Biotechnological aspect of LAMP in Agriculture

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LAMP has gained a significant interest in research across a diverse field of Agriculture. This technology is considered an established method for amplifying nucleic acid and also for diagnosis of field diseases. Due to its cost effectiveness when compared to PCR, LAMP method is considered to be fast and precise and large amount of DNA is produced. This technology was invented in 1998 by Eiken Chemical Company Tokyo. LAMP amplification is carried out at constant temperature and there is no need for the use of thermal cycler. Target DNA sequence is amplified at 60-65°C.Two or three set of primers along with polymerase like Bst Klenow fragment with high strand displacement activity in addition to replication activity is used. Amplification in LAMP can be detected using photometry and by measuring turbidity which is caused by Magnesium pyrophosphate precipitate in solution it is formed as a result of byproduct of amplification that allows easy visualization by naked eyes and also by simple photometric detection. Certain dyes recommended for LAMP that induce a visible colour change are SYBR green which allows visible colour change with naked this occurs as dye molecule intercalates DNA. Other methods for visualization by LAMP is by hybridization with complementary gold nanoparticle bound to SS DNA.

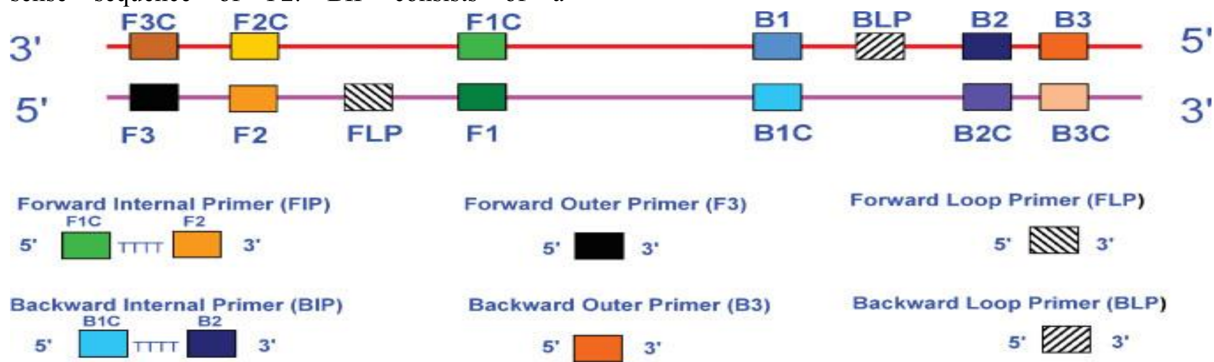
DESIGNING OF LAMP PRIMER

Designing of primer is specific which is accompanied by using Primer explorer which is a software program. Generally, we have six primers comprising two outer, two internal and two loop primer that recognise six distinct region of target sequence. Two outer primers were described as forward outer primer (f3) and backward outer primer (B3). These play a role in displacement during non-cyclic steps. Internal primer has both sense and antisense sequence and result in loop formation. Internal primer were 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) and are composed of sequence that are complementary to the sequence between F1 &F2 and B1 and B2 region respectively. Designing of primer is done in such a way that it matches with complementary regions of target site. LAMP offer excellent combination of sensitivity and specificity.

PRINCIPLE OF LAMP AMPLIFICATION

US hikubo et al., (2004) described chemistry for LAMP amplification which is based on autocyclic strand displacement when performed at constant temperature using DNA polymerase. There are two steps for amplification one is cyclic and other is non -cyclic step. Non cyclic step result in formation of stem loop that serve as starting point for amplification by LAMP cycling. One of primers in LAMP anneal to complementary sequence of double stranded target DNA that will initiate DNA synthesis by using DNA polymerase when taken in to consideration 3’ end of F2 region of Forward internal primer. F3 primer will anneal to F3C region outside of FIP on target DNA and will initiate strand displacement of DNA synthesis releasing FIP. FIP strand released as a single strand due to displacement of DNA strand synthesis from F3 primer which forms a stem loop at 5 ‘end due to complementary FIC and F1 region released single strand. Notomi et al., (2000) described design 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 30 side and the B1, B2, B3 and BLP regions at the 50 side which consists of the F2 region (at the 30 end) that is complementary to the F2c region, and the same sequence as the F1c region at the 50 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 30 end) that is complementary to the B2c region, and the same sequence as the B1c region at the 50 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 as given in figure below.



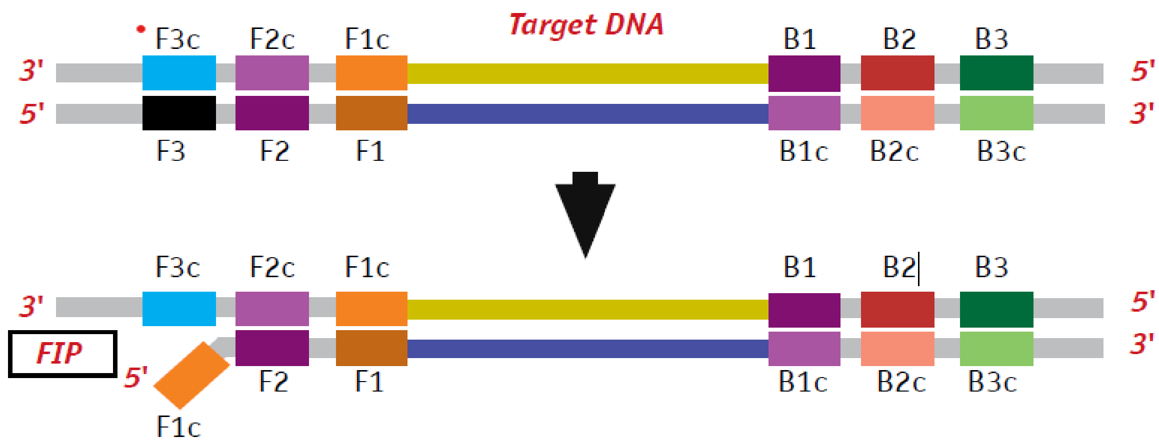
Mori et al., (2004) noticed turbidity in form of OD at 400nm and it was found that DNA yield greater than 4µg can notice the turbidity in form of white precipitates.

ELECTROPHORESIS IN LAMP

It is done on agrose gel i.e. Nusieve 3:1 normally incubation of 63°C is carried for 30 minutes and 10 µl aliquot of LAMP product is used . Normally dyes used for intercalating purpose are Etbr, SYBR green I for visualization purpose UV lamp is used. A change in colour is noticed from orange to green when observed by UV lamp torch by using SYBR green.when calcein is used and shows quenching effect. The amplification generates the by-product, pyrophosphate ions, which will bind and remove manganese ions from calcein to irradiate fluorescence. The fluorescence is further intensified as calcein combines with magnesium ions. From this feature, the presence of fluorescence can indicate the presence of target gene and visual.

LAMP IN AGRICULTURE

Many methods of GMO are based on nucleic acid and protein composition. LAMP has attracted agriculturist interest as it has overcomes the use of expensive equipments and is alternative to PCR, ISAAA (2019) has revealed use of 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 saturation, many of GM product now a days are labelled and screened GMO product are detected on the basis of specific protein that is synthesized in transgenic plant and nucleotide sequence of organism that is obtained from genetically modified organism. The developed enzyme immunoassay methods allow the determination of proteins encoded in transgenic plants by cp4-epsps, cry1Ab, cry1Ac, cry2A, cry2Ab, cry3A, cry9C, nptII, pat, gox, cpti Fraiture (2015).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* it has high revertase activity . Usually, the reaction it self is carried out at a temperature in the range of 55–65 °C. Nagamine et al.,(2002) revealed that LAMP reaction was first described 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 method. LAMP study can be implemented in amplification reaction as there are no separate stages of denaturation, hybridization and synthesis. Loop-mediated amplification can be divided into the following conditional stages – initiation, cyclic amplification and elongation. For loop-mediated amplification DNA polymerase that can replace the strand during synthesis (B. stearothermophillus Bst DNA polymerase), Forward Inner Primer (FIP)and Backward Inner Primer, (BIP) and external primers (F3, B3) that recognize 6 different regions on the target are needed. Two looping primers are needed to form a loop, and two pairs of stripping primers are need 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 (stage 3).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 formed . Synthesis of the molecule from regions F2 and B2, and the synthesis, which is caused by primers that generate a structure in the form of a loop, leads to the formation of a large size reaction product containing nucleotide sequences that correspond to the target

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. LAMP's isothermal nature eliminates the need for complex thermal cycling equipment, further enhancing its practicality and accessibility in various settings. Therefore, LAMP technology stands out as a robust solution for GMO detection analysis.

Future thrust: he 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.

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