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Screening brinjal (*Solanum melongena*) accessions conserved in the National Genebank collected from states adjoining Bangladesh for adventitious presence of EE1 event

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ABSTRACT

Systematic management of Plant Genetic Resources (PGRs) is the key to sustainable agriculture for food and nutritional security and in mitigating climate change. The National Genebank (NGB) at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, acts as a repository of PGRs for future use. This study aimed at screening for the adventitious presence of transgenes in brinjal (*Solanum melongena* L.) accessions conserved in the NGB. The study targeted the collections made during 2007-2016 from areas adjacent to Bangladesh (Assam, Meghalaya, Mizoram, Tripura and West Bengal), where field trials of *Bt* brinjal event EE1 were conducted during 2005-2012 and commercial cultivation of this event was permitted in 2013.There could be an apprehension of both unintentional introgression and transboundary movement through borders. Adventitious presence of transgenes was checked in a set of 96 accessions of brinjal employing Polymerase Chain Reaction (PCR) and real-time PCR assays. As event EE1 carries *cry1Ac* gene for insect resistance with *Cauliflower Mosaic Virus* (CaMV) promoter (*P-35S*) and marker genes (*nptII* and *aadA*), so these genetic elements were targeted for qualitative GM testing. Based on the test results, transgenes were not detected in brinjal accessions conserved in NGB. Our study showed that brinjal and wild species collected from adjoining areas of Bangladesh, post field trials and release, do not contain the event EE1. The study presents an efficient and reliable method to ensure conservation of GM-free germplasm in the NGB.

Key words: Adventitious presence, GM-free Conservation, National Genebank, Polymerase Chain Reaction, Real time PCR, Transgenes

Brinjal or eggplant (*Solanum melongena* L.) is an important vegetable crop, belonging to family Solanaceae. It is cultivated across tropics, subtropics and temperate parts of the world **(**Kumar 2009**)**. In India, brinjal is cultivated in almost all states including West Bengal, Odisha, Andhra Pradesh, Gujarat, Bihar and Maharashtra **(**http://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf**)**. India has a diverse range of wild species and landraces of brinjal comprising *Solanum aethiopicum* L.*, S. anomalum* Thonn*, S. incanum* L.*, S. indicum* L.*, S. insanum* L.*, S. khasianum* C.B. Clarke*, S. macrocarpon* L.*, S. mammosum* L.*, S. nigrum* L.*, S. sisymbrifolium* Lam.*, S. torvum* Swartz*, S. viarum* Dunal and *S. xanthocarpum* Schrad. & H. Wendl. (Choudhary and Gaur 2009). The National Genebank (NGB) at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, is the second largest genebank in the world and conserves more than 0.44 million accessions of different agri-horticultural crops including 4496 brinjal accessions (http://www.nbpgr.ernet.in/). Brinjal is usually a self-pollinated crop, but the extent of cross-pollination varies from 0 to 46 % (Sekara *et al.* 2008).

The major constraint to brinjal cultivation is the infestation of brinjal fruit and shoot borer (FSB) (*Leucinodes orbonalis* Guenee), which can cause damage up to 80% throughout the vegetative and reproductive stages (Raina *et al.* 2018). To control FSB, Maharashtra Hybrid Seeds Company Limited (MAHYCO) developed *Bt* brinjal event EE1 with *cry1Ac* gene of *Bacillus thuringiensis* (*Bt*) subsp. Kurstaki strain HD7 (http://www.isaaa.org/gmapprovaldatabase). In India, the event EE1 was under

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field trials in 2006, 2007 and 2009 (http://www.geacindia.gov.in/). Bangladesh has approved *Bt* brinjal event EE1 in 2013 (APAARI 2018). It is essential for genebank curators to ensure genetic purity of conserved germplasm before sharing the genetic resources to the breeders to restrict unintentional transfer of alleles from genetically modified (GM) crops (Bairwa *et al.* 2016). Thus, consistent efforts need to be made to check possible adventitious presence of transgenes in genebank collections employing reliable GM diagnostics for ensuring GM-free conservation (Tiwari and Randhawa 2010). Efficient and reliable DNA-based diagnostics for *Bt* brinjal event EE1 have been reported (Randhawa *et al.* 2012; Randhawa and Singh 2016), which could be systematically employed to monitor adventitious presence of transgenes in brinjal germplasm. PCR-based studies to monitor adventitious presence of transgenes in cotton, maize, brinjal and okra conserved in the genebanks have been reported (Mezzalama *et al.* 2010; Gamarra *et al*. 2011; Parimalan *et al*. 2015, Bairwa *et al*. 2016), showing absence of transgenes.

The present study was designed to assess the GM-free status of NGB *Solanum* accessions comprising six different species, namely, *S. melongena* L.*, S. aethiopicum* L.*, S. incanum* L.*, S. macrocarpon* L.*, S. torvum* Swartz and *S. violaceum* Ortega collected during 2007 to 2016 from different states (Assam, Meghalaya, Mizoram, Tripura and West Bengal) bordering Bangladesh employing PCR and real-time PCR-based diagnostics.

MATERIALS AND METHODS

Seed material: Based on the passport data from the NGB, ICAR-NBPGR, 96 brinjal accessions were chosen, which were collected during 2007 to 2016 comprising *S. melongena* L. (79 acc.,), *S. aethiopicum* L. (12 acc.,), *S. incanum* L. (02 acc.,), *S. macrocarpon* L. (01 acc.,), *S. torvum* Swartz (01 acc.,) and *S. violaceum* Ortega (01 acc.,) from five states of India, viz. Assam, Meghalaya, Mizoram, Tripura and West Bengal, bordering Bangladesh (Table 1).

Table 1 Passport details of brinjal and wild species used in this study

Accession number	Botanical name	Collector No/Other identity	Vernacular name	District	State
IC0618069	S. melongena	GGBC-9-1	Bengena	Kokrajhar	Assam
IC0618070	*S. melongena*	GGBC-10	Bengena	Kokrajhar	Assam
IC0394735	*S. melongena*	SMBR-618	Kola Bengena	Sonitpur	Assam
IC0345758	*S. melongena*	AKS-1/43	Bengun	Dhubri	Assam
IC0599663	*S. melongena*	GB 09-12	Bengena	Jorhat	Assam
IC0618074	*S. melongena*	GGBC-18-1	Bengena	Kokrajhar	Assam
IC0394872	*S. melongena*	SMBR-741	Bengun	Nalbari	Assam
IC0599662	*S. melongena*	GB 09-05	Bengena	Jorhat	Assam
IC0394902	*S.melongena*	SMBR-769	Junuka Bengena	Barpeta	Assam
IC0427083	*S. melongena*	AAU04 Brinjal-2	Bengena	Kokrajhar	Assam
IC0345740	*S. melongena*	AKS-1/7	Begun	Nalbari	Assam
IC0345757	*S. melongena*	AKS-1/38	Bengun	Dhubri	Assam
IC0618072	*S. melongena*	GGBC-17	Bengena	Kokrajhar	Assam
IC0618071	*S. melongena*	GGBC-12	Bengena	Kokrajhar	Assam
IC0618068	*S. melongena*	GGBC-6	Bengena	Kokrajhar	Assam
IC0599664	*S. melongena*	GB 10-11	Bengena	Jorhat	Assam
IC0618067	*S. melongena*	GGBC-4	Bengena	Kokrajhar	Assam
IC0618075	*S. melongena*	GGBC-19	Bengena	Kokrajhar	Assam
IC0618076	*S. melongena*	GGBC-20	Bengena	Kokrajhar	Assam
IC0618077	*S. melongena*	GGBC-21	Bengena	Kokrajhar	Assam
IC0618081	*S. melongena*	GGBC-35	Bengena	Kokrajhar	Assam
IC0618080	*S. melongena*	GGBC-26	Bengena	Kokrajhar	Assam
IC0618079	*S. melongena*	GGBC-25-2	Bengena	Kokrajhar	Assam
IC0618078	*S. melongena*	GGBC-24	Bengena	Kokrajhar	Assam
IC0394868	*S. melongena*	SMBR-738	Bengun	Nalbari	Assam
IC0618082	*S. melongena*	GGBC-35-1	Bengena	Kokrajhar	Assam
IC0281102	*S. melongena*	PK-230	Baring	West Garo Hills	Meghalaya
IC0090063	*S. melongena*	H-1442	-	East Garo Hills	Meghalaya

Cond.

Table 1 (*Continued*)

Accession number	Botanical name	Collector No/Other identity	Vernacular name	District	State
IC0090070	*S. melongena*	H-1449	-	East Garo Hills	Meghalaya
IC0090937	*S. melongena*	KB-9	-	Kalyani	West Bengal
IC0090944	*S. melongena*	KB-18	-	Kalyani	West Bengal
IC0090949	*S. melongena*	KB-23	-	Kalyani	West Bengal
IC0098148	*S. melongena*	B-95	-	West Garo hills	Meghalaya
IC0111077	*S. melongena*	H-1639	-	Falkata	West Bengal
IC0111080	*S. melongena*	H-1642	-	Jalpaiguri	West Bengal
IC0136003	*S. melongena*	D-1777	-	Nadia	West Bengal
IC0136359	*S. melongena*	BDS-1377	-	M. Tripura	Tripura
IC0136364	*S. melongena*	BDS-1382	-	M. Tripura	Tripura
IC0136367	*S. melongena*	BDS-1386	-	N. Tripura	Tripura
IC0136380	*S. melongena*	BDS-1423	-	N. Tripura	Tripura
IC0136383	*S. melongena*	BDS-1432	-	N. Tripura	Tripura
IC0280281	*S. melongena*	PK/RS-OT56	Nol Bengena	Barpeta	Assam
IC0280913	*S. melongena*	PK/RS-OT133	Brinjal	Bongaigaon	Assam
IC0280961	*S. melongena*	PK/RS-OT181	Gol Begun	Dhubri	Assam
IC0280963	*S. melongena*	PK/RS-OT183	Lamba Begun	Dhubri	Assam
IC0281072	*S. melongena*	PK-200	Baring	West Garo Hills	Meghalaya
IC0281074	*S. melongena*	PK-202	Baring (Gol)	West Garo Hills	Meghalaya
IC0281099	*S. melongena*	PK-227	Baring	West Garo Hills	Meghalaya
IC0597000	*S. melongena*	RSR/BR-56	Begun	Jalpaiguri	West Bengal
IC0596998	*S. melongena*	RSR/BR-45	Begun	Coochbehar	West Bengal
IC0618073	*S. melongena*	GGBC-18	Bengena	Kokrajhar	Assam
IC0611550	*S. melongena*	JJKNE/2011-6	-	Dhalai	Tripura
IC0611549	*S. melongena*	JJKNE/2011-B3	-	Kolazib	Mizoram
IC0611548	*S. melongena*	JJKNE/2011-B2	-	Kolazib	Mizoram
IC0598458	*S. melongena*	BCB-40	Begun	Nadia	West Bengal
IC0597240	*S. melongena*	RY/SS/DY/BD/AJ/VV-111	-	Ri-Bhoi	Meghalaya
IC0597239	*S. melongena*	RY/SS/DY/BD/AJ/VV-110		Ri-Bhoi	Meghalaya
IC0596998	*S. melongena*	RSR/BR-45	Begun	Coochbehar	West Bengal
IC0566645	*S. melongena*	Brinjal-BCC-1	Brinjal-BCC-1	Coochbehar	West Bengal
IC0553604	*S. melongena*	Singnath	Singnath	Lembuehera	Tripura
IC0553603	*S. melongena*	Bholanath	Bholanath	Lembuehera	Tripura
IC0374516	*S. melongena*	RD-137	Bonbock	Lunglei	Mizoram
IC0281112	*S. melongena*	PK-240	Baring	West Garo Hills	Meghalaya
IC0280281	*S. melongena*	PK/RS-OT56	Nol Bengena	Barpeta	Assam
IC0280913	*S. melongena*	PK/RS-OT133	Brinjal	Bongaigaon	Assam
IC0280961	*S. melongena*	PK/RS-OT181	Gol Begun	Dhubri	Assam
IC0280963	*S. melongena*	PK/RS-OT183	Lamba Begun	Dhubri	Assam
IC0281072	*S. melongena*	PK-200	Baring	West Garo Hills	Meghalaya
IC0281074	*S. melongena*	PK-202	Baring (Gol)	West Garo Hills	Meghalaya
IC0281099	*S. melongena*	PK-227	Baring	West Garo Hills	Meghalaya
IC0281102	*S. melongena*	PK-230	Baring	West Garo Hills	Meghalaya
IC0281112	*S. melongena*	PK-240	Baring	West Garo Hills	Meghalaya
IC0285125	*S. melongena*	RSP-1/B-5	Begun	Burdwan	West Bengal

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Table 1 (*Concluded*)

Accession number	Botanical name	Collector No/Other identity	Vernacular name	District	State
IC0285126	*S. melongena*	RSP-1/B-6	Begun	Burdwan	West Bengal
IC0285138	*S. melongena*	RSP-1/B-8	Bengun	Burdwan	West Bengal
IC0345742	*S. melongena*	AKS-1/9	Begun	Nalbari	Assam
IC0345746	*S. melongena*	AKS-1/16	Begun	Barpeta	Assam
IC0345750	*S. melongena*	AKS-1/22	Bengun	Bongaigaon	Assam
IC0345756	*S. melongena*	AKS-1/36	Bengun	Dhubri	Assam
IC0326242	*S. aethiopicum*	RVN-129	Solanum	Saiha	Mizoram
IC0622458	*S. aethiopicum*	AD-16/97	Santu	North Tripura	Tripura
IC0394570	*S. aethiopicum*	SMBR-47	Tita Bhekuri	Tinsukia	Assam
IC0394992	*S. aethiopicum*	SMBR-85	-	Tinsukia	Assam
IC0611552	*S. aethiopicum*	JJKNE/2011-G1	-	Kolazib	Mizoram
IC0611553	*S. aethiopicum*	JJKNE/2011-G4	-	Aizwal	Mizoram
IC0611554	*S. aethiopicum*	JJKNE/2011-G5	-	Kolasib	Mizoram
IC0606839	*S. aethiopicum*	-	-	Shillong	Meghalaya
IC0446954	*S. aethiopicum*	SS/DRLT-120	-	Shillong	Meghalaya
IC0419703	*S. aethiopicum*	FDI/RS/43	-	Shillong	Meghalaya
IC0419701	*S. aethiopicum*	FDI/RS/41	-	Shillong	Meghalaya
IC0419692	*S. aethiopicum*	FDI/RS/33	-	Shillong	Meghalaya
IC0284536	*S. incanum*	DRLT-345	Bengene	Sonitpur	Assam
IC0427630	*S. incanum*	SBC-2/34	Bengun	24 Paragana (S)	West Bengal
IC0587758	*S. macrocarpon*	BKS/KAP/YR-1	Satinrem	Kolasib	Mizoram
IC0394385	*S. torvum*	SMBR-302	Tita Bagun	Dhemaji	Assam
IC0611814	*S. violaceum*	KP/SC-1583	Takuri Teetha	Karbi-Anglong	Assam

The rationale behind choosing these accessions from the NGB was that the collection sites of these accessions were in close proximity to the field trial sites of *Bt* brinjal event EE1 and commercial *Bt* brinjal growing regions of Bangladesh with majority of porous border**.**

DNA extraction and preparation of test samples: Approximately 40 seeds of each accession were homogenized into fine powder and DNA was extracted using modified Sodium dodecyl sulphate (SDS) method (Dellaporta *et al.* 1983). The quality and quantity of DNA was determined using UV spectrophotometer (Eppendorf, Hamburg, Germany) by measuring ratio of the absorbance at 260 and 280 nm. DNA samples were diluted to a working concentration of 20 ng/μL.

Internal controls: Before conducting GM tests, amplifiability of extracted DNA was ensured using a plant-specific chloroplast t-RNA gene assay as reported earlier (Taberlet *et al.* 1991). For PCR-based testing to check adventitious presence of transgenes in test samples, appropriate positive and negative controls were included to eliminate the chances of false negatives and false positives. The DNA of MON 531 event of *Bt* cotton was used as positive control, as it has similar genetic construct to that of *Bt* brinjal event EE1.

PCR assays targeting P-35S and cry1Ac: Singleplex PCR assays were performed using Veriti® Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 20 μL reaction mixture containing 100 ng of DNA template, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.25 μM each of forward and reverse primers and 0.5 U *Taq* DNA polymerase (Thermo Scientific, Walthan, Massachusetts, USA). PCR was performed with following thermal profile: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s; annealing at 60 °C for 50 s and extension at 72 °C for 1 min followed by final extension at 72 °C for 7 min. PCR products were resolved on 2% agarose gel and visualized by UV gel documentation system (Alpha Innotech, USA). Details of primers used are given in Table 2.

Multiplex PCR assays targeting marker genes (nptII and aadA): Duplex PCR was standardized for simultaneous detection of two marker genes, namely *nptII* and *aadA,* to facilitate rapid screening. Multiplex PCR was standardized on Veriti® Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 25 μL reaction mixture containing 100 ng of template DNA, 2X Multiplex PCR Mastermix (Qiagen® Multiplex PCR Plus Kit), 0.25 μM each of forward and reverse primers of *nptII* and *aadA* with PCR profile of initial denaturation at 95 ˚C for 5 min; 35 cycles of denaturation at 95 ˚C for 30 s, annealing at 60 ˚C for 90 s, extension at 72 ˚C for 30 s and final extension at 68 ˚C for 10 min. PCR products were resolved on 2% agarose gel

Table 2 Primers employed in this study

Target	Marker	Primer sequence (5'-3')	Amplicon size (bp)	Reference
PCR-based markers				
Chloroplast specific endogenous t-RNA gene	Plant A1/ Plant A2	F-CGAAATCGGTAGACGCTACG R- GGGGATAGAGGGACTTGAAC	≈610	Taberlet *et al.* (1991)
CaMV 35S *promoter*	*P-35S* F/R	F- GCTCCTACAAATGCCATCA R- GATAGTGGGATTGTGCGTCA	195	Lipp *et al*. (1999)
cry1Ac	*cry1Ac* F/R	F-TGACCGCTTACAAGGAGGGATACG R-CACGGAGGCATAGTCAGCAGGAAC	230	Randhawa *et al.* (2010)
nptII	*nptII* *F/R*	F-CTC ACC TTG CTC CTG CCG AGA R-CGC CTT GAG CCT GGC GAA CAG	215	Randhawa *et al.* (2009)
aadA	*aadA* *F/R*	F-TCCGCGCTGTAGAAGTCACCATTG R-CCGGCAGGCGCTCCATTG	406	Randhawa *et al.* (2009)
Real-time PCR based markers				
cry1Ac	*cry1Ac* F/R	F-TGACCGCTTACAAGGAGGGATACG R-CACGGAGGCATAGTCAGCAGGAAC	230	Randhawa *et al.* (2010)

and visualized using UV gel documentation system (Alpha Innotech, USA).

Real-time PCR assay: Real-time PCR using SYBR® Green chemistry was performed targeting *cry1Ac* gene in all the selected 96 brinjal accessions to further confirm the results. Real-time PCR was performed in 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The reaction was carried out in 20 µl reaction volume containing 100 ng of template DNA, 1X QuantiFast SYBR® Green PCR master mix (Qiagen, Germany), 0.1 µM each of forward and reverse primers of *cry1Ac* (Table 2). The thermal profile of real-time PCR used was: 50 ˚C for 2 min, single cycle of pre-activation of DNA at 95 ˚C for 10 min followed by 40 amplification cycles of denaturation at 95 ˚C for 15 s and annealing-extension at 60 ˚C for 1 min. After amplification, melting curve analysis of the PCR products was carried out as per the standard conditions mentioned in the system.

All the PCR and real-time PCR tests were performed in two replications.

RESULTS AND DISCUSSION

Effective management of PGRs poses a challenge for genebank curators in the diversity rich regions, where field trials of large number of GM crops are being conducted. After large scale commercial cultivation of *Bt* Brinjal in Bangladesh, it is important to consistently monitor for adventitious presence of transgenes in brinjal from the adjoining areas with porous borders. The best strategy is to monitor adventitious presence of transgenes in *ex-situ* collections employing DNA-based GM diagnostics for relevant GM events in proximity to the collection sites (Tiwari and Randhawa 2010). Some reports of monitoring adventitious presence of transgenes in *ex-situ* conserved germplasm are available (Mezzalema *et al.* 2010; Gamarra *et al*. 2011; Randhawa *et al*. 2016; Bairwa *et al*. 2016), showing absence of transgenes in *ex-situ* collection of maize, cotton and okra. The present study screening 96 selected

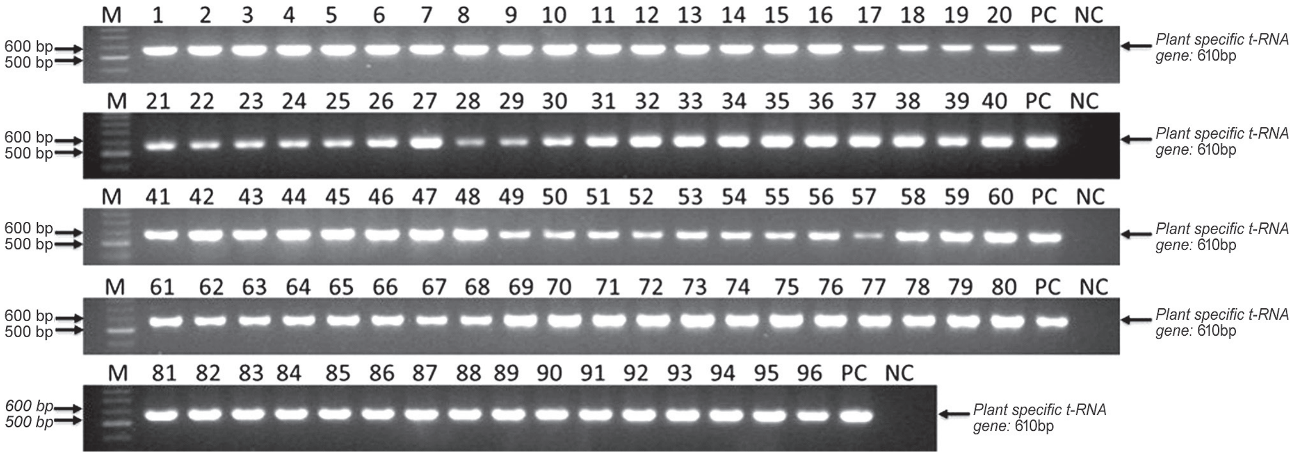

Fig 1 PCR amplification profile targeting 610 bp region of endogenous chloroplast t-RNA gene to confirm the amplifiability of DNA samples. Lane M=100 bp DNA ladder; 1-96=ex situ brinjal accessions; PC= Positive control and NC=Negative control.

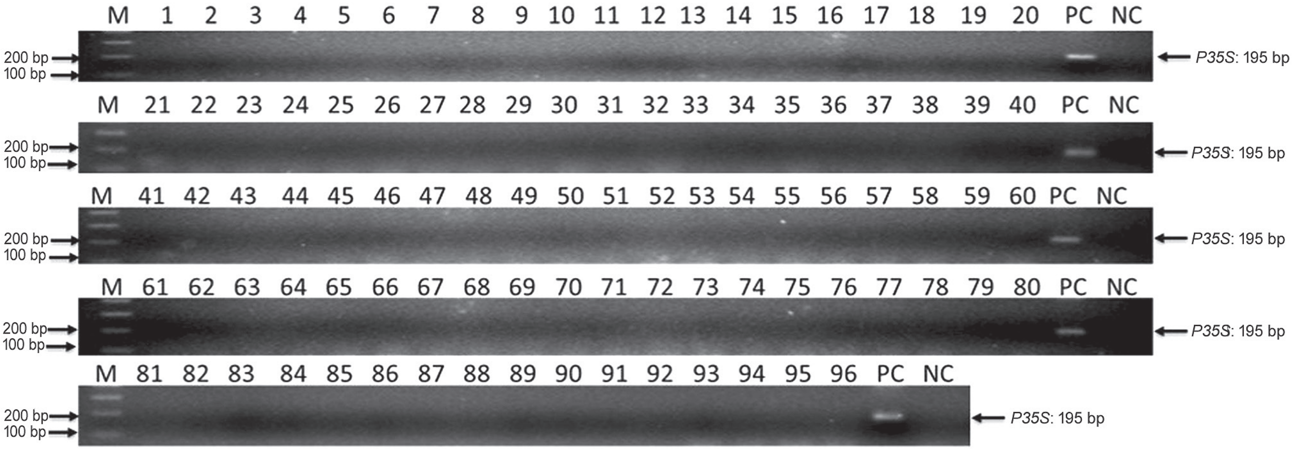

Fig 2 PCR amplification profile targeting 195 bp region of CaMV 35S promoter (*P-35S*). Lane: M=100 bp DNA ladder; 1-96= brinjal accessions; PC= Positive control and NC=Negative control.

accessions of brinjal targeted to monitor the adventitious presence of transgenes in brinjal germplasm collections.

PCR assay to check amplifiability of DNA samples: PCR assay targeting endogenous chloroplast t-RNA gene was performed to confirm the amplifiability of extracted DNA samples, for eliminating the chances of false negatives in the results. Amplifications were detected in all the brinjal test samples with expected amplicon size of 610 bp and no amplification was detected in negative control (Fig 1)**.**

PCR assay targeting P-35S and cry1Ac using singleplex PCR: The GM status of a sample can be checked by screening for the presence of control elements such as *P-35S* and *T-nos* (Waiblinger *et al.* 2008; Mezzalama *et al.* 2010; Bonfini *et al.* 2012; Block *et al*. 2013; Wu *et al.* 2014). The gene-specific assays target specific transgenes expressed in GM crops, e.g. *cry1Ac* gene of *Bt* brinjal event EE1 for FSB resistance. Singleplex PCR targeting *P-35S* and *cry1Ac,* was performed in all 96 accessions. Positive control showed amplification of respective targets with expected amplicon size of 195 *bp* for *P-35S* and 230 bp for *cry1Ac*. The *ex situ* genebank accessions, including negative control did not show amplification for *P-35S* and *cry1Ac* (Fig 2, 3).

Duplex PCR assay targeting marker genes: For rapid and simultaneous detection, duplex PCR targeting *nptII* and *aadA* marker genes was performed for all the samples. Only positive control amplified for these genes with amplicon size of 215 bp for *nptII* and 406 bp for *aadA*. The *ex situ* genebank accessions, including negative control did not show any amplification (Fig 4).

Real-time PCR assay targeting cry1Ac: Real-time PCR is more robust, specific and sensitive for GM detection (Debode *et al.* 2017). Real-time PCR was performed targeting *cry1Ac* gene. The amplification curve with mean Ct value of 20.76 and 18.90 was detected in the positive control in two set of reactions, *i.e.* 1-43 and 44-96 brinjal accessions respectively (Fig 5). The *ex situ* genebank accessions, including negative control did not show any amplification signals.

With the results of PCR and real-time PCR tests, it was confirmed that the brinjal and wild species collected during 2007-2016 from close proximity sites of India (Assam, Meghalaya, Mizoram, Tripura and West Bengal), bordering Bangladesh do not contain transgenes from *Bt*

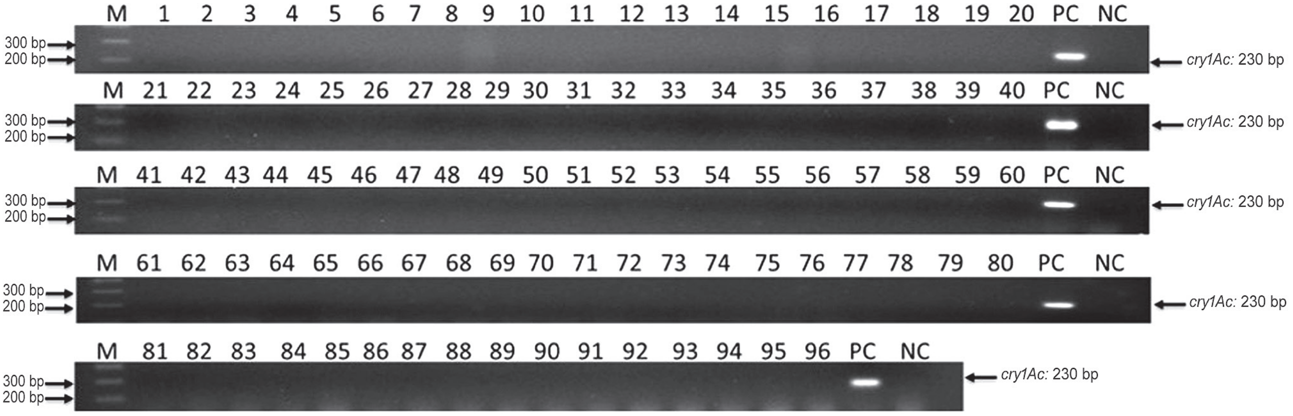

Fig 3 PCR amplification profile targeting 230 bp region of *cry1Ac* transgene. Lane: M=100 bp DNA ladder; 1-96 = brinjal accessions; PC= Positive control and NC=Negative control.

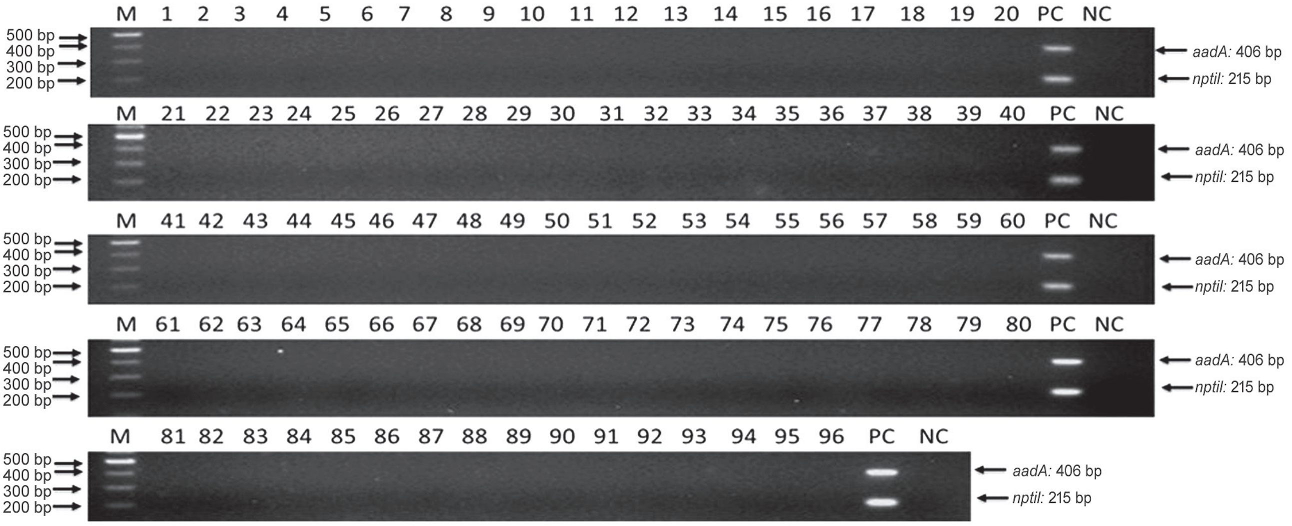

Fig 4 PCR amplification profile targeting 406 bp region of *aadA* and 215 bp of *nptII* marker genes. Lane: M=100 bp DNA ladder; 1-96=ex situ brinjal accessions; PC= Positive control and NC=Negative control.

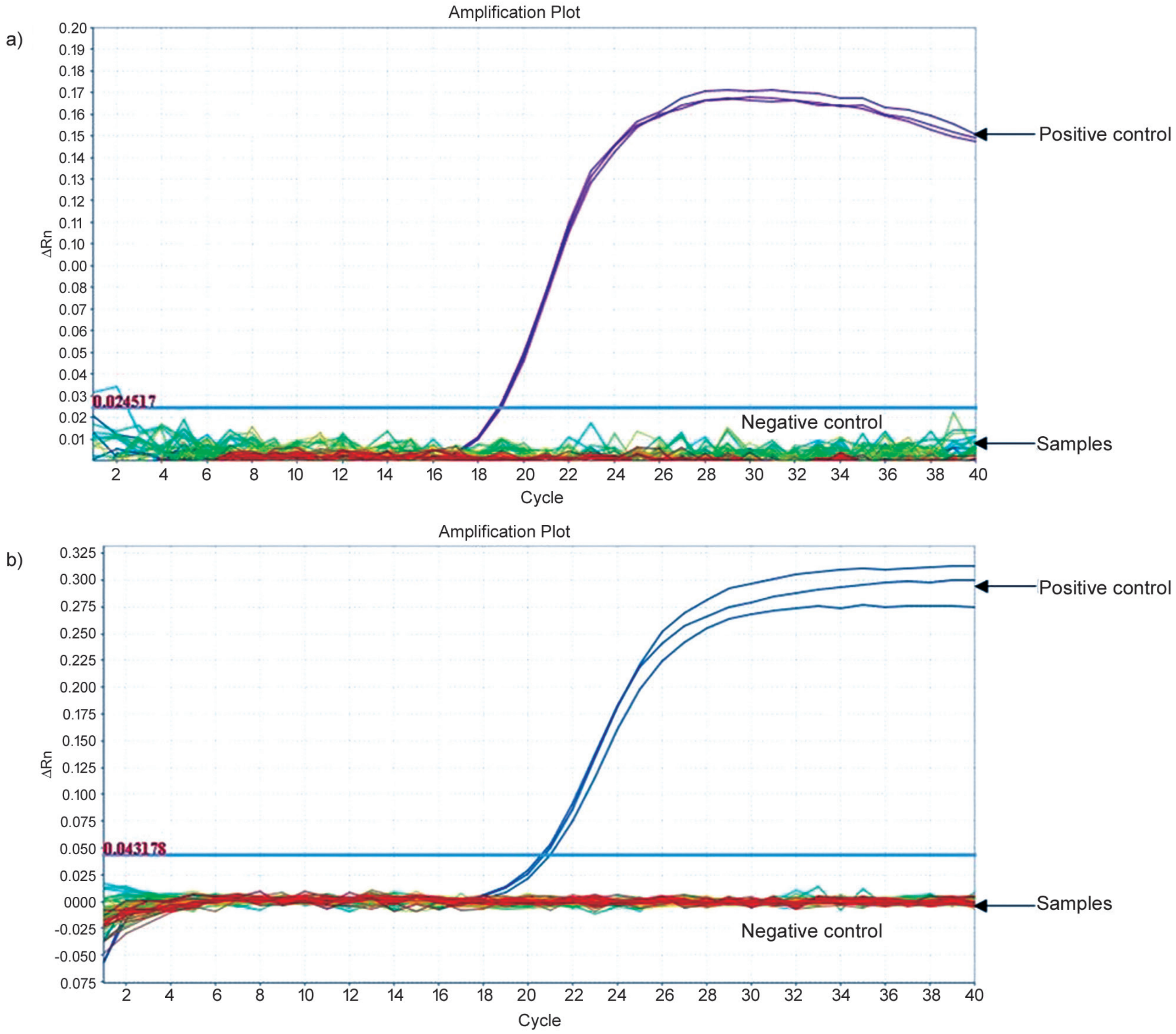

Fig 5 Real time PCR amplification profiles targeting *cry1Ac* transgene in brinjal accessions, a) 1-43 and b) 44-96, to confirm absence of adventitious presence of transgenes.

brinjal event EE1, thus eliminating the apprehension of either unintentional introgression or transboundary movement through borders. This study could serve as a model to detect adventitious presence of transgenes in commercial varieties, local cultivars including wild and weedy species to monitor intentional/unintentional introgression of transgenes, unapproved cultivations of GM events and their transboundary movement. Furthermore, it would help researchers and genebank curators for GM-free management of germplasm in different crops for which large scale field trials of their GM events have been conducted in their respective agro-climatic zones.

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REFERENCES

Bairwa R K, Singh M, Bhoge R K, Devi C and Randhawa G J. 2016. Monitoring adventitious presence of transgenes in *ex situ* okra (*Abelmoschus esculentus*) collections conserved in genebank: a case study. *Genetic Resources and Crop Evolution* **63**(2): 175–184.

Block A, Debode F, Grohmann L, Hulin J, Taverniers I and Kluga L. 2013. The GMOseek matrix: adecision support tool for optimizing the detection of genetically modified plants. *BMC Bioinformatics* **14**: 256.

Bonfini L, van den Bulcke M H, Mazzara M, Ben E and Patak A. 2012. GMOMETHODS: The European Union Database of reference methods for GMO analysis. *Journal of AOAC International* **95**(6): 1713–1719.

Choudhary B and Gaur K. 2009. The Development and Regulation of *Bt* Brinjal in India (Eggplant/Aubergine). ISAAA Brief No.38. ISAAA: Ithaca, NY.

Debode F, Janssen E, Bragard C and Berben G. 2017. Detection by real-time PCR and pyrosequencing of the *cry1Ab* and *cry1Ac* genes introduced in genetically modified (GM) constructs. *Food Additives & Contaminants*: *Part A* **34(**8): 1398–1409.

Dellaporta S L, Wood J and Hicks J B. 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter* **1**(4): 19–21.

Gamarra L F R, Delgado J A, Villasante Y A and Ortiz R. 2011. Detecting adventitious transgenic events in a maize center of diversity. *Electronic Journal of Biotechnology* **14**(4): 9–9.

Kumar P A. 2009. *Bt* brinjal: a pioneering push. *Biotechnology News* **4**(6): 108–111.

Lipp M, Bluth A, Eyquem F, Kruse L, Schimmel H, Van den Eede G and Anklam E.2001. Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. *European Food Research and Technology* **212**: 497–504.

Mezzalama M, Crouch J H and Ortiz R. 2010. Monitoring the threat of unintentional transgene flow into maize gene banks and breeding materials. *Electronic Journal of Biotechnology* **13**(2): 6–7.

Parimalan R, Bhoge R, Randhawa G J and Pandey C D. 2015. Assessment of transgene flow in eggplant germplasm conserved at National Genebank. *Indian Journal of Biotechnology* **14**(3): 357–363.

Raina J and Yadav G S. 2018. Brinjal shoot and fruit borer: Bio-ecology and management. *Journal of Pharmacognosy and Phytochemistry* **7**(4): 444–449.

Randhawa G J, Sharma R and Singh M. 2009. Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for the screening of genetically modified crops. *Journal of Agricultural and Food Chemistry* **57**(12): 5167–5172.

Randhawa G J, Sharma R and Singh M. 2010. Decaplex and real-time PCR based detection of MON531 and MON15985 *Bt* cotton events. *Journal of Agriculture and Food Chemistry* **58**(18): 9875–9881.

Randhawa G J, Sharma R and Singh M. 2012. Qualitative and event-specific real-time PCR detection methods for *Bt* brinjal event EE-1. *Journal of AOAC International* **95**(6): 1733–1739.

Randhawa G J and Singh M. 2016. Qualitative and quantitative diagnostics for EE-1 event of *Bt* eggplant. *Genetically Modified Organisms in Food* - 1st Edition, pp 87-96. Watson R R and Preedy V R (Eds). Academic Press Publication (Elsevier), USA.

Sekara A and Bieniasz M. 2008. Pollination, fertilization and fruit formation in eggplant [*Solanum melongena* L.]. *Acta Agrobotanica* **61**(1): 107–113.

Taberlet P, Gielly L, Pautou G and Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**(5): 1105–1109.

Tiwari S P and Randhawa G J. 2010. Strategies to monitor the adventitious presence of transgenes in *ex situ* collections. *Indian Journal of Agricultural Sciences* **80**(5): 351–356.

Waiblinger H U, Ernst B, Anderson A and Pietsch K. 2008. Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modified organisms in food products. *European Food Research Technology* **226**: 1221–1228

Wu Y, Wang Y, Li J, Li W, Zhang L, Li Y, Li X, Li J, Zhu L and Wu G. 2014. Development of a general method for detection and quantification of the P35S promoter based on assessment of existing methods. *Science Reports* **4**: 7358

http://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf) (last accessed on 18.11.2019)

http://www.nbpgr.ernet.in/ (last accessed on 17.11.2019)

http://www.indiaenvironmentportal.org.in/files/brinjal.pdf (last accessed on 18.11.2019)

http://www.isaaa.org/gmapprovaldatabase (last accessed on 15.11.2019)

http://www.geacindia.gov.in/decisions-of-GEAC-meetings.aspx (last accessed on 15.11.2019)

http://www.apaari.org/web/wpcontent/uploads/downloads/2018/Bt%20Brinjal%20in%20Bangladesh%20final.pdf (last accessed on 17.11.2019)