result. Figure 6 *b* shows the zoomed-in view of the target corresponding to the red dashed area in Figure 6 *a*. It can be clearly observed that the target in the sediment layer is well-reconstructed. This means that the presented system can be used for the sub-bottom profiler.

In order to visually evaluate the imaging performance, the azimuth and range slices were analysed in detail. Figure 7 *a* shows the azimuth slice. Figure 7 *b* shows the zoomed-in view of the azimuth slice. Figure 7 *b*, the azimuth resolution is about 0.15 m, which is not equal to the theoretical resolution of 0.08 m. In general, the synthetic aperture technique can theoretically promise high resolution of 0.08 m. In practice, it is always difficult to achieve full theoretical resolution, because the SAS system suffers from motion error, multipath in shallow water, and so on. Considering these factors, a simple rule of thumb is that the practical resolution would be 1.5–2 times lower[11]. At this point, the resolution shown in Figure 7 *b* is approximately close to the theoretical resolution.

Figure 8 *a* depicts the range slice and Figure 8 *b* shows the zoomed-in view of the range slice. The theoretical resolution in the vertical dimension is directly determined by the signal bandwidth and it is 0.11 m. From Figure 8 *b*, the vertical resolution is about 0.12 m. This means that the vertical resolution shown agrees well with the theoretical resolution.

Based on the processing results of real data, we conclude that the downward-looking SAS with Mills cross configuration can obtain high-resolution image of the sediment layer. It can also be used for the exploration of buried objects in the sediment layer. Some buried objects such as mines and chemical ammunition are hazardous. With the presented system, these objects can be efficiently detected and cleared. It can also be used to detect buried relics by the archaeologists and has great potential in exploring the sediment layer below the seafloor. Using this system, the morphology and sediment structures in the sub-bottom such as steep slopes, faults, reefs, landslide areas, ridges and rocky outcrops can be characterized. The results can be used for underwater engineering such as pipeline deployment, mineral resources investigations, bridge-building, shore protection and waterway dredging.

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ACKNOWLEDGEMENTS. We thank the National Natural Science Foundation of China and the National Key Laboratory Foundation, China for support. We also thank the anonymous reviewers for useful suggestions that helped improve the manuscript.

Received 25 November 2019; revised accepted 11 January 2022

doi: 10.18520/cs/v122/i4/461-464

Efficient molecular method/s for detection of *Bt* brinjal Event 142

Ruchi Sharma, Monika Singh and Gurinderjit Randhawa*

Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012, India

Fruit and shoot borer (FSB)-resistant *Bt* brinjal Event 142 with *cry1Fa1* gene has been approved for biosafety research level (BRL)-II field trials by the Genetic Engineering Appraisal Committee (GEAC), the competent authority in India. For regulatory compliance, systematic detection of genetically modified (GM) crops would facilitate monitoring for the presence of a GM event in the supply chain. Polymerase chain reaction (PCR) and real-time PCR are widely used for GM detection globally. Qualitative PCR and real-time PCR assays targeting *cry1Fa1* transgene were developed and validated with acceptable specificity and sensitivity up to 0.01%. A multiplex (hexaplex) PCR-based screening assay simultaneously detecting five transgenic elements and an endogenous gene was developed for Event 142. Construct-specific PCR reported herein could be employed for more specific

*For correspondence. (e-mail: gurinder.randhawa@icar.gov.in)

detection. These detection assays would facilitate efficient regulatory compliance by checking unapproved *Bt* brinjal event in the supply chain and also address the concerns of the consumers to an extent.

Keywords: *Bt* brinjal, detection assays, field trials, genetically modified crops, polymerase chain reaction.

THE adoption of genetically modified (GM) crops has increased globally, with 190.4 million hectares under GM crop cultivation in 2019 (ref. 1). The major GM crops approved globally include canola, cotton, maize and soybean. GM events of more than 30 crops, including food crops have been approved in different countries[2]. With the commercialization of GM food crops, many countries have adopted stringent regulations pertaining to GM crops and derived products to check for unapproved or unauthorized GM events in the supply chain.

Brinjal is an important vegetable crop of the tropical and temperate parts of the world. It is highly susceptible to brinjal fruit and shoot borer (FSB) causing losses of up to 60–70% (ref. 3). The transgenic technology, particularly *Bt* technology, has opened up opportunities to provide resistance against a range of insect pests. *Bt* technology refers to genetic transformation of plants with the gene constructs harbouring *cry* gene(s) derived from *Bacillus thuringiensis* for imparting insect resistance.

Bt technology has been employed in the development of *Bt* brinjal for imparting resistance to FSB[4–6]. *Bt* brinjal event EE1 was developed by Maharashtra Hybrid Seeds Company (Mahyco, Jalna, India). Event EE1 has been approved in the neighbouring country of Bangladesh.

The entry or cultivation of unapproved GM events can be checked using efficient GM diagnostics. Polymerase chain reaction (PCR) or real-time PCR is a widely used analytical approach for GM detection[7]. PCR and real-time PCR-based event-specific assays were developed for detection of *Bt* brinjal event EE1 (ref. 8). Recently, a systematic study for utilization of GM diagnostics has been reported for checking adventitious presence of transgenes of EE1 in 211 brinjal collections from the Indian states sharing porous borders with Bangladesh where EE1 is commercially grown[9]. The GM diagnostics for event EE1 has also been employed for checking adventitious presence of transgenes in GeneBank accessions[10].

Another event of FSB-resistant *Bt* brinjal, namely Event 142 has been approved for confined field trials in India[11]. Event 142 expressing *cry1Fa1* gene was developed by ICAR-National Research Centre on Plant Biotechnology (now ICAR-National Institute for Plant Biotechnology), New Delhi[12]. The technology was licensed to Bejo Sheetal Seeds Private Limited (now Beej Sheetal Research Private Limited, Jalna, India).

Appropriate GM detection strategies could facilitate checking for presence/absence of a particular GM event (whether approved or not in a country) for regulatory compliance.

In the present study, GM diagnostics for detection and quantification of *Bt* brinjal Event 142 is reported. An efficient GM detection method was developed employing multiplex PCR, construct-specific PCR and transgene-specific PCR/real-time PCR. The developed PCR and real-time PCR assays targeting *cry1Fa1* gene were found specific and sensitive enough with limit of detection (LOD) up to 0.01%.

Leaf sample of Event 142 procured from ICAR-National Institute for Plant Biotechnology, New Delhi was used as reference material for development and validation of GM diagnostics. Non-GM seeds of brinjal were purchased from the National Seeds Corporation Limited, New Delhi. For specificity experiments, commercialized *Bt* cotton events, namely MON531 and MON15985 samples from Mahyco. Event 1 from J. K. Agri Genetics Ltd (Hyderabad, India) and GFM from Nath Seeds (Aurangabad, India) were used. Lyophilized leaf of event EE1 was procured from Mahyco.

Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Hildon, Germany), according to the protocol provided with the kit, with minor modifications. DNA extracts were quantified using UV spectrophotometer (DU 650 Beckman, USA) and then diluted to a concentration of 20 ng/µl for further use. Table 1 provides details of primers used in the PCR/real-time PCR assays. Singleplex PCR targeting *cry1Fa1* gene was performed in 20 µl reaction mix containing 100 ng of template DNA, 1× polymerase buffer (10 mM *Tris*-HCl, pH 9.0, 50 mM KCl), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.5 units of *Taq* DNA polymerase (MBI Fermentas) and 0.2 µM each of forward and reverse primers. Amplification was carried out on a thermal cycler (PTC-200 Programmable Thermal Cycler; MJ Research, MA, USA) with the programme comprising initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 8 min. Hexaplex PCR was performed in 20 µl reaction with 100 ng of template DNA, 1× hot start buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTPs and 1 unit hot start polymerase (MBI Fermentas, USA). Primer concentration used for forward and reverse sets was 0.4 µM for *cry1Fa1*, 0.2 µM for *P-35S* promoter, 0.2 µM for *P-nos* promoter, 0.3 µM for *nptII* marker gene, 0.3 µM for *T-ocs* terminator and 0.25 µM for endogenous *β-fructosidase* gene. The thermal profile used was initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 50 sec, 59°C for 1 min, and at 72°C for 1 min, and final extension at 72°C for 8 min.

Sensitivity of *cry1Fa1* gene-specific PCR assay was determined using dilutions of Event 142 DNA prepared by mixing with the non-GM counterpart, having GM contents of 100%, 10%, 1.0%, 0.1%, 0.05% and 0.01%.

Construct-specific PCR was performed to specifically amplify the junction sequence of the 2.2 kb region of *P-35S* and *cry1Fa1* gene using the forward primer of promoter *P-35S* and reverse primer of gene *cry1Fa1*. PCR

Table 1. Primers employed in the present study

Target	Primer	Sequence (5′–3′)	Product size (bp)	Reference
Cry1Fa1	CryF-1/2	ACTTAGGGACGCTGTGTCGT GTCCTCCCCAGACAGTTTGA	449	Present study
NptII	Npt-F/R	GGGCGCCCGGTTCTTTTTG ACACCCAGCCGGCCACAGTCG	515	Present study
P-35S	35S-F/R	GAAGGTGGCTCCTACAAATGCC GTGGGATTGTGCGTCATCCC	199	18
P-nos	Pnos-F/R	ACAAGCCGTTTTACGTTTGG TTGGATACCGAGGGGAATTT	206	Present study
T-ocs	Tocs-F/R	TGCGAGACGCCTATGATCGCA ACCGAAACCGGCGGTAAGGA	102	Present study
β-fructosidase	PomtomF/R	CTGCCTCCGTCAAGATTTGGTCACT CTCTTCCCTTTCTTGATGG	141	19

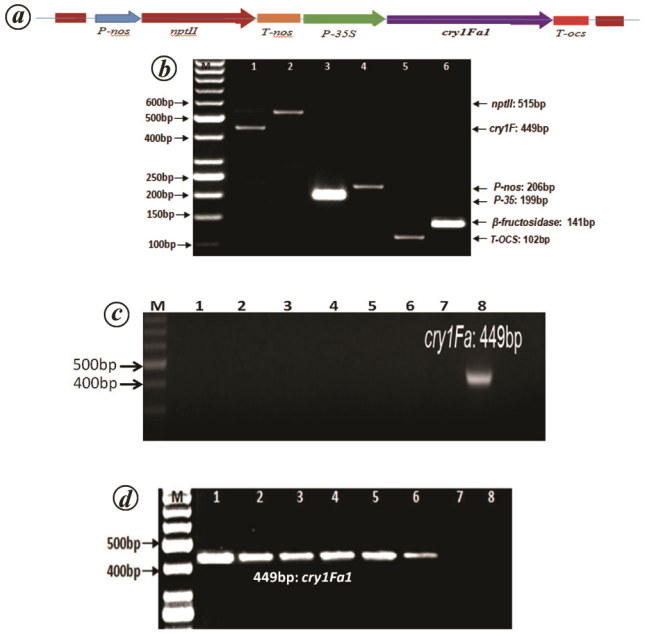

Figure 1. ***a***, Schematic representation of transgenic construct of *Bt* brinjal Event 142. ***b***, Singleplex PCR profile for *Bt* brinjal Event 142 targeting *P-35S* and *P-nos* promoters, *T-ocs* terminator, *cry1Fa1* transgene, *npt II* marker gene and endogenous *β-fructosidase* gene (lane M, 50 bp DNA ladder; and lanes 1–6, Sample of Event 142). ***c***, Specificity test for PCR assay targeting *cry1Fa1* gene (lane M, 100 bp ladder; lane 1, Non-template control; lane 2, MON531; lane 3, MON15985; lane 4, GFM; lane 5, Event 1; lane 6, *Bt* brinjal EE1 event; lane 7, Non-GM brinjal, and lane 8, *Bt* brinjal Event 142). ***d***, Amplification profile of sensitivity test for *cry1Fa1* gene-specific PCR assay (lane M, 50 bp ladder, lanes 1–6, Test samples of Event 142 with 100%, 10%, 1.0%, 0.1%, 0.05% and 0.01% GM content respectively; lane 7, Non-GM counterpart; and lane 8, Non-template control).

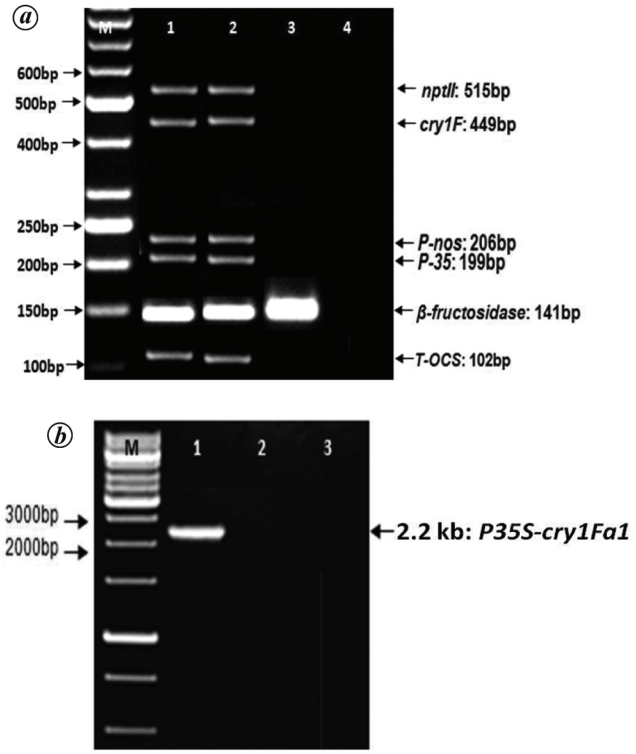

Figure 2. ***a***, Hexaplex PCR profile for detection of *Bt* brinjal Event 142 (lane M, 50 bp ladder; lanes 1–2, Sample of Event 142; lane 3, Non-GM brinjal and lane 4, Non-template control). ***b***, Amplification profile of construct-specific PCR targeting ~2.2 kb region of *P35S–cry1Fa1* (lane M, 1 kb ladder; lane 1, Sample of Event 142; lane 2, Non-GM brinjal, and lane 3, Non-template control).

was carried out in 25 µl reaction volume comprising 100 ng template DNA, 1× long-run PCR buffer, 2.0 mM $MgCl_2$, 200 µM dNTPs, 0.3 µM of each forward and reverse primers, and two units of PCR enzyme mix (MBI Fermentas, USA). The thermal profile used was initial denaturation at 95°C for 5 min followed by 10 cycles with 95°C for 20 sec, 59°C for 50 sec and 68°C for 3 min and then 30 cycles with 95°C for 20 sec, 59°C for 50 sec and 68°C for 3 min, with auto-extension at 2 sec/cycle. Final extension was performed at 68°C for 10 min.

Products of singleplex and construct-specific PCR were separated on 2.0% (w/v) agarose gel (Lonza, Rockland, ME, USA) using running buffer 1× TBE stained with ethidium bromide, visualized and photographed (using Gel Documentation Imaging System) (Alpha Innotech, USA). For hexaplex PCR, metaphor® agarose gel (Cambrex Bio Science Rockland, Inc., ME, USA) at the concentration of 4.0% (w/v) was used.

Real-time PCR targeting *cry1Fa1* gene was performed employing SYBR® Green chemistry on 7500 real-time PCR system (Applied Biosystems, CA, USA). The reaction

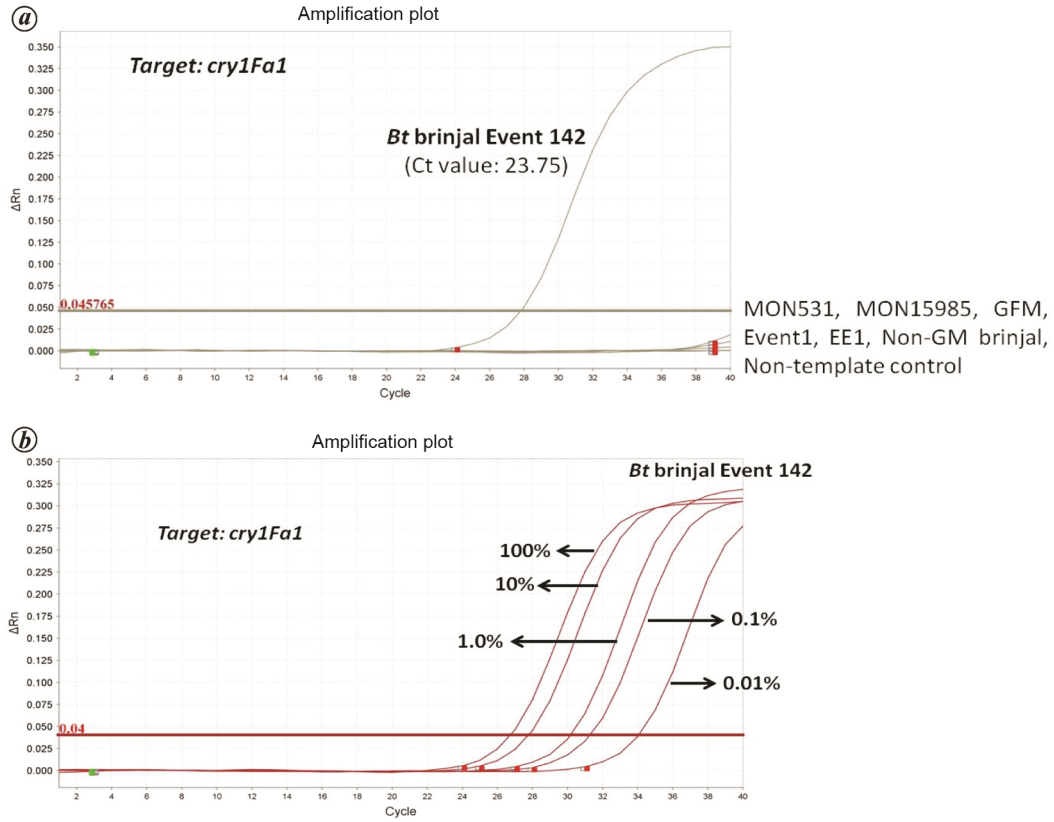

Figure 3. Amplification profile of SYBR® Green based real-time PCR assay for detection of *cry1Fa1* gene in Event 142: (***a***) specificity test and (***b***) sensitivity test.

was carried out in 20 µl volume containing 100 ng of template DNA, 1X QuantiFast SYBR® Green PCR master mix (Qiagen), and 0.1 µM each of forward and reverse primers of *cry1Fa1*. The thermal profile comprised of 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 step at 95°C for 15 sec and at annealing–extension step at 60°C for 1 min. After completion of amplification cycles, melting curve analysis was performed by heating to 95°C for 15 sec, then cooling at 60°C for 1 min followed by heating back to 95°C for 30 sec and 60°C for 15 sec.

Efficient GM diagnostics for GM crops/events approved for commercialization or for confined field trials in a country is required for regulatory compliance as well as post-release monitoring. PCR/real-time PCR-based GM detection assays were developed for approved events of *Bt* cotton in India[13,14]. GM detection assays were also developed for a range of GM crops under field trials such as *Bt* brinjal event EE1, *Bt* rice, *Bt* cauliflower and *Bt* okra[8,15–17].

Recently *Bt* brinjal Event 142 has been approved for BRL-II field trials. A DNA-based method for the detection of Event 142 is reported herein. Singleplex PCR assays for each transgenic element of Event 142 were first performed to confirm the amplifiability of each assay (Figure 1 *a* and *b*). Specificity of gene-specific PCR assay targeting *cry1Fa1* was confirmed using a set of seven test samples, namely Event 142, *Bt* brinjal Event EE1 with *cry1Ac* gene, non-GM brinjal and four approved *Bt* cotton events in the country, viz. MON531, MON15985, GFM and Event 1 and specific product targeting 449 bp region of *cry1Fa1* gene was amplified in the Event 142 sample whereas no amplification product was detected in the non-template control and other test samples, thereby confirming the specificity of the assay (Figure 1 *c*). Sensitivity of the developed gene-specific PCR assay was evaluated using dilutions of Event 142 DNA with different contents of GM target, i.e. 100%, 10%, 1.0%, 0.1%, 0.05% and 0.01%. The product of expected size (449 bp) was amplified in the test samples with GM content up to 0.01% (Figure 1 *d*). The results indicate that LOD of the PCR assay targeting *cry1Fa1* gene was up to 0.01%.

Multiplex PCR represents a cost- and time-efficient GM detection method, as in a single reaction the transgenic elements present in a GM event can be simultaneously detected. In the developed hexaplex PCR assay, products of expected size, i.e. 449 bp for *cry1Fa1* gene, 199 bp for *P-35S* and 206 bp for *P-nos* promoters, 515 bp for *npt II* marker gene, 102 bp for *T-ocs* terminator and 141 bp for *β-fructosidase* gene (endogenous gene) were detected in the sample of Event 142 (Figure 2 *a*). Product of 141 bp of

β-fructosidase gene was observed in a sample of non-GM brinjal as expected, being a housekeeping gene, whereas no amplified product was detected in the non-template control. The cost of singleplex PCR reaction detecting single target is approximately Rs 250. However, the cost of multiplex PCR reaction simultaneously detecting six targets in a run is approximately Rs 300. The cost calculation may vary from company to company and country to country.

Construct-specific PCR targeting junction of the promoter *P-35S* and gene *cry1Fa1* was also developed for more specific GMO screening. The long-run PCR targeting construct region produced approximately 2.2 kb-sized product in the sample of Event 142 and no amplified product was detected in the sample of non-GM brinjal and non-template control (Figure 2 *b*).

Real-time PCR-based GM detection assay was also developed for *cry1Fa1* gene. Specificity of the assay was evaluated and confirmed emploting seven test samples as used for PCR. Amplification was detected in the *Bt* brinjal Event 142 sample, whereas no amplified product was detected in rest of samples and non-template control (Figure 3 *a*). Sensitivity of the developed gene-specific real-time PCR was assessed using dilutions of DNA of Event 142 with the non-GM counterpart having 100%, 10%, 1.0%, 0.1% and 0.01% GM content. LOD of assay was established up to 0.01%.

Both the PCR and real-time PCR assays targeting *cry1Fa1* gene in Event 142 showed acceptable specificity and sensitivity with LOD up to 0.01%. Moreover, hexaplex assay facilitates screening for Event 142 and construct-specific PCR allows more specific detection of Event 142. The reported assays could facilitate efficient detection for checking adventitious presence/unintentional introgression of transgenes in the samples/seeds of brinjal.

Conflict of interest: The authors declare that they have no conflict of interest.

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ACKNOWLEDGEMENTS. We thank the Indian Council of Agricultural Research (ICAR), New Delhi and the Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for support, and the Department of Biotechnology, Government of India for funds. We also thank Dr P. Ananda Kumar (former Director, ICAR-National Institute for Plant Biotechnology, New Delhi) for a critical review of the manuscript.

Received 7 January 2021; revised accepted 14 November 2021

doi: 10.18520/cs/v122/i4/464-468