Chapter-9

Implementation of Transgenic Plants and PCR Technology for crop Improvement.

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ABSTRACT

Unlike conventional plant breeding methods, transgenic crops are developed through genetic engineering and have proven to meet the food security needs of the growing global population. To date, many transgenic plants have been commercialized, with many more undergoing research trials to address their biosafety concerns. Genetically modified plants also offer enhanced nutritional quality in food alongside their high resistance traits. The development of PCR has revolutionized the field of biological research. Over time, PCR technologies have advanced significantly. The PCR technique is now applied in almost every scientific area and is extremely beneficial. Some of its applications include the production of innovative biomedicines, early detection of genetic disorders, clinical treatments and also in screening transgenic plants.

KEYWORDS: Transgenic plants, crop improvement, genetically modified plants, PCR.

INTRODUCTION

Transgenic plants or genetically engineered plants are those plants in which their gene(s) have been modified by introducing foreign genes (transgenes) from another plant, which is done based on our desired traits. This artificial insertion of a foreign gene is done by various biotechnological methods. These transgenic plants carry the desired genes and show economic and commercial importance, such as enhanced quality, greater yield, resistance against biotic and abiotic stress, in vaccine production, etc. In 1982, the 1st commercial transgenic plant i.e., a virus-resistant tobacco plant, was reported. Some of the examples of transgenic plants are golden rice, roundup ready soybean, etc. In recent years, various pharmaceutical products have been produced with the help of transgenic plants, which is done by using transgenic plants as bioreactors. Transgenic seeds or fruits are now easily transported and stored without being degraded. Such seeds are stored for further hybridization in crop breeding programs based on the need of the products to be produced. Several immunotherapeutic drugs are being produced using these bioreactors or biofactories. Biodegradable plastics are also being prepared using PHB (Polyhydroxybutyrate), a compound derived from transgenic plants. Various antibodies, edible vaccines (1990) started to appear commercially, which provided immunization. IgG, IgA, and serum albumin genes were successfully incorporated into plants. Polymerase chain reaction (PCR) plays a critical role in the analysis of transgenic genes present in transgenic plants. It is an *in-vitro* molecular technique used to amplify the no. of a specific DNA region, including the transgenes, and confirms the presence of transgenes in a short period, and helps in the understanding of transgene function and potential silencing. Identification and detection of the transgenes in transgenic plants have been reported globally. The PCR technique uses an internal standard i.e., endogenous gene which acts as a

binding site for one primer and the other primer is designed to bind to the specific sequence of the target DNA sequence of the transgenic plant, thus differentiating the transgenic plants from the non-transgenic ones.

1. IMPORTANCE OF TRANSGENIC IN CROP IMPROVEMENT

Transgenics is a technology that allows the introduction of specific genes from foreign organisms to manipulate traits like quality, yield, and resistance (drought, salinity, and pest infections) shown in **Figure 1.** The importance of transgenic in response to resistance to pests and harmful diseases to improve food production are in high demand and work towards sustainability. Here are some important implications of transgenics.

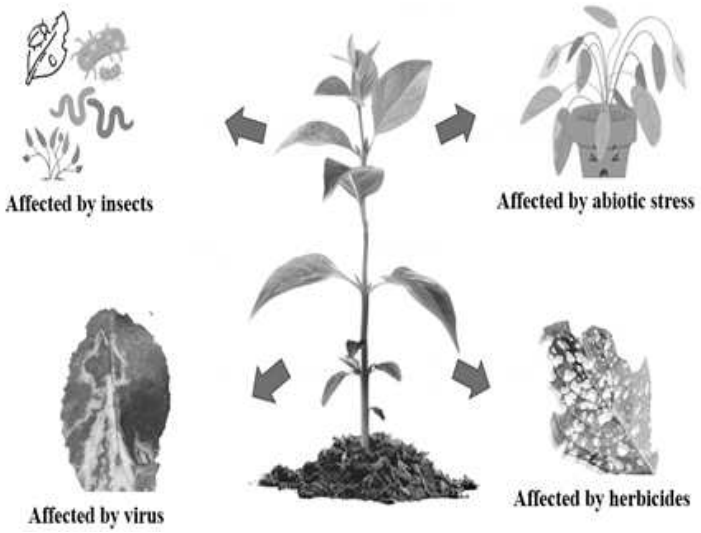

Figure 1. shows the plants affected by different kinds of both biotic and abiotic stresses.

1.1 INSECT-RESISTANT TRANSGENIC PLANTS

Bacillus thuringiensis contains the *Bt/cry* gene, which

encodes beta and delta endotoxins, which are proteinaceous toxins that destroy the gut epithelium of harmful insects. There are mainly four groups of *cry* genes based on their toxin-producing activities- *cry* I, *cry* II, *cry* III, and *cry* IV. The *Bt* genes are isolated and introduced into the Ti-plasmid of *A. tumefaciens* and are finally introduced into the plant cells. *Bt* cotton was commercialised in India on March 2003. Some other examples include *Bt* corn, *Bt* maize, etc.

1.2 VIRUS-RESISTANT TRANSGENIC PLANTS

Plant viruses cause harm to a large no. of plants resulting in the decrease of crop yield. Such plants are produced by introducing CP (coat protein) gene of TMV into the desired plant. Some of the examples include tomato (using CP gene of TMV), papaya (using CP gene of PRSV), etc.

1.3 HERBICIDE-RESISTANT TRANSGENIC PLANTS

These plants use different mechanisms for resistance against glyphosate, sulphoylurea and imidazolinone herbicides. For resistance against glyphosate, RPSPS genes were introduced in plants, and similarly, a mutant *als* gene was used to provide resistance against the other two herbicides mentioned above.

1.4 NUTRITIONAL CONTENT IN RESPONSE TO TRANSGENICS

Vitamin A is a very important fat-soluble vitamin needed by our body. Its deficiency causes night blindness and skin disorders. This deficiency is mainly found in children. Rice, being the staple food in many countries, has been found to contain low vitamin A levels. Thus, golden rice was engineered, which contained high levels of beta-carotene, a precursor for Vit-A for overcome the problems regarding Vitamin A deficiency.

2. HOW ARE TRANSGENIC PLANTS PRODUCED?

1) First, we need a piece of DNA that encodes our desired trait, including a promoter which helps in the gene expression. This all happens in a plasmid vector that replicates in a bacterial cell to make large no. of DNA copies.

2) Now, we need a way to select the cells containing our DNA containing the plasmids. This is achieved by adding a selectable marker, most commonly an antibiotic resistance gene.

3) Then we need to incorporate the target DNA inside the plant, which is done by:

a) **BRUTE FORCE METHOD: A GENE GUN**-Here, a gene gun fires the DNA coated gold particles (including promoter, sequence for the desired trait and a selection marker) into the plant cells.

b) **AGROBACTERIUM MEDIATED GENE TRANSFER**- *Agrobacterium tumefaciens* is a gram-negative soil bacterium. It causes crown gall disease by infecting the wound sites of plant, caused as a result of gene transfer. It has Ti-plasmid which consists of T-DNA (containing the genes to be transferred) and vir genes (help in gene regulation and T-DNA processing) into the plant naturally. Thus T-DNA expresses itself in the transgenic plants.

4) At last, the transformed plant that has the integrated DNA is grown under sterile conditions using tissue culture method.

3. PCR AND ITS APPLICATIONS IN TRANSGENIC CROPS

As the global population rises, the demand for the production of transgenic plants is also increasing rapidly. With the rate of new transgenic plants being introduced on the rise, there is a need for a molecular tool to help us distinguish between transgenic plants containing exogenous or foreign genes and non-transgenic plants. Several modern tools have been introduced to detect these foreign genes, but most of them are time-consuming and expensive, making them less convenient for our purpose. Polymerase Chain Reaction (PCR) is one of the widely used analytical tools for detecting foreign genes. PCR, being a fast, simple, highly specific, sensitive, reliable, and inexpensive method, has been found suitable to meet our needs. Detecting transgenes becomes crucial for screening a large number of transgenic plants due to the interference of the endogenous genes of plants with transgenes.

3.1 PCR TECHNIQUES

PCR techniques have been used in transgene detections and analysis of transgenic plants like rice, chickpea, soy, maize, etc. These techniques are helpful in amplifying and detecting specific sequences such as regulatory gene or structural genes in the transgene. PCR can also be combined with other techniques like ELISA, southern blotting, northern blotting, etc. for enhanced screening of transgenes.

1)PRIMER DESIGN- Binds with the specific sequences of the transgene.	**2)AMPLIFICATION**- Target DNA sequence is amplified using primers to detect transgene.	**3) QUALITATIVE PCR**- Confirms whether transgenes are present or not.	**4) qPCR**- Quantifies the copy no. and expression level in transgenic plants.

3.2 APPLICATIONS OF PCR IN TRANSGENIC CROP

There are two main ways to detect the presence of transgenic

genes in plants. They are as follows:

3.2.1 QUANTITATIVE PCR (qPCR)

This method is used to determine the measure of the transgene's copy number precisely along with its level of expression that have been introduced into the genome of plant. Here, the target sequence of transgene is amplified and compared with endogenous reference gene. Then a standard curve is produced to quantify copy no. of transgenes. Expression levels are quantified using mRNA levels. It is a more efficient substitute to southern blotting for quantifying transgenes to screen large no. of plants. It also tells us about the effects of transgenes regarding the stability, morphological traits, etc. in the plant genome. RT-PCR along with qPCR is also used for this method where cDNAs are produced using mRNA which are amplified and then quantified **(Figure 2)**.

3.2.2 QUALITATIVE PCR

This method is divided into two ways: screening and event-specific PCR method. In screening, sequences that are common in transgenic plants are monitored, such as the CaMV 35S promoter of CMV (cauliflower mosaic virus). In the event-specific method, transgenic events are detected to differentiate between the transgene and the plant's genome. Here, designed primers bind to the specific DNA sequence of the plant sample and amplify it. The PCR will produce a detectable product, indicating the presence of the sequence in the sample and vice versa. Using the designed primer that binds to a specific sequence of the transgene helps in differentiating the transgene from the plant genome **(Figure 2)**.

Isolated mRNA

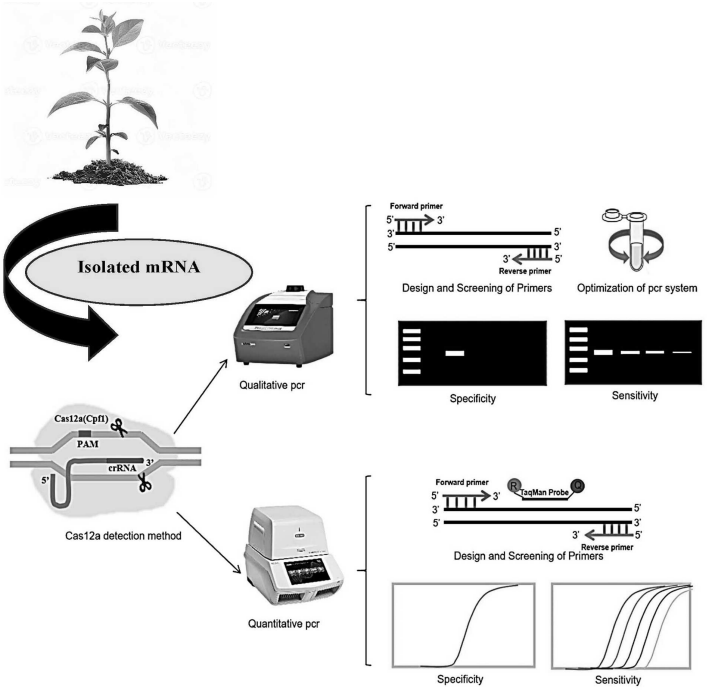

Figure 2. Shows the qualitative and quantitative PCR for transgenic crop improvement

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