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SHORT COMMUNICATION

Detection Method for Tracking Unauthorized Genetically Modified Organisms (GMOs) in Selected Fruit and Vegetable Crops

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Abstract

A detection method for checking unauthorized GMOs in fruit and vegetable crops grown in the North-Western Himalayan (NWH) Region was reported. Using common transgenic elements identified with GMO matrix of apple, capsicum, plum, potato, and tomato, multiplex PCR targeting *CaMV 35S* promoter, *nos* terminator, *nptII* marker gene and *ctp2-cp4epsps* gene, was developed.

Keywords: Fruit crops, GM detection, Multiplex PCR, NWH Region, Vegetable crops

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Introduction

The cultivation of fruit and vegetable crops plays a pivotal role in the global agricultural industry, contributing to food and nutritional security, and the economic revenue in many countries. The plant genetic resources (PGR) of a country, their conservation and maintaining genetic purity play a crucial role in sustainable agriculture and food security (Srivastava *et a*l., 2024). Temperate fruits and vegetables refer to those adapted to the temperate zone climates including that of the North-Western Himalayan (NWH) Region. Some of the examples grown in the NWH Region include apple, plum, pear, peach, grapes, strawberry, potato, tomato, cauliflower, and capsicum. The advent of genetically modified (GM) crops has revolutionized agriculture through increased yields, tolerance to biotic and abiotic stresses and nutritional enhancement. While reaping the benefits of GM crops with desirable traits, it is crucial to safeguard genetic diversity (Randhawa, 2016). Moreover, the acceptability of these GM crops varies widely across the nations due to differing regulatory frameworks (Singh *et al*., 2023a). For instance, except for *Bt* cotton, no GM food crop has been approved in India (Singh and Aminedi, 2024). Stringent regulation governing GM fruits and vegetables in our country following extensive global trade underscores the critical requirement for reliable GM detection in order to track unauthorized GMOs in the supply chain. In the NWH Region, known for its unique biodiversity and cultivation of crops of temperate nature, the implementation of robust GM detection mechanisms is crucial for promoting trade compliance, and preserving ecological balance.

Polymerase chain reaction (PCR) and real-time PCR are widely used methods for GM detection. Common transgenic elements, such *P-35S* and *T-nos*, are the preferred targets for GMO screening (Lipp *et al*., 1999; Weiblinger *et al*., 2008). However, these two transgenic elements are not sufficient to maximally screen GMOs in all crops. In India, gene-specific PCR assays in singleplex format were reported for the detection of *cry1Ac* in GM cotton, *cp4epsps* in maize and soybean, *barnase* and *barstar* in mustard (Firke and Randhawa, 2005). Further, a crop-wise GMO matrix approach was used to choose more appropriate targets for GM detection, and add more screening targets related to approved GM events of a range of GM crops, which may improve the GM coverage (Singh *et al*., 2016; Singh *et al*., 2023b).

In view of the stringent regulation of food crops in the country, we herein report on the multiplex PCR method developed for checking the GM status of selected fruit and vegetable crops grown in the NWH Region. The information of the GMO matrix of globally approved GM events of apple, capsicum, plum, potato and tomato (Singh *et al*., 2023b) was updated using databases, http://bch.cbd.int/database/organisms and http://www.isaaa.org/gmapprovaldatabase/ for selection of common transgenic elements in these crops. The identified screening targets were *CaMV 35S* promoter (*P-35S*), *nos* terminator (*T-nos*), *nptII* marker gene and *ctp2-cp4epsps* gene. Among a total of 149 GM events of these crops, *P-35S*, *T-nos* and *nptII* altogether could screen for the presence or absence of all the GM events of apple, capsicum, plum, and tomato and all the four elements (*P-35S*, *T-nos, nptII, ctp2-cp4epsps*) could cover the screening of 87% of GM potato events.

Seeds of GM cotton (positive control) and non-GM seeds/tissue (apple, plum, tomato, capsicum, potato) were ground to fine powder. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany)

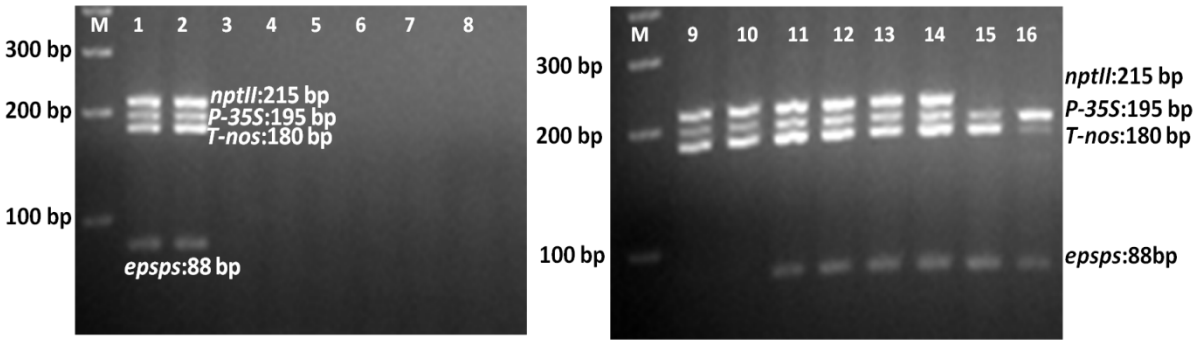

Fig. 1: Confirmation of specificity of multiplex PCR targeting *P-35S*, *T-nos*, *nptII* and *ctp2-cp4epsps*. M: 100 bp DNA ladder, 1, 11: Bollgard®III Roundup® Ready Flex, 2, 12: *Bt* Roundup® Ready Flex, 3: Non-GM apple, 4: Non-GM plum, 5: Non-GM tomato, 6: Non-GM capsicum, 7: Non-GM potato, 8: Negative control (Non-template control), 9: Bollgard®I, 10: Bollgard®II, 13: Roundup® Ready cotton, 14: Roundup® Ready Flex cotton, 15: Roundup® Ready II maize, 16: Roundup® ready Soybean

Table 1: Test material used for specificity studies along with the distribution of selected transgenic elements in these events/ crops

Test Sample		*Targets (Transgenic element tested)*			
Event Name	*Trade Name*	*P-35S*	*T-nos*	*nptII*	*ctp2-cp4epsps*
MON531	Bollgard®I Cotton	+	+	+	-
MON15985	Bollgard®II Cotton	+	+	+	-
MON15985 x MON88913 x Cot102	Bollgard®III Roundup® Ready Flex Cotton	+	+	+	+
MON15985 x MON88913	*Bt* Roundup® Ready Flex Cotton	+	+	+	+
MON1445	Roundup® Ready Cotton	+	+	+	+
MON88913	Roundup® Ready Flex Cotton	+	+	+	+
NK603	Roundup® Ready II Maize	+	+	-	+
40-3-2	Roundup® Ready Soybean	+	+	-	+
Non-GM Apple	--	-	-	-	-
Non-GM Capsicum	--	-	-	-	-
Non-GM Plum	--	-	-	-	-
Non-GM Potato	--	-	-	-	-
Non-GM Tomato	--	-	-	-	-

(+) present, (-) absent

protocol. Multiplex (tetraplex) PCR was performed in thermal cycler (Analytik Jena) in 20 µL volume containing 5 µL of 20 ng/µL of genomic DNA, 1x Muliplex PCR master mix (Qiagen), 0.25 µM each of forward and reverse primers for *P-35S* (Lipp *et al*., 1999), *nptII* (ISO21569:1–69, 2005), *ctp2-cp4epsps* (Grohmann *et al*., 2009) and 0.5 µM for each of the forward and reverse primers for *T-nos* (Lipp *et al*., 1999). PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, and 40 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 50 seconds, and extension at 72°C for 1-minute; and a final extension at 72°C for 7 minutes. The PCR products were analyzed on 4% (w/v) metaphor agarose gels in 1x TAE buffer, and visualized using UV Gel Imaging System (Alpha Innotech, USA).

This tetraplex PCR exhibited acceptable specificity across the range of target and non-target analyzed. Amplification for four transgenic elements, *i.e*., 195 bp for *P-35S,* 180 bp for *T-nos*, 215 bp for *nptII* and 88 bp for *ctp2-cp4epsps* gene was detected in the DNA samples of respective targets (Table 1, Fig. 1) whereas no amplification was detected in respective non-targets and negative control.

Conclusion

The developed multiplex PCR method can serve as a guide to monitor unauthorized GM entries in food crops of the NWH Region as a precautionary approach, thereby safeguarding biodiversity and fostering sustainable agriculture. This study provides valuable insights for researchers, stakeholders, policymakers, and regulatory bodies, aiding in the enforcement of GM crop regulations in the NWH region.

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