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Polymerase Chain Reaction (PCR): Principle and Applications in Agriculture

(*Prashant Vasisth and Mohit Sharma)

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi

*pvasisth555777@gmail.com

Polymerase chain reaction (PCR) was invented by Mullis in 1983 and patented in 1985. Its principle is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. This method can generate tens of billions of copies of a particular DNA fragment (the sequence of interest, DNA of interest, or target DNA) from a DNA extract (DNA template). he introduction of thermostable Taq DNA polymerase from Thermus aquaticus has facilitated automation of this technology. he use of this invention for crop improvement was widely accepted in the areas of research like detection of plant pathogens, development of genetic or physical map of crop genome, cloning and characterization of existing or new trait responsive genes and their expression pattern analysis, genetic diversity analysis and marker assisted breeding for development of new crop varieties.

Principle of the PCR

PCR makes it possible to obtain, by in vitro replication, multiple copies of a DNA fragment from an extract. It is a technique for obtaining large amounts of a specific DNA sequence from a DNA sample. This amplification is based on the replication of a double-stranded DNA template. It is broken down into three phases: a denaturation phase, a hybridization phase with primers, and an elongation phase. In practical, all reaction components are assembled on ice and added one by one in a single reaction tube. Generally, polymerase is added at the last and final reaction volume is made by doubled distilled water or MiliQ water. Immediately reactions are transferred to thermos cycler machine for starting the reactions.

Steps of PCR

1. Denaturation Temperature and Duration: Initial denaturation at 95°C for 3-10 minutes is recommended prior to PCR cycling to fully denature the DNA. Subsequently, 10-30second of denaturation at same temperature is sufficient in start of cycle.
2. Annealing Temperature and Duration: At this temperature both primers anneal to complementary sequence at the template DNA. The optimum annealing temperature is 5°C lower temperature than the primer with lowest melting temperature (Tm) and difference between Tm of both primers should not be more than 5 °C. Primers are designed so that the annealing temperature fall in the range of 50-60 °C and annealing times are 15-30 seconds.
3. Extension Temperature and Duration: Extensions are normally performed at 72°C. At this temperature, nucleotides are added at the 3'-OH ends of both primes by Taq DNA polymerase and extended up to the desired length. Generally, for extension of 1000 bases one minute is sufficient (e.g., 2 minutes for a 2 kb product). For products less than 1

kb,45-60 seconds is advised. Products greater than 3 kb, practically it is difficult to amplify by using normal Taq DNA polymerase. Special kind of high-fidelity polymerase enzymes are required.

Applications of PCR Technology in Crop Improvement

1. To increase the number of very few number of DNA template.
2. Isolation of a orthologous gene sequence by using degenerate primers designed from closely related plant species gene sequence alignments.
3. Amplification and isolation of potential trait responsive genes from crops and their transcript expression pattern analysis under different stress conditions.
4. Synthesis of complementary DNA (cDNA) from RNA isolated from the crop using modified process of PCR called Reverse-transcriptase PCR.
5. Amplification of gene from cDNA.
6. PCR is used for DNA sequencing to determine unknown PCR-amplified sequences, which helps in gene discovery.
7. PCR remains as an integral tool for cloning, vector construction, and transformant identification into bacterial cells.
8. Identification of genetically modified crops for the presence of transgene using PCR
9. PCR amplification and sequencing for marker genes like 16S and 18S ribosomal RNA genes in prokaryotes and eukaryotes, respectively for phylogenetic analysis.
10. Screening and differentiation of diseased with viral pathogen and healthy plants using viral gene specific primers.