 1996).

APPENDIX-III

GROWTH MEDIA

1. Potato dextrose agar (PDA)

Peeled potato	: 400gm
Dextrose	: 20gm
Agar	: 20gm
Water	: 100ml

Required amount of peeled potato was boiled in distilled water and filtered through cheese cloth. Then 2% dextrose and 2% agar powder was added to it and melted by heating before the medium was sterilized at 15 lb p.s.i. for 15 minutes.

2. Oat meal agar (OMA)

Oat meal	: 40 g
Agar agar	: 15 g
Distilled water	: 1000 ml

Required amount of powdered oat was boiled in distilled water in a water bath, stirred occasionally and strained through cheese cloth. Then agar powder was added to it and melted by heating before the medium was sterilized at 15 lb p.s.i. for 15 minutes.

3. Czapek dox agar (CDA)

Sodium Nitrate ($NaNO_3$)	: 3 g
Potassium hydrogen phosphate (K_2HPO_4)	: 1 g
Potassium Chloride (KCI)	: 0.5 g
Magnesium sulfate ($MgSO_4$, $7H_2O$)	: 0.5 g
Ferrous Sulphate ($FeSO_4$)	: 0.01 g
Sucrose	: 30 g
Agar agar	: 15 g
Distilled water	: 1000 ml

All the ingredients except agar and K_2HPO_4 were dissolved. Then agar was added and dissolved by boiling. Finally K_2HPO_4 was added to the molten solution, mixed thoroughly and sterilized at 15 lb p.s.i. for 15 minutes.

4. Richard's agar (RA)

KNO_3	:10 g
KH_2PO_4	:5 g
$MgSO_4$, $7H_2O$	:2.5 g
$FeCl_3$	:0.02 g
Sucrose	:50 g
Agar agar	:20 g
Distilled water	:1000 ml

Required amount of all the constituents were taken and dissolved by stirring with required distilled water. The agar was melted by heating the medium before sterilization at 15 lb p.s.i. for 15 minutes.

5. Yeast extract mannitol agar (YEMA)

Yeast extract	: 2 g
Mannitol	: 10 g
Potassium Dihydrogen Phosphate (KH_2PO_4)	: 0.5 g
Magnesium sulfate ($MgSO_4$, $7H_2O$)	: 0.2 g
Sodium Chloride (NaCl)	: 0.1 g
Agar agar	: 20 g
Distilled water	: 1000 ml

All the ingredients except agar were dissolved in distilled water. Finally, agar was added and dissolved by boiling before the medium was sterilized at 15 Ib p.s.i. for 15 minutes.

6. Malt extract agar (MEA)

Malt extract	: 20 g
Agar	: 20 g

Distilled water : 1000 ml

Malt extract was dissolved in distilled water by boiling. Then, required amount of agar powder was added. Finally the solution was boiled with constant shaking till the agar was dissolved. Sterilization was done at 15 lb p.s.i. for 15 minutes.

7. Lagenaria dextrose agar (LDA)

Potato	: 200gm
Carrot	: 200gm
Lagenaria fruit extract	: 200gm
Dextrose	: 20gm
Agar	: 20gm
Water	: 1000 ml

Required amount of peeled potato, carrot and lagenaria fruit extract was boiled in distilled water and filtered through cheese cloth. Then 2% dextrose and 2% agar powder was added to it and melted by heating before the medium was sterilized at 15 lb p.s.i. for 15 minutes.

8. Potato dextrose broth (PDB)

Peeled potato	:	40 g
Dextrose	:	2 g
Distilled water	:	100 ml

Required amount of peeled potato was boiled in distilled water. The potato broth was taken by straining through cheesecloth and required amount of dextrose was added. Finally, the medium was sterilized at 15 lb p.s.i. for 15 minutes.

APPENDIX IV

NUCLEOTIDE SEQUENCES

Nucleotide sequences of rRNA genes of the pathogenic fungi isolated from diseased bottle gourd fruits.

1. F/A/1 (*Colletotrichum gloeosporioides*) (Submission code SILIGU 1)

Accession No. KC355249

GACTCCTCCTAGGGGAACCTGCGGAGGGATCATTACTGAGTTTACGCTCTA
CAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTCTCCG
CGACCCTCCCGGCCTCCCGCCTCCGGGCGGGTCGGCGCCCGCCGGAGGA
TAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATC
AAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCA
TTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAGGC
CCTCAAAGGTAGTGGCGGACCCTCTCGGAGCCTCCTTTGCGTAGTAACTTTA
CGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTC
CAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCA
ATAAGCGGGGGAA

2. F/A/2 (*Fusarium incarnatum*, submitted as *Fusarium* sp., Submission code SILIGU 2)

Accession No. KR263845

GTAGGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCC
CTGTGAACATACCTATACGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAA
ACGGGACGGCCCGCCCGAGGACCCCTAAACTCTGTTTTTAGTGGAACTTCT
GAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCG
GGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTGTTGG
GACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTCACGTCGAGCTT
CCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTCGCGGCCACGCC
GTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCT
GAACTTAAGCATATCGATAACCCAGGAGAAAC

APPENDIX V

LIST OF PUBLICATIONS

1. **Saha A.**, Das S., Chakraborty P., Saha B., Saha D. and Saha A. (2016) Two new bottle gourd fruit rot causing pathogens from sub-Himalayan West Bengal. *International Journal of Agricultural Technology* **12(2):** 321-332.
2. Mandal H., Chakraborty P., Das S., **Saha A.,** Sarkar T., Saha D. and Saha A. (2017) Biocontrol of virulent *Ralstonia solanacearum* isolates by an indigenous *Bacillus cereus*. *International Journal of Agricultural Technology* **13(1):** 19-30.
3. Chakraborty P., Das S., Saha B., Sarkar P., Karmakar A., **Saha A.**, Saha D. and Saha A. (2015) Phylogeny and synonymous codon usage pattern of *Papaya ringspot virus* coat protein gene in sub-Himalayan region of north-east India. *Canadian Journal of Microbiology* **61(8):** 555-564.