INTERNATIONAL JOURNAL OF PROCESSING AND POST HARVEST TECHNOLOGY (December, 2010); 1 (2) : 118-124

A REVIEW

Received : Oct., 2010; Revised : Nov., 2010; Accepted : Dec., 2010

Prospects of transgenic technique for the improvement of vegetables

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KEY WORDS :

Dhumal, S.S., Sane, S.C. and Gaikwad, A.G. (2010). Prospects of transgenic technique for the improvement of vegetables, *Internat. J. Proc. & Post Harvest Technol.,* **1** (2) : 118-124.

INTRODUCTION

Genetic engineering is the most potent biotechnological approach for the transfer of specially constructed gene assemblies through various transformation techniques.Development of transgenic crops during the 1990's is an important landmark in the history of crop improvement.Since the first commercial release in 1994, transgenic crops have registered a steady increase in area (67.7 million ha, 2003) and have slowly spread across nations (18 countries) (James, 2003). A 'transgenic' plant contains a gene or genes that has been artificially inserted. Transgenic plants are also called as 'genetically modified' (GM) crops. The inserted gene sequence is known as the 'transgene'. It may come from an unrelated plant, or from a completely different species. 'Transgenic technology refers to the technique capable of transferring genes from donor organisms to recipient organisms without the involvement of sexual reproduction between them (Rissler and Mellon, 1996). The global area under the cultivation of transgenic crops has increased from 1.6 million ha in 1996 to 81.00 million ha in 2004. The first, and as yet the only transgenic crop permitted for the commercial cultivation in India is cotton, for bollworm resistance, popularly known as Bt cotton (Rai, 2006).

Applications of transgenic technology:

– For Herbicide resistance : e.g. Plants have been obtained tolerant to herbicides like glyphosate, sulfonylurea and imidazolinones.

– For disease resistance.
  Virus resistance.
  Fungal resistance.
  Bacterial resistance.
– Insect pest resistance.
– For obtaining parthenocarpic plants.
– For improving the post harvest traits.
– For obtaining male sterile plants.
– For development of vaccines.

(Source : ISVS Souvenir : Silver Jubilee National Symposium, Dec.1998.)

Few milestones in the history of transgenics

1953	-	Watson and Crick determine the double helix structure of DNA.
1970 s	-	Recombinant DNA technology developed.
1983	-	Foreign gene inserted into tobacco (First transgenic plant).
1991	-	Foreign gene inserted into petunia (First transgenic ornamental).
1994	-	Calgene's Flavr savr transgenic tomato approved for sale in the United States.
1996	-	U.S. market introduction of transgenic corn, cotton and soybean : 3 million acres planted. Market introduction of transgenic carnation in Australia.
1998	-	More than 700 requests to move, import or field release transgenic plants approved.
2002	-	Bt-Cotton was released for commercial cultivation in March in India.
2006	-	(24th Sept.) Indian Government brings a stay on the trials carried out on GM plants.

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Advantages of transgenic plants:

Resistant to insects and pests :

The excessive use of pesticides can cause health hazards. Growing transgenic plants can help to reduce the application of pesticides since they are internally resistant to pests.

Resistant to diseases:

Same is true for diseases also.

Tolerant to herbicides:

Transgenic plants have been developed tolerant to herbicides.

Examples of successful transgenic vegetables against viruses

Name of viruses	Transformed plant
Tomato mosaic virus (TV)	Tomato
Cucumber mosaic virus (CMV)	Cucumber
Potato virus X (PVX)	Potato (Russet Burbant)
Potato virus Y (PVY)	Potato
Cucumber mosaic virus (CMV)	Tomato
Tomato spotted wilt virus (TSWV)	Tomato
Tomato yellow leaf curl virus (TYLCV)	Tomato

(Source : ISVS; Souvenir)

Examples of successful transgenic vegetables for parthenocarpic fruits

Transgene	Origin of transgene	Transformed plant
Rol B gene	*Agrobacterium rhizogens*	Tomato
iaah gene	*Pseudomonas syrinagae* pv. *savastonai*	Brinjal

(Source : ISVS; Souvenir)

Examples of successful transgenic vegetables against bacteria

Resistance against	Origin of transgene	Transformed plant
Erwinia carotovora	T_4 bacteriophage	Potato
E. carotovora	Horseshoe crab	Potato
Pseudomonas syringae pv. Phaseolicola	*P. syringae* pv. phaseolicola	Bean
E. carotovora	*Aspergillus niger*	Potato

(Source : ISVS, Souvenir)

Cold tolerance:

An antifreeze gene isolated from cold water fish has been introduced and transferred into plants to make them cold tolerant.

Heat tolerance:

Similarly heat tolerant plants can be produced.

Drought or salinity tolerance:

Plants can be grown on sodic soils and waste lands.

Increased nutritional value:

Transgenic plants having a nutritious value can play a very important role in fulfilling the need of undernourished countries.

Can be used in pharmaceuticals:

Edible vaccines can be made from tomatoes and potatoes.

Transgenic plants expressing insect resistant genes

Genes	Origin of transgene	Target insects	Transformed plants
Cry1 AB	*Bacillus thuringiensis*	Lepidoptera	Tomato
Cry3 A	*Bacillus thuringiensis*	Coleoptera	Egg plant
Cp T1 (Cowpea trypsin inhibitor)	Cowpea	Coleoptera, Lepidoptera	Tomato
Tomato proteinase inhibitor I	Tomato	Lepidoptera	Tomato
Tomato proteinase inhibitor II	Tomato	Lepidoptera	Tomato
α Al-Pv.	Common bean	Coleoptera	--
Bovine pancreatic trypsin inhibitor (BPT-1)	--	Lepidoptera	Lettuce

(Source : ISVS, Souvenir)

Examples of successful transgenic vegetables for post harvest traits

Purpose of genetic manipulation	Transgene product	Transformed plant
Improved shelf / Storage life	Antisense polygalac-turonase	Tomato
Ripening	Antisense ACC oxidase	Tomato
	Antisense ACC synthase	Tomato
Fruit pigmentation	Phytoene synthase gene	Tomato

(Source : ISVS, Souvenir)

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(Source : ISVS, Souvenir)

Increased yield:

Transgenic plants have been reported to give a high yield.

Improved quality:

Transgenic crops of tomato give fruits which have good colour, flavour and shelf life.

(Source : Invention Intelligence, 2004)

Agro-bacterium mediated gene transfer:

In this method, a soil borne bacterium called *Agrobacterium fumefaciens* is used as a mediator to transfer genes into plants. This method includes :

– Co-culture with tissue explants

– In-planta transformation

Direct gene transfer:

In this method, there is no involvement of such biological agent like *Agrobacterium*. This method includes :

Transformation techniques

I)	Agrobacterium-mediated gene transfer	II)	Direct gene transfer
i	Co-culture with tissue explants.	i	Chemical methods
ii	In planta transformation	ii	Electroporation
		iii	Particle gun method
		iv	Lipofection
		v	Microinjection
		vi	Fibre mediated DNA delivery
		vii	Laser inducted DNA delivery
		viii	Pollen transformation
		ix	DNA delivery via growing pollen tubes
		x	Macroinjection
		xi	Direct DNA uptake by mature zygotic embryos.

(Source : Biotechnology – B.D. Singh).

Chemical methods:

In this, chemicals like polyethylene glycol, polyvinyl alcohol, calcium phosphate are used, which enhance the DNA uptake by protoplast.

Electroporation :

Introduction of DNA into plant cells by exposing them to high voltage electric pulses.

Particle gun method/Bolistic method :

1-2 mm diameter tungsten or gold particles are coated with DNA and are accelerated to velocities so that they enter plant cells.

Lipofection :

Introduction of DNA into cells via liposomes. Liposomes are small vesicles prepared from a suitable lipid.

Microinjection :

DNA is directly injected into plant cells.

Fibremediated DNA delivery :

DNA is transferred into cells by using silicon carbide fibres.

Laser induced DNA delivery :

Laser is used for transfer of DNA.

Pollen transformation :

Pollens are soaked in DNA solution before pollination.

DNA delivery via growing pollen tubes :

The stigma is cut after pollination and DNA solution is applied on the cut ends.

Macroinjection :

DNA solution is injected on the developing inflorescence.

Direct DNA uptake by mature zygotic embryos:

The mature zygotic embryos are imbibed in DNA solution.

Discontinued transgenic products:

Flavr savr tomatoes :

Introduced in 1994 by Calgene. Developed by using antisense RNA to regulate the expression of polygalacturonase (PG). It is responsible for fruit ripening. (Softening enzyme). First commercial product.

Zeneca tomato paste :

Here also activity of PG was reduced. But instead of anti-sense technology, Zeneca tomatoes were engineered with a non-functional, shortened version of the gene. Withdrawn in 1999 because of negative public opinion about transgenics in general.

Some of the genetically transformed horticultural crops

Category	Crop
Fruits and nuts	Citrus, melon, papaya, banana, apple, pear, peach, cherry, walnut, grape, strawberry, kiwifruit, plum, almond, apricot
Vegetables	Tomato, pea, cowpea, beans, cucumber, carrot, cabbage, cauliflower, lettuce, asparagus, brinjal, sweet pepper, chilli, broccoli
Roots and tubers	Potato, cassava, sweet potato, yam, sugarbeet
Plantation	Cocoa, Coffee
Ornamental	Orchid, petunia, chrysanthemum, carnation, gladiolus and antirrhinum
Spices	Black pepper, ginger, onion and garlic

(Source : Naik *et al*., 2000)

Genetically transformed vegetables in India

Crop	Gene and characteristics	Research organization
Brinjal	Cry 1A(c), resistance to stem and fruit borer	NRC on plant biotechnology (NRCPB), IARI, New Delhi.
Cabbage	Cry 1(b), resistance to diamond back moth	NRCPB, IARI, New Delhi.
Cauliflower	Cry I A (c), insect resistant	NRCPB, IARI, New Delhi.
Chilli	npt II, Kanamycin resistant	Indian Institute of Sciences, Bangalore.
Potato	Cry 1A(c), resistance to potato tuber moth, Osmotin, resistance to late blight and stress. Amal, novel Amaranthus storage proteins.	Central potato research Institute, Shimla and NRCPB, IARI, JN University, New Delhi.
Tomato	Cry 1A(c) resistance to Heliothis ACC synthase, delayed ripening	NRCPB, IARI, New Delhi.

(Source : Naik *et al*., 2000)

Some important genes modified/clones in India

Trait	Gene	Institute
Insect resistance	Cry 1 I A 5	ICGEB, New Delhi
	Cry 1A (C)	Bose Institute, Calcutta
	Cry 1 A (b)	
	Cry III A	
	Cowpea trypsin inhibitor	NBRI, Lucknow
	Bt. VIP and cowpea lectin	NRCPB, IARI, New Delhi.
Virus resistance	CP – Potato virus Y	IARI, New Delhi.
	CP – Cucurbit mosaic virus	NBRI, Lucknow
	CP – Tomato leaf curl virus.	
	CP–Tomato leaf curl virus and	IIHR, Bangalore.
	CP – Water melon bud necrosis virus.	
Quality characters	Amaranthus protein Amalgene	JNU, Delhi
	ACC synthase and fatty acid elongase	NRCPB, IARI, New Delhi
Male sterility	Barnase and Barstar	Delhi University, Delhi.

(Source : Naik *et al*., 2000)

Private companies in India working on transgenic crops (Lines in advanced stage of development for field trials)

Name of company	Crop	Transgene	Aim of Project
Ankur seeds Ltd., Nagpur	Cotton	Cry 1 A (c)	To generate plants resistant to lepidopteran pests.
Indo-American Hybrid seeds, Bangalore	Tomato	Alfalfa glucanase and Tomato leaf curf virus genes	Generate plants resistant to viral and fungal attack
Proagro PGS (India) Ltd.,	Tomato	Cry 1 A (b)	Plants resistant to lepidopteran pests.
Gurgaon	Brinjal	Cry 1 A (b)	Plants resistant to lepidopteran pests.
	Cauliflower	Cry 1 H /Cry 9 C	Plants resistant to lepidopteran pests.
	Cabbage	Cry 1 H /Cry 9 C	Plants resistant to lepidopteran pests.

(Source : Current Science Vol. 84, 2003).

Major Indian developments in transgenic research and application in Public sector

Institute	Plants	Transgene inserted	Aim of the project
Central Potato Research Institute Shimla	Potato	Bt Cry 1 A (B)	To generate plants resistant to lepidopteran pests.
Delhi University, South Campus, New Delhi	Tomato	Ctx and Tcp antigens of *Vibrio cholerae*`	Edible vaccine development.
	Brinjal	Chitinase, glucanase and thaumatin encoding genes	To generate plants resistant to diseases.
Indian Agricultural Research	Brinjal	Bt cry 1A (b)	To generate plants resistant to lepidopteran pests.
Institute, New Delhi.	Tomato	Bt cry 1A (b)	To generate plants resistant to lepidopteran pests.
	Cauliflower	Bt cry 1A(b)	Generate plants resistant to *Plutella xylostella* (Diamond back moth)
	Potato	ACC synthase	To control fruit ripening
	Tomato	ACC Synthase	To control fruit ripening
	Banana	ACC Synthase	To control fruit ripening
Indian Institute of Horticultural	Musk melon	Rabies Glycoprotein gene	To develop edible vaccines
Research, Bangalore	Tomato	Leaf curl virus sequence	To generate plants resistant to leaf curl virus
	Tomato	Chitinase and glucanase	To generate plants resistant to fungal diseases.
	Citrus	Coat protein gene of citrus triesteza virus	To generate plants resistant to citrus triesteza.
Jawaharlal Nehru University,	Potato	Ama-1	To generate nutritionally enriched plants.
New Delhi	Tomato	OXDC	Generate plants resistant to fungal infection.
Madurai Kamaraj University, Madurai	Coffee	Chitinase,β-1, 3-glucanase and osmotin genes	To develop plants resistant to fungal infection.
University of Agricultural Sciences, Bangalore	Musk melon	Rabies glycoprotein gene	To develop edible vaccines.

(Source : Current Science, Vol. 84, 2003)

New leaf potatoes :

Contained Bt gene resistant to insects and pests. Marketed in 1996 by Monsanto Co. Never commanded the market because fast food chains and chip makers did not accept them. Withdrawn in 2001.

Triffid flax :

Developed by University of Saskatchewan.

Starlink corn :

Developed by Aventis.

Bt-176 corn :

Sold as Knockout by Novartis and Naturegard by Mycogen.

(Source : Transgenic crops : An introduction & research guide, 2006.)

The hazards of GM plants/transgenic plants:

Poisoning the environment :

The Bt toxin in the Bt plants like cotton and corn persists in the soil for at least 234 days and is supposed to be toxic to animals and humans.

The Terminator gene:

Some GM plants have engineered to produce sterile or non-viable seeds. This trait, labelled the 'terminator gene' was engineered to force farmers to buy new seeds from agribusiness giants every year.

Allergens:

The novel proteins in GM plants can cause unexpected allergic reactions such as vomiting, diarrhea and anaphylactic shock.

Creation of superweeds and superbugs:

GM plants are exotic species that can take over a new landscape, creating new 'superweeds or 'superbugs' that may in turn require more toxic chemicals

Contamination of organic crops:

The pollen from transgenic plants can drift onto neighbouring farms and cross pollinate with similar crops and thus contaminate them.

Toxins:

Transgenic plants contain unexpected high concentrations of plant toxicants.

Antibiotic resistance:

Scientists used a marker gene to help them determine whether the gene they are trying insert into the organism has actually made. Frequently, the marker gene used is one that codes for antibiotic resistance. Due to the use of this humans are building up dangerous levels of resistance to modern antibiotics that could leave them vulnerable to killer diseases.

Cancer risks:

Bovine growth hormone leads to increased levels of a potent chemical hormone that has been linked to cancer in humans when at elevated levels.

Nutrition:

There is evidence that some GM crops have reduced nutritional quality.

Socioeconomic disparity:

The big companies only think of their own profit. While trying to bring in resistance to herbicides and insects, the public health or environmental benefits are being ignored causing socio-economic disparity.

Worsening world hunger:

The biotech industry claims that this technology is needed to feed the growing world population. But the transgenic crops fail to give good yields thus worsening world hunger.

Controversy	
Indian farmers defied laws to obtain illegal Bt-cotton seed on the black market because of its superior yields and the significant profits. (R. Ray-Social movements and Poverty in India.)	Top biotech scientists say, "It is an absolute scandal for us to allow further trials of Bt-cotton despite its failure. (Frontline June 2006)
Reduced pesticide application and expenditure and better product quality were the most important adoption factors of Bt cotton by the farmers. (Economic and political weekly)	Deccan Development society disproved many claims made about advantages of Bt-cotton. The study found that organic farmers had higher net returns & lower pest management costs than Bt-cotton farmers. (Frontline June 2006).
Farmers using Bt cotton in India reported decline in soil productivity. (Frontline : June 2006)	Mahyco said that its tests had ruled out the possibility of any such adverse impact on the environment. (Frontline).
Studies worldwide have shown that eating GM food can result in wasteful growth of gut tissues, intestinal tumors, immune system supression.	Mahyco denied. "Bt is no more immunogenic than any other protein that human and animals are exposed to"
Scientists demonstrate that recombinant cry 1 A (c), protoxin in the Bt gene is a powerful immunogen and when fed to mice binds the inner surface of the mouse's small intestine. (Ambumani Ramdoss)	Cry proteins do not affect mammals since they supposedly do not have receptors that bind the truncated toxin in the gut. (Ambumani Ramdoss)
If humans eat Bt brinjal, it is possible that the Bt toxin can inter the digestive system and interfere with the bacteria in the intestines.	There is no harmful effect of Bt brinjal since all the safety tests have been completed and there are no reasons to stop the large scale field trials.

(Source : Agrobios Newsletter, Vol. IV May 2006.)

Why BAN GMO crops?

Irreversible harm to the environment:

The transgenic crops will cross-pollinate with the non-GM crops and will produce new weeds. This is irreversible harm to the environment which cannot be recovered.

Irreversible harm to the economy:

The livelihood of organic farmers will be threatened by creation of new weeds *i.e.* the GMO pollution will make the produce unmarketable which will prove to be a loss to the economy.

Genetic pollution of private properties:

In some regions where nearby GMO crops are grown, wind drift has blown GMO pollen to organic farms, cross contaminating the resulting seeds and fruits. So, the individual property owners who wish to keep their properties GMO free, may be unable to do so.

(Source : Agrobios Newsletter, Vol. IV, May 2006).

Conclusion:

– The concept of transgenic technology is extremely controversial. It can prove to be a boon or bane.

– Technological innovations bring their own sets of benefits and risks to the environment and no technology is 100 per cent safe. The same is true for transgenic plants. If scientists keep generating transgenic plants harmful to the environment, then the future of this technology will be dark.

So co-ordinated efforts should be undertaken to investigate the potential environmental effects, both positive and negative, of transgenic plant technologies in their specific applications.

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