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Genomics Approach for Detection of Genetically Modified Organisms in Agriculture

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Abstract: ***Upcoming challenges in Genetically Modified (GM) plants through Loop mediated isothermal amplification (LAMP) assay have been studied in food and feed. The agricultural crops well diagnosed through this technology are plant species of rice, banana, potato, wheat, mustard, eggplant, chickpea, pea, melons, flax, beans, tomato etc. Application of LAMP assays were made for rapid visual detection of cry2Ab and cry 3A genes in GMO crops. The development was applied for detection of nptII and GUS genes in Nicotiana tabacum, GM potato EH92 - 527 - 1 and single copy detection of 35Sp and N0St in transgenic maize using Bioluminescent Assay in Real Time. Genomics Approach for detection of Genetically Modified Organisms in Agriculture was studied and discussed through LAMP assays.***

Keywords: Genomic, LAMP assay, GMO crops

1. Introduction

Loop mediated isothermal amplification (LAMP) assay has gained a significant interest in research across the world in Agriculture. This technology is considered an established method for amplifying nucleic acid and also for diagnosis of field crop diseases. Due to its cost effectiveness, LAMP method is considered to be the fast and precise method in DNA amplification. This technology was invented in 1998 by Eiken Chemical Company in Tokyo. In LAMP target sequence is amplified at constant temperature of 60 - 65°C and there is no need for the use of thermal cycler. Target DNA sequence is amplified using either two or three set of primers along with polymerase like Bst Klenow fragment with high strand displacement activity in addition to a replication activity. Generally 4 different primers are used to amplify 6 distinct regions on the target gene of interest. DNA amplification through LAMP can also be detected using photometry by measuring turbidity which is caused by magnesium pyrophosphate. It precipitates in solution as a by product of amplification that allows easy visualization by the naked eye or simple photometric detection. Certain dyes recommended for LAMP that induce a visible colour change are SYBR green which allows visible colour change that can be seen with a naked eye. This occurs as dye molecule intercalates or directly labels the DNA. Other methods for visualization are by detection of the amplicon by the unaided eye based on their ability to hybridize with complementary gold nanoparticles bound ssDNA.

Designing of LAMP primer

Designing of primer is specific which is accompanied by using Primer Explorer which is a software program. Generally, there are six primers comprising two outer, two inner and two loop primers that recognise eight distinct regions of the target sequence. Two outer primers are described as forward outer primer (F3) and backward outer primer (B3). These play a role in strand displacement during the non - cyclic step. Internal primers are described as forward internal primer (FIP) and backward internal primer (BIP). Sense and antisense sequence in internal primer result in loop formation. Notomi et al., (2000) described forward internal primer (FIP) and backward internal primer (BIP) which are composed of sequence that are complementary to the sequence between F1 and F2 and B1 and B2 region, respectively. Designing of primer is done in such a way that it matches with complementary regions of the target site. LAMP offers excellent combination of sensitivity and specificity. There is thus no requirement for thermocycler, electrophoresis equipment and a UV trans - illuminator.

Principle of LAMP amplification

Ushikubo et al., (2004) described chemistry for LAMP amplification which is based on autocyclic strand displacement reaction when performed at a constant temperature using a DNA polymerase. There are two steps of amplification comprising of cyclic and non - cyclic steps. Non cyclic step results in formation of DNA with stem loops at each end that serve as a starting point for the amplification by LAMP cycling. One of primers in LAMP anneal to the complementary sequence of double stranded target DNA that initiates DNA synthesis by using DNA polymerase with strand displacement activity by displacing and releasing a single stranded DNA. Starting from the 3' end of F2 region of Forward internal primer (FIP). The F3 primer will anneal to the F3c region outside of FIP on the target DNA and will initiate strand displacement of DNA synthesis releasing FIP linked complimentary strand. In this way, double strand is formed from the DNA strand synthesized from the F3 primer and the template DNA strand. This forms a stem loop structure at the 5' end due to complementary FIc and F1 regions. Notomi et al., (2000) described design of six types of primers which are based on the following eight distinct regions of the target gene. The F3c, F2c, F1c and FLP regions at the 3' side and the B1, B2, B3 and BLP regions at the 5' side. FIP consists of the F2 region (at the 3' end) that is complementary to the F2c region, and the same sequence as the F1c region at the 5' end. Forward outer primer (F3) consists of the F3 region that is complementary to the F3c region. BIP consists of the B2 region (at the 3' end) that is complementary to the B2c region, and the same sequence as the B1c region at the 5' end. Backward outer primer (B3) consists of the B3 region that is complementary to the B3c region. FIP consists of a complementary sequence of F1 and a sense sequence of F2. BIP consists of a complementary sequence of B1 and a sense sequence of B2. Mori et al.,

(2004) measured turbidity in form of OD at 400nm and it was found that DNA yield was greater than 4µg and turbidity noticed in form of white precipitates.

Electrophoresis in LAMP Assay

It is done on agarose gel following incubation at 63ºC for 30 min.10µL aliquot of LAMP amplified products are electrophoresed on 3% NuSieve 3: 1 agarose gel. Normal dyes used for intercalating purpose are Ethidium Bromide, SYBR green I, Calcein, etc. for visualization purpose using UV lamp. A change in colour is noticed from orange to green when observed by UV lamp torch by using SYBR green. Calcein combines with Mg++ to achieve quenching effect. The amplification generates the by - product pyrophosphate ions which binds and remove manganese ions from calcein to irradiate fluorescence. The fluorescence is further intensified as calcein combines with Mg++. From this, the presence of fluorescence can indicate the presence of target gene and visual detection can be achieved without opening the tube thus preventing the carryover of contamination with post amplified products.

Usefulness of LAMP in Agriculture

LAMP has attracted the agriculturist interest as it overcomes the use of expensive equipment and is alternative to PCR. ISAAA (2019) has revealed its use now in Genetically Modified (GM) plants. GM technology is used in US, Brazil, Argentina, Canada and India. International service for the acquisition of Agri - biotech applications assume that the level of GM has reached its saturation. Many plant species of rice, banana, potato, wheat, mustard, eggplant, chickpea, pea, melons, flax, beans, tomato etc. can be monitored through this technology. Many GM products now a day in market are labelled and screened by LAMP technology. GMO products are detected on the basis of specific protein that is synthesized in transgenic plants and nucleotide sequence of an organism thus is obtained. The developed enzyme immune - assay methods allow the determination of proteins encoded in transgenic plants by cp4 - epsps, cry1Ab, cry1Ac, cry2A, cry2Ab, cry3A, cry9C, nptII, pat, gox, cpti. Fraiture et al., (2015) has narrated current and new approaches in GMO detection. This technique of LAMP was developed by Japanese scientist as described by Notomi (2000) which is based on unique feature of DNA polymerase isolated from *Bacillus stearothermophilus.* Nagamine et al., (2002) revealed that LAMP reaction was first described by using 4 primers, however, later it was found that the use of an additional pair of primers for loop formation significantly increases the sensitivity of the method. LAMP study can be implemented in amplification reaction as there are no separate stages of denaturation, hybridization and synthesis at the same time. Loop - mediated amplification can be divided into initiation, cyclic amplification and elongation conditional stages. For loop - mediated amplification, DNA polymerase (*B. Stearothermophillus* Bst DNA polymerase) can replace the synthesis; Forward Inner Primer (FIP) and Backward Inner Primer (BIP) and external primers (F3, B3) recognize 6 different needed regions on the target site. Two looping primers are needed to form a loop and two pairs of stripping primers are needed to synthesize linear nucleic acid strands.

In beginning of the reaction, the primers forming the loop hybridize with F2 or B2 regions in order to initiate the synthesis of complementary DNA strands. After that, primers necessary for the synthesis of linear strands of nucleic acids hybridize with loci F3 or B3, and amplification of complementary DNA strands begins which further leads to the release of the synthesized chains of the molecule. At this stage, the single - stranded chain of the nucleic acid molecule already has a nucleotide sequence that allows the formation of a loop - like structure. The F1 and B1regions at the 5' - end act as primers for generating a double - stranded loop. The regions containing the loop (F1 and B1) are single stranded, so new primers that generate the loop can hybridize with these regions. New complementary DNA strand is thus formed. Synthesis of the molecule from regions F2 and B2 is caused by primers that generate a structure in the form of a loop leading to the formation of a large size reaction product containing nucleotide sequences that correspond to the target gene.

2. Conclusion

Loop - mediated isothermal amplification (LAMP) is a highly suitable method for GMO detection due to its sensitivity, rapid reaction time, ease of implementation, and cost - effectiveness. It also allows for the simultaneous analysis of multiple samples, making it a versatile choice for laboratories and industries involved in GMO testing. The agricultural crops well diagnosed through this technology are plant species of rice, banana, potato, wheat, mustard, eggplant, chickpea, pea, melons, flax, beans, tomato etc. Application of LAMP assays were made for rapid visual detection of cry2Ab and cry 3A genes in GMO crops. The development was applied for detection of nptII and GUS genes in *Nicotiana tabacum*, GM potato EH92 - 527 - 1 and single copy detection of 35Sp and NOSt in transgenic maize using Bioluminescent Assay in Real Time etc. LAMP isothermal assay technique eliminates the need for complex thermal cycling equipment that further enhances its practicability and accessibility in various settings. It stands out as a robust solution for GMO detection analysis in biotechnology. Future thrust of LAMP technology lies in its continued innovation and adaptation to meet the evolving demands of various industries and research fields requiring rapid, sensitive, and cost - effective molecular diagnostics.

References

[1] Fraiture, M. - A., Herman, P., Taverniers, I., De Loose, M., Deforce, D., and Roosens, N. H. (2015). Current and New Approaches in GMO Detection: Challenges and Solutions. BioMed Res. Int., Art.392872. doi: 10.1155/2015/392872.
[2] ISAAA. (2019). Global Status of Commercialized Biotech/GM Crops in 2019: Biotech Crops Drive Socio - Economic Development and Sustainable Environment in the New Frontier. ISAAA Brief No.55. Ithaca, NY: ISAAA.
[3] Mori, Y., Kitao, M, Tomita, N. and Notomi, N. (2004). Real - time turbidimetry of LAMP reaction for quantifying template DNA. J. Biochem & Biophysic. Meth.59: 145 - 157.
[4] Nagamine, K., Hase, T., and Notomi, T. (2002). Accelerated reaction by loop - mediated isothermal

amplification using loop primers. Mol. Cell. Probes, 16 (3): 223–229.
[5] Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., (2000). Loop mediated isothermal amplification of DNA. Nucleic Acids Res.28.12 e63.
[6] Ushikubo H. (2004). Principle of LAMP method - a simple and rapid gene amplification method. Uirus 54 (1): 107–112.