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Event-specific analytical methods for six genetically modified maize events using visual and real-time loop-mediated isothermal amplification

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ABSTRACT

Currently 138 genetically modified (GM) maize events have been authorized for commercial cultivation, comprising more than 65 per cent stacked events. With the increase in number of GM maize events globally, cost- and time-efficient diagnostics with on-site applicability are required to check for authorized GM events. Six GM maize events, namely, *Bt*11, GA21, MON810, MON89034, NK603 and TC1507, also present in 89 stacked events, are being widely commercialized in more than 17 countries. Visual and real-time loop-mediated isothermal amplification (LAMP) assays targeting these six GM maize events are being reported in the present study. Specificity of the developed LAMP assays was confirmed using fourteen commercialized GM maize events. Limit of detection of visual and real-time LAMP assays targeting *Bt*11, GA21, MON810, MON89034 and TC1507, was up to 0.01%, detecting 8 target copies, and for NK603 event-specific assays, was up to 0.1% detecting 73 target copies. Practical applicability of developed LAMP assays was verified using a set of five stacked GM maize events, namely, *Bt*11 × GA21, MON89034 × NK603, MON89034 × NK603 × TC1507, TC1507 × NK603 and TC1507 × MON810; and six powdered maize samples of proficiency testing. The reported LAMP assays can be efficiently employed for screening for presence of selected GM maize events in single or stacked form.

1. Introduction

Maize (*Zea mays* L.) is an important food and feed crop in most of the countries. Genetically modified (GM) maize is being commercially cultivated in an area of 57.3 million hectares, comprising more than 30 per cent of total area under GM cultivation (James, 2013). GM maize has highest number of events, *i.e.*, 138 events followed by cotton, soybean and canola. GM maize for diversified traits such as insect resistance, herbicide tolerance, enhanced product quality, abiotic stress tolerance and male sterility or/and fertility restoration system, have been developed (http://www.isaaa.org/gmapprovaldatabase/). For better performance for imparting insect resistance and herbicide tolerance, more than 70 per cent of the commercial GM maize events are stacked (http://www.isaaa.org/gmapprovaldatabase/). In India, more than 70 imports of GM maize for research purposes, the highest number has been made through the Indian Council of Agricultural Research - National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, the designated nodal agency to issue import permits and undertake quarantine processing of transgenics as per the Government of India Notification No. GSR 1067 (E) dated 05.12.1989 and Plant Quarantine (Regulation of Import into India) Order, 2003. Molecular testing of imported GM material is being undertaken on regular basis by GM detection laboratory at NBPGR. Increase in number and complexity of GM events necessitates the development of efficient and rapid detection assays (Randhawa, Morisset, Singh, & Žel, 2014; Randhawa, Singh, Sood, & Bhoge, 2014).

Polymerase chain reaction (PCR) and real-time PCR are being commonly employed for detection of GM maize events with higher sensitivity and specificity (Akiyama et al., 2012; Fernandes, Amarala, Oliveira, & Mafraa, 2014; Holst-Jensen et al., 2012; Mano et al., 2011,

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Table 1
GM events used for testing specificity and practical utility of LAMP assays.

Event	*Bt*11	GA21	MON810	MON89034	NK603	TC1507
For specificity testing						
*Bt*11	x					
*Bt*176						
GA21		x				
MIR162						
MIR604						
MON810			x			
MON863						
MON88017						
MON89034				x		
NK603					x	
T25						
TC1507						x
For testing practical utility						
*Bt*11 × GA21	x	x				
MON89034 × NK603				x	x	
MON89034 × NK603 × TC1507				x	x	x
TC1507 × NK603					x	x
TC1507 × MON810			x			x

(x) shows the presence of event-specific target(s) in particular single/stacked GM maize event (s), as verified experimentally.

2013; Kim, Zhang, & Kim, 2014; Noguchi et al., 2014; Oguchi et al., 2010; Shin et al., 2013). Quantitative real-time PCR assays for nineteen GM maize events are available at http://gmo-crl.jrc.ec.europa.eu/gmomethods/. Multitarget real-time PCR-based system has been reported for detection of GM maize events (Cottenet, Blancpain, Sonnard, & Chuah, 2013; Kluga, Folloni, Van den Bulcke, Van den Eede, & Querci, 2012; Querci et al., 2009; Randhawa, Singh, et al., 2014). Loop-mediated isothermal amplification (LAMP), an isothermal nucleic acid amplification technique developed by Notomi et al. (2000), is also being employed in GM detection due to its efficiency and on-site applicability. In LAMP assays, target DNA is amplified under isothermal conditions using DNA polymerase, such as *Bacillus stearothermophilus* (*Bst*) polymerase with strand displacement activity; and can be on-site performed using portable systems such as conventional heating block and isothermal real-time system.

Table 2
Primers employed in the study.

Target	Primer code	Primer	Amplicon size (bp)
MON89034	MON89034-F3	CATGCTACACTGCCTACAC	255
	MON89034-B3	GCACCTGAATTTGAATGGC	
	MON89034-FIP	GTACTCGTGCTCACGTCGTAGATTATTATTGGAACATGAGGCT	
	MON89034-BIP	ATCCGCCCTCTCTGTCCCTGCTGTTTCATCTGACAACA	
	MON89034-LoopF	CCGTGTGGGAGGAGAAAT	
	MON89034-LoopB	CTGCTACTCCAGCCACTG	
NK603	NK603-F3	AACAGATCAGCATCAGCG	246
	NK603-B3	ATGAATGACCTCGAGTAAGC	
	NK603-FIP	AGGAGGAGTCTGGCCGAATTCGAAAGTTTCGTCAAAGGA	
	NK603-BIP	TCTCTGGCATTTCCAACCCTCCGCTACAAGGCTTGC	
	NK603-LoopF	GGCGGCTGGAAACAGT	
	NK603-LoopB	AGAGACGTGCGTCCCT	
GA21	GA21-F3	TCGAAGCGGACAAAGC	267
	GA21-B3	TCACTTTGGGCCACCT	
	GA21-FIP	TCTAGAGCTGCACTTCCTCTGCTGTAGTTGTTGGCTGT	
	GA21-BIP	CCAGCTTGCATGCCTGCTTGGACTATCCCGACTCTC	
	GA21-LoopF	GCATCCTCAACTGGGAACT	
	GA21-LoopB	GGTCGAGGTCATTCATATGC	
*Bt*11	*Bt*11-F3	GCCTCGTGATACGCCTA	293
	*Bt*11-B3	TGTACGAGCCTCTGGTC	
	*Bt*11-FIP	TCCCTCCATGAGCGGATACAACGTCAGGTGGCACTT	
	*Bt*11-BIP	TGGTGGAGACCATTTCTTGGTAGCCATGAGCGACCAT	
	*Bt*11-LoopF	GTTCCGCGCACATTTCC	
	*Bt*11-LoopB	ATCTGTAGGTGTTAGCCTCT	
TC1507	TC1507-F3	CCTCTAGAGTCGACCTGC	193
	TC1507-B3	ACTGCACTGCAAATGAA	
	TC1507-FIP	GCCGAAGACTACTGAGTGTCCGCCTCTAGTTGAAGACAC	
	TC1507-BIP	CAGAATGGCCTAACTCAAGGTGCGTCAAATATCTTTGCCA	
	TC1507-LoopF	CTTACGATGAAGACATGAAC	
	TC1507-LoopB	CCTCACTCCGCTTGATCT	
MON810	MON810-F3	GGTACTGGTTCCCTCTGG	263
	MON810-B3	GGTGGTCTTACATCTAAGAAGG	
	MON810-FIP	TCGGAAATGAAAGAAGGCTACCCACTTCTCCTTGGACATCG	
	MON810-BIP	GCTTGCGAGCAGTCAGGTAAGCACTAGTACTGCCAA	
	MON810-LoopF	AAGTCCTCGTTCAGGTCG	
	MON810-LoopB	TTTGATTCATCTGAGTTTGGCT	