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Research Article

Transgenic Research in Horticultural Crops an Overview

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Abstract

India ranks second in world after China in the production of vegetables and fruits worldwide and these crops play an important role in human diets because they provide vitamins, minerals, dietary fiber, and phytochemicals. Flower market in India growing at increasing rate and export potential of such crops are very high in global market. Horticulture production suffers from many abiotic and biotic stresses caused by pathogens, pests, and weeds and requires high amounts of plant protection products per hectare. Transgenic crops (genetically modified/GM), crops enable breeders to bring favorable genes, often previously inaccessible, into already elite cultivars, improving their value considerably and offer unique opportunities for controlling insects and other pathogens. This novel "molecular farming" offers enormous possibilities, but will require stringent regulations and control mechanisms to avoid new, potentially serious risks human health, though possible contamination of the food supply. Transgenic plant breeding therefore provides genetically enhanced seed embedded technology that contributes to integrated pest management in horticulture by reducing pesticide sprays as well as improving food safety by minimizing pesticide residues. 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. Transgenic horticulture crops could make important contributions to sustainable horticulture production.

Keywords: GMOs; Horticulture; Transgenic horticulture crops; herbicide tolerance; insect resistance; Plant Breeding

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Introduction

India is currently producing about 283 million tones of horticulture produce and horticulture production has surpassed the food production in the country. It has proven beyond doubt that productivity of horticulture crops is much higher compared to productivity of food grains. Horticulture contributes about 30% of GDP in agriculture, using only 17% land area. Horticulture production increased from 167 million tones in 2004- 05 to 283 million tones in 2014- 15 or 69% increase in 9 years [1]. Vegetable and fruit consumption is rising in India enormously, reflecting the consumer's increased income, desire of diversity, and awareness of nutritional benefits. Some phytochemicals of vegetable and fruit crops are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumor cells [2-3].Ornamental plants play a fundamental part in the way humans interact with and modify the environment. Now, thousands of varieties of cut flowers, pot plants, hanging plants, bedding plants, shrubs, lawn and turf, ornamental tree and aquatic plants are available to the public. The lawn and turf industry is an excellent target for GM technology with the potential to improve turf grass quality whilst significantly reducing chemical inputs [4].

Biotechnology is a new powerful tool that has been added in most of the horticulture crops breeding programs. Transgenic crops, commonly referred to as genetically modified (GM) crops enable plant breeders to bring favorable genes, often previously inaccessible, into elite cultivars, improving their value considerably and offer unique opportunities for controlling insects, viruses and other pathogens, as well as nutritional quality and health benefits. More than half of the cultivated area is located in the United States, followed by Argentina, Brazil, Canada, China, Paraguay and India. Four major crops account for practically all the transgenic production worldwide: soybean, for which 64% of the total cultivated area is used to grow transgenic varieties, cotton (43%), maize (24%) and rapeseed (20%). GM papayas, poplars, tobacco, tomato and sweet pepper are also cultivated in China, transgenic rice in Iran and brinjal in Bangladesh [5]. The "first generation" of genetically modified crops, those which are now growing in the fields, were designed for the improvement of agronomic characteristics, mostly herbicide tolerance and insect resistance. The "second generation" of transgenics focused on quality improvement, for example of nutritional value, but has not yet lived up to expectations. Now, the "third generation" of engineered plants is about to enter the market, designed to be used as biofactories for the production of a wide variety of high value, pharmacological and industrial compounds [6]. This review article highlights advances in breeding transgenic horticultural crops and their usefulness.

Research on genetically modified horticultural crops

Genetic modification has been incorporated into the development of herbicide and insect resistant varieties of *Zea mays* (maize), *Glycine max* (soybean), *Brassica napus* (canola), *Gossypium spp.* (cotton) and other important horticulture crop species for two decades (**Tables 1** and **2**). This development has been supported by significant public and private research in many countries and has been proven to increase the profitability of growers and to reduce impacts on the environment [9].

Table 1 List of genes used in the development of GM vegetable and fruit crops

Institute	Crop	Transgenic	Target
CPRI, Shimla	Potato	*Bt cry 1Ab*	Resistance to potato tuber moth
		Osmotin	Water stress tolerance
		Coat protein	Resistance to PVY
Delhi University, Delhi	Tomato	*Ctx-B* and *Tcp* antigens of *Vibrio cholera*	Edible vaccine
	Brinjal	*Chitinase, glucanase* and thaumatin encoding genes	Disease resistance
IARI, New Delhi	Brinjal	*Cry 1 Ab*	Lepidopteran resistance
	Tomato	*Cry 1 Ab*	Lepidopteran resistance
	Cauliflower	*Cry 1 Ab*	Diamond black moth resistance
	Cabbage	*Cry 1 Ab*	Diamond black moth resistance
	Tomato	*ACC synthase*	Controlling fruit ripening
		Replicase gene	TLCV resistance
IIHR, Bangalore	Muskmelon	*Rabies glycoprotein gene*	Edible vaccine
	Tomato	*Leaf curl virus sequence*	LCV resistance
	Tomato	*Chitinase and glucanase*	Fungal disease resistance
JNU, New Delhi	Tomato	*OXDC*	Fungal disease resistance
	Potato	*Ama1*	Protein rich potatoes
Cornell University, University of California/ Davis, USDA ARS.	Apple	Reduced polyphenol oxidase Ethylene suppression Resistance to fire blight	PPO suppression transgene, ACC oxidase, ACC synthase NPTII attE, nptII, gusA
University of Hawaii.	Banana	Bunchy top resistance Tolerance to Sigatoka Resistance to virus	Replicase associated protein, replicase inverted repeat, nptII pAB6, pAHC17, pH1
Hawaii Agriculture Research Center, Cornell U. and U. Florida.	Papaya	Female to male or hermaphrodite PRSV resistant	FSH19, Gene11Y, Gene5, GM183, nptII Coat Protein gene
University of Florida.	Grapevine *(Vitis vinifera)*	Powdery mildew resistance	Endogenous grapevine antifungal gene
Okanagan Specialty Fruits.	Plum	Non-browning; resistance to Plum pox virus (PPV)	PPV coat protein

Source: Modified from Sharma, N. *et al.*, 2016 [7].

Table 2 List of genes used in the development of GM ornamentals

Gene	Source(s)	Result(s)
F3'5'h gene	*Petunia/Pansy*	Overexpression produces blue flowers in combination with a silenced *dfr* gene in Carnation (Petunia) and Roses (Pansy)
CHS	*Gentian*	Gene silencing produces white flowers in Gentian
Ls	*Chrysanthemum*	Less branching in Chrysanthemum
Ipt	*Agrobacterium tumefaciens*	Increased branching and reduced internode length in Chrysanthemum
MADS-Box	Orchid/Lily	Ectopic expression changes the second round of petals into calyx in orchids and lilies
AP1	Chrysanthemum	Speeds up time to flowering in Chrysanthemum
Rdr1	Rose	Resistance to black spot in Roses
ERS1	Chrysanthemum	Mutated gene slows down yellowing of leaves in Chrysanthemum

Source: Modified from http://www.isaaa.org/kc [8]

Flower colour modification

Several ornamental plants, including carnation, rose and gerbera, have been engineered for modified flower color. Research has focused on the manipulation of either anthocyanins (red and blue colors) or carotenoids (yellow and orange colors), with the intent of creating a wider range of flower colors than occurs naturally, as well as to produce natural dyes for industrial purposes. GM of flower colour was first demonstrated by Mayer and co worker in 1987 and in 1993 the gene encoding flavonoid was isolated, providing the tool to allow development of the colour modified *Dianthus caryophyllus* and *Rosa hybrida* [10, 11].The keys genes of the anthocyanin, flavonoid and carotenoid biosynthesis and metabolism pathways have been identified, allowing modification of flower colour in many ways [12-14].

Fragrance modification

Key genes related to the production and regulation of fragrance has been identified and this presents the possibility of transferring fragrance from one species to another [15-16]. Putting the scent back into flowers that have “lost” this trait over years of traditional hybridization and selection, or creating new fragrances in plants, has considerable potential and appeal. Fragrance is also important in certain pot and bedding plants, and there is an example where potentially improved products have been obtained after the transformation process [17].

Abiotic stress resistance

Abiotic stresses, including salinity, temperature, and water stresses, are among the most limiting factors, lowering crop yield and quality worldwide**.** Most commonly used strategies in transgenic vegetables for abiotic stress are the overexpression of LEA genes and overproduction of glycine betaine [18]. Carrot accumulates low levels of glycine betaine naturally, but chloroplastic overexpression of endogenous BADH resulted in enhanced glycine betaine levels [19]. These transgenic carrots were able to grow on soil containing physiologically very high salt levels. Another strategy used by drought and salinity resistant plants is to compartmentalize the abundant but toxic Na+ ions to utilize them for osmotic adjustment by transporting the ions into the vacuole using tonoplastic Na+/H+ antiporters [20]. Transgenic tomato plants, overexpressing the *Arabidopsis* Na+/H+ antiporter (*Atnhx*) gene, were able to grow and produce fruit at relatively high levels of Na+, without accumulating excess Na+ in the fruit [20]. The *Atnhx* gene has also been overexpressed in sugar beet, which became more resistant to drought stress, in addition to increasing the natural level of sucrose in the taproot [21]**.** Frost tolerance in Petunia hybrida (petunia) may be increased by transfer of the CBF3 gene from Arabidopsis thaliana by Warner in the year 2011and this would potentially increase the range of environments in which this bedding plant could be grown [22].

Disease resistance

Fungal, bacterial and viral pathogens can have a devastating effect on horticulture crops during production, storage, distribution and end consumer use. To engineer resistance to this pathogen, a binary vector plasmid containing inverted repeats of portions of the *iaaM* and *ipt* genes was constructed and transformed into walnut [23]. Constitutive expression of this construct induces RNAi-mediated degradation of the *iaaM* and *ipt* transcripts, demonstrating the use of RNAi to generate resistance to a major bacterial disease [24]. Resistance to fire blight in apple and pear has been engineered using several transgenic strategies. Expression of the lytic peptide attacin E in transgenic apples and pears provided good resistance to the pathogenic bacterium [25, 26]. Transgenic orange plants have been engineered using the coding region of the tomato pathogenesis related protein PR-5, a chitinase with antifungal activity [27]. Tang et al. (1999) reported that overexpression of the *Pto* gene in transgenic tomato plants activated defense responses and conferred broad resistance to several bacterial pathogens [28]. *Rosa hybrida* has been genetically modified for mildew resistance and caffeine production in transgenic *Dendranthema grandiflorum* (chrysanthemum) was shown to confer resistance to grey mould [29, 30]. Clarke et al. (2008) reported GM virus resistant lines of the pot plant *Euphorbia pulcherrima* (poinsettia) and Chang et al. (2005) reported virus resistant GM lines of the pot and cut flower orchids *Phalaenopsis* spp. and *Dendrobium* spp [31 32].

Pest resistance

Horticulture crop grower faces a continuous threat of insect infestation. Populations of aphids, thrips, leaf miners, caterpillars, moths, spider mites and other pests can explode in the open field, nursery and greenhouse, where plants are at their optimal attractiveness to an insect pest [33]. Pests not only reduce the attractiveness and marketability of foliage and flowers but are potential vectors of pathogens and viruses. The insect resistance genes currently utilized in GM food crops are primarily based on the cry endotoxin genes from *Bacillus thuringensis.* Though these are effective against a relatively narrow range of pests, tolerance to susceptible insects has been demonstrated in transgenic plants of *Dianthus grandiflorum* carrying the cry1Ab of *Bacillus thuringiensis* var. *kurstaki* HD-1 [34, 35]. The demonstration of aphid resistance in *D. grandiflorum* modified to produce caffeine is a recent significant development. Codon optimized synthetic versions of *cry*IAc have been introduced into apple, where they confer high levels of mortality to *Cydia pomonella* larvae both under greenhouse and field conditions. Similar experiments have also been done in walnut and persimmon where chemically synthesized versions of *cry*IAc provided excellent protection against target insect larvae [36, 37]. *Bt*-potato cultivars expressing resistance to Colorado potato beetle (*Leptinotarsa decemlineata*), the most destructive insect pest of potato and aphids associated with *Potato virus Y* and *Potato leafroll virus*, were approved for sale in the United States in 1995 [38]. NewLeaf®, NewLeafY®, and NewLeafPlus® were the trade names of the transgenic potato cultivars sold by NatureMark®, a subsidiary of Monsanto®. The most damaging pest of eggplant is fruit and shoot borer (FSB, *Leucinodes orbonalis*) [39]. Losses have been estimated to be between 54 and 70% in India [40]. FSB-resistant *Bt*-eggplant was genetically engineered by Mahyco under a collaborative agreement with Monsanto® and the first *Bt*-eggplant with resistance to FSB was produced in 2000. This transgenic eggplant incorporates the *cry1Ac* gene expressing insecticidal protein to confer resistance against FSB. This *Bt*-eggplant was effective against FSB, with 98% insect mortality in *Bt*-eggplant shoots and 100% in fruits compared to less than 30% mortality in non-*Bt* counterparts [41].

Edible Vaccines

In recent years, successful fruit and vegetable crops based edible vaccines have been produced against common viruses such as measles, rabies and hepatitis B, common bacterial diseases such as cholera and HIV which are extremely difficult to treat. Mc Garvey and coworker engineered tomato plants of cultivar “UC82b” to express a gene encoding a glycoprotein (G-protein), which coats the outer surface of the rabies virus [42]. Hepatitis B surface antigen (HBsAg) was expressed in banana plants. Hepatitis B is the major cause of persistent viremia in humans, and banana is an ideal host for expression of HBsAg due to its palatability to infants and year round availability. By expressing an HBsAg “*s*” gene-ER retention sequence fusion under the control of the banana ethylene forming enzyme promoter, Sunil-Kumar et al., successfully produced antibody-reactive antigen in banana leaves and fruits [43]. Ma, *et al*. (2001) overexpressed hepatitis E virus (HEV) open reading frame 2 partial gene in tomato plants, to investigate its expression in transformants, the immunoactivity of expressed products, and explore the feasibility of developing a new type of plant-derived HEV oral vaccine [44].

Vase life and 'keeping' quality

In climacteric fruits, ethylene triggers a rapid increase in respiratory rate and initiates a cascade of biochemical and physiological changes associated with ripening**.** Enhanced vase life in horticultural crops could be obtained by the introduction of resistance to ethylene or by the inhibition of expression of endogenous ethylene biosynthesis genes. Introduction of a mutated ethylene receptor gene also reduced ethylene sensitivity in the orchids *Oncidium* spp. and *Odontoglossum* spp [45]. The first commercially grown transgenic crop was Flavr Savr™ tomato, which was released by Calgene in 1994. This tomato contains an antisense version of the *polygalacturonase* (*PG*) gene. 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 [46]. Delayed ripening in transgenic fruit trees has been demonstrated most convincingly in apple and papaya. Silencing of ACC oxidase in papaya resulted in a 40% reduction in fruit ethylene production, with a corresponding delay in fruit softening and the retention of green peel color [47].

Improving Fruit Quality and Nutritive Value

Several recently published studies have focused on the generation of transgenic fruit, vegetables and nut tree crops with enhanced nutritive value or increased levels of specific phytochemicals beneficial to human health. For example, the grape stilbene synthase gene, which is responsible for the synthesis of the phytoalexin resveratrol, has been introduced into apple and kiwifruit [48, 49]. Sorbitol is synthesized from glucose-6-phosphate through the action of the enzyme sorbitol-6-phosphate dehydrogenase (S6PDH) and Teo and co workers generated transgenic apple plants expressing an antisense copy of the S6PDH gene [49, 50]. Fraser and co worker reported also an increase in tomato fruit carotenoids phytoene, lycopene, *β*-carotene and lutein in cultivar "Ailsa Craig". Phytoene synthase from the bacterium *Erwinia uredovora* (crtB) has been overexpressed in tomato cultivar Ailsa Craig. Total fruit carotenoids of primary transformants were 2-4 fold higher than the controls, whereas phytoene, lycopene, *β*-carotene, and lutein levels were increased 2.4, 1.8 and 2.2 fold respectively [51, 52]. Cho and co worker developed transgenic lettuce plants of the cultivar "Chung-chima" expressing a cDNA encoding *γ*-tocopherol methyl- transferase from *Arabidopsis thaliana* to improve tocopherol composition [53]. When onions are cut, two compounds are formed: pro-panethial sulphoxide also known as the lachrymatory factor and 1-propanesulphenic acid. The lachrymatory factor reacts with nerve cell membranes in the eye to produce tears. Recently, Eady and co worker silenced the gene for the lachrymatory factor enzyme by using RNA interference, to produce tearless onions [54, 55]**.** This feat of genetic engineering reduces levels of lachrymatory factor up to 30-folds. These "tearless onions" have potential health benefits for consumers as they do not produce tears, but retain their health-promoting properties. The first successful study conducted to engineer genetically the taste of tomato fruit involved transformation of tomato with the thaumatin gene (Thaumatin is a sweet-tasting protein) from the African plant katemfe (*Thaumatococcus daniellii*) [56]**.**

Looking ahead

Modern gene transfer based on recombinant DNA technology is a rapidly growing area of research and offers vast opportunity for manifesting the utility of this technology in economic terms. Commercialization of GM food and industrial crops will continue to outpace horticulture [57]. However, commercialization of such applications has been largely stymied to date, and additional research in both scientific and policy arenas is needed to expand opportunities for horticultural biotechnology. Strategies for improving the health functionality of different fruits and vegetable crops that rely on transgenic approaches, offer great scientific promise, but have so far been met with public scepticism, and even fear. Thus far, none of horticulture transgenic crops has been approved and sold commercially in India except cotton. Nutritionally improved horticultural products could appeal to consumers and create demand. Transgenic fruits and vegetable crops can be coupled with conventional breeding as a powerful tool for making available better produce. There is need to develop cost-efficient benefit/risk analysis systems for products of biotechnology [58, 59]**.**

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