Genetic Engineering Techniques in Fruit Crops

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Introduction

Genetic engineering is a powerful method for plant development that has the potential to allow desirable features to be integrated into existing genomes. Transformation technology has paved the way for crucial genes to be transferred into plant genomes for improving resistance to fungal, viral and other pests, drought and salinity, as well as silencing undesired genes and improving nutrient acquisition (Mallikarjuna *et al*., 2016).

Advantages

- GM Technology reduces the number of backcross generations.
- The ideal scenario being targeted gene editing with no alteration of the genetic background (no off-target effects). Thus GM breeding has the potential to be extremely fast.

Disadvantages

- One of the most significant drawbacks of GM breeding is that the target gene must be identified and sequenced.
- It necessitates specialist laboratories and is costly, while less expensive, simpler alternatives are being developed (Forster *et al*., 2015).

Methods of genetic transformation are usually divided into two categories:

- Indirect transformation.
- Direct transformation methods.

Direct Gene Transformation Methods

The methods by which foreign DNA is directly inserted into the plant genome then it is known as direct gene transformation methods. The introduction of naked DNA into plant cells is used in direct DNA transfer procedures. The majority of direct DNA transfer procedures are recognized to be simple and efficient. These methods have resulted in the development of several transgenic plants.

a. Particle bombardment

In biolistics technology, DNA is coated onto gold or tungsten micro-particles and bombarded at high velocity in a stream of helium into intact cells or tissues. The biolistic procedure is divided into two stages: 1. Coating metal particles (microprojectiles) with nucleic acid, and 2. Accelerating the coated microprojectiles to velocities suitable for penetrating target cells or tissues without causing severe biological disturbance. Because it does not require the manipulation of genetically engineered organisms like *Agrobacterium*, biolistic transformation is simple and safe. It also enables for the co-transformation of numerous constructs (Agrawal *et al*., 2005 and Naqvi *et al*., 2009) and the integration of larger transgenes (Alpeter *et al*., 2005). The co - transfer of large portions of the vector backbone DNA, which can severely affect transgenic expression, is one negative aspect of biolistics (Hammond *et al*., 2001 and Tassy *et al*., 2014).

b. Electroporation

The treatment of plant cells with short high-voltage electric pulses is known as electroporation. For high molecular particles like DNA, the electric pulse shock generates a temporary permeability of the plasmalemma (Bates *et al.*, 1989). The transport of DNA occurs through pores generated in the cytoplasmic membrane as a result of electric pulses (Sowers *et al.*, 1992). The pores have a temporal nature and are linked to the enhanced dipole moment of hydrophilic heads that make up cell membrane lipids. Phospholipid dipole heads dislocate in the direction of the electric field, causing breaches in the cell membrane's integrity. The specific effect of the electric field on tissues cultured in vitro was determined by analyzing the growth of isolated protoplasts as well as with protoplast-derived calli of Colt cherry (*Prunus avium* × *P. pseudocerasus*). Analyzing the growth of isolated protoplasts as well as protoplast-derived calli of Colt cherry (*Prunus avium* × *P. pseudocerasus*) was used to evaluate the specific influence of the electric field on tissues cultivated in vitro. The ability of electroporated tissues to regenerate plants was also examined. The callus made from protoplasts and exposed to three exponential pulses at 250 V or 500 V demonstrated the greatest fresh weight gains between subcultures (Ochatt *et al.*, 1988). Plant regeneration was achieved through secondary somatic embryogenesis when embryo and somatic embryos at the torpedo stage of coffee were electroporated with DNA containing the *gus* and *bar* genes.

A. PEG (Poly Ethylene Glycol)

Rearrangement of transgene sequences has been seen during protoplast transformation employing PEG transgenes, which can be integrated in single copies as well as multiple copies linked together or at independent loci. Protoplasts are advantageous as a starting material because they are totipotent, allowing transgenic plants to be regenerated from single cells without chimaeras. The protoplast's' cell cycle stage appears to play a role in the integration pattern, with protoplasts in M phase (mitotic phase) producing transgenic plants with more copies of the transforming plasmid, frequently at different loci. High copy numbers and frequent plasmid sequence rearrangement occur in protoplasts during the S phase (DNA synthesis phase) (Kartzke *et al*., 1990). PEG has often resulted in low transformation frequencies (less than 1 per cent of treated cells). Using effective selection techniques, however, a high number of transgenic plants can be created due to the availability of a large number of cells in such systems.

B. Sonication

The temporary permeability of the plasma membrane can be altered by sonication (ultrasound) to enhance uptake (Tachibana *et al.*, 1999). The ultrasound treatment may be easier to perform than other direct DNA delivery methods such as particle gun bombardment, electroporation and microinjection. Sonication, however, could cause cell damage or ever rupture. Regardless of the nature of the plant material to be transformed, gene transfer by ultrasonication follows the same simple procedure (Liu *et al.*, 2006).

Indirect Gene Transformation Methods

The bacteria of the genus *Agrobacterium* are mostly soil-dwelling and plant-associated. Crown gall disease is caused by phytopathogenic strains that have a tumor-inducing (Ti) plasmid in their genome, whereas hairy root disease is caused by strains that have a root-inducing (RI plasmid in their genome. The T-DNA region, which is flanked by left and right repetitions on the TI (tumor-inducing) plasmid, enables the transfer of DNA encompassed by these border sections. On their Ti plasmids, some *Agro bacterium species* contain more than one T-

DNA, resulting in more than two T-DNA boundaries from which T-DNA can be processed.

a. Agrolistics/ Agrolytics

The agrolistics strategy combines the benefits of efficient biolistic delivery with the precision of the *Agrobacterium* T-DNA insertion mechanism, reducing homologous areas that cause genetic and/or epigenetic instability. For some plant species, biolistic transformation is the preferred strategy, although many of the integration events that arise from these changes are undesirable. It is possible to achieve relatively predictable inserts in plants that are not ordinarily transformable using *Agrobacterium* by combining aspects of *Agrobacterium*-mediated transformation (Sharma *et al*., 2005).

b. Sonication-Assisted Agrobacterium-Mediated Transformation (SAAT)

In the presence of *Agrobacterium*, plant tissue is subjected to brief periods of ultrasound, which is an important modification in *Agrobacterium*-mediated transformation. SAAT treatment causes a large number of small and uniform wounds throughout the tissue, allowing easy access to the *Agrobacterium* and improving transformation efficiency in a variety of plant tissues, including immature cotyledons, leaf tissue, suspension cultures, somatic and zygotic embryos (Sharma *et al*., 2005).Many experiments have recently shown that SAAT significantly increased the efficiency of *Agrobacterium* infection by introducing a large number of micro-wounds into the target plant cells or tissues. In the *Agrobacterium*-mediated transformation of various fruit crops, SAAT improved transformation efficiency. For example, *Prunus mume* and *Vitis vinifera*.

Modern Genetic Engineering Techniques

Several innovative strategies have been created and are being used to enhance the breeding of superior crop varieties over the last 15 years. These procedures, when compared to traditional breeding, increase the precision with which changes in the genomes are made, reducing the time and effort required to develop varieties that meet new requirements. The employment of a GM phase is a common denominator throughout these approaches, but the end result is products with no foreign genes (i.e., genes not from the species itself or from cross-compatible species).GM is typically defined as a change in genotype caused by the insertion or alteration of a specific DNA sequence using artificial delivery systems and recombinant DNA technology. Earlier, GM technology centered on inserting DNA from a foreign species, but there has been a shift away from transgenics (foreign DNA insertion) to cisgenics (same species DNA insertion) and, more recently, targeted mutagenesis (genome editing) of a preferred genotype. These innovative methods includes TALENs, CRISPR/Cas9, Zinc Finger Nucleases (ZFNs) and RNAi and micro RNA technology.

Conclusion

Many fruit and vegetable crops, such as strawberry (*Fragaria* × *ananassa*), apple (*Malus* × *domestica*) and sweet orange (*Citrus sinensis*), are developed through hybridization and selection. The development of seedless horticultural crops such as watermelon using diploid and tetraploid parents is another application of hybridization breeding. Crop hybridization breeding, on the other hand, has some constraints that are quite difficult to overcome. Although fast track breeding techniques and genetic engineering approaches may speed up breeding and selection procedures, this takes tremendous amount of manpower and land resources. Recently, in addition to classical gene transfer technology, modern genetic engineering methods also have been started to apply for many plant species. It seems that the techniques illustrated in the present literature will be more important with combination of classical plant breeding.

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