STUDY OF TRANSGENIC TECHNIQUES IN VEGETABLE CROPS

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Abstract - Genetically modified crop or transgenic crop enable breeder to bring favorable genes into elite cultivars and offer unique opportunities for controlling insects and other pathogens. FLAVR SAVR tomato was developed through the use of antisense RNA to regulate the expression of the enzyme polygalaturonase in ripening tomato fruit. Antifreeze proteins in the blood of some polar fishes have been shown to inhibit ice recrystallization at low concentrations. The afa3 antifreeze gene was expressed at high steady-state mRNA levels in leaves from transformed plants. Amount of Bt protein was present in all transgenic lines and plants expressing Cry2Ab could be used for management of the target lepidopteran insect pests. AmA1 coding sequence was successfully introduced and expressed in potato tuber in constitutive manner. It was found that production and growth of tubers was increased, as well as protein content with an increase in most essential amino acids. Recently, a purple tomato highly enriched with anthocyanin was produced by the ectopic expression of two selected transcription factors from the ornamental flower snapdragon. In addition to being enriched with anthocyanin, these fruits also prolonged the life of cancer-susceptible mice, they have additional health-promoting effects. Transgenic crops basically improving production stability, nutritional benefits, reduce environmental impacts and increase the availability of pharmaceuticals and vaccines.

I. INTRODUCTION

Transgenic crops, generally referred as genetically modified crops, refers to the transfer of desirable gene between organisms through recombinant DNA techniques for cloning gene, splicing DNA segment together and inserting genes into cells. A transgenic crop is a plant whose genetic characteristics have been altered by human (Chahal and Gosal, 2010). The aim is to introduce new trait into a plant which does not occur naturally in the species. A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the transgene, it may come from an unrelated plant or from a completely different species (Rani and Usha, 2013).

The whole process of introduction, integration and expression of foreign genes in the host is called genetic transformation (Chahal and Gosal, 2010). The purpose of inserting a combination of genes in a plant is to make it as useful and productive as possible. This process provides advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses (Herbers and Sonnewald, 1999).

What is transgenesis?

Transgenesis, as the word denotes, means transfer of genetic material i.e. DNA fragment carrying known genes from across the biological systems.

Why we make transgenic crops?

Traditionally, a plant breeder tries to exchange genes between two plants to produce offspring that have desired traits. This cross breeding, however, is limited to exchanges between the same or very closely related species. It can also take a long time to achieve desired results. GM technology enables plant breeders to bring together in one plant useful genes from a wide range of living sources, not just from within the crop species or from closely related plants. This powerful tool allows plant breeders to do faster what they have been doing for years.

Importance of transgenics in agriculture

The biotechnological approaches can be used in the following conventional crop improvement programs:-

- Integrated gene management
- Integrated nutrient management
- Integrated pest management
- Water management
- Soil health care
- Post-harvest management

How are transgenic crops made?

Gene transfer methods have broadened the available gene pool as the gene may come to plants from unrelated plants, viruses, bacteria, fungi, insects, animals, human beings and even from chemical synthesis in the laboratory. Depending upon the mode of transfer of foreign gene to plants two types of methods i.e. vector mediated and direct gene transfer are usually applied.

Vector mediated gene transfer

- Agrobacterium tumefaciens
- Agrobcacterium rhizogenes

Direct gene transfer (DGT)

- Physio-chemical uptake of DNA
- Liposome encapsulation
- Electroporation of protoplasts
- Microinjection
- DNA injection into intact plants
- Incubation of seeds with DNA

- Pollen tube pathway
- Laser microbeam
- Electroporation into tissues/embryos
- Silicon carbide fibre
- Particle gun method

Agrobacterium-mediated transformation

Agrobacterium is naturally occurring gram negative, soil bacterium with two common species viz. A. tumefaciens and A. rhizogenes. These are known as natural genetic engineers for their ability to transform plants. In its natural environment, wild type Agrobacterium tumefaciens causes crown gall disease. Genes for recognition of susceptible cells by the bacterium and its binding to these cells are located on bacterial chromosomes. However, the capability of bacterium to transfer DNA that is incorporated into plant's chromosome, resistance to antibiotics and pathogenicity are encoded on a plasmid. A plasmid is a segment of DNA which is separate from bacterial chromosome and can replicate independently of the chromosome. Wild type plasmid of A. tumefaciens is responsible for inducing tumor and hence termed as tumor inducing (Ti) plasmid. The Ti plasmid has two major regions of interest of transformation i.e. T-DNA (transfer DNA) and vir region. The T-DNA region of the Ti plasmid of Agrobacterium is the part which is transferred to plant cell and is incorporated into nuclear genome of cells in an infected wound. Transfer of T-DNA is mediated by genes in another region of Ti-plasmid called vir (virulence) genes. So after infection of plants by bacteria, the single stranded T-DNA gets transferred to plant cells which actually express the plasmid genes to result in galls i.e. disease symptoms. If disease-inducing gene are improved from T-DNA region of plasmid i.e. plasmid is disarmed, the bacterium does not cause disease. On the other hand, if some other gene is placed in the T-DNA region, it is transferred to plant.

Direct gene transfer methods

Particle bombardment/Biolistics

The process of particle bombardment or biolistics involves acceleration of DNA into cells with sufficient force such that a part of its gets integrated into DNA of the target cells. The process of transformation employs foreign DNA coated minute 0.2-0.7 μm gold or tungsten particles to deliver into target plant cells. The original equipment used a gunpowder charge behind a packed cartridge to accelerate the particle that gave it name of particle gun. Particle gun accelerated particles penetrate even deep into the tissue.

Achievements in transgenic crops

The potential advantages of genetically engineered foods include improved nutrition and taste, decreased use of pesticides, increased tolerance to drought, increased yield and decreased production costs. The main aim is the production of transgenic plants with disease and pest resistance because of the worldwide importance for the grower despite the present difficulty in their public acceptance (Miia et al. 2013).

Transgenics for quality improvement

FlavrSavr tomato

The first commercially grown transgenic crop was FlavrSavr™ tomato. . It was produced by the Californian Company Calgene, and submitted to the U.S. Food and Drug Administration (FDA) in 1992.Through genetic engineering, Calgene hoped to slow the ripening process of the tomato and thus prevent it from softening, while still allowing the tomato to retain its natural color and flavor. The tomato was made more resistant to rotting by adding an antisense gene (Arctic Tuna fish) which interferes with the production of the enzyme polygalacturonase. The enzyme normally degrades pectin in the cell walls and results in the softening of fruit which makes them more susceptible to being damaged by fungal infections (Mathew et al. 1994). However, FlavrSavr™ tomato failed in the market since this cultivar was considered inferior by growers, and was rapidly withdrawn from the market. Tomato fruit ripening manipulation has been however achieved by introducing anti-ripening genes (rin and nor) in heterozygous genotypes. These genes have been incorporated in many fresh and processing tomatoes (Dias and Ortiz, 2012).

Blue tomatoes

Blue tomatoes are referred as purple tomatoes. In 2012, Jonathan D. G. Jones and Cathie Martin of John Innes Centre developed blue tomato. The fruit are rich in antioxidant pigment called anthocyanin which have anti-cancer properties (Gonzali et al. 2009). A team from the John Innes Centre, Norwich, created the tomatoes by incorporating genes from the snapdragon flower,which is high in anthocyanin.

Transgenic potato

Protein deficiency is the most crucial factor that affects physical growth and development and increases mortality. GM potato rich in protein was developed by team scientists by Dr. Subhra Chakraborty of the national institute of plant genome research (NIPGR) in close collaboration with central potato research institute (CPRI) of India.Gm potato is called "Protato" which contains 60% higher protein content developed by introducing AmA1 (Amaranth Albumin 1) from edible amaranth plant (Chakraborty et al. 2010).

Colorful cauliflowers

The orange cauliflower has higher than normal levels of beta carotene, a form of vitamin A that encourages healthy skin. The purple colour comes from anthocyanin, which may help prevent heart disease by

slowing blood clotting. Tests of the orange cauliflowers in America found that they contained 25 times the concentrations of beta carotene in normal cauliflowers.

Tearless onion

Dr. Colin Eady and his collaborators develop tearless onion. As onions are sliced, cells are broken and sulphoxides generate sulphenic acids which is unstable and rearrange into volatile gas and reaches the eye where it reacts with water to form diluted solution of sulphuric acid, tear glands produce tears to dilute and flush out the irritant. They proved that it was controlled by an enzyme. Insert DNA into onions by using gene silencing technology and switches off the tear-inducing gene in onion so it doesn't produce tears (Highfield, 2008).

Transgenics for biotic stress
Transgenic crops approved for multi-locational trials

Crop	Trait	Institute/Industry	Transgene
Eggplant	Insect resistance	Mahyco, Mumbai IARI, New Delhi Sungro seeds Ltd, New Delhi	Cry1Ac, Cry1Aabc and Cry 2Ab
Cabbage	Insect resistance	Sungro seeds Ltd, New Delhi	Cry1Ba, Cry1Ca
Cauliflower	Insect resistance	Sungro seeds Ltd, New Delhi	Cry1Ac, Cry2Ab
Potato	Late blight resistance	CPRI, Shimla	RB gene derived from Scianum
Okra	Insect resistance	Mahyco, Mumbai	Cry1Ac, Cry2Ab
Tomato	Virus resistance	IARI, New Delhi Mahyco, Mumbai	Antisense replicase gene for tomato leaf curl virus, Cry2Ab

(Ram, 2012)

Transgenic Brinjal

The Bt brinjal is a suite of transgenic brinjal (also known as an eggplant or aubergine) created by inserting a crystal gene (Cry1Ac) from the soil bacterium Bacillus thuringiensis into the genome of various brinjal cultivars. Bt brinjal, a genetically modified strain created by India's number one seeds company Mahyco (Maharashtra hybrid seeds Company) in collaboration with American multinational Monsanto. The Bt brinjal has been developed to give resistance against lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (Leucinodes orbonalis). The Bt varieties of brinjal are: Malpur local, Manjari gota, Kudachi local, Udupi local, and Pabkavi local (Ram, 2012).

Mechanism of Bt

- Insect eats Bt crystals and spores.
- The toxin binds to specific receptors in the gut and insects stop eating.
- The crystals cause the gut wall to break down, allowing spores and normal gut bacteria to enter the body.
- The insect dies as spores and gut bacteria proliferate in the body.

Bt potato

Cry3A gene has been inserted into potato plant that has toxic effect on Colorado Potato Beetle (Douches et al. 2004).

Bt tomato

Cry2Ab gene has been inserted into tomato plant, showed resistance to tobacco hornworm, tomato fruitworm, tomato pinkworm and tomato fruit borer (Sakeret al. 2011).

Transgenics for abiotic stresses

Anti-freeze protein in tomato

Placing the anti-freeze gene "afa3" from a polar fish in tomatoes so that they can grow in cold weather. Anti-freeze protein was discovered by Arthur De Vries and colleagues in 1969 (Hightower et al. 1991).

Transgenics for human benefits

- Malarial vaccine has been developed in many varieties of tomatoes.
- Researchers and Scientists have produced Transformed Potatoes expressing vaccine against diarrhea caused by Escherichia coli bacteria.
- Researchers and Scientists have produced Transformed Potatoes expressing Human vaccine against Hepatitis B
- Researchers and Scientists are working towards developing Banana to produce vaccine against diarrhea caused by Escherichia coli bacteria.
- HIV-1 Vaccine candidate Tat protein has been produced in Spinach.
- In near future we can expect edible vaccines in the form of Tomato, Potato, Lettuce, Watermelon, Muskmelon and many more.

CONCLUSION

Transgenic crop research and development should focus on plants that will improve production stability, give nutritional benefits to the consumer, reduce the environmental impacts, increases the availability of pharmaceuticals and vaccines. Genetic engineering has the potential to address some of the most challenging biotic constraints faced by crop growers, which are not easily addressed through conventional plant breeding alone. Furthermore, herbicide-tolerant transgenic crops can help reducing plough in fields, thereby saving fuel because of less tractor use, which also protects the structure of the soil by reducing its

erosion. Evaluation of the transgenic plants under stress conditions, and understanding the physiological effect of the inserted genes at the whole plant level remain as major challenges to overcome. Develop the protocols and regulations to ensure that transgenic crops designed for purposes other than food, such as pharmaceuticals, industrial chemicals etc. do not spread or mix with either transgenic or non-transgenic.

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