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Transgenic plants resistant to viruses- A perspective

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Introduction

Transgenic plants used in agriculture, the DNA of which has been modified using genetic engineering methods. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species.

Examples: In food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation. Farmers have widely adopted GM technology. Between 1996 and 2015, the total surface area of land cultivated with GM crops increased by a factor of 100, from 17,000 km^2 (4.2 million acres) to 1,797,000 km^2 (444 million acres). 10% of the world's arable land was planted with GM crops in 2010. In the US, by 2014, 94% of the planted area of soybeans, 96% of cotton and 93% of corn were genetically modified varieties.

There is a scientific consensus that currently available food derived from GM crops poses no greater risk to human health than conventional food, but that each GM food needs to be tested on a case-by-case basis before introduction. Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe. The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation (Timmons and Fire, 1998).

Virus diseases are significant threat to modern agriculture and their management remains a challenge in the crop production. The phenomenon of virus resistance is based on either natural

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resistance or engineered virus–resistant plants. Transgenic crops contributing 10.8 m ha of area in India (ISAAA, 2016). Recent progress in understanding the molecular mechanisms underlying the roles of resistance genes has promoted the development of new anti-viral strategies. In genetically engineered plants, RNA- silencing nucleotides and Coat Protein (CP) mediated resistance are becoming increasingly important and are likely to provide more effective strategies in future. Various kinds of genome manipulation were done in order to overcome virus infection in host by means of classical breeding mechanism, targeted genome editing and transgenic approaches (Voinnet and Baulcombe, 1997) (Fig. 1)

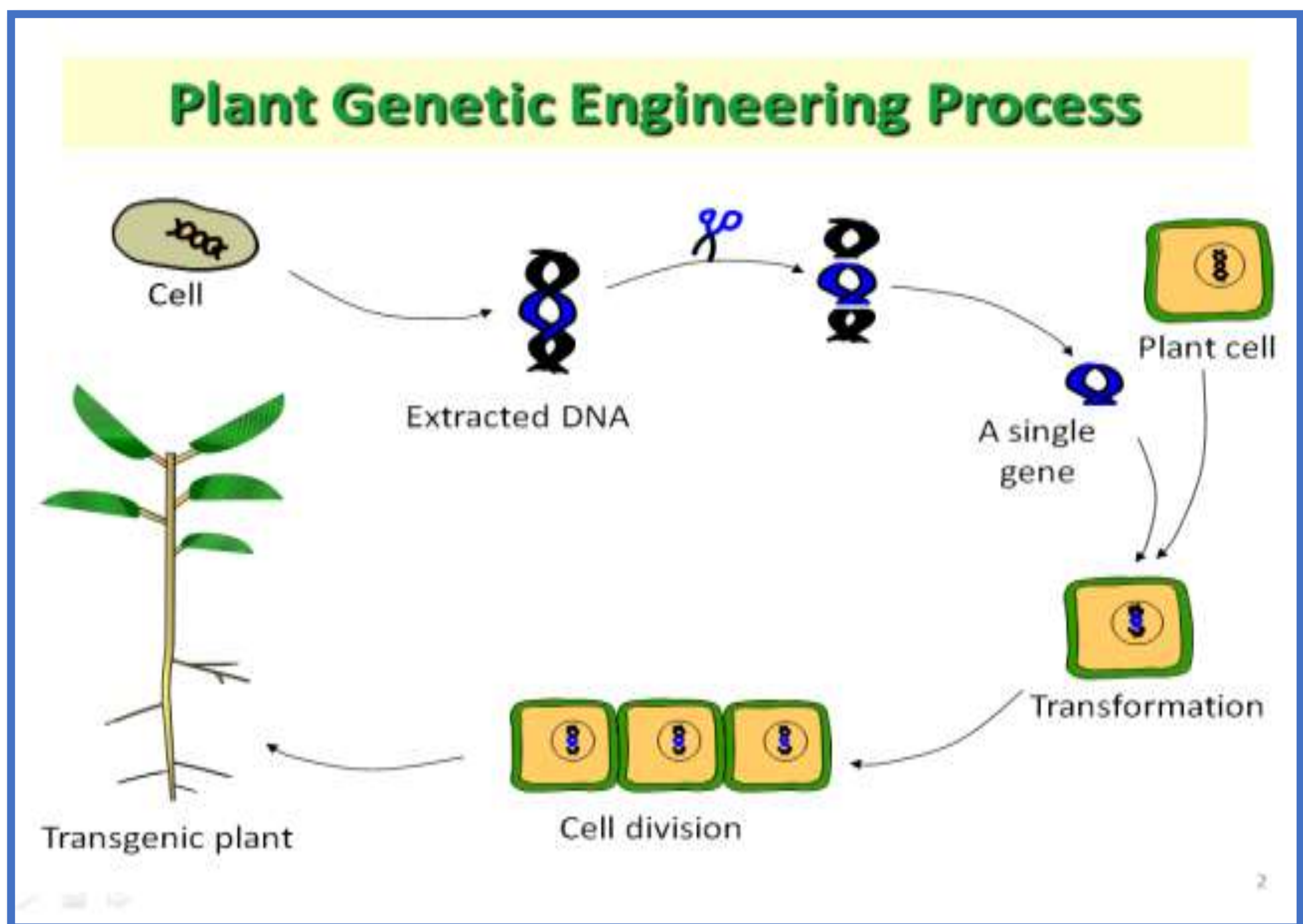

Fig. 1: Plant genetic engineering process

Transgenic developmental steps include

- Clone the gene of interest
- Add DNA segments to initiate gene expression

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- Add selectable markers
- Transformation
- Select transformed cells or tissues
- Regenerate whole plants

- ***Clone the gene of interest***

Construct the recombinant DNA initially, later transport to *E coli* for multiplication. The multiplied cells then shifted to the host cell. Public and private labs are directing more efforts to locate, identify, characterize, and clone genes of agricultural importance.

- ***Recombinant DNA***

The bacterial restriction enzymes cut the DNA molecules at specific DNA sequences called restriction sites. A restriction enzyme usually makes many cuts, yielding restriction fragments. The most useful restriction enzymes cut DNA in a staggered way, producing fragments with "sticky ends. Sticky ends can bond with complementary sticky ends of other fragments (Fig. 2).

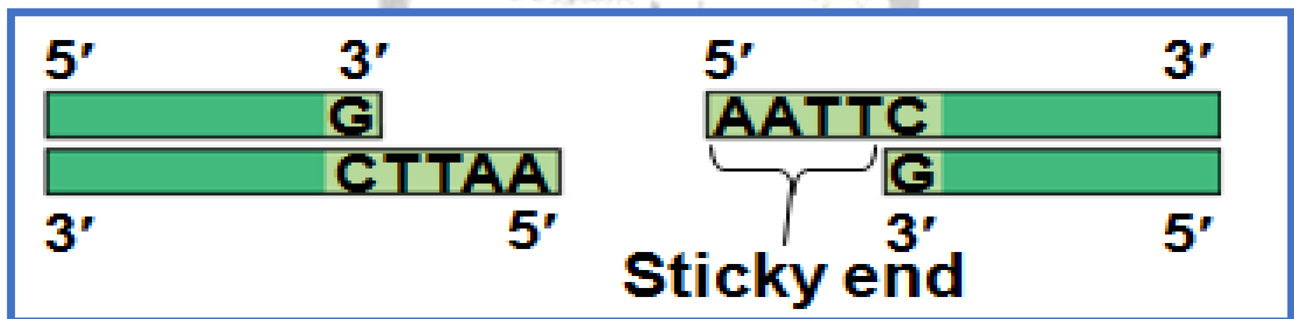

Fig. 2: Recombinant DNA

DNA ligase - seals the bonds between restriction fragment.

- ***Add DNA segment to initiate gene expression (Fig. 3)***

Promoter: CaMV 35S promoter

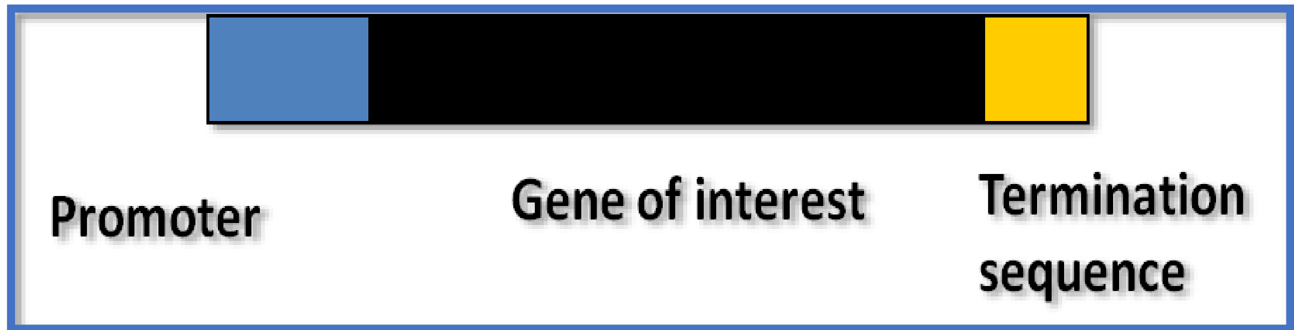

Fig. 3: Gene expression

Terminator sequence: Nopaline synthase (*nos*) transcription terminator sequence from Agrobacterium *tumefaciens* (Haywood, 2002).

- ***Add selectable marker***

Gene transfer marker is needed to identify cells with the new genes. Typically, antibiotic resistance genes are used as markers.

Properties of good host

Easy to transfer, support the replication of recombinant DNA. Free from elements which interfere in the replication of recombinant DNA. Lacks active restriction enzyme.

Advantages

Genome integration is precise. Simple transgene insertions with defined ends. Low copy number. Reproducible and highly efficient protocol.

Disadvantages

Not easy to handle, Slow process. Introduction of unnecessary vectors that produce unknown genetic expressions into the plant. Requires sterile protocols.

Gene Gun (Microprojectile bombardment method/Particle acceleration method and Biolistic method)

DNA is bound to tiny particles of gold or tungsten which are subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant DNA inside the nucleus. Used in monocots like wheat or maize.

Advantages

No pre-treatment of the cell wall required, Independent of the physiological properties of the cell. Transformation with multiple transgenes is possible.

Disadvantages

Expensive, DNA can be damaged. Produces multiple copies of introduced genes which can lead to various unprofitable effects. Low transformation efficiency

Electroporation

DNA enters the plant cells through miniature pores which are temporarily caused by electric pulses. Low voltage- long pulses, 300-400V/cm. High voltage- short pulses, 1000-1500V/cm.

Advantages

It can be applied to any plant protoplasts. Different cell types can be used. Simple, fast and cheap.

Disadvantages

Laborious protocols, it requires protoplast formation. Depends on electrophysiological characteristics of the plant. Low transformation efficiency.

Microinjection

Under a microscope, gentle suction holds cell in place. DNA or RNA is injected directly into nucleus or cytoplasm. The protoplast is cultured from 1-5 days before injection for partial regeneration of cell wall.

Advantages

Extremely high transformation efficiency, Allows introduction of plasmids and whole chromosomes.

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Disadvantages

Expensive, tedious and slow. Expression of gene products that are: Directly toxic or which reduce growth of the pathogen. Destroy the pathogen components. Potentially enhance the structural defences in the plant. Release signals that can regulate plant defences. Resistance gene(R) products involved in R/Avr interactions and hypersensitive response.

Activation of plant defence responses

Elicitor molecules from invading pathogen trigger a network of signalling pathways that coordinate defence responses of the plant, including hypersensitive response, PR protiens and phytoalexins.

Ex- *INF1*, act as an avirulence factor in tobacco -*Phytophthora infestans*

AVR9 peptide elicitor from *Cladosporium fulvum* in transgenic tomatoes containing *cf9* gene – necrotic defences response

- ***Sap inoculation:*** A rapid and efficient sap inoculation method for tobacco streak virus (TSV) was developed in sunflower. Sap from TSV-infected sunflower plants was freshly extracted in phosphate buffer and diluted serially from 10(-1) to 10(-8). This methodology can also be extended for the analysis of resistance against other viruses.
- ***ELISA:*** A novel form of indirect enzyme-linked immunosorbent assay (ELISA) has been devised for the detection of viruses in plants. The method uses protein A in two applications to sandwich antibody-antigen-antibody layers. The first applied layer of protein A prepares the plate for the coating antibody layer. The second layer of protein A is conjugated to the enzyme and detects the second antibody layer. The orientation of the IgG induced in the coating layer of antibody prevents later unwanted reaction with the conjugated protein A.
- ***Microprecipitation tests:*** A microprecipitation test (MPT) for the detection of adenovirus antibody has been developed. The new procedure combines precipitation of virus particles with specific antibody, separation of unreacted components from the resulting electroneutral virus-antibody complexes by electrophoresis, and detection of these complexes with a protein stain.
- Examination of specific and amorphous inclusions.

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- Immune-specific electron microscopy.
- Gel diffusion tests.

Defense through genetically engineering disease resistant plants

1) With Plant - Derived Genes
2) With Pathogen- Derived Genes

With Plant - Derived Genes'R' genes (Table 1)

Gene	Host	Pathogen
Hm1	corn	*Cochliobolus carbonum*
Pto	Tomato	*Pseudomonas syringae* pv. *tomato*
RPS2	Arabidopsis	*P. syringae* pv. *Tomato, P. syringae* pv. *maculicola*
N	Tobacco	*Tobacco Mosaic Virus*
Cf9	Tomato	*Cladosporium fulvum*
L^6	Linseed	*Melampsora lini*

Table. 1: List of R genes

With Pathogen- Derived Genes

Pathogen resistance genes can be derived from a pathogen 's own genetic material (Sanford and Johnston, 1985).

I. Coat protein-mediated resistance
II. RNA-mediated resistance
III. Replicase-mediated resistance
IV. Movement protein mediated resistance

Coat protein-mediated resistance (CPMR)

First reported by Powell-Abel *et al*. (1986) TMV "Coat protein-mediated resistance" is used to refer to the resistance caused by the expression of a virus coat protein (CP) gene in transgenic

plants. Controls +ve sense, ssRNA virus infection. Degree of protection to crop ranges from delay in symptom expression to absence of disease symptoms and viral accumulation. The transgenic plant providing coat protein-mediated resistances to virus are rice, potato, peanut, sugar beet, alfalfa, etc. CPMR can provide either broad or narrow protection (Han, 2004).

Ex: Broad protection- Potato Virus Y (PVY): N605 and N0803 strains. Narrow protection- Tobacco: Papaya ringspot virus (PRV).

RNA-mediated resistance (RNAi)

Occurs in cytoplasm rather than the nucleus. Key feature- production of 21-24 nt short interfering RNA (si RNA) by Dicers. Formation of RISCs (RNA –induces silencing complex) which contain Argonaute (Ago) proteins that directly carry out silencing. Silencing at transcriptional and post-transcriptional level. 2 kinds of RNAi: 1) siRNA, 2) miRNA.

siRNA produced from action of dicers on dsRNA. miRNA- transcribed from their own genetic material/introns/fold back structures with ds regions. Targets specific sequences of RNA and degrading them. RNAi acts as interconnected pathways for RNA surveillance and cell defence and also called as post transcriptional gene silencing, RNAi or antisense RNA technique.

Movement protein mediated resistance

Movement proteins (MPs) are encoded by plant viruses and enable infections to spread between adjacent cells (local spread) as well as systemically. Act as dominant negative inhibitors to block the local and systemic spread of many different viruses with high efficiency. A movement protein is a non-structural protein which is encoded by some plant viruses to allow their movement from one infected cell to neighbouring cells. Movement proteins modify the plasmodesmata by one of two well-understood molecular mechanisms.

Mechanisms

The movement proteins of many plant viruses form a transport tubule within the pore of the plasmodesmata that allows the transport of mature virus particles. Ex: cowpea mosaic virus (CPMV) and tomato spotted wilt virus (TSWV).

It is associating with genome of the virus, causing the ribonucleoprotein complexes to be transported through plasmodesmata into neighbouring cells. Ex: TMV's 30 K Da.

Conclusion

The various fluctuations in the environment results in changes in the plant virus existence. So, the basic plant virus management strategies in virus management will not be so effective. So advanced molecular tools may help to overcome losses and also enhances the economic yield.

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