



Review Article

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## Transgenics in Fruit Crops Research- A Review

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### ABSTRACT

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Present increasing trend of population is alarming for food shortage. It was estimated that by the year of 2050 the population rate may increase up to 9 billion. Rapid increase of human population together with global climate variability resulted in increased demand of plant based food and energy sources. Conventional breeding is still limited due to genetic restrictions (high heterozygosity and polyploidy), long juvenile periods, self-incompatibility, resources restricted to parental genome and exposed to sexual combination. Thus, there is an urgent need for the biotechnology-assisted crop improvement, which ultimately aimed to obtain novel plant traits. Plant genetic engineering has opened new avenues to modify crops, and provided new solutions to solve specific needs. Therefore, this paper reviews the recent trends in the field of transgenic in fruit crops.

### Introduction

Genetically modified organisms are the one which the genetic material has been altered in such a way to get the required quality. This technology is called as Recombinant DNA technology or Genetic engineering. Genetically engineered organisms are referred to as Transgenic or cisgenic organisms. The process of transferring, integration and expression of transgene in an organism are called transgenic transformation. A transgenic crop plant contains a gene which has been

artificially inserted instead of the plant, acquiring them through fertilization or pollination. The inserted gene sequence (known as the transgene) may come from another unrelated plant. Improvement of the plant characteristics by transfer of selected genes into fruit plant cells is possible mainly through two principal methods: *Agrobacterium*-mediated transformation and micro projectile bombardment. Genetic transformation and genetic engineering contribute to an overall increase in crop productivity. Contrary to conventional plant

breeding, this technology can integrate foreign DNA into different plant cells to produce transgenic plants with new desirable traits. These biotechnological approaches are a great option to improve fruit genotypes with significant commercial properties such as increased biotic (resistance to disease of virus, fungi, pests and bacteria) or abiotic (temperature, salinity, light, drought) stress tolerances, nutrition, yield and quality (delayed fruit ripening and longer shelf life) and to use as directed to produce proteins, edible vaccines and biodegradable plastics (Khandelwal *et al.*, 2011).

### **Transgenic fruit crops**

#### **Papaya**

The transgenic papaya has been developed against papaya ring spot virus using coat protein mediated resistant in university of Hawaii by Dennis Gonsalves. The coat protein gene from PRSV was isolated, cloned and used for transforming papaya to provide resistance against the severe strain of the same virus. The target cultivars used in transforming papaya were the Red fleshed, Sunset and the Yellow fleshed Kopoho. Transformation with the coat protein gene is done using a micro projectile bombardment technique using embryonic tissues of papaya. Two transgenic lines Sun UP from Sunset and UH Rainbow from Kapoho were developed which have shown excellent resistance to PRSV. Papaya ring spot virus (PRSV) reduces both fruit quality and edible yield. Transgenic papaya with a pathogen derived resistance carrying the coat-protein gene provides effective protection against PRSV strains over a significant period of the cycle of this perennial fruit crop.

#### **Banana**

Transgenic banana resistant to bunchy top disease

Symptoms Typical symptoms of BBTD include the appearance of dark green broken streaks of leaf veins, midribs, petioles and pseudostem with a group of clustered leaves on the top of the plant looks bunchy appearance (Borth *et al.*, 2011). Reported BBTV resistant banana clone development through RNAi technology Agrobacterium-mediated transformation of embryogenic banana cell suspensions with constructs that may prevent the replication of BBTV has been favoured by many research groups, as there is a much better chance of a plant developing that is not a chimera. In 2011, it was reported that some transformed clones of 'Dwarf Brazilian' (AAB, Pome subgroup) were resistant to BBTV under experimental conditions in Hawaii.

Shekhawat *et al.*, (2012) have published an account of tests that showed that transformed 'Rasthali' (AAB genome, syn. 'Silk') did not develop symptoms when exposed to aphids carrying BBTV. They have explored the concept of using intron-hairpin-RNA (ihpRNA) transcripts corresponding to viral master replication initiation protein (Rep) to generate BBTV-resistant transgenic banana plants.

Elayabalan *et al.*, (2013) reported on the Agrobacterium-mediated transformation on a highly valued hill banana cultivar Virupakshi (AAB) for resistance to BBTV disease. The target of the RNA interference (RNAi) is the rep gene, encoded by the BBTV-DNA1. The presence of the transgenes was conformed in the selected putative transgenic hill banana lines by PCR and reverse transcription PCR analyses. Transgenic hill banana plants expressing RNAi- BBTV-rep were obtained and shown to resist infection by BBTV. The transformed banana plants were symptomless, and the replication of challenge BBTV almost completely suppressed.

Klopez (2012) reported that Banana *Xanthomonas* wilt (BXW), caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Xcm), is the most devastating disease of banana in the Great Lakes region of Africa. IITA, in partnership with NARO-Uganda and the African Agricultural Technology Foundation, has developed transgenic banana by constitutively expressing the Hypersensitive Response Assisting Protein (*Hrap*) or plant ferredoxin-like protein (*Pflp*) gene from sweet pepper (*Capsicum annuum*). The transgenic plants have exhibited strong resistance to BXW in the laboratory and screen house tests. The best 65 resistant lines were planted in a confined field trial at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda, for further evaluation.

### **Plum**

Plum is one of the tree fruits threatened by Plum pox poty virus (PPV) a quarantine disease that causes fruit loss to plums and other stone fruits.. As an enhancement to classical breeding, genetic engineering was used to produce transgenic clones that contain the PPV coat protein (CP) gene, applying the principle of pathogen derived resistance. Gene for PPV virus coat protein inserted into plant genome and result of this effort was the development of a transgenic clone designated as C5 (cv.Honey Sweet) Developed by Gene silencing or RNA interference (RNAi) at USDA-ARS Appalachian Fruit Research Station, which is resistant to Plum Pox Virus (PPV).

### **Apple**

The "non browning" apple is genetically engineered to keep from going brown after being cut. When apple flesh is cut and exposed to oxygen, it begins to brown. But the genetically modified apple or "Arctic Apple,"

is resistant to browning. The "nonbrowning" genetically modified apples are designed to look fresh when they're not. It was developed by silencing a gene in the apple (that controls browning) by inserting modified apple DNA. It is approved for sale by USDA in 2015.

### **Pineapple**

The "extra sweet pink flesh pineapples" are set to be launched by Del Monte. The fruit has been genetically engineered to produce fewer enzymes that are naturally responsible for converting lycopene to beta carotene. The suppression of enzymes will leave the flesh of the fruit pink, as the genes retain the pink pigment lycopene instead of beta carotene.

### **Grapes**

Nirala *et al.*, (2010) reported that to enhance the antifungal potential of grapevine, transgenic plants were generated by transferring a rice chitinase gene under a maize-ubiquitin promoter along with its first intron into the leaf disc-induced somatic embryos via *Agrobacterium* mediated transformation. After co-cultivation for 2 days with recombinant *Agrobacterium*, somatic embryos were transferred onto WPM medium containing BAP 1.5  $\mu$ M and NAA 0.1  $\mu$ M supplemented with 25 mg/L hygromycin. Secondary or tertiary embryos were selected and the antibiotic resistant transgenic plantlets were analyzed. The integration and stability of the transgene were confirmed by PCR, RT-PCR, Southern blotting and by Western blot analyses. The transgenic plants exhibited higher chitinase activity than the non-transformed plants.

### **Sweet orange**

Omar *et al.*, (2008) stated that 'Hamlin' sweet orange (*Citrus sinensis* (L.) Osbeck) is one of the leading commercial cultivars in Florida

because of its high yield potential and early maturity. 'Hamlin' also has a high regeneration capacity from protoplasts and is often used in transformation experiments. A Citrus canker disease caused by the bacterial pathogen *Xanthomonas axonopodis pv.citrii* becoming a worldwide problem in sweet orange. Plasmid DNA (pARS108) encoding the non-destructive selectable marker EGFP (Enhanced Green Fluorescent Protein) gene, and the plasmid cDNA of the Xa21 gene (pXa21-mtaq) were co-transformed into 'Hamlin' orange protoplasts using polyethylene glycol. More than 150 transgenic embryoids were recovered. Over a thousand transgenic plantlets GFP positive were regenerated from 150 independent transformation events. PCR analysis revealed the presence of the cDNA of the Xa21 and the GFP genes in some of the transgenic plantlets. The recovery of transgenic plants was expedited by in vitro grafting. The transgenic plants have shown normal growth and stable GFP expression for over a year in the greenhouse. Southern blot analysis is showing 0-5 copies of the transgene per transgenic plant.

### **Grape fruit**

Febres *et al.*, (2008) reported that grapefruit (*Citrus paradisi*) transgenic plants transformed with a variety of constructs derived from the Citrus tristeza virus (CTV) genome were tested for their resistance to the virus. Most transgenic lines were susceptible (27 lines), a few were partially resistant (6 lines) and only one line, transformed with the 3' end of CTV was resistant.

### **Guava**

Mishra *et al.*, (2014) reported that guava wilt disease is a severe threat to guava growers all over the world. It is caused by the soil-borne fungus *Fusarium oxysporum f.sp. psidii*. To

control the disease, the Trichoderma-endochitinase gene was first introgressed into guava (*Psidium guajava L.*). The transgenic plantlets were screened in vitro for resistance against the wilt pathogen. Six-months-old genetically transformed plants raised in cocopeat under in vitro conditions were inoculated with a 7-days old culture of *F. oxysporum f.sp. psidii*. The presence of the pathogen in the cocopeat medium was confirmed by cultural as well as PCR analysis using species-specific primers. The roots of transgenic plants were wounded to facilitate the entry of the pathogen. The histopathological analysis revealed the presence of mycelium in vascular bundles. However, none of the plants showed symptoms of wilt disease during the investigation. Transgenic plants could not develop any symptoms of wilt disease due to overexpression of endochitinase.

### **Kiwi**

The kiwifruit (*Actinidia chinensis* Planch.) is an economically and nutritionally important fruit crop that has remarkably high vitamin C content and is popular throughout the world. However, kiwifruit plants are vulnerable to attack from pests, and effective pest control is urgently required. Zhang *et al.*, (2015) reported that transgenic kiwifruit plants containing the synthetic chimeric gene SbtCry1Ac that encodes the insecticidal protein btCry1Ac were obtained through an Agrobacterium-mediated transformation of kiwifruit leaf discs. The kanamycin resistance of the transgenic plants was then analyzed. Results of polymerase chain reactions and genomic DNA Southern blot analyses indicated that SbtCry1Ac had been integrated into the genomes of these plants. The results of insect bioassays revealed that the average *Oraesia excavate* inhibition rate of plants tested at 10 days post-infestation was 75.2%.

Table.1

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)
Transgenic banana	Banana Bunchy top Virus	Replicase- associated gene (Rep gene)	RNAi technology <i>Agrobacterium</i> -mediated transformation	Dwarf Brazilian (AAB) Pome sub group	Borth <i>et al.</i> , (2011)
Transgenic banana	Banana Bunchy top Virus	master replication initiation protein (Rep)	<i>Agrobacterium</i> -mediated transformation	Rasthali' (AAB genome)	Shekhawat <i>et al.</i> , (2012)
Transgenic banana	Banana Bunchy top Virus	Replicase- associated gene (Rep gene) RGA2 gene from banana and Ced9 gene, is derived from a nematode	<i>Agrobacterium</i> -mediated transformation	Virupakshi (AAB)	Elayabalan <i>et al.</i> , (2013)
Transgenic banana	<i>Xanthomonas</i> wilt	Plant ferredoxin-like protein ( <i>Pflp</i> ) gene from sweet pepper ( <i>Capsicum annuum</i> ).	<i>Agrobacterium</i> -mediated transformation	'SukaliNdiizi', and 'Nakinyika',	Klopez (2012)
Transgenic Pear	Fire blight ( <i>Erwinia amylovora</i> ) produces (desferrioxamine protein	Exogenous ferritin gene which acts as iron chelator from pea	<i>Agrobacterium</i> -mediated transformation	'Conference' and 'Passe-Crassane'	Djennane <i>et al.</i> , (2009)
Grapefruit ( <i>Citrus paradisi</i> )	citrus tristeza	closterovirus genes	<i>Agrobacterium</i> -mediated transformation	-	Febres <i>et al.</i> , (2008)
Guava ( <i>Psidium guajava</i> )	guava wilt	Trichoderma-endochitinase gene	<i>Agrobacterium</i> -mediated transformation	-	Mishra <i>et al.</i> , (2014)
Guava ( <i>Psidium guajava</i> )	cold temperature tolerance	cold hardiness genes (CBF1, CBF2 and CBF3)	<i>Agrobacterium</i> -mediated transformation	-	Biswas <i>et al.</i> , (2005)
Kiwifruit	Resistance to the insect <i>Oraesia excavate</i>	synthetic chimeric gene SbtCry1Ac that encodes the insecticidal protein btCry1Ac	<i>Agrobacterium</i> -mediated transformation	-	Zhang <i>et al.</i> , (2015)
Trifoliolate orange	salinity tolerance	Betaine aldehyde dehydrogenase gene ( <i>AhBADH</i> ) gene	<i>Agrobacterium</i> -mediated transformation	-	Fu <i>et al.</i> , (2011)

		cloned from <i>Atriplex hortensis</i> (Mountain spinach			
American cranberry ( <i>Vaccinium macrocarpon</i> Ait.)	conferring tolerance to the phosphinothricin-based herbicide glufosinate	<i>bar</i> gene	<i>Agrobacterium</i> -mediated transformation	-	Zeldin <i>et al.</i> , (2002)
<i>Vitis vinifera</i> ( <i>V.berlandieri</i> x <i>V. riparia</i> ) and 41B ( <i>V.v</i> x <i>V. berl.</i> )	Virus resistance	LBA 4404 GFLV cp gene	Anther-derived embryogenic callus	-	Mauro <i>et al.</i> , (1995., 2000)
<i>V. rupestris</i>	Virus resistance	CP of ArMV	Somatic embryos	-	Spielmann <i>et al.</i> , (2000)
<i>V. vinifera</i>	Fungal resistance	Rice chitinase (RCC2)	Somatic embryos	'NeoMuscat'	Yamamoto <i>et al.</i> , (2000)
<i>V. vinifera</i>	Cold resistance	SOD from Arabidopsis	<i>A. tumefaciens</i>	'Cabernet Franc'	Rojas <i>et al.</i> , (1996)
<i>V. vinifera</i> 'x'	Modified fruit traits	DefH9/iaaM	<i>A. tumefaciens</i>	'Silcora', 'Thompson Seedless'	Mezzetti <i>et al.</i> , (2002)
<i>Vitis vinifera</i>	Modified fruit traits	UDP:flavonoid 3-O-glucosyltransferase (UFGT)	<i>A. tumefaciens</i>		Thomas <i>et al.</i> , (2001)

### American cranberry

Zeldin *et al.*, (2002) reported that American cranberry (*Vaccinium macrocarpon* Ait.) was genetically transformed with the *bar* gene, conferring tolerance to the phosphinothricin-based herbicide glufosinate.

### Trifoliolate orange

Trifoliolate orange (*Poncirus trifoliata* L. Raf.), a rootstock widely used for citrus species, is salt-sensitive. Worldwide, salinity is a major abiotic stress affecting citrus growth and yield. Fu *et al.*, (2011) reported that a betaine aldehyde dehydrogenase gene (*AhBADH*) cloned from *Atriplexhortensis* was introduced

into the trifoliolate orange by means of *Agrobacterium*-mediated transformation. RT-PCR analysis on three selected transgenic lines showed that the *AhBADH* gene was overexpressed in each of them. GB levels in these lines were also higher than those in untransformed wild-type (WT) plants. His data suggest that overexpression of the *AhBADH* gene in transgenic trifoliolate orange enhanced salt stress tolerance.

It is concluded as genetically-modified fruit crops in addition to food grain crops have the potential to solve many of the world's hunger and malnutrition problems. However, transgenic research in fruit crops is very low due to difficulty in regeneration and

transformation procedure being perennial and woody in nature. The long term effects of GMOs are not certain. Most of the fruit genetic transformation protocols integrated the new genes randomly and in unpredictable copy numbers influencing negatively its expression. Public concerns and reduced market acceptance of transgenic crops have promoted the development of alternative marker free technologies in fruit species. Most of the fruit transgenic plants are generated by introducing just one single new character (gene of interest), however, multigene transfer technology (MGT) needs to be developed to obtain new traits related at the same time.

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