The Pharma Innovation Journal 2023; SP-12(10): 1301-1312

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(10): 1301-1312
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www.thepharmajournal.com
Received: 24-08-2023
Accepted: 29-09-2023

Scope and status of Transgenics in fruit crops

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Abstract
Fruit crops have traditionally been improved through conventional breeding methods that involve selecting plants with desirable traits over many generations to achieve incremental improvements. This approach aims to enhance various phenotypic traits, such as fruit size, yield, nutritional properties, and aroma/taste. The introduction of new traits, such as disease resistance, requires crossing an elite line with a disease-resistant variety, followed by repeated backcrossing with the elite parent to preserve as much of the genetic material from the elite variety as possible while introducing the new resistance allele. This process takes many generations and can take several decades in species with a long juvenile phase for the selectable phenotype to emerge in each generation. The outcrossing nature of many fruit- bearing crops makes it impossible to recover the original genotype and phenotype, which makes the chance selection of a desirable phenotype in an elite cultivar highly valuable. The development of resistance to apple scab is a good example of the difficulties of conventional breeding, as crosses between a wild type and an elite line of apple initiated in the 1950s have not yet resulted in derivative cultivars with desired fruit quality traits. Marker-assisted selection, which involves selecting molecular markers linked to the desired trait at an earlier developmental stage, has been developed to some extent, but it still requires generations of backcrossing to achieve successful introgression.

Keywords: Transgenic, fruit crops, speed breeding, marker assisted selection

Introduction
Fruit crops have traditionally been improved through conventional breeding methods that involve selecting plants with desirable traits over many generations to achieve incremental improvements. This approach aims to enhance various phenotypic traits, such as fruit size, yield, nutritional properties, and aroma/taste. The introduction of new traits, such as disease resistance, requires crossing an elite line with a disease-resistant variety, followed by repeated backcrossing with the elite parent to preserve as much of the genetic material from the elite variety as possible while introducing the new resistance allele (Kaur *et al*. 2020; Singh *et al*. 2021) [36, 37]. This process takes many generations and can take several decades in species with a long juvenile phase for the selectable phenotype to emerge in each generation. The outcrossing nature of many fruit- bearing crops makes it impossible to recover the original genotype and phenotype, which makes the chance selection of a desirable phenotype in an elite cultivar highly valuable. The development of resistance to apple scab is a good example of the difficulties of conventional breeding, as crosses between a wild type and an elite line of apple initiated in the 1950s have not yet resulted in derivative cultivars with desired fruit quality traits (Hough *et al*. 1953; Schouten *et al*. 2006) [15, 28]. Marker-assisted selection, which involves selecting molecular markers linked to the desired trait at an earlier developmental stage, has been developed to some extent, but it still requires generations of backcrossing to achieve successful introgression (Semagn *et al*. 2006) [6].

However, genetic engineering offers a faster approach for developing improved fruit varieties, and the ability to propagate fruit trees vegetatively allows for the engineered cultivars to cover larger areas compared to those relying on sexual reproduction. The production of genetically engineered fruit crops has been achieved through either Agrobacterium-mediated transformation or direct DNA transfer, although the efficiency of transformation depends on the species and cultivar, necessitating the development of specific optimized methods that include efficient gene delivery, selection, and regeneration from transformed explants.

With the population expected to grow by 40% in the next few decades, farmers will need to produce as much food in the next 50 years as has been produced in the past 10,000 years. To ensure an abundant food supply, new techniques are needed, and it is necessary to move away from conventional breeding methods and increase the use of transgenic Fast-Track breeding.

Also, the current and futuristic patterns of climate change have been a major concern for the entire ecosystem. The effects of climate change have significantly impacted crops, especially the fruit industry. Climate change has significant effects on fruit crops, including altered flowering and fruiting times, increased incidence of pests and diseases, and changes in fruit quality and quantity. These changes in fruit production could potentially lead to food insecurity and economic losses for farmers. Adaptation strategies, such as breeding for heat tolerance and pest resistance, and shifting to more climate-resilient crop varieties, are crucial for ensuring sustainable fruit production in the face of climate change.

Transgenic fruit breeding has the potential to address some of the challenges posed by climate change in the agriculture sector. The changes in temperature and rainfall patterns can shift the geographical distribution of fruit crops and alter the timing of flowering and fruiting. Transgenic fruit breeding can help to mitigate some of these impacts by developing fruit varieties that are more resilient to climate change. For example, fruit trees that are genetically engineered to be drought-tolerant could help to maintain crop yields during periods of water scarcity. Similarly, transgenic fruit varieties with increased resistance to pests and diseases could reduce the risk of crop losses due to climate-related factors. Another potential benefit of transgenic fruit breeding in the context of climate change is the ability to develop fruit varieties with improved nutritional content. Climate change can affect the nutrient content of fruits and vegetables, with some studies showing that increased atmospheric CO_2 levels can reduce the levels of key nutrients in crops such as apples, grapes, and strawberries. By developing transgenic fruit varieties with enhanced nutrient content, it may be possible to counteract some of the negative impacts of climate change on food quality and nutrition. Transgenic technology has been utilized to manipulate the genes of fruit crops to improve their productivity, quality, and resistance to biotic and abiotic stresses. The technology involves the introduction of foreign genes or silencing of target genes to improve the traits of interest. Fruit crops are a significant source of nutrition and livelihoods for millions of people around the world. The development and utilization of transgenic technology in fruit crops have the potential to increase production and reduce the environmental impact of farming practices.

Transgenic technology is not a substitute for traditional plant breeding, but rather a complementary tool that can be used in combination with classical breeding methods to develop economically viable products. (Visarada *et al*. 2009) [35]. Although there are numerous reports available on the use of transgenic technology in different crops, there are only limited reports on research and development for genetically modified (GM) varieties. The main purpose of this report is to identify the key steps involved in transgenic breeding programs, including gene designing, methods involved in gene transfer, achievements of transgenic breeding in fruit crops and limitations encountered in the direct adoption of transgenic lines. The review also highlights breeding strategies that can be used to overcome these limitations and to develop commercially viable products. This report emphasizes the importance & prospects of transgenic breeding methods in fruit industry.

Conventional Vs Transgenic breeding

S. No.	Conventional Breeding	Transgenic Breeding
Genetic material	Recombination of genetic material through crossing	Transfer of specific gene sequences
Methods	Germplasm utilization, pedigree methods, genotype x environment interactions	Accelerated introgression of genes, marker-assisted selection
Selection process	Selects for desirable recombination among large populations	Selects for defined trait phenotype and then introgressed transgenic event into desirable genetic backgrounds
Site of gene integration	Random integration of transgene, sometimes resulting in alteration of DNA sequence or disruption of recipient genome	Random integration, but site-specific integration is under development
Testing and release	Performance-testing of new cultivar	Performance-testing of transgenic event, followed by testing in broad range of desirable genetic backgrounds

History of Transgenics in Fruit Breeding

Transgenic fruit breeding is a relatively recent development in the field of fruit breeding. The history of transgenic fruit breeding can be traced back to the 1980s when researchers first started exploring the potential of genetic engineering to improve fruit crops. The first genetically modified (GM) fruit, the Flavr Savr tomato, was approved for commercial production in 1994. This tomato was genetically modified to delay the ripening process, which allowed it to remain on the vine longer and have a longer shelf life. Since then, transgenic fruit breeding has made significant progress. In 1985, the first transgenic fruit tree was created when researchers from the University of California, Davis, inserted a gene from a virus into a plum tree to create resistance to the plum pox virus. The success of this experiment opened the doors to further research and development in transgenic fruit breeding. (Scorza *et al*. 2013) [29]. In the 1990s, transgenic breeding of fruit crops gained more momentum, with researchers focusing on developing disease-resistant crops. One notable example is the development of the Rainbow papaya, which was genetically engineered to resist the papaya ringspot virus that had devastated the papaya industry in Hawaii. In the 2000s, the use of transgenic breeding in the fruit industry experienced growth with the development of several new genetically modified fruit varieties. For instance, the first genetically modified apple variety, the Arctic Apple, was approved for commercialization by the USDA in 2005. The Arctic Apple was engineered to resist browning. Currently 3 commercial varieties of arctic apples are available in the markets of USA i.e., Arctic Golden Delicious, Arctic Granny Smith recently released Arctic Fuji variety.

The Pinkglow pineapple, was approved by the FDA in 2013. Pinkglow pineapples are modified to have a pink colour instead of the typical yellow ones and produce less of the enzyme that causes fruit to rot. (Gomez *et al*. 2021.) [22] Nevertheless, the use of transgenic breeding in the fruit industry has been a controversial topic due to concerns over the safety of GM foods and the potential environmental impact. This has resulted in slower adoption of transgenic fruit breeding in some parts of the world, and in some countries, the cultivation of GM crops is banned entirely.

What Is Transgenic Breeding?

Transgenic breeding is a process of genetic modification in

which foreign DNA is introduced into the genome of an organism to create a new trait. This is achieved by transferring genes from one organism to another, resulting in the creation of a transgenic organism that possesses a specific desired trait. The foreign gene or modified gene is known as transgene. This transgene can be transferred from any related plant species, unrelated or wild plant species, micro-organisms like viruses & bacteria or even from animals.

Steps involved in developing a transgenic plant

1. Identification & isolation of desired gene.
2. Designing the transgene.
3. Introduction of transgene into plant cells.
4. Selection of transgenic cells/tissue (screening).
5. Regeneration of transgenic plant.

Isolation of desired gene

The process of isolating a desired gene from a tissue involves several steps. When it is known that the gene of interest is expressed in a particular tissue, a crude extract of that tissue is prepared and then purified to remove contaminants. Then, the desired gene can be identified by using various techniques such as PCR, DNA sequencing or hybridization. One of the methods used is the cDNA method, which involves the conversion of mRNA into cDNA using reverse transcriptase. The cDNA is then amplified by PCR using specific primers that match the desired gene sequence.

Once the PCR product has been generated, it can be purified and cloned into a vector for further study or manipulation. This process allows for the isolation of a specific gene of interest from the complex genomic material of a tissue sample.

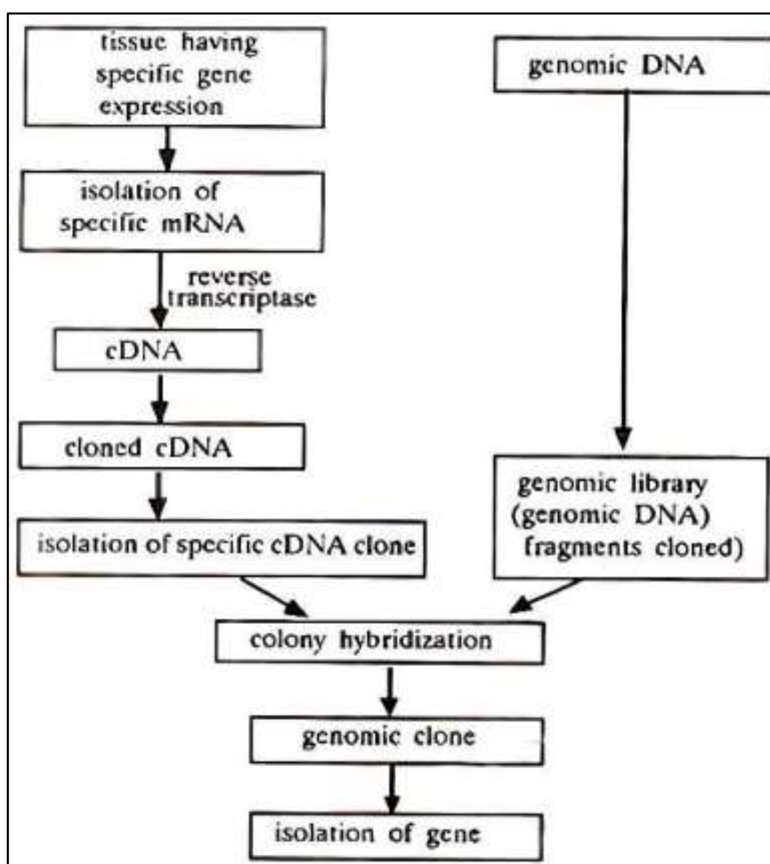


Fig 1: Isolation of desired gene from specific tissue by C-DNA method source: Gene cloning and DNA analysis An Introduction: T.A. BROWN

Designing the transgene

Designing a transgene involves several steps, starting with the identification of the gene of interest and its regulatory elements (as shown above). The gene of interest is usually identified based on its known function or through a screen of the relevant genetic material. The promoter region is the region of DNA that controls the expression of the gene, and it determines when and where the transgene is expressed. It is a DNA sequence in a transgene that initiates gene expression by binding to transcription factors that regulate transcription. Typically situated upstream of the coding region, it plays a vital role in determining the transgene's temporal and spatial expression pattern. In transgenic breeding, the selection of promoters is based on their capacity to drive strong, constitutive expression or tissue-specific expression. The CaMV 35S promoter and the ubiquitin promoter are commonly used promoters in transgene design.

Fruit-specific promoters: These are promoters that drive gene expression specifically in fruit tissues. Examples include the tomato polygalacturonase promoter and the apple *MADS-box* promoter The promoter selected depends on the transgene's intended use and the desired level and pattern of expression. The enhancer region is a regulatory element that can enhance the activity of the promoter, resulting in increased expression of the gene. The choice of promoter and enhancer can have a significant impact on the level and specificity of gene expression. Once the regulatory elements have been identified, they are combined with the gene of interest to create the transgene. The transgene is typically cloned into a vector, which is a small piece of DNA that can be easily manipulated in the laboratory. The vector can also contain other elements, such as selectable markers and reporter genes, to aid in the selection and characterization of transgenic cells.

A reporter gene is incorporated into a transgene to track the expression of the transgene in an organism. It is commonly used to study gene expression patterns, providing researchers with a way to visualize and quantify the expression of the transgene. The Green Fluorescent Protein (GFP) gene is a frequently used reporter gene as it produces a fluorescent protein that can be easily observed under a fluorescence microscope. Other examples of reporter genes include luciferase, beta-galactosidase, and alkaline phosphatase, with the choice depending on the experimental requirements and the detection method used. For instance, luciferase is used for non-invasive imaging studies, while beta-galactosidase (GUS) is preferred for histochemical assays (Kumar *et al*. 2019) [19].

A marker gene is a gene that is inserted along with the desired transgene in order to select the organisms that have successfully incorporated the transgene. These genes often encode for resistance to antibiotics or herbicides, such as the neomycin phosphotransferase (*nptII*) gene & glyphosate oxidoreductase (*gox*) respectively. However, the use of marker genes has raised concerns about their potential impact on non- target organisms and the environment. As a result, alternative methods for selecting transgenic organisms that do not involve marker genes have been developed (Chandran R *et al*. 2019) [8].

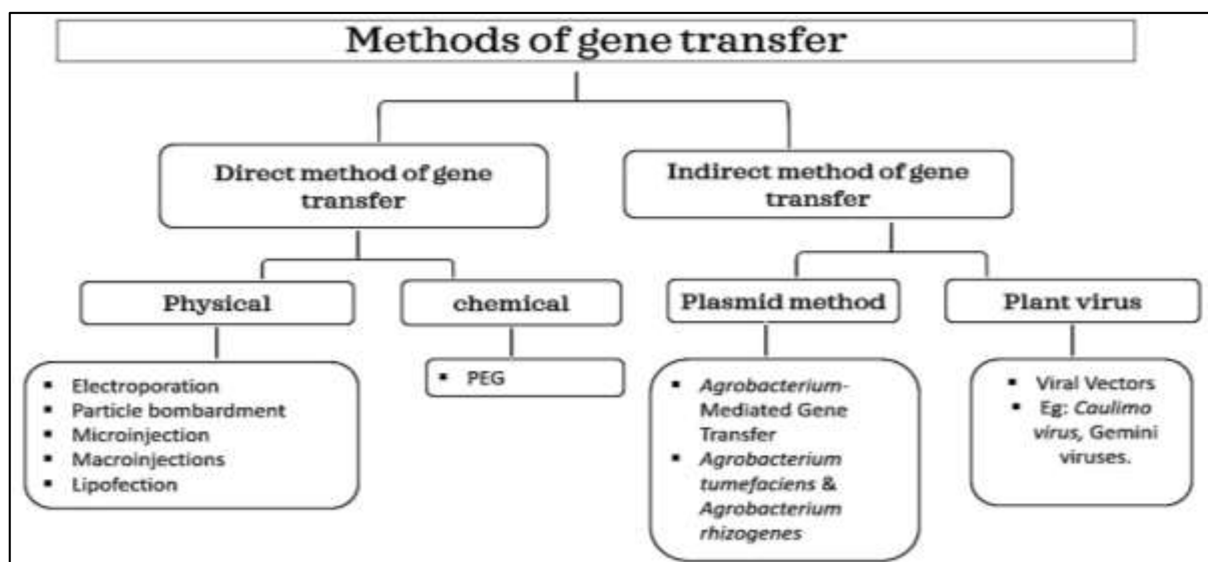
The design of a transgene can also involve the modification of the gene of interest to optimize its expression or function. For example, the addition of introns, which are non-coding regions of DNA, can enhance gene expression by increasing the stability and efficiency of mRNA production. Alternatively, the gene of interest may be modified to alter its activity or specificity, such as through the addition of a tag or fusion protein.

Fig 2: A modified transgene

Introduction of transgene into plant cells:

The process of introducing a transgene into a host plant involves the transfer of the designed transgene into the plant's genome. This can be accomplished through two different methods i.e., (i) Direct method of gene transfer (ii) Indirect method of gene transfer.

These methods are further classified into following categories.



Direct method of gene transfer

Electroporation

Electroporation is a gene transfer method that involves the use of an electric pulses of 1000-1500 V/cm for 15-20 milliseconds to temporarily create pores in the cell membrane, facilitating the entry of DNA into the cells. It is a versatile technique that can be used to introduce DNA into various types of cells such as mammalian, bacterial, and plant cells. Compared to other methods of gene transfer, electroporation has benefits such as high efficiency, minimal toxicity, and the capacity to transfer large DNA fragments. Nevertheless, the process can be difficult to optimize for different cell types and may result in cell damage or death when high voltages are applied. (Zhang *et al*. 2015) [20].

Electroporation offers numerous advantages over other methods such as high efficiency, low toxicity, and the capability to transfer larger DNA fragments. However, optimizing the method for different cell types can be difficult, and it may cause cell damage or death when high voltages are applied.

Transgenic sweet orange was obtained by electroporation (Niedz *et al*. 1995) [26].

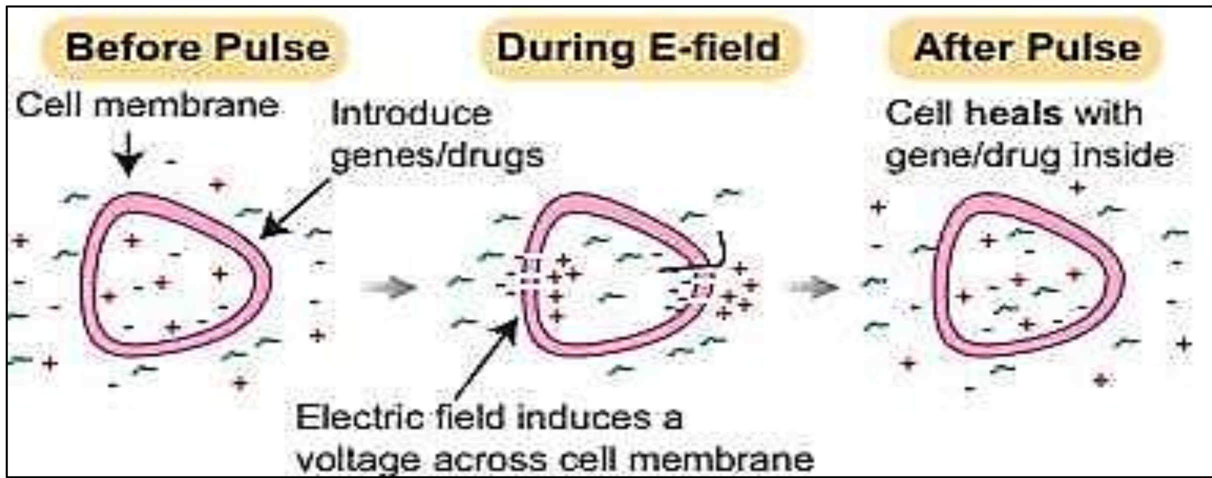


Fig 3: Electroporation method, Source NCERT XII biology

Particle bombardment method:

The biolistic method, which is also called particle bombardment, is a gene transfer technique that introduces foreign DNA into cells. This method involves coating microscopic particles like gold or tungsten with the DNA of interest, and then accelerating them to high velocities (1000 ft/s) using a gene gun. These particles are then shot into the target cells where they penetrate the cell wall and reach the cell nucleus. Inside the nucleus, the foreign DNA can integrate into the host genome and be expressed by the host cell. The biolistic method offers several advantages over other gene transfer methods. It can introduce large DNA fragments, and it is applicable for a wide range of cell types and organisms including both plant and animal cells. Despite its advantages, this method can cause damage to the target cells and is less efficient compared to other gene transfer methods. To enhance the efficiency of the method, optimization of factors such as particle size and composition, gene gun velocity and angle, and target cell conditions is required.

Ex. Transgenic papaya Rainbow and SunUp were developed using particle bombardment method Fitch *et al*. (1992) [5].

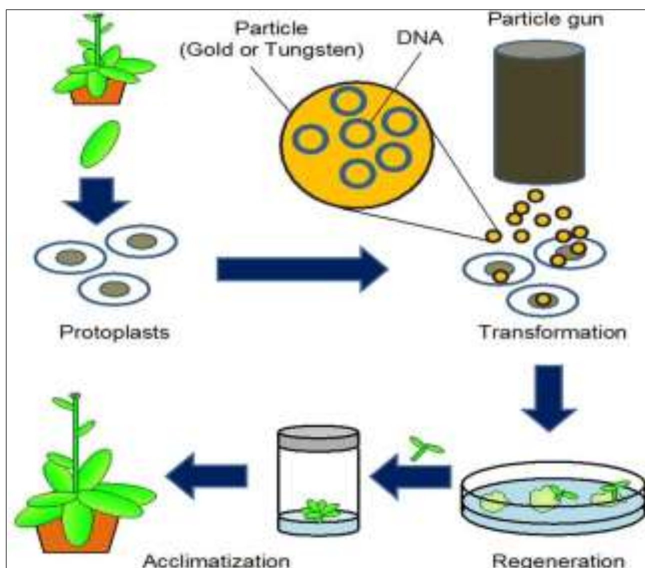


Fig 4: Particle bombardment method

Micro-injection method

Microinjection uses micro capillaries and microscopic devices to deliver DNA into defined cells in such a way that the injected cell survives and can proliferate. Plant cells are transformed by directly injecting DNA into the nucleus using a micro pipette or fine-tipped glass needle. During this process, protoplasts can be immobilized on a solid support, such as agarose on a microscopic slide, or held in place with a holding pipette using suction.

Lipofection method

This technique involves the transfer of genes or DNA from a liposome into the vacuole of plant cells. This method protects the introduced DNA from damage caused by the acidic pH and protease enzymes present in the vacuole by encapsulating the DNA. Gene transfer is achieved through the fusion of the liposome and tonoplast of the vacuole. This technique is also known as liposome-mediated gene transfer or lipofection.

Chemical method

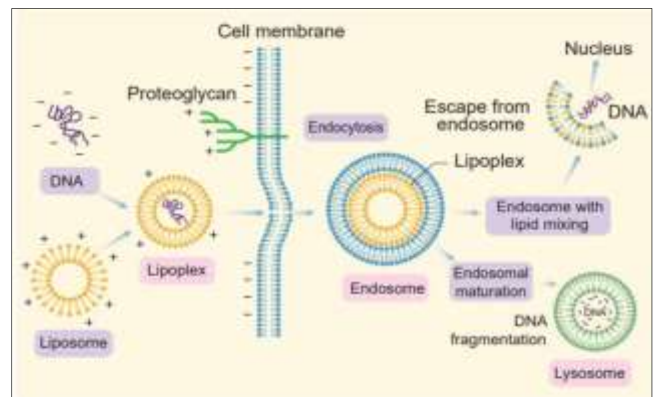


Fig 5: Lipofection method, Source NCERT XII biology

Chemical methods of gene transfer utilize chemicals to introduce foreign DNA into cells. Various methods are available, such as Polyethylene glycol, calcium phosphate transfection, DEAE-dextran transfection. In calcium phosphate transfection, the DNA and calcium phosphate form a complex that is taken up by the cell via endocytosis. DEAE-dextran transfection utilizes DEAE-dextran, a polycationic compound that neutralizes DNA's negative charge, allowing it to enter the cell via endocytosis. Chemical methods have several benefits, including their ability to transfect many cell types, low cost, and relative ease of use compared to other methods. However, their effectiveness can vary depending on cell type and conditions, and the chemicals employed may be

harmful to target cells. Optimal conditions such as the lipid-DNA ratio, the size and composition of the lipid particles, and the cell type utilized can improve efficiency.

Indirect method of gene transfer

Indirect methods of gene transfer in plants are performed through the use of vectors, such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, which naturally transfer DNA into plant cells. These bacteria possess the ability to integrate their own DNA into the host plant genome, leading to the formation of tumor-like growths in infected plants. Scientists have utilized this natural trait of *Agrobacterium* to develop methods for introducing foreign DNA into plants. This method has numerous advantages, such as the capacity to transfer large fragments of DNA, and it is widely utilized in plant genetic engineering.

Some other important vectors

1. **Plasmids:** plasmids are circular DNA molecules that have self-replicating ability and are often used for gene cloning and expression in both prokaryotic and eukaryotic cells.
2. **Bacterial artificial chromosomes (BACs):** BACs are large, artificial chromosomes derived from bacterial plasmids and commonly used for genomic research and gene mapping.
3. **Yeast artificial chromosomes (YACs):** These are derived from yeast cells and can carry large inserts of DNA, often used in genomic research and for cloning and expressing large genes.
4. **Viral vectors:** These modified viruses that infect cells and introduce foreign DNA into the host genome, commonly used for gene therapy and gene transfer.
5. **Cosmids:** These are hybrid plasmid vectors that carry large fragments of DNA, commonly used for cloning and mapping large genomic regions.
6. **Retroviral vectors:** These are viral vectors that use retroviruses to introduce foreign DNA into host cells, commonly used for gene therapy and gene transfer in eukaryotic cells. Transposons are DNA sequences that can move within the genome, used as vectors for gene transfer and tools for genetic manipulation.

***Agrobacterium* mediated gene transfer in plants**

Agrobacterium tumefaciens mediated gene transfer is a process of genetic modification that employs *Agrobacterium tumefaciens*, a natural plant pathogen, as a vector to introduce foreign DNA into plant cells. *Agrobacterium tumefaciens* is a gram-negative bacterium that causes tumor/crown gall disease in plants. This bacterium is known as a natural genetic engineer and is widely used in agriculture for genetic transformation. The method involves the modification of a plasmid vector, which induces the formation of tumor-like growths in plants. The T region of the plasmid of this bacterium has oncogenes that causes tumor. These oncogenes are removed, and our desired gene is inserted into this disarmed Ti plasmid. This bacterium containing recombinant plasmid is co-cultured with the protoplast of host cells that are to be transformed. During co-culture, plant cells release acetosyringone which enables the *vir* genes to release T-DNA and transfer it in plant cells. The transgene is then expressed in the plant, resulting in the desired trait.

Agrobacterium tumefaciens mediated gene transfer is a widely used method for genetic engineering in plants due to its high efficiency, ability to transfer large fragments of DNA, and ability to transfer genes to a wide range of plant species. However, the method has some limitations, such as a limited host range, and the possibility of disrupting the plant's natural genetic makeup.

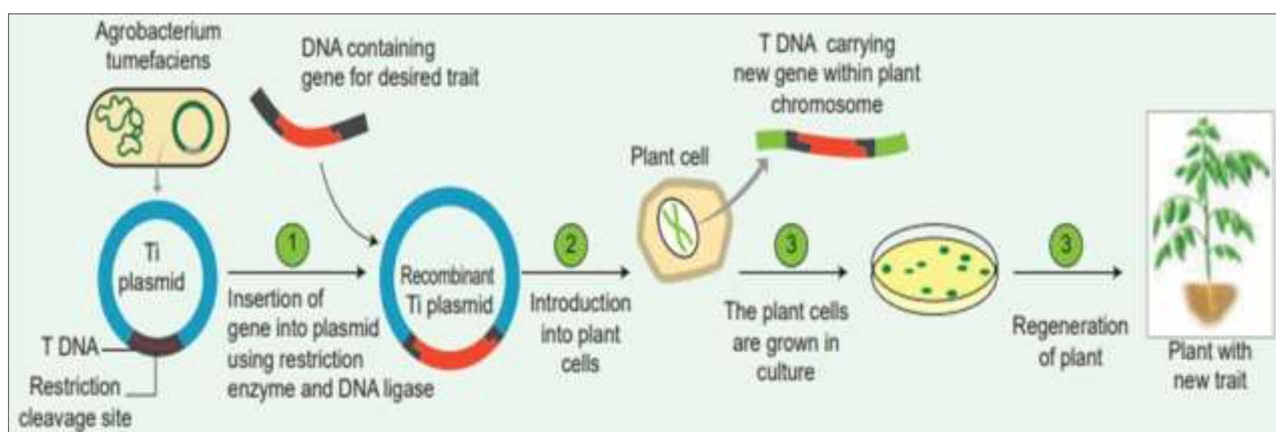


Fig 6: *Agrobacterium* mediated gene transfer in plants TN botany class XII

Selection of transgenic cells/tissue (screening)

After the foreign gene has been introduced into the plant cells using a vector, the next step is to identify the cells or tissues that have successfully incorporated the gene. This process is known as selection or screening. Selection is necessary because not all cells or tissues that have been exposed to the foreign gene will actually incorporate it into their genome. Therefore, it is important to identify the ones that have incorporated the gene so that they can be used for further experiments or for generating transgenic plants. Selection can be achieved using a variety of methods, including the use of selectable markers, such as antibiotic or herbicide resistance genes, that are co-introduced with the gene of interest. These markers enable the selection of transformed cells or tissues by providing a means of identifying and eliminating non-transformed cells.

Another common selection method is the use of visual markers, such as fluorescent or coloured proteins, which enable the identification of transformed cells or tissues under a microscope. In some cases, the gene of interest itself may confer a visual or physiological trait that can be used for selection. Once the transformed cells or tissues have been identified, they can be cultured or regenerated to produce transgenic plants. The selected cells or tissues are placed in

culture media containing hormones and nutrients to promote growth and development. After a few weeks, the resulting plantlets can be transferred to soil and grown to maturity.

Regeneration of transgenic plant

The regeneration of transgenic plants is a crucial step in plant genetic engineering. After the selection of transgenic cells/tissues, the next step is to regenerate whole plants from the transformed cells. The regeneration process varies depending on the plant species, the tissue used for transformation, and the method of transformation. In general, the regeneration process involves inducing the transgenic cells to form a callus, which is an undifferentiated mass of cells that can be induced to differentiate into various plant tissues. The callus is then subjected to a series of steps to induce the formation of shoots, which can then be rooted to form whole plants. The regeneration process can be a major bottleneck in plant genetic engineering, as not all cells will regenerate into whole plants, and the process can be time-consuming and labour-intensive. However, the development of new techniques, such as tissue culture and bioreactor systems, has greatly improved the efficiency and speed of plant regeneration.

Status of Transgenic Fruit Crops

According to a report published by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) in 2018, genetically engineered fruits occupied less than 0.01% of the total 185.43 million hectares of land cultivated with genetically engineered crops. The most widely cultivated genetically engineered fruit was the PRSV-resistant papaya, followed by Arctic® apples, and Pinkglow™ pineapple.

Fig 7: Achievements of Transgenic Breeding in Fruit Crops

Table 1: Production and adoption rates of genetically engineered fruits on the market. Adoption rate = ha of transgenic crop (dark orange)/total ha of crop (light orange). Source: Maria Lobato Gómez *et al.* 2021 [21].

Fruit crops	Traits	Research	Reference
Apple	• Reduced polyphenol oxidase • Juvenile stage reduced. • Resistance to fire blight • Apple scab resistance	• PPO suppression. • *BpMADS4,* silencing *MdTFL1* • *FB_mr5* fire blight resist. gene from crab apples • Endo-chitinase gene	Ko. K *et al.* (2000) [24] Faize M, Malnoy, Dupuis F, Chevalier M, Parisi L *et al.* (2003) [3] Broggini *et al.* (2014) [39] Bolar JP *et al.* (2000) [2]
Citrus	• Enhanced resistance to citrus canker • Resistance to tristeza virus (in Mexican lime & Duncan grapefruit) • Resistance to citrus psorosis virus (Duncan grapefruit)	• cDNA of *XA21* (*Xanthomonas* resistance gene in rice) • CTV-CP • CPV Coat protein	Moore *et al.* 1992 [40]; Gutierrez *et al.* 1997 [41] Grosser *et al.* (2009) [42] (Fagoaga *et al*, 2001) [43]
	• Resistance to *Phytophthora citrophthora* • (Pineapple)	• *PR-5* gene	
Papaya	• PRSV resistance	• Coat Protein gene	Fitch *et al.* (1992) [5]
Grapes	• Grapevine fanleaf nepovirus resistance • Improved rooting	• Coat protein gene • *rolB* gene from *A.rhizogenes* inserted into cv. "Richter 110"	Geier *et al.* (2008) [12]
Plum	• Resistance to Plum pox virus (PPV)	• PPV coat protein	Scorza *et al.* (2001) [38]

Non-browning transgenic apples: Arctic apples

Arctic® apples are a type of genetically modified apple that do not turn brown when sliced or bruised, due to the suppression of the genes responsible for producing polyphenol oxidase (PPO), the enzyme that causes browning. The Arctic® apple was developed by Okanagan Specialty Fruits, Inc. using biotechnology techniques, specifically RNA interference (RNAi), to silence the expression of the PPO

genes. The suppression of apple PPO genes was achieved by using RNA interference (RNAi) technology. A chimeric sense RNA containing partial coding sequences of four PPO genes (*PPO2, GPO3, APO5, and pSR7*) was expressed, which generated dsRNA and led to the suppression of homologous genes through post-transcriptional silencing.
These apples were initially planted in 2016 on 80 hectares of land, with 70,000 trees. By 2018, the number of trees had increased to 300,000 over 101 hectares. In 2019, the area under cultivation exceeded 500 hectares. Although the profitability of growing Arctic® apples is not publicly known, Okanagan Specialty Fruits claims that they are more suitable for mechanical harvesting and suffer less superficial damage, resulting in higher yields and less waste. The Arctic® Golden variety does not require warm packing, which lowers the production cost. The non-browning trait of Arctic® apples has several potential benefits, including reducing food waste by extending the shelf life of sliced apples, and making them more appealing to consumers.

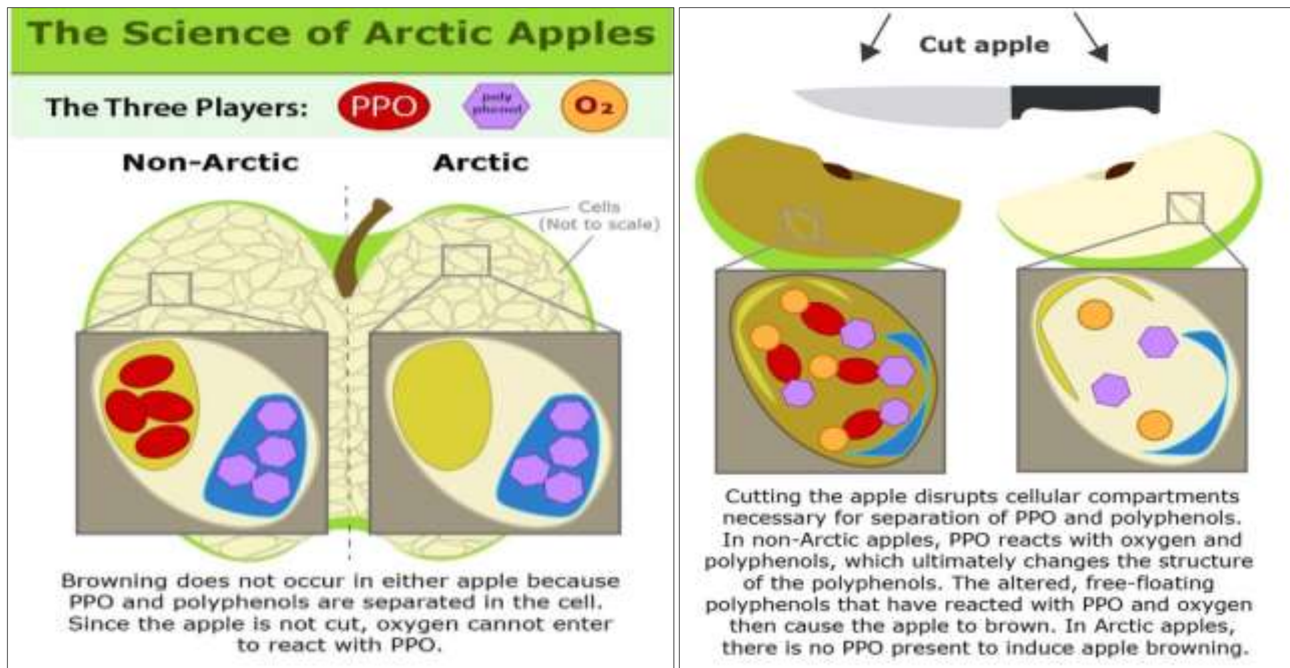


Fig 7: The science of arctic apple Source: Stowe & Dhingra. 2021

PRSV resistant transgenic papaya:
The science of transgenic papaya involves the genetic modification of papaya plants to make them resistant to the devastating Papaya ringspot virus (PRSV). The genetic modification involves the insertion of a coat protein gene from the PRSV virus into the papaya genome, which allows the papaya plant to produce the coat protein that can provide immunity to the virus. This method was developed in the 1990s by Denis Gonsalves and other scientists at the University of Hawaii in response to the widespread destruction of papaya crops in Hawaii due to the PRSV virus.
Papaya ringspot virus raised havoc on papaya farms from the time it first appeared in early 1990s in Puna, Hawaii's major papaya-growing region. Transformation of embryogenic cultures of papaya was carried out in 1988 using the particle bombardment method in commercial cultivars of 'Sunrise', its sib 'Sunset', and 'Kapoho'.
A coat protein gene from the ringspot virus was inserted into the papaya, where it acts like a built-in vaccine against the virus. Two new transgenic cultivars were developed 'SunUp' and 'Rainbow' in the year 1991.
'SunUp' is a transgenic, red-fleshed Sunset that is homozygous for the coat protein gene. 'Rainbow' is a yellow-fleshed F1 hybrid developed by crossing 'SunUp' and non-transgenic yellow-fleshed 'Kapoho.'
The transgenic papaya was first approved for commercial use in Hawaii in 1998, and since then, it has been widely adopted by farmers in Hawaii and other countries where the virus is a significant threat to papaya production.
The development of transgenic papaya has been hailed as a significant success story in the field of genetic engineering, as it has helped to save the papaya industry in Hawaii and other parts of the world. The Rainbow papaya, a popular cultivar of transgenic papaya, is now widely available in supermarkets and grocery stores in the United States and other countries.

The transgenic Pinkglow pineapple
The Pinkglow™ pineapple was developed by Del Monte using RNA interference (RNAi) technology to modulate the carotenoid pathway. The company used their commercial variety, 'MD2' or Del Monte Gold, as the starting material. The Pinkglow™ pineapple expresses the tangerine *PSY* gene, a rate-limiting enzyme in carotenoid biosynthesis, and suppresses the endogenous lycopene β and ε cyclase genes (*βLYC* and *εLYC*) by RNAi. Additionally, a meristem-specific ACC synthase (*ACS*) was suppressed by RNAi to inhibit flowering. (Gomez *et al*. 2021) [22].
Del Monte patented the transformation method, which involved the cultivation of organogenic pineapple cells with A. tumefaciens. The conventional pineapple has yellow flesh, whereas the Pinkglow™ pineapple has pink flesh due to the modulation of the carotenoid pathway.

Honey Sweet Plum
The Honey Sweet plum is a plum variety that has been genetically modified to be resistant to the Plum Pox Virus (PPV), which can cause serious damage to stone fruit crops. This was achieved using the "coat protein gene" approach,

where the virus-resistant gene from PPV was inserted into the plum genome. The inserted gene produces a protein similar to the virus coat protein, which activates the plant's immune system to resist the virus.
Since being deregulated in the US in 2004, the Honey Sweet plum has been approved for commercial cultivation in the US and Canada. It possesses other desirable traits such as high sweetness, juicy texture, and a longer shelf life. The Honey Sweet plum has been proven safe for consumption and does not have any negative effects on the environment. Research shows that it contains similar levels of nutrients and antioxidants as non-genetically modified plums.

Other successful genetic transformation in fruit trees
Strawberry
The modern cultivated strawberry is a hybrid of two wild species and is susceptible to diseases and environmental stresses. Genes for disease resistance are found in related wild species. Organogenesis and somatic embryogenesis have been achieved from various plant parts. Genetic transformation has been mediated by *A. tumefaciens*, biolistic transformation, and protoplast electroporation. The factors involved in *Agrobacterium*-mediated transformation have been studied. (Husaini *et al*. 2010) [16].

Banana
Classical breeding methods pose challenges for banana and plantain due to their triploid nature and sterility, except for a few genotypes. Biotechnologies such as mutation breeding and genetic transformation have been explored to achieve specific breeding objectives for these crops, especially targeting disease resistance against Panama disease and Black Sigatoka.
Researchers have focused on *Agrobacterium*-mediated transformation of embryogenic cultures using microprojectile bombardment, and the transformation of *Musa* protoplasts by electroporation has been replaced by these methods. Different banana cultivars have been transformed using embryogenic suspension cultures, scalped shoot cultures, and meristems of *in vitro* plantlets, and the efficiency of plant regeneration varies depending on the transformation method used Sagi *et al*. (1994) [27].

Apple
Researchers have successfully utilized genetic modification techniques to improve various traits in fruit trees. For instance, the *BpMADS4* gene from silver birch has been introduced to shorten the juvenile period of 'Pinova' trees, which could reduce the time needed for conventional breeding programs. Another gene, *MdTFL1*, has been silenced to achieve the same result. Additionally, the transcription factor *MdMYB10* has been used to enhance the accumulation of anthocyanin, which determines the red colour of fruit, potentially leading to the development of new cultivars. Finally, by altering the levels of the gene for sorbitol-6-phosphate dehydrogenase, researchers have studied the relationship between sorbitol synthesis in leaves and glucose, fructose, starch, and malic acid accumulation in apple fruit. (Flachowsky *et al*. 2008) [10].

Pear
Mourgues *et al*. (1996) [25] reported the first successful transformation of pear by targeting wounded leaves of micro propagated 'Conference', 'Doyenné du Comice', and 'Passe Crassane' cultivars with *A. tumefaciens* strain EHA101 containing the binary vector pFAJ3000, which carried the *npt II* and *uidA* genes.

Passion fruit
Passion fruit genetic transformation is being used to combat disease problems such as passion fruit woodiness, Fusarium wilt, and bacterial blight. The first reported A. tumefaciens-mediated transformation of passion fruit was done by Manders *et al*. (1994) [46] using the pMON200 vector. Alfenas *et al*. (2005) [44] recovered transgenic plants resistant to CABMV from hypocotyl segments using the pBI121 binary vector. Trevisan *et al*. (2006) transformed passion fruit with the full-length CP of CABMV in pCAMBIA 2300, and Takahashi (2002) [45] and Monteiro (2005) [23] used biolistic method to transform passion fruit with the *attA* gene to confer resistance to bacterial blight.

Limitations of Transgenic Breeding
Instable performance: The term "instable performance of transgenic crops" refers to the inconsistency or loss of the desired traits of a transgenic crop over time, which can result in lower yields or other unintended effects. This may be caused by factors such as genetic instability or environmental factors and can lead to unexpected outcomes. For instance, a transgenic crop that was developed to be resistant to a particular pest or disease may lose its resistance over time due to genetic instability or the evolution of the pest or disease. Due to the unpredictability of the long-term performance of transgenic crops, it is important to carefully monitor and evaluate their performance.

High cost: Transgenic breeding involves complex procedures, including genetic engineering and transformation, which can be expensive.

Pleiotropic effects: Pleiotropic effects are when a single gene or genetic modification affects multiple traits in an organism. In transgenic breeding, this can result in unintended consequences, where a gene added to enhance one trait may also impact other traits. For example, a gene that increases fruit size and yield may also make the plant more susceptible to pests or diseases, or less able to withstand environmental stress. These unintended effects may only become apparent after the transgenic plant is released or consumed, making it challenging to predict and manage potential risks. It is crucial to conduct thorough testing and evaluation of transgenic plants before commercial use to prevent these unintended consequences.

Regulatory issues: The use of transgenic breeding is strictly regulated in many countries, which can limit its use and increase the time and cost of obtaining regulatory approval.

Public acceptance: There is often public concern and opposition to genetically modified organisms (GMOs), which can limit the commercial success of transgenic crops.

Limited trait selection: Transgenic breeding is currently limited to the introduction of a small number of genes, which can limit the range of traits that can be introduced into crops.

Risk of creating new pests: The introduction of new genes into crops can create new pest and disease problems that were not present before.

Intellectual property issues: The use of transgenic breeding can raise intellectual property issues, as companies may patent the genes used in their transgenic crops, which can limit access to these genes by other researchers and breeders.

Future Challenges in Transgenic Breeding

Addressing public concerns: One of the major challenges in transgenic breeding is addressing the public's concerns and perceptions about genetically modified organisms (GMOs) and their potential impacts on human health and the environment. This requires effective communication and outreach strategies to help build trust and understanding of the technology.

Increasing trait diversity: The success of transgenic breeding depends on the availability of genes that confer desirable traits. To expand the range of traits available for manipulation, efforts are needed to discover and characterize new genes, as well as to develop tools and techniques for their efficient transfer.

Overcoming regulatory hurdles: Transgenic breeding is subject to strict regulatory oversight, which can be time-consuming and costly. To facilitate the development and commercialization of new transgenic crops, efforts are needed to streamline regulatory processes and establish international standards for safety assessment.

Addressing intellectual property issues: Intellectual property rights are a critical aspect of transgenic breeding, as they determine who has the right to use and commercialize specific genes or traits. To ensure fair and equitable access to transgenic technologies, efforts are needed to develop clear and transparent licensing and royalty arrangements.

Improving the efficiency of gene transfer: One of the challenges in transgenic breeding is the low efficiency of gene transfer and integration. To improve the efficiency of transgene insertion, efforts are needed to develop new delivery methods and optimize gene transfer protocols.

Mitigating unintended effects: Unintended effects of transgenic crops on non-target organisms or the environment can have negative consequences. To minimize these effects, it is important to conduct thorough biosafety assessments and to design transgenic crops that have minimal impacts on the environment and non-target organisms.

Addressing ethical and social issues: The development and commercialization of transgenic crops raises a range of ethical and social issues, such as concerns about the impact of transgenic crops on small-scale farmers, food security, and access to genetic resources. To address these issues, efforts are needed to engage stakeholders and ensure that the benefits of transgenic breeding are distributed equitably.

Future Strategies for Genetic Engineering & Transgenics

Gene editing: Gene editing tools such as CRISPR-Cas9 have the potential to revolutionize transgenic breeding by allowing for precise modification of genes without introducing foreign DNA. This could enable the creation of transgenic fruits with desired traits while avoiding some of the concerns associated with traditional transgenic breeding.

Multi-trait stacking: Multi-trait stacking involves the simultaneous incorporation of multiple desirable traits into a single plant. This could lead to the development of transgenic fruits that exhibit multiple beneficial traits such as increased yield, improved flavour, and resistance to multiple pests and diseases.

Epigenetic modification: Epigenetic modifications can alter gene expression without changing the DNA sequence itself. This could allow for the modification of gene expression in transgenic fruits without introducing foreign DNA.

Transcriptomics and proteomics: Advances in transcriptomics and proteomics have made it possible to study gene expression and protein function at a large scale. This could lead to a better understanding of the molecular mechanisms underlying desirable fruit traits and could facilitate the identification of new targets for transgenic breeding.

Sustainable production: Transgenic breeding can be used to develop fruits that are more resilient to climate change and require less water and fertilizer. This could help to ensure the sustainability of fruit production in the face of increasing environmental pressures.

Conclusion

Transgenic breeding has the potential to revolutionize the fruit industry by offering precise and targeted genetic modifications that can improve fruit quality, yield, and disease resistance. However, the commercialization and acceptance of transgenic fruits have faced significant challenges due to regulatory hurdles, consumer perception, and the lack of clear benefits for growers. Despite the challenges, the increasing demand for sustainable and efficient agricultural practices, along with the need to meet global food demands, necessitates the exploration of alternative breeding strategies, including transgenics. To this end, researchers and breeders continue to invest in advancing transgenic technology on *Agrobacterium-mediated* transformation, such as trans-grafting, Fast-Track breeding, *intra-genesis*, and genome editing, which may provide solutions for the problems faced by the global tree fruit industry. Furthermore, the use of transgenic technology in fruit breeding should not be viewed as a replacement for conventional breeding techniques but rather as a complementary approach that can enhance the efficiency and precision of fruit breeding programs. Collaborative efforts between researchers, breeders, and regulatory agencies are essential to ensure the safe and effective development of transgenic fruit varieties and their commercialization. In conclusion, transgenic technology offers immense potential for improving fruit crops' genetic traits and enhancing their productivity and quality. The technology's development and deployment will depend on the effectiveness of the regulatory frameworks, public acceptance, and the continued investment in research and development. Transgenic fruit breeding has a bright future, and with continued support and advancement, it can contribute to achieving sustainable agriculture and food security.

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