Review Article

Advancing Fruit Crop Improvement through Transgenic Strategies

ABSTRACT

India is second in the world after China in production fruits around the world and these crops play one Important role in human diet because they provide Vitamins, minerals, diet fiber and phytochemicals. The flower market in India is growing at the rising rate and the export capacity of such crops is very high in global market. Horticulture production suffers from many abiotic and biotic stress due to pathogens, pests and weeds and per large amount of plant protection products are required Hectares. Transgenic crops (genetically modified/GM), Crops enable breeders to bring favorable genes, often Already inaccessible, already in elite farming, improving their value significantly and providing unique Opportunities to control insects and other pathogens. This novel "molecular farming" provides very large Possibility, but stringent rules will be required and Control mechanisms to avoid new, potentially serious risks Human health, although possible contamination of food supply. This is why transgenic plant provides breeding Generally seed-ambed technology has increased Insect contributes to management Horticulture with gardening by reducing pesticides Food security improves by reducing pesticides remain. In addition, Herbicide-Toller Transgenic Crop can help reduce the plow in the fields, so that Save fuel trust for the use of less tractor, whatever it is It reduces the soil structure by reducing it Eater. Transgenic horticulture makes crops cool an important contribution to permanent gardening Production.

Keywords: molecular farming, abiotic and biotic stress, Vitamins, minerals, diet fiber and phytochemicals

INTRODUCTION

Genetically modified plants are those whose genetic material has been altered in such a way that the quality of the products is increased. This technology is known as recombinant DNA technology or genetic engineering. Genetically modified plants are called transgenic plants. This process of translational integration and expression of a transgene in a plant is called transgenic transformation. Incorporating these traits into the genetic background of a species by conventional breeding requires overcoming some major disadvantages, including long juvenile periods and the incorporation of suitable traits into commercially relevant varieties(Song *et al.*, 2019). However, the current use of new technologies based on high throughput platforms for sequencing and genotyping has contributed profoundly to

accelerating the association of these relevant traits with molecular markers and key genes, in addition to those associated with causal factors(Shelton *et al.,* 2017).These species have complex characteristics associated with cross-breeding, such as delayed flowering, failure to set fruit due to embryo destruction, massive fruit drop, and self-incompatibility barriers. Improvement of plant characteristics by transfer of selected genes into the cells of fruit plants is mainly possible through various major methods such as vector-mediated gene transformation, microprojectile bombardment and direct DNA transfer, to generate transgenics for desired characteristics and techniques(Limera *et al.,* 2017). which can be used for the production of plants. Foreign DNA can be integrated into cells with new desirable properties. These biotechnological methods improve the genotype of the fruit for important commercial properties such as biotic (disease resistance to viruses, fungi, pests and bacteria) or abiotic (temperature, salinity, light, drought) stress tolerance, nutrition, making it a turn out to be a good product. The choice becomes available and tends to be of quality (fruit ripens later and lasts longer). Among various genetically modified fruit crops, GM papaya shows resistance to papaya ring spot virus, contributing approx 53% of the total share of GM fruit crops cultivated globally. Some phytochemicals derived from fruit crops are strong antioxidants and are believed to influence processes by protecting against free radical damage, regulating metabolic activation and detoxification of carcinogens, or even altering the course of tumor cells Play a role in the treatment of chronic disease risk can be reduced. Genetic modification provides a means of adding a desired trait to existing varieties without modifying their commercial characteristics. Fruit tree species face challenges in conventional breeding due to their long generation times and juvenile periods, complex reproductive biology, high levels of heterozygosity, scarce genetic resources and the association of unwanted traits from wild relatives. This ability is especially helpful in these species. Fruit trees have to be genetically improved to improve fruit production. New cultivars created for most of these species take into account horticultural properties related to fertility, yield, attractiveness, quality, disease and pest control, abiotic stress, and shelf-life(Khandelwal *et al.,* 2011).

History

The first genetically engineered fruit product was regulated in 1992 and introduced to the market in 1944 (Baranski *et al.,* 2019). A gene that triggers pectin solubility was reduced in the transgenic fruits, resulting in delayed fruit softening and increased shelf-life. Several

additional fruit crops that have been improved by genetic engineering have received regulatory approval for commercialization in various parts of the world, and are targeted for cultivation as human food or animal feed. These are tomato (*Solanum lycopersicum*), papaya (*Carica papaya* L.), jujube (*Prunus domestica*), apple (*Malus domestica* Borkh.), watermelon (Cucumis melo L.), and pineapple (*Ananas comosus* L.) (Firoozbady *et al.,* 2015). Most transgenic fruits were developed to improve agricultural productivity due to pest or disease resistance or delayed ripening. However, more recent products have addressed quality traits by eliminating browning of the fruit or adding new visual traits such as pulp color. Some engineered fruit crops have been withdrawn from the market because they were not commercially viable and were never commercialized (Lobato *et al.,* 2021).

Advantages of transgenic crop.

Breeding Rapid method of crop improvement Stable transgenic plants can be developed in 3- 4 years, whereas it takes 12-15 years to develop a new variety through conventional methods of breeding (Alvarez *et al.,* 2021). Overcome crossing barriers Transgenic breeding permits gene transfer between unrelated species and even between unrelated organisms. Evolution of new genotypes Sometimes transgenic breeding may lead to the evolution of new plant species because it permits gene transfer between various plant species. Thus, it will affect the process of natural evolution. The application can be used for the genetic improvement of both autogenous and allogamous crop plants. Effectiveness Transgenic breeding has been found effective for only the genetic improvement of monogenic characters. It has been found very effective in developing plants with resistance to various diseases, and insects (Menz *et al.,* 2020).

Disadvantages of transgenic crop breeding

The main drawback of this method is that there is no control over the copy number and side of integration of foreign genes. The high cost of equipment prohibits the use of this method by many researchers for DNA transfer.

Mechanism of Transgenic Plant

Numerous types of plant transformation techniques have now been made accessible to the public. These plant transformation techniques can be categorized into two groups: indirect or direct gene transfer. Indirect gene transfer (also known as vector-mediated gene transfer) involves the introduction of exogenous DNA into the plant genome biological vectors, whereas direct gene transfer methods involve the introduction of exogenous DNA directly into the plant genome through physical or chemical reactions.

Plasmid vectors. (Ti plasmid)

The Ti plasmid is the most commonly used vector in the production of a transgenic plant. The Ti plasmid has an estimated size ranging between 200 and 800 kbp depending on the classes of the Ti plasmid. The Ti plasmid is divided into three main regions: the transfer DNA (T-DNA) region, virulence region, and opine catabolism region. The T-DNA region that is transferred into the plant genome is about 24 kbp in size (Baker *et al.*, 1983). This region is bordered by repeat sequences on each end commonly known as the left border and right border. The right border is the critical part essential for the transfer of DNA-causing tumorigenesis. The virulence region, however, is responsible for encoding the *vir* genes, which aids in the transfer of the T-DNA. The T-DNA sequence also codes for opine and phytohormones (auxin and cytokinin) biosynthesis. The three oncogenes (opine, cytokinin, and auxin biosynthesis gene) within the T-DNA are the main causes of tumor formation in plant, leading to the crown gall disease (Christie *et al.*, 2014).

***Agrobacterium*-mediated gene transfer**

Agrobacterium-mediated transformation is the most common technique used in plant transformation as it is efficient and effective in a wide range of plants. Agrobacteria are indigenous to the soil ecosystem. They are pathogenic Gram-negative bacteria that cause crown gall or hairy root disease in plants. The genetic information for tumor growth is encoded on a tumor-inducing plasmid (Ti plasmid) or hairy root-inducing plasmid (Ri plasmid) in the genome of these bacteria.

Direct gene transfer

Direct gene transfer, as the name suggests, involves the direct introduction of exogenous DNA (naked DNA) into the plant nucleus. In order to introduce foreign DNA into the plant cell, the outer membrane of the cell is first disrupted, permeating it for foreign DNA to enter. Most of the methods under direct gene transfer are simple and effective. However, gene expression in these transgenic plants can be transiently or stably transformed. Direct gene transfer can be categorized into two main groups: physical gene transfer and chemical gene transfer.

Physical gene transfer

Physical gene transfer disrupts the cell wall and cell membrane via mechanical means. Among these methods, particle bombardment biolistic is the most common one used in plant transformation since it was first introduced by Sanford. The DNA coated with gold or

tungsten particles are shot into the target plant cell under high pressure using a "Gene Gun". The fast-moving particles allow for the penetration of coated DNA through the thick plant cell wall, directing the foreign DNA into its nucleus. The coated DNA will then separate from the metal particles and integrate itself into the chromosomes within the nucleus of the plant cell (Lai *et al*., 1997).

Chemical gene transfer

Chemical gene transfer approaches involve the use of chemicals to disrupt cell membranes enabling the entry of foreign DNA. This particular method is not preferable in plant transformation as it is only effective when applied to protoplasts. One of the most prominent chemicals used in this approach is polyethylene glycol (PEG) which is used for destabilizing the cell membrane in the presence of a divalent cation, thus increasing the permeability of the cell membrane, allowing for the uptake of foreign DNA. The exact mechanism for chemical gene transfer is not fully understood, but it was postulated that PEG increases the osmotic pressure and causes contraction in the protoplast; this facilitates endocytosis of the divalent cation/DNA complex (Lazzeri *et al*., 1991). Besides those, liposome is another chemical method that is used in the transformation of a plant's protoplast cells. Liposomes act as vehicles to encapsulate and deliver foreign genetic materials into the protoplast. The lipophilic attribute of liposomes provides easy access to the protoplast in transforming the cell (Caboche *et al*., 1990).

Table 1. Use of Transgenic Approach in Fruit Crops

Crop	Character	Gene transferred	Method of gene Transfer	Variety	Reference
Transgenic Papaya	PSRV Resistant	coat protein gene from PRSV	micro projectile bombardment technique	Sun UP from Sunset and UH Rainbow from Kapoho	(Gonsalves 1992)
	Banana Bunchy top Virus	Replicase-associated gene (Rep gene)	RNAi technology *Agrobacterium*mediated	Dwarf Brazilian (AAB) Pome sub group	(Borth *et al*., 2011)

Transgenic Banana			Transformation		
		master replication initiation protein (Rep)	*Agrobacterium*mediated transformation	Rasthali' (AAB genome)	(Shekhawat *et al.*, 2012)
		Replicase-associated gene (Rep gene) RGA2 gene from banana and Ced9 gene, is derived from a nematode	*Agrobacterium*mediated transformation	Virupakshi (AAB)	(Elayabalan *et al.*, 2013)
	Xanthomonas wilt	Plant ferredoxin-like protein (*Pflp*) gene from sweet pepper (*Capsicum annuum*).	*Agrobacterium*mediated transformation	'SukaliNdiizi', and 'Nakinyika',	(Klopez, 2012)
Transgenic Pear	Fire blight (*Erwiniaamylovora)* produces (desferrioxamine protein	Exogenous ferritin gene which acts as iron chelator from pea	„	'Conference' and 'Passe-Crassane'	(Djennane *et al.*, 2009)
Grapefruit (*Citrus paradisi*)	citrus tristeza	closterovirus genes	„	-	(Febres *et al.*, 2008)

Trifoliate Orange	salinity tolerance	Betaine aldehyde dehydrogenasegene (*AhBADH*) gene cloned from *Atriplex hortensis*	„	-	(Fu *et al*., 2011)
Guava (*Psidiumguaja va*)	guava wilt	Trichoderma-endochitinase gene	„	-	(Mishra *et al*., 2014)
	cold temperatures tolerence	cold hardiness genes (CBF1, CBF2 and CBF3)	„	-	(Mishra *et al*.,2014)
Kiwifruit	Resistance to the insect Oraesia excavate	synthetic chimeric gene SbtCry1Ac that encodes the insecticidal protein btCrylAc	”	-	(Fu *et al*., 2011)
Vitis vinifera	Virus resistance	CP of ArMV	Somatic embryos	-	(Spielmann *et al*., 2000)
	Fungal resistance	Rice chitinase (RCC2)	Somatic embryos	‘NeoMuscat’	Yamamoto *et al*.,2000)
	Cold resistance	SOD from Arabidopsis	*A. tumefaciens*	‘Cabernet Franc’	(Rojas *et al*., 1996)
	Modified fruit traits	DefH9/iaaM	*A. tumefaciens*	Silcora’, ‘Thompson Seedless’	(Mezzetti *et al*., 2002)
Apple	Non browning	PPO suppression transgene, ACC oxidase, ACC synthase	*Agrobacterium rhizogenes*	Golden Delicious, Fuji, Gala	(Zhu *et al*., 2001)
	Resistance to	NPTII attE, nptII,	Replicase	Gala	(Kost *et al*.,

	fire blight	gusA	associated protein		2015)
Strawberry	Powdery mildew	Chitinase, rice PpMlo1	A. tumefaciens	-	(Jiwan *et al.*, 2013)
Pear	Fire blight	Cecropin genes *SB-37* and Shiva-1 and Attacin E	A. tumefaciens	-	(Malnoy *et al.*, 2005)
Plum	Plum pox virus	Coat protein, plum pox virus	A. tumefaciens	-	(Malinowski*et al.*, 2006)

Future Prospects

Advances in genetic engineering, particularly the development of genome editing technologies have provided new tools for the generation of improved fruit varieties. Many proof-of-concept examples involving fruit crops have been reported and the further development and marketing of such varieties could have a major socioeconomic impact. Though considerable progress has been made in transgenic research in many fruit crops, a lot needs to be done to make the transgenic approach the preferred method of fruit improvement. In recent years, several countries have amended their current regulations or have developed new guidelines to regulate genome-edited plants and their products. This may make it possible that genome-edited fruits, similarly to all other genome-edited crops, to reach the market faster in countries with a genome-editing-friendly policy. Here, we first discuss fruit varieties that have already been approved for commercialization, focusing on those that are on the market. We then consider fruit varieties developed more recently using transgenic fruit crops, and their potential socioeconomic impact. Major concerns such as consumer acceptance and environmental safety need to be addressed. It will be useful to identify new and better candidate genes for different traits within the same species and/or genus. Manipulation of target genes and is expected to bring much needed impact us to fruit improvement programs.

Conclusion

From above the discussion, it is concluded as genetically-modified fruit crops have the potential to solve many of the world's hunger and malnutrition problems. According to

many researchers it concludes that transgenic techniques help to improve in the genetically development of fruit crops by gene transfer. There is no doubt that transgene removal will eventually be routine as some of the techniques reviewed here are perfected. The accumulated information derived from this scaling-up process fused to the characterization of some of the kinetic parameters involved in grapevine SE, have enabled design of new experimentation focused on the development of SE protocols for genotypes cultivars such as ´Red Globe´. The results indicate that grape genetic transformation can be considered as a model system in which efficiency is not necessarily an issue and the possibility for high through-put candidate gene evaluation is plausible.

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