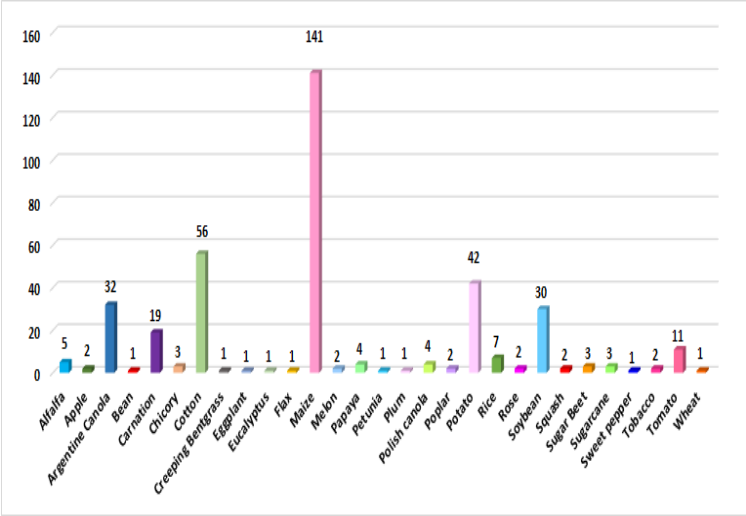
DNA-based Diagnostics for Genetically Modified Maize Events

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7th Indo-Global Summit and Expo on Food & Beverages

Scenario of GM Maize

- Genetically Modified (GM) maize has **highest number of globally approved events, i.e., 141 GM events** and its adoption would play a role in nutritional security and increasing crop productivity



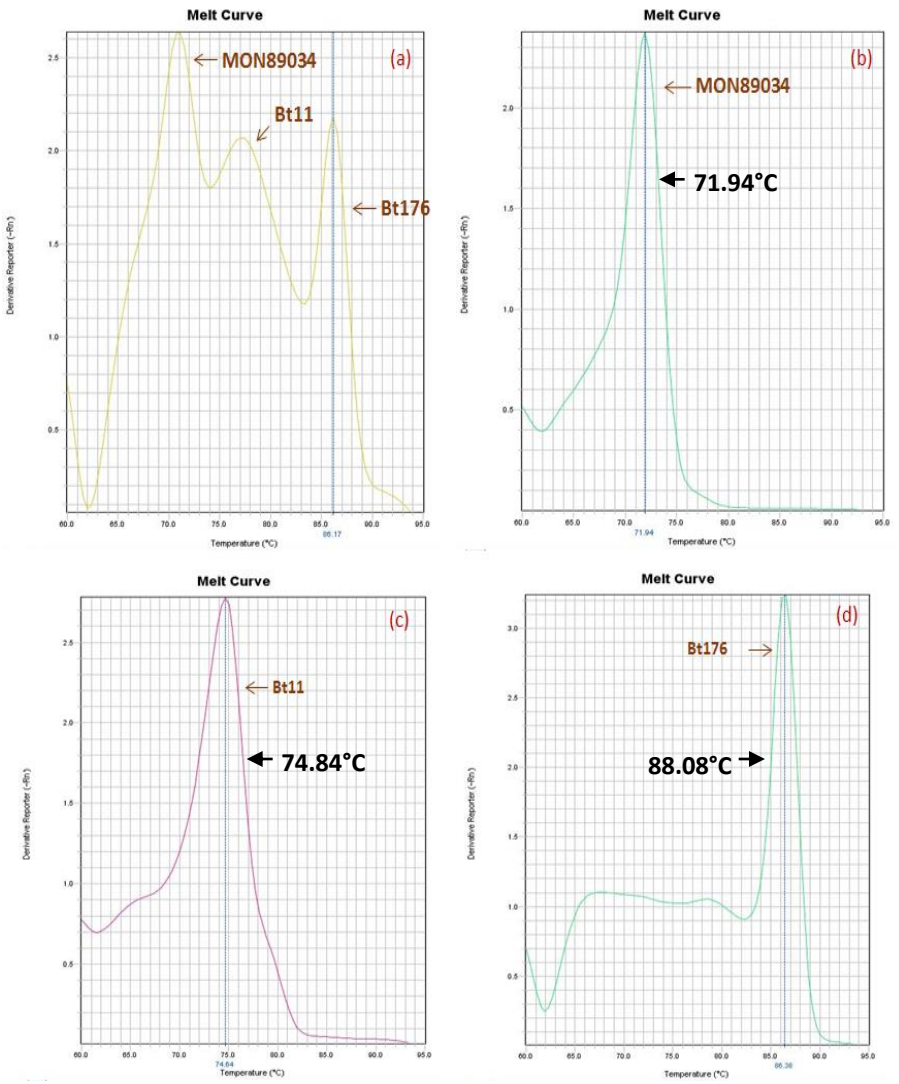
- In India, **more than 35 percent of imports for research purposes are of GM maize**, predominantly for insect resistance or/and herbicide tolerance. Several other GM maize events have been **under field trials at Biosafety Research levels I and II** since 2006
- Maize is **highly cross-pollinated** crop, so there may be **chances of introgression of transgenes into the wild or weedy relatives**

What is a need of GM Detection???

- To fulfill the legislation along with **biosafety issues relating with the GM crops**
- approval and introduction of GM crops and GM-derived food/feed products in supply chain are regulated in different countries by respective regulatory frameworks
- Several countries have implemented **labelling thresholds for unintentional presence of approved GM crops** defined as 0.9% in the European Union (EU) and Russia, 3% in Korea, 5% in Japan, Indonesia, Thailand and Taiwan, and 1% in Brazil (Guere and Rao 2007)
- So far**, no labelling threshold has been implemented in India**. efficient detection strategies would assist to check the unauthorized GM events and to address consumers' choice for opting GM or GM-free food

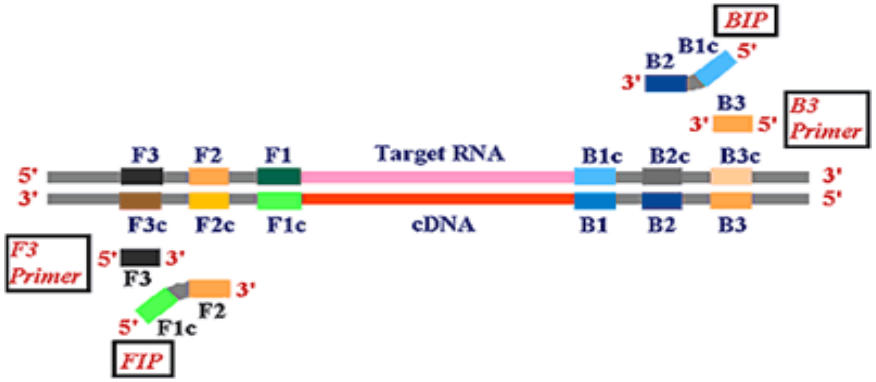
Event-specific SYBR® Green I based multiplex real-time PCR assays for maize event *Bt*11, *Bt*176 and MON89034

- high throughput testing and minimum risk of contamination due to elimination of post-PCR analysis
- saving time and resources
- In multiplex real-time PCR using SYBR® Green I chemistry, differentiation of events can be done based on melting curve analysis
- These assays could be utilized for screening of GM events, which can be further quantified using singleplex or multiplex TaqMan® real-time PCR assay



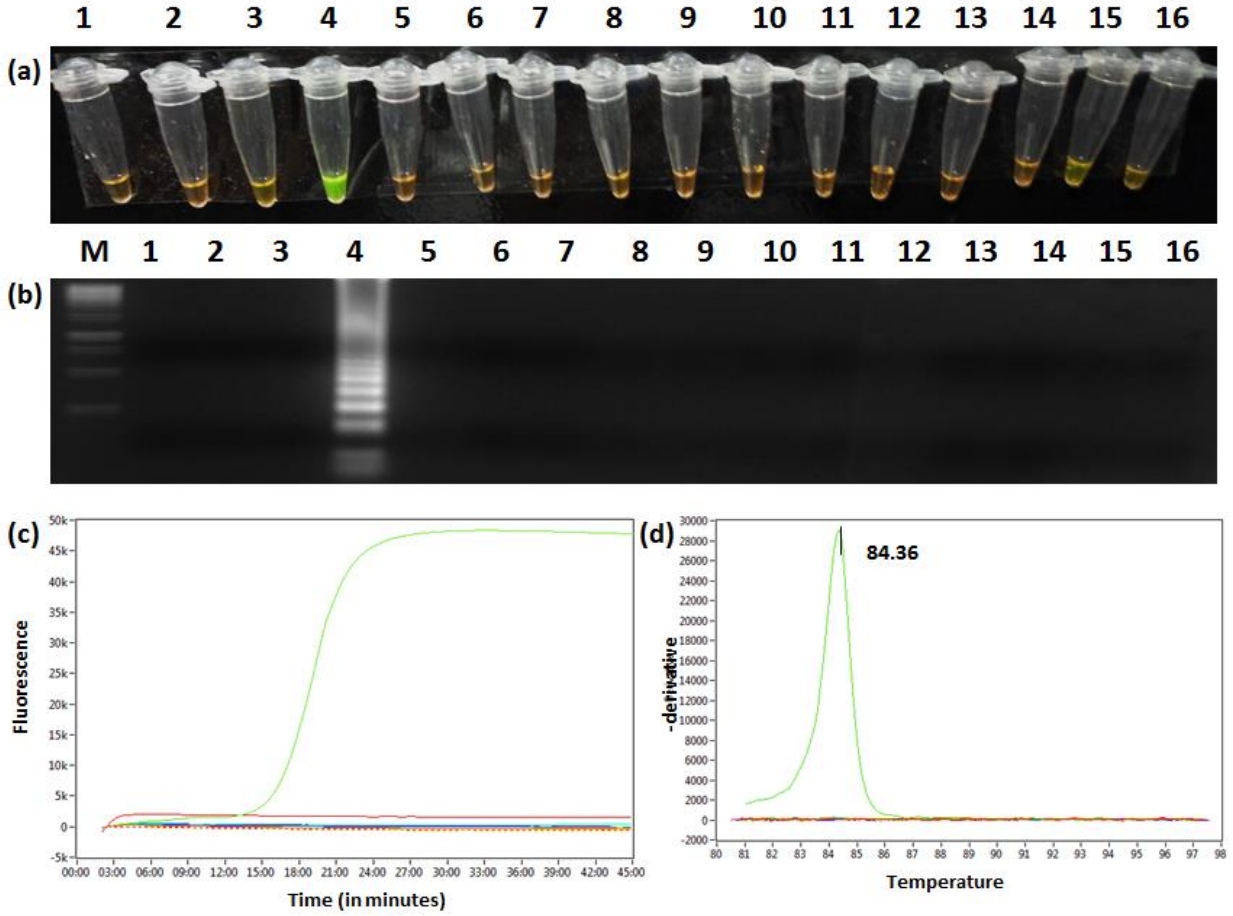
Loop Mediated Isothermal Amplification (LAMP)

- It is a nucleic acid isothermal amplification technique, which can detect the product with high specificity, efficiency and rapidity
- This technique has been widely employed for pathogen detection; nowadays, LAMP is also gaining popularity for GM detection due to its specificity and sensitivity
- This method is performed with *Bst* polymerase (derived from *Bacillus stearothermophilus*) and a set of four primer (two outer and two inner), which recognize six distinct regions on the target DNA
- An advantage of LAMP is that it can amplify the DNA isothermally (60-65^{o}C)



EVENT-SPECIFIC LAMP FOR DETECTION OF GM MAIZE EVENT BT11, GA21, MON810, MON89034, NK603 AND TC1507

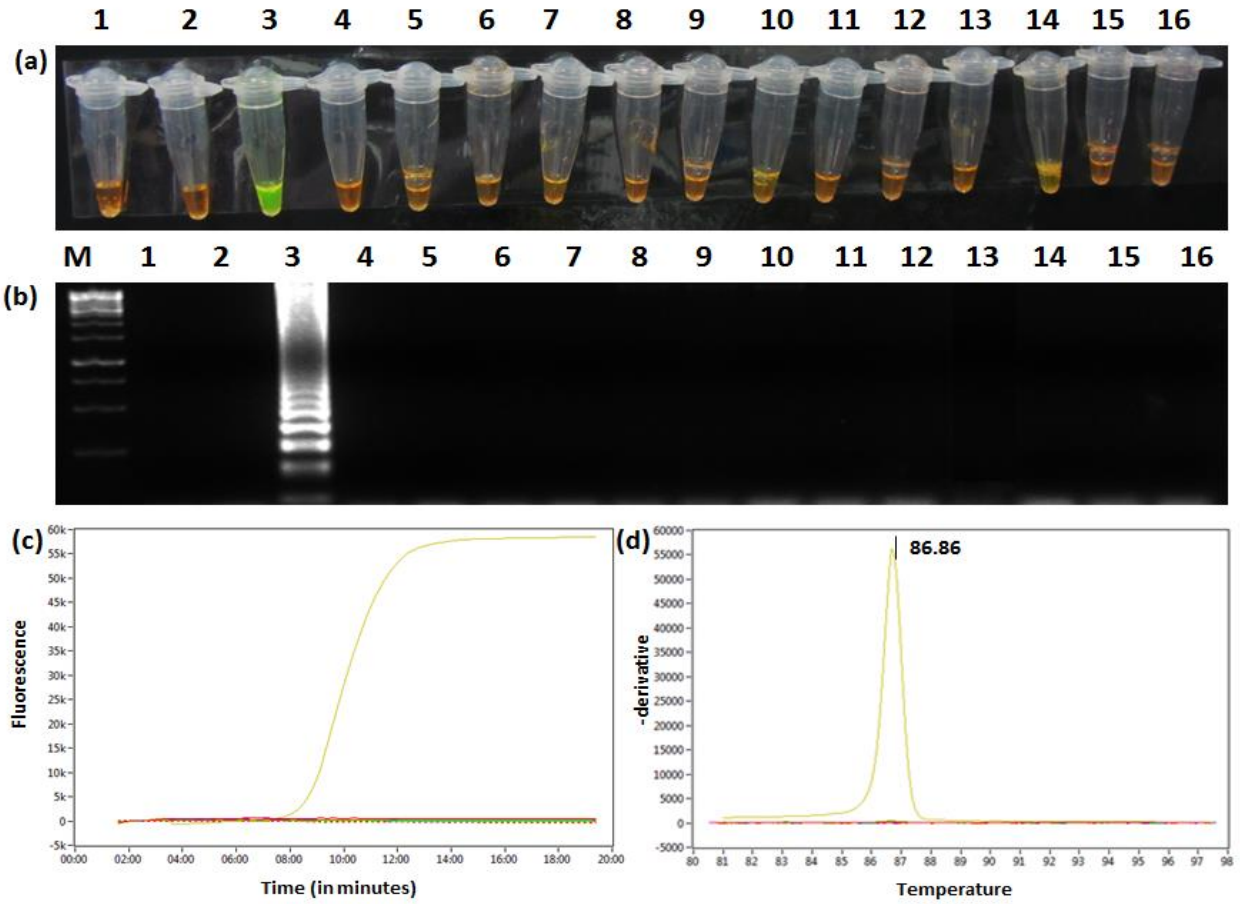
Event-specific LAMP for detection of GM maize event *Bt*11



(a) Visual event specific *Bt*11 LAMP assay, (b) Electrophoretic event specific *Bt*11 LAMP assay, (c) Real-time based LAMP assay for *Bt*11, (d) Melt curve of real-time based LAMP assay for *Bt*11
Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.01%

Event-specific LAMP for detection of GM maize event GA21

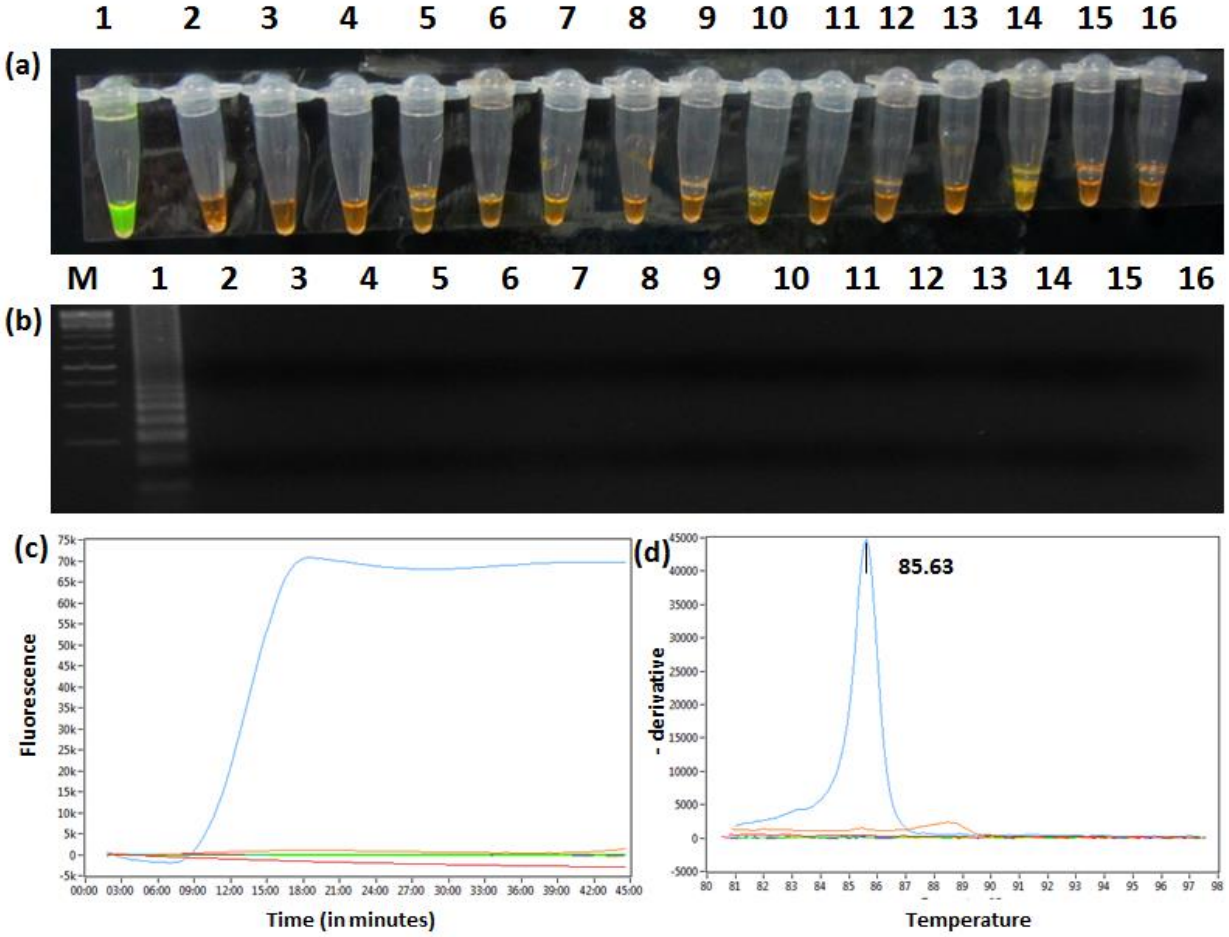


(a) Visual event specific GA21 LAMP assay, (b) Electrophoretic event specific GA21 LAMP assay, (c) Real-time based LAMP assay for GA21, (d) Melt curve of real-time based LAMP assay for GA21

Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.01%

Event-specific LAMP for detection of GM maize event MON810

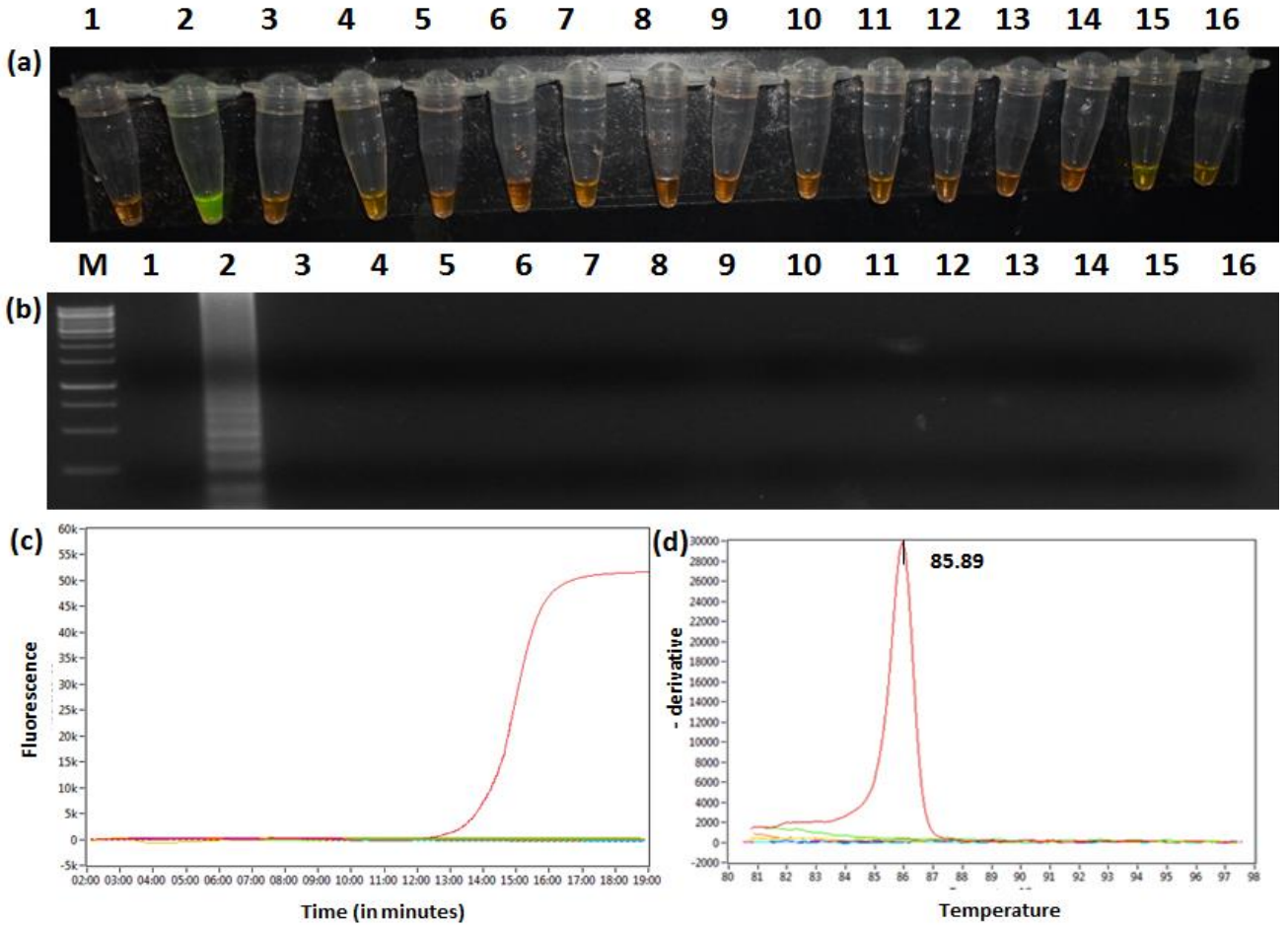


(a) Visual event specific MON810 LAMP assay, (b) Electrophoretic event specific MON810 LAMP assay, (c) Real-time based LAMP assay for MON810, (d) Melt curve of real-time based LAMP assay for MON810

Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.01%

Event-specific LAMP for detection of GM maize event MON89034

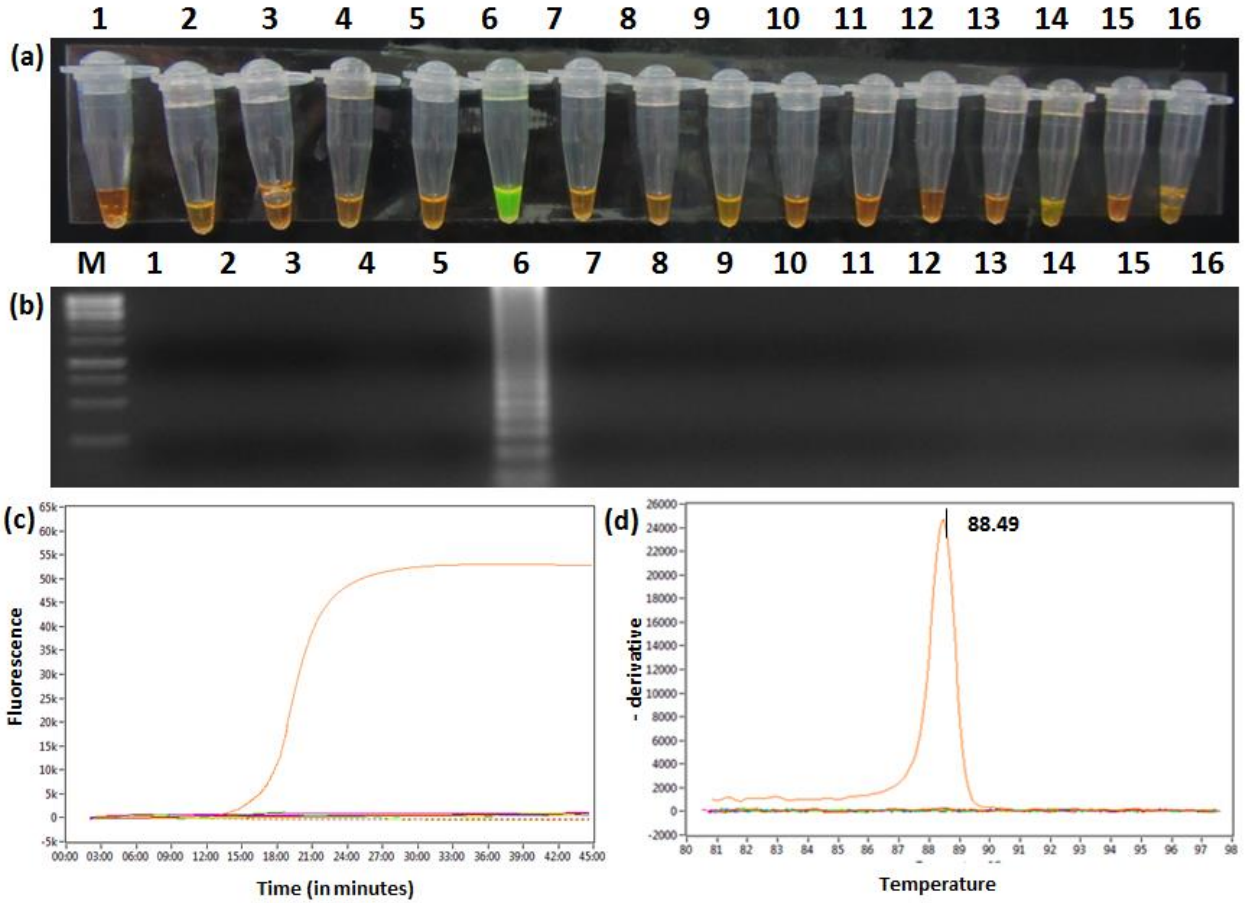


(a) Visual event specific MON89034 LAMP assay, (b) Electrophoretic event specific MON89034 LAMP assay, (c) Real-time based LAMP assay for MON89034, (d) Melt curve of real-time based LAMP assay for MON89034

Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.01%

Event-specific LAMP for detection of GM maize event NK603

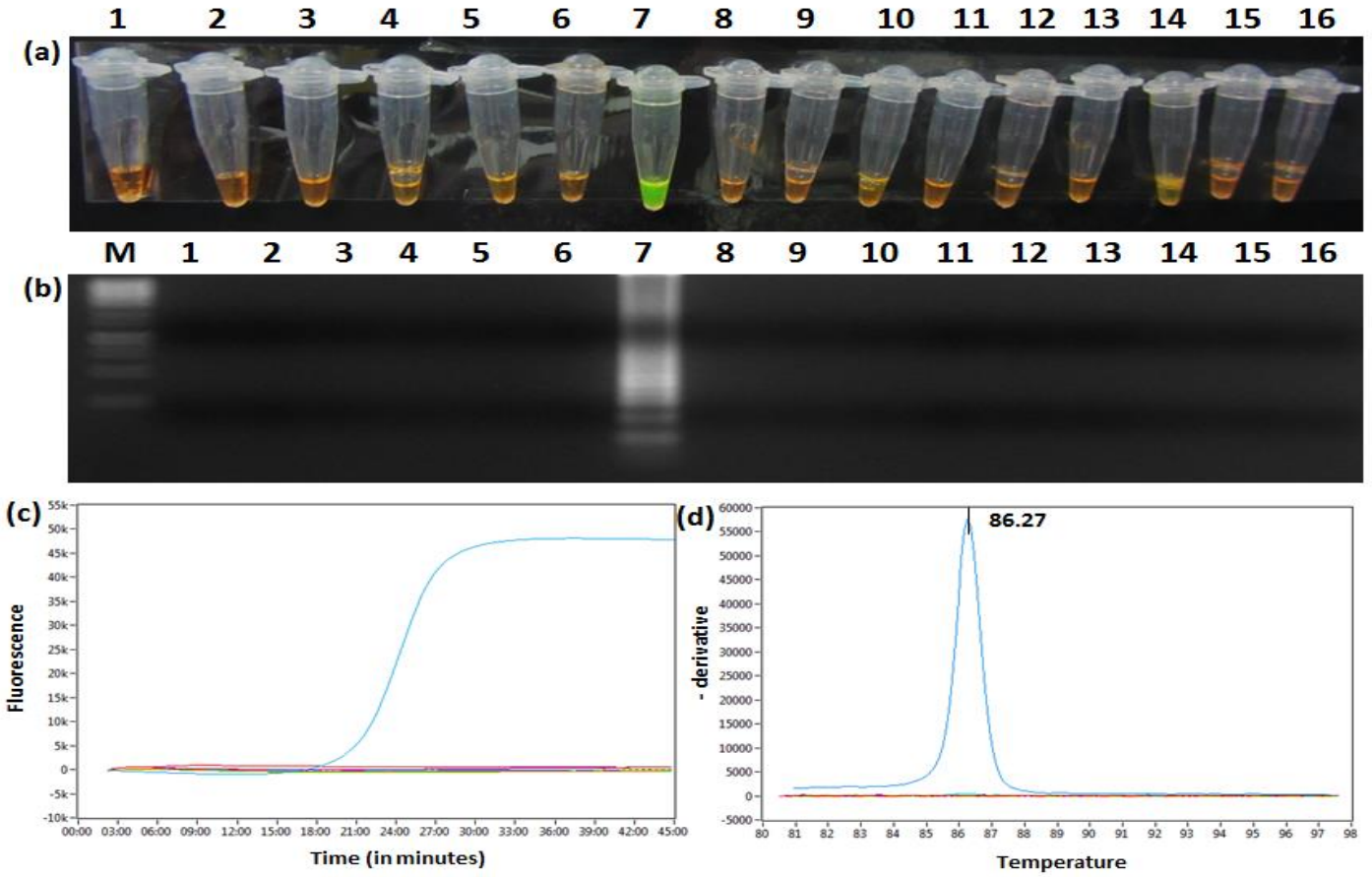


(a) Visual event specific NK603 LAMP assay, (b) Electrophoretic event specific NK603 LAMP assay, (c) Real-time based LAMP assay for NK603, (d) Melt curve of real-time based LAMP assay for NK603

Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.1%

Event-specific LAMP for detection of GM maize event TC1507



(a) Visual event specific TC1507 LAMP assay, (b) Electrophoretic event specific TC1507 LAMP assay, (c) Real-time based LAMP assay for TC1507, (d) Melt curve of real-time based LAMP assay for TC1507

Lane M: 1 kb ladder, Lane 1-16: MON810, MON89034, TC1507, *Bt*11, *Bt*176, NK603, GA21, 59122, MON863, 3272, MIR604, MON88017, T25, MIR162, non-GM maize and non-template control.

Limit of detection of developed LAMP assay is 0.01%

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

Event	Bt11	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 practical utility						
*Bt*11 x GA21	x	x				
MON89034 x NK603				x	x	
MON89034 x NK603 x TC1507				x	x	x
TC1507 x NK603					x	x
TC1507 x MON810			x			x

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

Conclusion

- These assay would assist in systematic risk assessment and post release monitoring of GM crops
- These assay would be benefiting the stakeholder, to ensure the public confidence on GM status of crops
- Developed LAMP assay useful for event specific detection and differentiation of GM maize events
- The developed assays, when combined with a fast DNA Extraction method, will facilitate on-site detection to check the GM status of a sample or product at ports of entry and in farmers‘ fields.


Thank you!