

## TRANSGENIC TOMATOES – A REVIEW

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### ABSTRACT

Transgenic plants have gained popularity in few years. The tomatoes grown by tradition method soften during the ripening process and are intolerant to many biotic and abiotic stresses. Thus genetic engineering provides a solution by increasing productivity by enhancing efficiencies in metabolic and photosynthetic pathways. Generally transformation in tomatoes is achieved by co culturing with *Agrobacterium tumifaciens*. Approval of FDA has made clear that transgenic tomatoes are as safe as tomatoes bred by conventional means and would not require any special labeling.

Key words: transgenic, *Agrobacterium tumifaciens*, biotic, FDA, metabolic.

### INTRODUCTION

A person living in a Westernized culture often takes for granted the hard work, resource usage, and waste that occur to bring food to him. Tomatoes, for example, currently follow a long and difficult route to the supermarket. To begin with, field workers must pick the tomatoes by hand while they are still green. The unripe tomatoes are then trucked to facilities where they are gassed with ethylene to artificially induce ripening [8]

Normal tomatoes grown commercially cannot be allowed to ripen on the vine because they soften during the ripening process. Picking them while they are still hard allows them to be shipped, but it also prevents the development of natural flavors. Therefore, supermarket tomatoes generally have little flavor. Tomato, potato, squash and papaya are among a variety of crops that have been modified to resist infection by viruses or insect pests. Plant productivity is influenced by abiotic factors such as herbicides, soil composition, water supply, and temperature. Therefore, conferring plants with genes that

will help them withstand a wider range of environmental conditions could increase productivity. Plants are also being genetically modified to withstand drought, heat, cold temperatures and poor soil conditions such as salinity and aluminum contamination. [1, 7, 11, 14, 16]

Increased productivity also can be achieved by enhancing efficiencies in the metabolic and photosynthetic pathways. Some pathways that could be improved to increase crop yield include nitrogen assimilation, starch biosynthesis, and modification of photosynthesis. Traditionally to prevent delivering spoiled fruit, mature tomatoes are harvested while still green and ripened during delivery by exposure to ethylene, a ripening hormone in tomatoes. In 1994 the Food and Drug Administration approved a brand of tomato that had a genetic solution to this processing problem.

To utilize potentially fertile land, scientists have been trying to use new tools provided by molecular biology to see if plants could be genetically engineered to grow well in saline soils.

### METHODS OF TRANSFORMATION

There are various methods of transformation available which are followed by different laboratories.

To create the transgenic tomato, a gene from *E. coli* (a bacterium which occurs naturally in the mammalian gut) called kan(r) and the FLAVR SAVR gene (from a tomato) were inserted into a plasmid (a circular ring of DNA) and plasmids like these were inserted into a group of tomato cells in a growth medium containing an antibiotic (Engel 77). The Kan(r) gene, when established in the cell, produced a substance called APH (3') II that gave the cell resistance to the antibiotic. The antibiotic killed cells that did not receive the plasmid. The purpose of the bacterial gene was, therefore, to identify the cells that were genetically transformed. The FLAVR SAVR gene coded for a strand of RNA that was the reverse of a strand of RNA that naturally occurs in the plant. The original RNA strand in the plant is responsible for the production of the enzyme polygalacturonase. Polygalacturonase breaks down pectin in the cell walls of the tomato during the ripening process and causes the entire tomato to become soft [8]. The complementary strand of RNA from the FLAVR SAVR gene binds to the polygalacturonase RNA and the two strands "cancel each other out," preventing the production of polygalacturonase and the softening of the tomato [8].

Tomato transformation and regeneration were analyzed and optimized by Carolina Cortina and coworkers [5]. They infected Cotyledon explants from *Lycopersicon esculentum* cv. UC82B with *Agrobacterium tumefaciens* strain LBA4404 harbouring the neomycin phosphotransferase (*NPTII*) reporter gene. They found that on increasing concentration of thiamine(vitamin) from 0.1 mg l<sup>-1</sup> in standard medium to 0.4 mg l<sup>-1</sup> decreased the chlorophyll lost that accompanied the

expansion of necrotic areas in cotyledon explants. They observed Optimal shoot regeneration rate with a balanced concentration of 0.5 mg l<sup>-1</sup> auxin indolelactic acid (IAA) and 0.5 mg l<sup>-1</sup> cytokinin zeatin riboside. Finally, when the phenolic acetosyringone was present in the co-culture medium at 200 µM, they confirmed transgenic lines reached 50% of antibiotic resistant shoots. The efficiency of transformation reached 12.5% with this protocol.

Chyi Y.S. and coworkers [6] investigated the genetic behavior of DNA sequences in the backcross progeny of 10 transformed *Lycopersicon esculentum* x *L. pennellii* hybrids. They used Isozyme and restriction fragment length polymorphism (RFLP) markers to test linkage relationships of the insertion in each backcross family. The T-DNA inserts in 9 of the 10 transformants were mapped in relation to one or more of these markers, and each mapped to a different chromosomal location. Because only one insertion did not show linkage with the markers employed, it must be located somewhere other than the genomic regions covered by the markers assayed. They concluded that *Agrobacterium*-mediated insertion in the *Lycopersicon* genome appears to be random at the chromosomal level. Backcross progeny of two nopaline negative transformants showed incomplete correspondence between the T-DNA genotype and the kanamycin resistance phenotype. Two kanamycin resistant progeny plants of one of these two transformants possessed altered T-DNA restriction patterns, indicated genetic instability of the T-DNA in this transformant

McCormick S. [10] modified leaf disc transformation system in tomato. They used leaf explants and hypocotyls sections can be used to regenerate transformed plants. They

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found evidences for both single and multi-copy insertions of the T-DNA, and have demonstrated inheritance of the T-DNA insert in the expected Mendelian ratios. A reduced efficiency of transformation was observed with binary T-DNA vectors as compared to co-integrate T-DNA vectors.

Table 1: Comparison of Ranges of Nutrients between Transgenic and Normal Tomatoes (per 100 g Fruit)

Nutrient	Normal Range	Transgenics	Controls
Protein	0.85 g	0.75-1.14 g	0.53-1.05 g
Vitamin A	192-1667 IU	330-1660 IU	420-2200 IU
Thiamin	16-80 µg	38-72 µg	39-64 µg
Riboflavin	20-78 µg	24-36 µg	24-36 µg
Vitamin B6	50-150 µg	86-150 µg	10-140 µg
Vitamin C	8.4-59 mg	15.3-29.2 mg	12.3-29.2 mg
Niacin	0.3-0.85 mg	0.43-0.70 mg	0.43-0.76 mg
Calcium	4.0-21 mg	9-13 mg	10-12 mg
Phosphorus	7.7-53 mg	25-37 mg	29-38mg
Sodium	1.2-32.7 mg	2-5 mg	2-3 mg

Note: For Table 1, the "Normal Range" represents values that the researchers looked up in standard references. The "Controls" column represents actual amounts of nutrients found in non-transgenic (traditional) varieties grown by the researchers alongside the transgenic varieties.

### VARIOUS BENEFITS OF TRANSFORMED PLANTS

The increased consumption of fruits and vegetables is associated with reduced cardiovascular disease. D. Rein and coworkers studied the health effects of wild-type tomato (wtTom) and flavonoid-enriched tomato (flTom). Human C-reactive protein transgenic (CRPtg) mice express markers of cardiovascular risk. They analyzed markers of general health (bodyweight, food intake, and plasma alanine aminotransferase activities) and of cardiovascular risk (plasma CRP, fibrinogen, E-selectin, and cholesterol levels). CRPtg mice were fed a diet containing 4 g/kg wtTom, flTom peel, vehicle, or 1 g/kg fenofibrate for 7 weeks which reduced cardiovascular risk.

A.L.E.Lopez and coworker [2] showed that transgenic tomato expressing interleukin-12 has a therapeutic effect on progressive pulmonary tuberculosis. They observed that transgenic tomato L-12 administration resulted in a reduction of bacterial loads and tissue damage compared with wild-type tomato (non-TT). In the late infection, a long-term treatment with TT-IL-12 was essential. They successfully demonstrated that TT-IL-12 increases resistance to infection and reduce lung tissue damage during early and late drug-sensitive and drug-resistant mycobacterial infection.

Konijeti R and coworkers [11] found dietary lycopene combined with other constituents from whole tomatoes have greater chemopreventive effects against prostate cancer as compared to pure lycopene provided in a beadlet formulation in mice. They fed mice with lycopene in form of tomato paste and lycopene beadlets. The incidence of prostate cancer was significantly decreased in the lycopene beadlets LB group relative to the control group up to 95%.

S. K. Raj and coworkers [13] investigated the expression of coat protein gene of Tomato leaf curl virus (TLCV) into an expression vector and mobilized to *Agrobacterium tumefaciens* through triparental mating. Cotyledon leaf explants of Pusa Ruby tomato were transformed by co-cultivation with *Agrobacterium* containing TLCV-CP constructs.

Kanamycin-resistant transformants were regenerated and established in glasshouse. They observed that in T1 generation transformed plants showed disease tolerance when compared to non transformed ones.

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### REFERENCES:

1. Alia Hayashi, et al, (1998) Enhancement of the tolerance of *Arabidopsis* to high temperatures by genetic engineering of the synthesis of glycinebetaine. *Plant J* 16:155-161.
2. A L Elias Lopez et al, (2008), transgenic tomato expressing interleukin-12 has a therapeutic effect in a murine model of progressive pulmonary tuberculosis, [PMC free article. [Pubmed]
3. Available from <http://www.hos.ufl.edu/meteng/HansonWebpagecontents/Tomatotransformatioprocol>
4. Buxdorf K, et al, (2010), Identification and characterization of a novel miR159 target not related to MYB in tomato. *Planta*. PMID: 20661587 (Pubmed)
5. Carolina C., et al., (2004), *Plant Cell, Tissue and Organ Culture*, Vol-76, No. 3, pg 269-275
6. Chyi Y.S. et al, (1986) locations and stability of *Agrobacterium* – mediated T-DNA insertions in *Lycopersicon* genome. *Mol. Gen. Genet.* 204:64-69
7. D.L. Fuente, et al, (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science* 276:1566-1568.
8. Engel, Karl-Heinz et al., editors. (1995). *Genetically Modified Foods: Safety Aspects*, Washington, DC: American Chemical Society.
9. Food and Drug Administration Center for Food Safety and Applied Nutrition (1994) First biotech tomato marketed 1994 Accessed 12/20/2001. Available from: <http://vm.cfsan.fda.gov/~1rd/biotech.html>.
10. McCormick S., et al, (1986), *Plant Cell Reports*, Vol-5, No-2, pg 81-84.
11. Kasuga, M., et al, (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress inducible transcription factor. *Nat. Biotechnol.* 17:287-291.
12. Konijeti R, et al, (2010), Chemoprevention of prostate cancer with lycopene in the TRAMP model. *Prostate*, PMID: 20687227, (Pubmed)
13. Raj S.K. et al (2005), *Agrobacterium*-mediated tomato transformation and regeneration of transgenic lines expressing Tomato leaf curl virus coat protein gene for resistance against TLCV infection, *current science*, VOL. 88, NO. 10, 1674- 1679
14. Thomashow, M. F. (2001) so what's new in the field of plant cold acclimation? *Lots. Plant Physiol* 125:89-93.
15. Yoder JI, et al, (1988) Ac transposition in transgenic tomato plants. *Mol. Gen. Genet.* 213:291-296.
16. Zhang, H.-X. & Blumwald, E. (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat. Biotechnol.* 19:765-768.