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An Insight into Transgenic Development Activities in Fruit Crops

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A B S T R A C T

Fruits and vegetables are often referred to as "protective foods" because they are rich in minerals and vitamins. Fruit production is severely impacted by various biotic and abiotic stresses and during the past, efforts have been made by conventional breeding programs to mitigate these problems. However, classical breeding has had little success in improving fruit plants and is constrained due to long juvenile period, genetic erosion, genetic drag and reproductive barriers that limit the transfer of favourable genes from diverse genetic resources. A transgenic crop plant harbors an additional, stably integrated and expressed, foreign gene(s) from trans-species by the process called genetic transformation. In the last two decades, genetic transformation of fruit crops has focused mainly on enhancing resistance to biotic stresses and increasing tolerance to abiotic ones. However, it is worthwhile to mention that field evaluation and commercialization of these crops is very limited. Advances in genomics in the next few years is to put a major impact on this field. While it is difficult to determine the changes in public acceptance of transgenic fruits in the future, the advances in alternatives like cisgenics, genome editing etc. may considerably affect public opinion.

Introduction

Fruits alongside, vegetables are known for their health-promoting assets because of their concentrations of vitamins, minerals, electrolytes, antioxidants and dietary fibre (Slavin and Lloyd, 2012). With an ever-increasing population, there is a growing demand in the global fruit production, which stress on the necessity of improving the economically important fruit crops (Tanuja and Kumar, 2017). Classical breeding techniques are being used since the twentieth century for improvement of fruit crops but are still limited due to high heterozygosity, polyploidy, long juvenile periods, self-incompatibility, resources delimited to parental genome and wide-open to the sexual combination (Litz and Padilla, 2012; Tanuja and Kumar, 2017). With the recent advances in the field of biotechnology, exciting scopes of modifying crops with desired trait are coming up and these biotechnology-assisted crop improvement can radically change the picture of fruit breeding (Kumar and Kumar, 2000). 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 bioreactor to produce proteins, edible vaccines and biodegradable plastics can also be achieved with the aid of biotechnology (Kuruganti and Ramanjaneyulu, 2011). Transformation with genes that regulate the horticulturally significant traits implies that superior cultivars can be improved for a specific trait without otherwise changing the integrity of the clone (Litz and Padilla, 2012).

Genetically Modified Crops

Genetically modified crops of GM crops or Biotech crops are crops, who's DNA has been modified using genetic engineering techniques. In most cases, they carry additional, stably integrated and expressed, foreign gene(s) from trans-species. The process involves introduction, integration and expression of a foreign gene(s) in the host and is called genetic transformation. The combined use of rDNA technology, gene transfer methods and tissue culture techniques has led to an efficient transformation and production of transgenics (Kumar and Mina, 2016). Apart from transgenics, cisgenics and subgenics also comes under genetically modified crops.

Cisgenics are developed from genes found within the same species or closely related ones, where conventional breeding can take place. This technique is believed to be useful for plants that are difficult to crossbreed by conventional techniques (MacKenzie, 2008). On the other hand subgenics are developed using gene knockdown to alter the genetic makeup of the plant without incorporation of genes from other plants (Holme *et al.*, 2013).

Features of Gm Plants/Transgenic Plants

Genes can now be transferred into plants from a wide range of organisms, including unrelated plant species, microbes, animals, and from DNA synthesized from laboratory. In the year ahead, transgenic variety will be produced that have modification in a wide range of characters and have genetic changes not achievable through the conventional breeding methods. The features of transgenic plants are briefly discussed beneath.

Contain transgenes

Transgenic plants contain transgenes (foreign genes). The foreign genes may be utilized from unrelated plants, microbes and animals. Microbial genes are utilized from fungi, bacteria and viruses. Sometimes, genes from DNA synthesized in the laboratory are also used for development of transgenic plants.

Involve Biotechnology

Development of transgenic plants involves plant biotechnology, which refers to the combination of tissue culture and genetic engineering. In other words, transgenic plants are developed through tissue culture and genetic engineering.

The genetic engineering helps in manipulation of foreign gene (DNA) and tissue culture is essential for genetic transformation.

The foreign gene cannot be inserted into whole plant. It can only be inserted into single cells. Thus, tissue culture is essential for transfer of foreign gene into single cells.

The genetic transformation can be achieved either through cell culture or protoplast culture.

Moreover, tissue culture is essential for regeneration of genetically transformed single cells into whole plant. Thus combination of tissue culture and molecular genetics is essential for development of transgenic plants.

Bypass Sexual Process

In the development of transgenic plants, the sexual process (reproduction) is bypassed. In other words, transgenic plants are developed without involving sexual fusion between donor and the recipient parents.

As stated above, transgenic plants are developed by the techniques of tissue culture and genetic engineering. Once a transgenic plant is developed, the transgenic trait can be transferred to other genotypes through backcross method.

Low Frequency

In most of the field crops, transgenic plants are recovered at a very low frequency. Hence, huge single cells or protoplasts have to be screened in the culture medium for identification and isolation of transgenic cells. The transformed cells are identified by polymerase chain reaction (PCR) technique.

Utility

Transgenic plants are developed to solve specific problems in crop plants such as development of plants having resistance to diseases, insects, drought, frost, salinity and metal toxicity; improvement in keeping quality of some vegetables, and fruits; and development of male sterility, etc

Applications of Transgenic Crop Breeding

Some of the applications of transgenic crop breeding as

Fast method of crop improvement

Stable transgenic plant can be developed in 3-4 years, whereas it takes 12-15 years to develop a new variety through conventional methods of breeding (Southgate *et al.,* 1995).

Overcome crossing barriers

Transgenic breeding permits gene transfer between unrelated species and even between unrelated organisms. For example, a freezing resistant gene has transfer from fish to cultivated tomatoes.

Evolution of new genotypes

Sometimes transgenic breeding may lead to evolution of altogether new plant species, because it permits gene transfer between various plant species. Thus, it will affect the process of natural evolution.

Insect-pests and disease resistance

Crop losses from insect- pests and diseases are of big concern due to devastating financial loss for farmers and starvation in developing countries. Applications of chemical pesticides are increasing annually. This is a serious cause for potential health hazards, and even run-off of agricultural wastes from excessive use of pesticides and fertilizers can poison the water supply and cause harm to the environment. Therefore, growing insect-pest and disease resistance GM fruits such as papaya, grapes, and apple etc. not only reduces the economic losses but also ensure to provide chemical free fruits.

Herbicide resistance

For some fruit crops, it is not cost-effective to remove weeds by physical means such as tilling, so farmers will often spray large quantities of different herbicides to destroy weeds, a time-consuming and expensive process that requires care so that the herbicide doesn't harm the crop plant or the environment.

Crop plants genetically engineered to be resistant to one very powerful herbicide could

help prevent environmental damage by reducing the amount of herbicides needed.

Cold tolerance

The unexpected frost can destroy sensitive seedlings in many fruit crops. Genetic transformation of guava with cold hardiness genes (CBF1, CBF2 and CBF3) make these plants able to tolerate cold temperatures that normally would kill unmodified seedlings.

Drought /salinity tolerance

The tailoring plants that can withstand long periods of drought or high salt content in soil and groundwater will help farmers to grow crops in formerly inhospitable places.

Pharmaceuticals medicines and vaccines

These are costly to produce and sometimes require special storage conditions. With the help of genetic engineering reaching that goal is becoming more realistic. In recent years, researchers have developed "edible vaccines," foods that contain the power to protect against disease and must only be eaten to be effective. These vaccines will be much easier to ship, store and administer than traditional injectable vaccines. These edible vaccines would certainly provide a more practical method of disease control than traditional immunizations and could come at much less cost and inconvenience for the consumer.

Methods of Genetic Transformation

Direct Methods

Direct methods are those methods which do not use bacteria as mediators for integration of DNA into host genome. These methods include micro projectile bombardment, electroporation and microinjection (Narusaka *et al.,* 2012).

Microprojectile/particle bombardment (biolistics)

Biolistics is a method where cells are physically impregnated with nucleic acids or other biological molecules. Abiolistic particle delivery system is a device for plant transformation where cells are bombarded with heavy metal particles coated with DNA/RNA.

This technique was invented by John Stanford in 1984 for introduction of DNA into cells by physical means to avoid the host-range restrictions of *Agrobacterium. Agrobacterium*-mediated genetic transformation system works well for dicotyledonous plants but has low efficiency for monocots. Biolistic particle delivery system provides an effective and versatile way to transform almost all type of cells. It has been proven to be a successful alternative for creating transgenic organisms in prokaryotes, mammalian and plant species.

Electroporation

Electoporation is a method of transformation via direct gene transfer. In this technique mixture containing cells and DNA is exposed to very high voltage electrical pulses (4000 – 8000 V/cm) for very brief time periods (few milliseconds). It results in formation of transient pores in the plasma membrane, thorough which DNA seems to enter inside the cell and then nucleus.

Microinjection

The process of using a fine glass micropipette to manually inject transgene at microscopic or borderline macroscopic level is known as microinjection. The transgene, in the form of plasmids, cosmids, phage, YACs, or PCR products, can be circular or linear and need not be physically linked for injection.

Micro injection involves direct mechanical introduction of DNA into the nucleus or cytoplasm using a glass microcapillary injection pipette. The protoplasts are immobilized in low melting agar, while working under a microscope, using a holding pipette and suction force. DNA is then directly injected into the cytoplasm or the nucleus. The injected cells are then cultured *invitro* and regenerated into plants. Successful examples of this process has been shown in rapeseed, tobacco and various other plants.

Chemical mediated gene transfer

Cells or protoplasts can be stimulated to take up foreign DNA using some chemicals. Polyethylene glycol (PEG) is the most commonly used chemical for this purpose. It helps in precipitation of DNA, which can then be taken up by the calls through the process of endocytosis.

Liposome mediated gene transfer

Plasmid containing foreign desired gene can be enclosed in small lipid bags called lipososmes, which can then be fused with protoplasts using chemicals like PEG.

Silicon carbide method

In this method, fibres of organic material like silicon carbide are used for gene transfer. These fibres, when mixed with plasmid DNA and plant tissue or cells, help in penetration of the foreign DNA into the plant tissue.

Indirect Methods

Agrobacterium Mediated Gene Transfer

Agrobacterium tumefaciens and *Agrobacterium rhizogenes* are common gram-negative soil borne bacteria causing induction of 'crown gall' and 'hairy root' diseases. These bacteria naturally insert their genes into the genome of higher plants. Virulent strains of bacteria introduce a part of their genetic material into the infected cells where it gets integrated randomly with the genetic material of the host cell. The bacterial genes are able to replicate along with the plant genome and uses the machinery of plants to express their genes in terms of the synthesis of a special class of compounds, called opines, which the bacterium uses as nutrients for its growth but are useless to the host cells. *A. tumefaciens* attracted to the wound site via chemotaxis, in response to a phenolic compound. Infected tumorous plant cells were found to contain DNA of bacterial origin integrated in their genome. The transferred DNA (named T-DNA) was originally part of a small molecule of DNA located outside the chromosome of the bacterium. This DNA molecule is called as Ti (tumor-inducing) plasmid (Gelvin, 2003).

Transgenic Research Activities on Various Fruit Crops

Papaya (*Carica papaya*)

Unlike the fruit crop species that have been addressed in this chapter, the papaya is not a perennial plant species. Generally seed-propagated, the papaya has a relatively short juvenile period, and conventional breeding has had considerable impact on its improvement. Commercial papaya production is based upon dioecious cultivars in the subtropics and hermaphroditic cultivars in the tropics. The latter group includes the 'Solo' type of papayas, which are highly inbred and highly susceptible to the disease caused by Papaya ringspot virus (PRSV). Because of their homozygosity, the 'Solo' papayas have mostly been targeted for genetic transformation; resistance to PRSV has been the major focus, although other traits are also

being addressed, including resistance to fungal and oomycete diseases, soil and climate stress tolerance, improved shelf life, etc.

PRSV Resistance

Papaya ringspot virus (PRSV) is often a limiting factor in the production of papaya worldwide. In 1992, PRSV was discovered in the district of Puna on Hawaii Island, where 95% of Hawaii's papaya was grown. Within two years, PRSV was widespread and caused severe damage to the papaya in that area. Coincidentally, a field trial to test a PRSV-resistant transgenic papaya had started in 1992. Transformation with coat protein gene is done by micro projectile bombardment technique using embryonic tissues of papaya. Two transgenic lines *UHSun UP* from Sunset and *UH Rainbow* from Kapoho were developed and they showed excellent resistance to PRSV (Gonsalves, 2004).

Developing resistance to mites in papaya

The transgenic PRSV-resistant cultivar Rainbow is susceptible to mites. To enhance papaya resistance to the carmine spider mite, McCafferty *et al.,* (2006) did transformation on a commercial variety of papaya with the gene for chitinase from *Manducasexta.* Embryogeniccalli of papaya were bombarded with the plasmid pBI121 containing the msch gene under the control of CaMV 35S promoter and the nptII gene under the control of the nopaline synthase promoter as selectable marker. Chitinase activity was higher by up to 52% in the transgenic leaf extracts compared to control. Under field conditions, the number of mites on most transformed lines was significantly lower than the control, Kapoho. Two lines, T-23 and T-14 had significantly lower number of mites than control. By the end of 10 weeks, the control plants died while lines T-23 and T-14 had grown new leaves. These results indicate a greater tolerance of the transgenic lines to the mites.

Developing resistance to *Phytophthora palmivora*

Papaya is highly susceptible to *Phytophthora palmivora* at the seedling and mature stages causing fruit and root rot particularly during the rainy season and in poorly drained soil. To improve the resistance of papaya to *Phytophthora*, Zhu *et al.,* (2007) introduced defensin gene from *Dahlia merckii*by particle bombardment in embryogeniccalli of papaya. The mycelial growth of *P. palmivora* was inhibited by 35%–50% by leaf extracts of the transgenic lines. Further, inoculation experiments in the greenhouse showed that defensin expressing transgenic papaya plants had increased resistance against *P. palmivora.*

Other transgenic research works on papaya includes, development of aluminum and herbicide tolerance (Fuente *et al.,* 1997), suppressing ethylene production strategy, reducing softening strategy and production of pharmaceuticals (Zhang *et al.,* 2003).

Apple (*Malus x domestica*)

The major breeding objectives that have been targeted by genetic transformation include resistance to scab (*Venturiainaequalis*) and fire blight (*Erwiniaamylovora*) diseases. Apple scab, caused by the ascomycete *Venturiainaequalis*, is the most damaging fungal disease in apple orchards. The earliest attempts to transform apple for enhanced resistance involved the pathogenesis-related chitinase gene from *Trichoderma* (Litz and Padilla, 2012).

Flachowsky *et al.,* (2008) transformed 'Pinova' with a gene encoding for an extracellular polysaccharide (EPS)-

depolymerase of the fire blight bacteriophage phi-Ea1h and evaluated its effects on the susceptibility to the disease. The regenerated transgenic plants were more resistant to fi re blight than the control plants.

Overexpression of the apple *MdNPR1* gene, an *Arabidopsis NPR1* homolog that plays a key factor in the SAR response, reduced *E. amylovora* symptoms in transformed plants of 'Galaxy' and M26 compared with the non-transformed control plants.

Some transgenic plants also showed some resistance to *Venturiainaequalis* and *Gymnosporangiumjuniperi-virginianae* (Malnoy *et al.,* 2007).

Plum (*Prunus domestica*)

Resistance to Plum Pox Virus (PPV)

"Honey Sweet" is a plum variety developed through genetic engineering to be highly resistant to plum pox potyvirus (PPV) the causal agent of sharka disease that threatens stone-fruit industries world-wide, and most specifically in Europe.

The gene that encodes the PPV protein was separated from the virus and inserted into the plum's DNA. The transgenic shoots (coded as C5) were placed in vitro to grow roots and were then propagated via grafts. To verify its resistance to Sharka, the trees were placed in greenhouses and studied for five years. Portions of infected plant tissue were grafted onto them, but they never developed the disease.

Based on evaluations of fruit quality and productivity 'Honey Sweet' is not only protected against PPV but also has the attributes of fruit quality and yield that make is suitable for commercial production (Scorza *et al.,* 2001).

Trifoliate orange (*Poncirus trifoliata*)

Salt stress tolerance

Trifoliate 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. Glycinebetaine (GB) is an important osmoprotectant involved in responses to salt stress.

In a quest to develop a transgenic trifoliate orange, tolerant to salt stress, Fu *et al.,* (2011) by means of *Agrobacterium*-mediated transformation introduced a *betaine aldehyde dehydrogenase* gene (*AhBADH*) cloned from *Atriplexhortensis* into trifoliate orange. *BADH* is involved in the production of glycine betaine (GB). GB levels in these lines were also higher than those in untransformed wild-type (WT) plants. In the transgenic lines, exposure to 200 mMNaCl resulted in significantly less serious leaf burning and defoliation, lower MDA accumulation, and higher chlorophyll contents than those in the WT plants. Moreover, when exposed to salt, shoots of transgenic plants contained lower levels of Na^+ and Cl^-, higher levels of K^+, and a higher K/Na ratio, while the same was true for the roots in most cases. RT-PCR analysis on three selected transgenic lines showed that the *AhBADH* gene was overexpressed in each of them. Overexpression of the *AhBADH* gene in transgenic trifoliate orange enhanced salt stress tolerance.

Banana (*Musa paradisiaca*)

Banana and plantain present unique problems for classical breeding. Both the dessert (AAA) and cooking bananas or plantains (AAB and ABB) are triploids and are sterile with the exception of a few genotypes. There has been considerable interest in the application of

biotechnologies such as mutation breeding and genetic transformation in order to target breeding objectives of specific cultivars. Most transformation reports have focused on disease resistance, primarily the real threats of Panama disease and Black Sigatoka.

Sagi *et al.,* (1994) reported the transformation of *Musa* protoplasts by means of electroporation; however, this procedure has been superseded by microprojectile bombardment and *Agrobacterium*-mediated transformation of embryogenic cultures.

'Rasthali' banana (AAB) has been transformed with a synthetic analogue (*MSI 99*) of the gene encoding the antimicrobial peptide magainin derived from the skinof the African clawed frog *Xenopuslaevis* (Ganapathi *et al.*, 2001).

Atkinson *et al.,* (2004) have transformed 'Grand Nain' banana with a rice cystatin as a means for conferring resistance to the nematode *Radopholussimilis*. The protocol involved a plasmid construct containing the rice cystatin*OcI D D86* under the control of the maize ubiquitin promoter UBA-1. Regenerated lines were assessed for resistance 8 weeks after challenge with the nematode, and 16 regenerated lines were putative positives.

Kumar *et al.,* (2005) reported the transformation of embryogenic cultures of 'Rasthali' (AAB) with the "s" gene of the hepatitis B surface antigen (HBsAg).

Cranberry (*Vaccinum macrocarpon*)

Herbicide resistance

Genetic transformation of American cranberry (*Vaccinium macrocarpon* Ait with the *bar* gene, conferring tolerance to the phosphinothricin-based herbicide glufosinate was carried out by Zeldin *et al.*, (2002). Plants of one 'Pilgrim' transclone grown under greenhouse conditions, though was injured by foliar treatments of 100 mg/L glufosinate, the injury was less severe compared to untransformed plants. Actively growing shoot tips were the most sensitive part of the plants and at higher dosages of glufosinate, shoot-tip injury was evident on the transclone. Injured transgenic plants quickly regrew new shoots. Shoots of goldenrod (*Solidago* sp.) and creeping sedge (*Carexchordorrhizia*), two weeds common to cranberry production areas, were seriously injured or killed at 400 mg/L glufosinate when grown in either the greenhouse. Stable transmission and expression of herbicide tolerance was observed in both inbred and outcrossed progeny of the cranberry transclone.

Orange (*Citrus sinensis*)

Resistance to greening

Commercial sweet orange cultivars lack resistance to Citrus Greening/Huanglongbing (HLB), a serious phloem limited bacterial disease that is usually fatal. In order to develop sustained disease resistance to HLB, transgenic sweet orange cultivars 'Hamlin' and 'Valencia' expressing an *Arabidopsis thaliana NPR1* gene under the control of a constitutive CaMV 35S promoter or a phloem specific *Arabidopsis* SUC2 (*AtSUC2*) promoter. Transgenic trees exhibited reduced diseased severity and a few lines remained disease-free even after 36 months of planting in a high-disease pressure field site (Dutt *et al.*, 2015).

Pineapple (*Ananascomosus*)

Herbicide tolerance

Sripaoraya *et al.,* (2001) transformed Thai pineapple variety 'Phuket' by microprojectile-

mediated delivery of the plasmid AHC25, carrying the β-glucuronidase (*gus*) reporter gene and the bialaphos resistance (*bar*) gene for herbicide tolerance. Transformed plants were regenerated from bombarded leaf bases on Murashige and Skoog-based medium. Integration and expression of the *bar* gene in regenerated plants was confirmed by Southern analysis and RT-PCR. Regenerated plants were assessed *in vitro* and under glasshouse conditions for their tolerance to the commercial herbicide Basta™, containing glufosinate ammonium as the active component.

Plants sprayed with Basta™ @1400 mg/L remained healthy and retained their pigmentation. The generation of herbicide-tolerant pineapple will facilitate more efficient weed control in this widely cultivated tropical crop.

Guava (*Psidium guajava*)

Resistance to fungal wilt disease

Genetic transformation was performed by MishraI *et al.,* (2014) using the *A. tumefaciens* strain LBA4404 harbouring the binary vector pIIHR-JBMch with endochitinase gene obtained from *Trichoderma harzianum,* neomycin phosphor transferase (nptII) gene under the control of CaMV 35S promoter and Nos terminator.

The highest transformation efficiency was achieved by wounding explants with tungsten particles (0.5 µm) through particle acceleration system.

Transformed explants regenerated shoots on selection medium stressed with 200 mg/L kanamycin for 12 weeks. Molecular analysis of transformants by PCR confirmed the integration of endochitinase and nptII gene in the plant nuclear genome.

Strawberry (*Fragaria* x *ananassa*)

Expression of Miraculin

Miraculin is a taste-modifying protein found in the miracle fruit (*Richadelladulcifica*), a native West African shrub. Sugaya *et al.,* (2008) introduced the gene encoding miraculin under the control of the CaMV 35S promoter into strawberry by *Agrobacterium*-mediated transformation to produce transgenic plants. Although, miraculin was detected in the leaves and fruits of the transgenic plants, the level of accumulation among the transgenic lines, which ranged from 0.5 to 2.0 µg/g fresh fruit which was lower than that in miracle fruits (145 µg/g fresh fruit). In conclusion, although the level of accumulation was not high enough for commercial production, miraculin was stably expressed and accumulated in the vegetative progeny of transgenic strawberry, suggesting that strawberry may be a viable platform for recombinant miraculin production.

Kiwifruit (*Actinidia chinensis*)

Insect resistance

The kiwifruit (*Actinidia chinensis* Planch.) is an economically and nutritionally important fruit crop that has a 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.

HY *et al.,* (2015) developed transgenic kiwifruit plants containing the synthetic chimeric gene *SbtCry1Ac* that encodes the insecticidal protein btCrylAc were obtained through an *Agrobacterium*-mediated transformation of kiwifruit leaf discs. Results from polymerase chain reactions and genomic DNA Southern blot analyses indicated that *SbtCrylAc* 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 day's post-infestation was 75.2%.

Grape (*Vitis vinifera*)

Improved cold resistance

Dehydration response element binding (*DREB*)*1b* is a cold-inducible transcription factor in *Arabidopsis thaliana*. Jin *et al.*, (2009) genetically introduced *DREB1b* driven by cauliflower mosaic virus 35S promoter into grape *Vitis vinifera* L. cv. Centennial Seedless through *Agrobacterium*-mediated transformation for improving its cold resistance.

After the cold treatment at –4 °C for 12 h, 26% of transgenic plants wilted among which 95% plants recovered once being placed under the condition of temperature 22 to 25 °C. However, subjected to the same treatment, 98% of non-transgenic plants wilted and only 2% recovered.

Possible Challenges

Genetically modified fruit crops are beneficial yet there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labelling. Therefore, we must proceed with caution to avoid causing unintended harm to human health and the environment. Some of the challenges are:

The transfer of pollen between modified and non-modified plants could also create health and ecological problems involves. There is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health on the whole, with the exception of possible allergenicity, scientists believe that GM foods do not present a risk to human health.

Bringing a GM food to market is a lengthy and costly process, and companies wish to ensure a profitable return on their investment.

Many new plant genetic engineering technologies and GM plants have been patented, and patent infringement is a big concern of agribusiness. Patenting these new plant varieties will raise the price of seeds so high.

Patent enforcement may also be difficult. One way to combat possible patent infringement is to introduce a "suicide gene" into GM plants. These plants would be viable for only one growing season and would produce sterile seeds that do not germinate. Farmers would need to buy a fresh supply of seeds each year.

However, this would be financially disastrous for farmers in third world countries who cannot afford to buy seed each year and traditionally set aside a portion of their harvest to plant in the next growing season.

Fruits are the rich sources of vitamins and the other nutrients, these sources can be improved by the means of this transgenic breeding. To date, most transformations have been concerned with enhancing resistance to diseases and extending the shelf life of fruit.

In the future, researchers hope to be able to provide vaccinations and medicines in GM foods, which can provide medications to people in developing countries more easily. With advances in science and technology, safety of the process and cost-effectiveness will be addressed.

Advances in genomics during the next few years will have a major impact on this area. While it is difficult to determine changes in

public acceptance of transgenic fruit in the future, the advancement of the alternative, cisgenics, may significantly affect public opinion.

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