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Transgenic Vegetable Crops

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Transgenic plants commonly referred to as genetically modified (GM) crops are the ones, whose DNA is modified using genetic engineering techniques aiding plant breeders to bring favorable genes, often lacking in elite cultivars, improving their value considerably by improving nutritional and health benefits and offer unique opportunities for ameliorating biotic stress as well as abiotic stress. Vegetables make up a major percentage of the human diet and play a significant role in human nutrition, especially as sources of phytonutriceuticals and phytochemicals. The Flavor Saver tomato was the first commercially grown, GE vegetable for human consumption in US in 1994. Development of Amflora potato, bruise-resistant potato, virus resistant squash, biopharming in tomato etc are some of the achievements of transgenics. Thorough a mechanism exists to ascertain the ethical and environmental issues, involvement of Farmers, Consumers and NGOs besides the scientific body is necessary to determine the true safety of transgenic crops and whether they are safe for both the environment and for those who consume these products over the ages.

Key words: GE vegetable, Genetically modified (GM), Recombinant DNA (rDNA) technology, Transgene, DNA splicing, Lipofection, Microinjection, Agrobacterium *tumefaciens*, EIQ (Environmental Impact Quotient), Tumor-inducing (Ti) plasmid,

Introduction

Transfer of the gene of interest in an organism became possible with the advent of recombinant DNA Technology in 1970s. A plant in which a foreign gene has been transferred through genetic engineering is called a transgenic plant and the gene so transferred is called transgene. With this technology it has become possible to modify the genetic information of living organisms in a new way, by transferring one or more fragments of DNA directly between organisms across species, genera or even kingdom. Organism thus developed contains a gene or genes which have been introduced artificially into its genetic makeup by using a set of several biotechnology techniques collectively known as recombinant DNA (rDNA) technology. Transgenic term has been synonymously refereed with other terms such as recombinant DNA technology, DNA splicing or Genetic engineering. The technology refers to plants containing genes from another organism that were not transferred via sexual crossing. However genetically modified plants refers to those wherein manipulation of genes of a living organism is carried out by any means which is often misused to describe organisms that were genetically modified using means other than traditional breeding. Several vegetable crops viz., Tomato, Brinjal, Potato, Squashes, Melons, Cassava etc., have been genetically transformed to include resistance to pests, pathogens and herbicides, and for improved features, such as slow ripening, higher nutritional status, reducing anti-nutritional factors, parthenocarpy etc., Genetic transformation is achieved in the target plants primarily through particle bombardment and *Agrobacterium* mediated transformation. *Agrobacterium* method is advantageous over biolistics because it can introduce larger segments of DNA with minimal arrangement and with fewer copies of inserted transgenes at higher efficiency and lower cost. 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. Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical applications.

Relevance of Transgenics Crops

Changing scenario of Indian Agriculture in view of the climatic changes viz., rise in temperature, elevated CO2 levels and unpredicted rainfall and need to sustain a burgeoning population with nutrient deficiencies has made imperative use of alternate technologies such as transgenics. Such a transformation of agriculture for reducing poverty and hunger for promoting equity in many of the world's poorer countries is inevitable. The existing food shortage as such is the principal cause of hunger and ignores to some extent the reasons for poverty, inequitable distribution of food, land tenure inequity, overpopulation, poor health, poor education etc. With the rapid adoption of transgenic crops within the agricultural sector, has resulted in sustained increases in agricultural productivity, contributed to economic growth and ensured an abundance of food. Biotech crops contributed to food security, sustainability and climate change solutions by: increasing crop productivity by 657.6 million tons valued at US$186.1 billion in 1996-2016; and 82.2 million tons valued at US$18.2 billion in 2016 alone; conserving biodiversity in 1996 to 2016 by saving 183 million hectares of land, and 22.5 million hectares of land in 2016 alone; providing a better environment by saving on 671 million kg. a.i. of pesticides in 1996-2016, and by 48.5 million kg in 2016 alone from being released into the environment; by saving on pesticide use by 8.2% in 1996-2016, and by 8.1% in 2016 alone; by reducing EIQ (Environmental Impact Quotient) by 18.4% in 1996-2016, and by 18.3% in 2016 alone reducing CO2 emissions in 2016 by 27.1 billion kg and helping alleviate poverty through uplifting the economic situation of 16-17 million small farmers, and their families totaling >65 million people, who are some of the poorest people in the world Brookes and Barfoot, 2018.

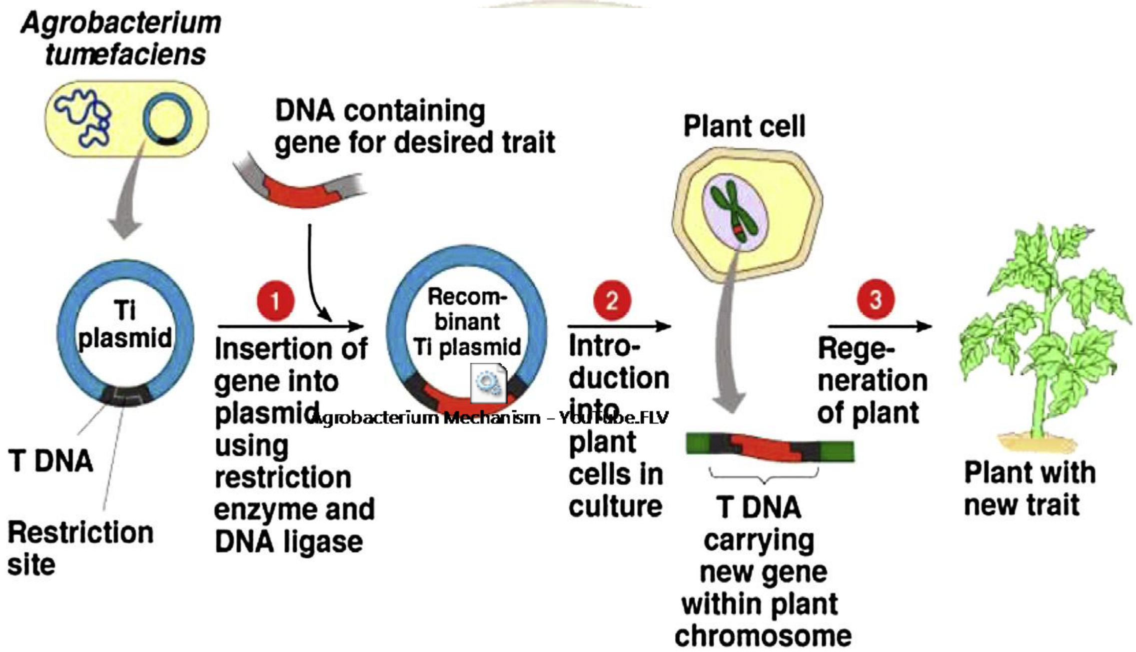
Development of Transgenic Crops

Transgenic crops are primarily developed though three methods viz., Physical, Chemical and Biological. Physical and chemical methods are also known as the direct method or vectorless method of genetic transformation. Biological method comprises of employing a vector viz., Agrobacterium *tumefaciens* or *A.rhizogenes*. In direct gene transfer methods, the foreign gene of interest is delivered into the host plant cell without the help of a vector. The physical methods are: (*a*) Protoplast transformation/fusion (*b*) Microprojectile bombardment c) Lipofection (*d*) Microinjection e) Sonication (*f*) Electroporation (*g*) Pollen-tube pathway (PTP) (*h*) Aerosol Beam Injection and the chemical methods include i) PEG method (j) Liposome mediated gene transfer.

Biological Method of Genetic Transformation

Commonly used vector for genetic transformation include the Ti plasmid of *Agrobacterium tumefaciens*. This *A. tumefaciens* is known as "natural genetic engineer" of plants because these bacteria have natural ability to transfer T-DNA of their plasmids into plant genome by the infection at wounding site. Ti plasmids are used as gene vectors for delivering useful foreign genes into target plant cells and tissues. *Agrobacterium* genetically transforms the plant cells by transferring a well-defined transgene segment from its tumor-inducing (Ti) plasmid to the host-cell genome (Gelvin, 1998). The native transfer DNA (T-DNA) carries a set of oncogenes and opine catabolism genes, whose expression in plant cells leads to neoplastic growth of the transformed tissue and the production of opines, amino acid derivatives that are used by the bacteria as a nitrogen source. The two 25–28 bp direct repeat borders are the only *cis*-acting elements essential for T-DNA transfer and for this reason T-DNA boarders are required to flank the gene of interest to be transferred.

The native wild-type oncogenes and opine synthase genes from the T-DNA can be replaced by genes of interest (Klee *et al.*,1987). As a result, any DNA placed between the borders will be transferred to the host cell. Because the T-DNA is not able to mediate its own transfer, other bacterial features need to be altered. The vir genes, residing on the virulence region of the Ti plasmid, are required for T-DNA transfer and integration. Altering their regulation (Ankebauer *et al.*, 1991) and copy number (Rogowsky *et al.*, 1987) proved to be useful for increasing transformation efficiency (Klee *et al.*,1987).



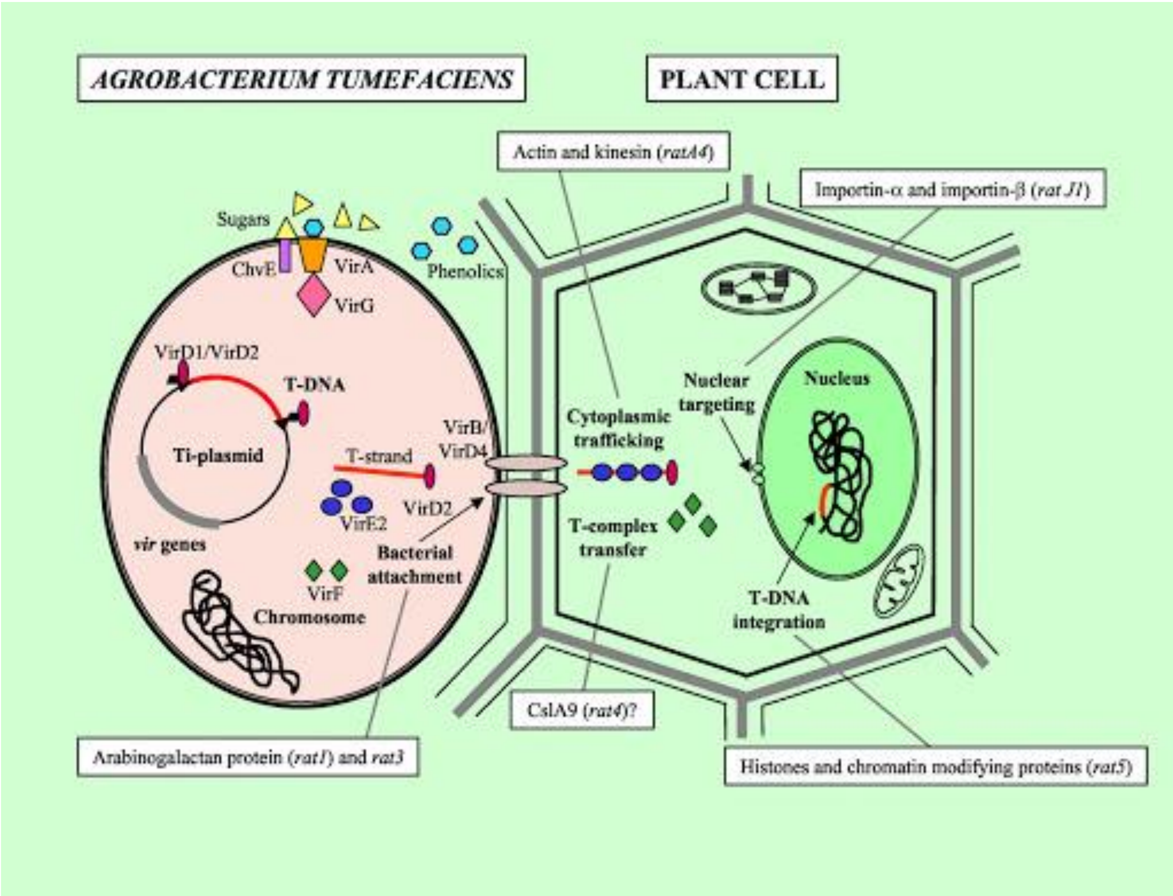
Agrobacterium mediated transformation

Thereby the size of the T-DNA that can be mobilized into plants could be enlarged (Hamilton *et al.*, 1996). The ability of vir genes to act in *trans* led to the development of binary and superbinary transformation vectors as a major step toward increasing the range of species that are amenable to *Agrobacterium*-mediated transformation (Lee *et al.*, 2008; Nester *et al.*, 2005). Many *Agrobacterium* strains, plasmids, and protocols have been developed and adapted for the genetic transformation of various plant species (Draper *et al.*, 1988). The molecular machinery needed for T-DNA production and transport into the host plant cell comprises proteins that are encoded by the bacterial chromosomal DNA as well as Ti plasmid vir genes.

In addition, various host plant proteins have been reported to participate in the *Agrobacterium*-mediated genetic transformation process (Tzfira and Citovsky, 2002; Gelvin, 2003), mostly during the later stages of the process (i.e., T-DNA intracellular transport, nuclear import, and integration). Recombinant *Agrobacterium* strains, in which the native T-

DNA has been replaced with genes of interest, are the most efficient cloning vehicles for the introduction of foreign genes into plants and for the production of transgenic plant species (Draper *et al.*, 1988). T-DNA transfer and its integration into the plant genome are controlled by various factors such as host plant genotype, type of explant, plasmid vector, bacterial strain, composition of culture medium, tissue damage, suppression, or elimination of *Agrobacterium* infection after cocultivation (Mohammad and Bagherieh-Najjar, 2009).The foreign gene is cloned in the T-DNA region of Ti plasmid in place of undesirable sequences.

Agrobacterium

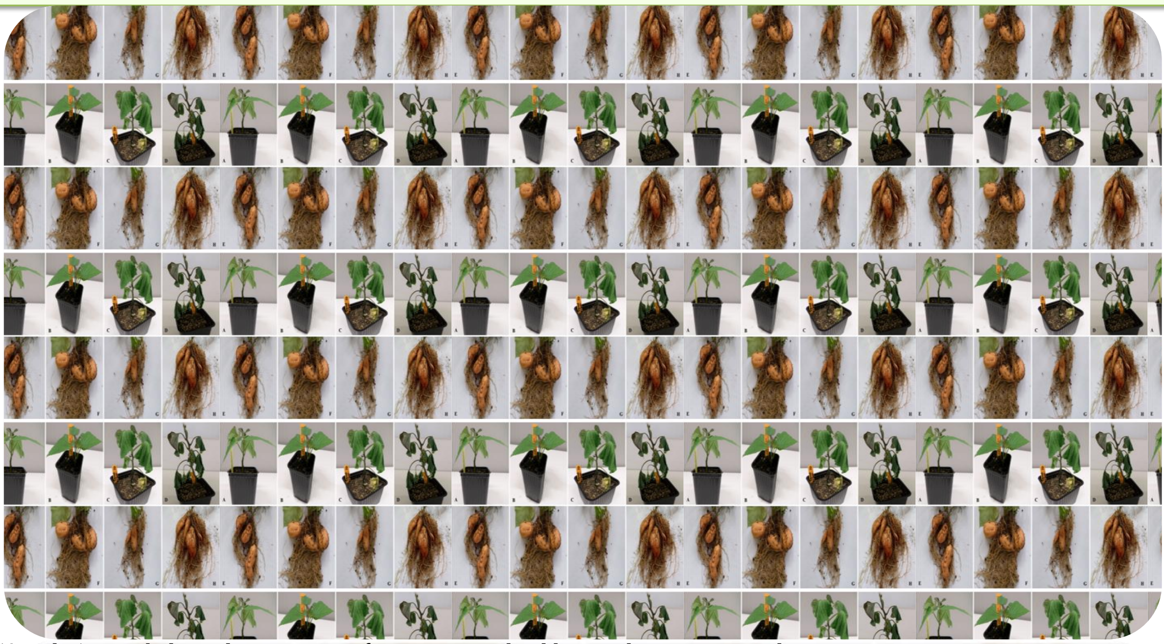


Applications of Transgenic Vegetable Crop

Applications of transgenics for welfare of mankind will include reducing poverty, hunger and malnutrition and for promoting equity in many of the world's poorer countries. GM Technology has been used to produce a variety of crop plants to date. As the global population continues to expand, food remains a scare resource. Genetically engineered foods offer significant benefits by improving production yield, lowering transportation costs and enhancing the nutritional content. Developments, resulting in commercially produced varieties in countries such as USA and Canada, have centred on conferring resistance to insect, pests or viruses and producing tolerance to specific herbicides. While these traits had benefits for the farmers, it has been difficult for the consumers to see any benefit other than these. In limited cases, a decreased price owing to reduced cost and increased ease of production is observed (Flack-zepeda *et al*, 1999).

Several GM crops for malnutrition are expected to be revealed for cultivation in the coming five to ten years. The existing food shortage as such is the principal cause of hunger and ignores to some extent the reasons for poverty, inequitable distribution of food, land tenure inequity, overpopulation, poor health, poor education etc. The multi-faceted nature of hunger and poverty criticized the assumption that transgenic have a moral head start on other technologies. With the rapid adoption of transgenic crops within the agricultural sector, has resulted in sustained increases in agricultural productivity, contributed to economic growth and ensured an abundance of food.(i) Improved storage (Shelf-life) ii) Increased nutritional value (iii)Resistance to abiotic stress (iv) Resistance to biotic stress (v) Bio-pharming (vi) Parthenocarpy (vii) Male Sterility

Fig: Responses to drought stress in transgenic and wild-type sweet potato plants



(A and B) Morphological appearance of transgenic and wild-type plants, respectively.
(C and D) Phenotypes of transgenic and wild-type plants grown in soil after 12 days of drought stress, respectively.
(E and F) Yield performance of wild-type and transgenic plants grown under control conditions, respectively.
(G and H) Yield performance of wild-type and transgenic plants grown under drought conditions for 90 days, respectively

Mbinda *et al.*, 2019

Transgenics Crops for Bio-Pharming

Bio-pharming consists of using plants to produce therapeutic molecules which will be further purified or introduced directly into the human or animal diet. Transgenic plants are being looked upon as a source of antibodies which can provide passive immunization by direct application. They provide as a tool for drug targeting. Gene technology has provided impetus to the utility of antibodies. Proteins of therapeutic importance, like those used in the treatment, diagnosis of human diseases can be produced in plants, using recombinant DNA technology. Scaling-up of these transgenic plants to fields, results in industrial production of proteins. The proteins produced in transgenic plants for therapeutic use, are of three types (a) antibodies, (b) proteins and (c) vaccines. Antibodies directed against dental caries, rheumatoid arthritis, cholera, E. coli diarrhea, malaria, certain cancers, HIV, rhinovirus, influenza, hepatitis B virus and herpes simplex virus are known to be produced in transgenic plants. Vaccines against infectious diseases of the gastro intestinal tract have been produced in plants like potato and bananas.

Future Prospects of Transgenic Crops

The future of transgenic vegetables will be the decided by developing countries, since nearly 60% of the world's vegetables are grown in China and India, which account for nearly 40% of the world's population and where pest and viruses are rampant. Both countries have readily adopted Bt-cotton, and it is likely that Bt-rice will be commercialized in China in the very near future, since China is the first country in the world to give biosafety approval (BSA) for the development of Bt-rice cultivars. Acceptance of GM field crops in these two large, highly populated countries will make it more likely they will adopt GM vegetables. This in turn likely will hasten their adoption in other parts of the world and allow farmers to use this technology. With the eventual acceptance of GM technology, it is expected that the costs associated with deregulation will become more affordable and that the biotechnology industry, in the hands of huge corporations, will become more interested in developing GM vegetables, especially for the developing world, where more than 80% of the world's 6.8 billion people live.

These populations will increase rapidly in the next several decades. Reasonable profit margins are necessary to pay back the research-for development costs, to fund future research on developing even better GM vegetable cultivars, and to stay competitive. It is further recommended that a tiered process for regulating new crop varieties should focus on a plant's characteristics rather than the process by which it was developed. Bio-fortified crops hold a very bright future as these have the potential to remove micronutrient malnutrition among billions of poor people, especially in the developing countries. Efficient transformation of plant cells will successfully incorporate the transgene. Better control of gene expression through more specific promoters, so that the inserted gene will be active only when and where needed. Transfer of multi-gene DNA fragments to modify more complex traits. Better marker genes to replace the use of antibiotic resistance genes. Last but not the least, emerging genetic engineering technologies have the potential to create novel plant varieties that are hard to distinguish genetically from plants produced through conventional breeding or processes that occur in nature.

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