ROLE OF GENETIC ENGINEERING IN HORTICULTURAL CROP IMPROVEMENT – A REVIEW

Ajay Kumar Thakur*, Devendra Kumar Chauhan†, Nehanjali Parmar and Vandana Verma‡

Dr. Y.S. Parmar University of Horticulture and Forestry,
Nauni, Solan-173 230, India

Received: 04-08-2011 Accepted: 30-02-2012

ABSTRACT

Biotechnology has offered tremendous scope and potential to conventional methods of crop improvement, crop protection, crop quality management and other horticultural traits. Biotechnology extends tremendous opportunities in fruit production by providing new genotypes for breeding purpose, supply of healthy and disease free planting material, improvement in fruit quality, enhancing shelf-life, availability of biopesticides, biofertilizers, etc. Integration of specially desired traits through genetic engineering has been possible in some horticultural crops. Recent advancements in molecular biology and genetic transformation have made it possible to identify, isolate and transfer desirable genes from any living organism to plants. The introduction or enhancement of desirable traits is traditionally done by breeding. This is time consuming and also not very precise. On the other hand, genetic engineering creates plants with specific changes in the background of a proven cultivar without disturbing their genetic constitution. Expression of undesirable genes can be blocked by the application of antisense gene technology and RNAi technology. Genetic transformation provides the means for modifying horticultural traits in various horticultural crops without altering their phenotype. Biotechnological interventions that could increase the efficiency of horticultural crop improvement are essential to generate plants with several desirable traits.

Key words: Genetic engineering, Horticultural crops, Insect resistance, Quality improvement, RNAi.

Genetic engineering consists of isolation of a gene of interest, ligating that gene with a desirable vector to form the recombinant-DNA molecule and then transferring that gene into the plant genome to create a new function. In contrast to conventional breeding, which involves the random mixing of tens of thousands of genes present in both the resistant and susceptible plants, recombinant DNA technology allows the transfer of only the desirable genes to the susceptible plants and the preservation of valuable economic traits. Moreover, the genetic sources for resistance are not limited to closely related plant species (Lurquin, 2002). In contrast to the increasing global adoption of biotech field crops, biotechnology has had limited commercial success to date in horticultural crops, including fruits, vegetables, flowers and landscape plants. Horticultural biotechnology has been a leading example in the following mentioned areas for more than two decades, right from the commercialization of the first ever transgenic crop in the form of Flavr-Savr transgenic tomato with enhanced shelf life trait. The main resistant traits introduced into horticultural plants and already commercialized are insect-pest resistance (Bt. toxin gene) and herbicide tolerance. Other studies concern virus resistance, male sterility etc. Amongst various genetically modified horticultural crops, GM papaya showing resistance to Papaya ring spot virus contributes to approx. 53% of the total share of GM horticultural crops cultivated globally. Herbicide tolerance trait is dominating the GM horticultural crop acreage followed by insect resistance and virus resistance traits.

*Corresponding author: DRMR, Bharatpur-321 303, Rajasthan, email: thakurak2010@gmail.com
†Mega Seed, Division of Plant Breeding & Genetics, SKUAST-Jammu-181 101, India.
‡Jaipur National University, Jaipur-302 025, India.
Also, the RNAi technology has found its most powerful expression in plant biology these days. The applications of this technology cover a wide range from producing insect, viral and disease resistant plants to developing designer flower colors by knocking down the expression of certain endogenous genes. It is being used as a potential tool in tweaking the regulation of various metabolic pathways in plants and assigning functions to the genes involved, thereof. A very little work has been carried out in the field of improvement of fruit quality and male sterility development. The most studied crop so far is tomato, but research activities had already been carried out on many horticultural crops such as fruits, vegetables and flowers. A brief review of the work done for genetic modification of horticultural crops is being presented here.

**Pest resistance:** A large number of insect species attacks plants and causes severe damage to yield. From a grower’s perspective, any genetic improvement that could reduce the cost of chemical application to combat pests would be of significant benefit. The bioinsecticidal δ-endotoxin gene (Bt. gene) isolated from *Bacillus thuringiensis* is currently in use to make the plants resistant to insect pests. Progress in engineering insect resistance in horticultural plants has been attained by the use of insect control protein genes of *Bacillus thuringiensis*. Insect resistance was firstly reported in tomato using Bt. gene in 1987. Transgenic Bt. tomato plants exhibited resistance against *Spodoptera litura* and *Heliothis virescens* (Fischhoff et al., 1987). Fruit trees like persimmon transgenic for *cry* gene were found resistant to *Plodia interpunctata* and *Monema flavescens* (Tao et al., 1997). Potato varieties engineered for resistance to Colorado potato beetle were in commercial production for several years and were technically and agronomically successful, allowing significant reductions in insecticide use (Shelton et al., 2002). Chakrabarty et al. (2002) transformed cauliflower var. Pusa Snowball K-1 with a synthetic *cry1A(b) gene* and the transgenic plants indicated the effectiveness of the transgene against infestation by diamondback moth (*Plutella xylostella*) larvae during insect bioassays. Paul et al. (2005) developed transgenic cabbage (*Brassica oleracea* var. *capitata*) with a synthetic fusion gene of *Bacillus thuringiensis* encoding a translational fusion product of *cry1B* and *cry1Ab* δ-endotoxins and found the transgenic plants resistant to diamondback moth (*Plutella xylostella*).

Transgenic technology has also been found to deliver resistance against various nematodes. Roderick et al. (2012) developed transgenic plantain (*Musa sp*) cv. Gonja manjaya plants expressing a maize *cystatin* gene that inhibits the digestive cysteine proteinases and a synthetic peptide that disrupts nematode chemoreception. The best level of resistance exhibited by the transgenic plants against the major pest species *Radopholus similis* was 84% for the cystatin, 66% for the peptide and 70% for the dual defence.

In our country, ICAR had supported crop biotech research under NAIP Project at several ICAR institutions (10 institutes, 7 national research centers and 6 directorates) and state agricultural universities for the development of insect-pests, viral and disease resistant horticultural crops. Bt. brinzal may become available as the first biotech vegetable food crop in India within next 1-2 years. Efforts are being directed for the development of various vegetable crops such as biotech tomato, broccoli, cabbage, cauliflower and okra, which require heavy application of insecticides (which can be reduced substantially by a biotech product) and some of them are currently in various stages of field trials and bio-safety testing.

**Disease resistance:** One of the major constraints limiting the production of fruit crops is diseases caused by several fungi, bacteria and viruses. Conventional breeding seems to have limited application due to non-availability of resistant gene(s) in gene pool of a particular crop. Genetic engineering of disease-resistance in crops has become popular and valuable in terms of cost and efficacy. In fruit crops, the coat protein mediated approach to engineer virus resistance has been in application to introduce resistance against diseases like Plum Pox Virus (PPV), Citrus Tristeza Virus (CTV) and Grape Fan Leaf Virus (GFLV) etc. Papayas are grown in many tropical countries, but its cultivation is being threatened by Papaya Ring Spot Virus (PRSV), a disease that is considerably lowering its yield. Using biotechnological interventions, the coat protein gene of the virus has been transferred to papaya to confer PRSV resistance. Since 1998, GM papayas have been
cultivated in Hawaii, USA, which had shown considerable resistance to PRSV. PRSV resistant transgenic papaya varieties ‘SunUp’ and ‘Rainbow’ have now occupied >80% shelf-space in the US market. Also, transgenic papaya plants with the mutated replicase (RP) gene from papaya ringspot virus (PRSV) showed high resistance or immunity against PRSV in the field (Xiangdong et al., 2007). Praveen et al. (2010) developed transgenic plants of tomato with AC4 gene-RNAi construct and the transgenic plants were found to show the suppression of tomato leaf curl virus activity. Yu et al. (2011) transformed commercial watermelon cultivars with an untranslatable chimeric construct containing truncated Zucchini Yellow Mosaic Virus coat protein (CP) and Papaya Ring Spot Virus W CP genes. Greenhouse evaluation of the selected ten transgenic lines of ‘Feeling’ cultivar revealed that two immune lines conferred complete resistance to ZYMV and PRSV-W, from which virus accumulation were not detected by western blotting 4 weeks after inoculation.

RNA-interference (RNAi) technology is being used these days quite successfully in controlling various bacterial and viral diseases in plants by switching off the expression of certain endogenous genes. Transgenic tomato plants expressing hpRNA constructs against Agrobacterium iaaM and ipt oncogenes were found to be resistant to crown gall disease (Escobar, 2001). The expression of a self-complementary hairpin RNA under the control of rolC promoter controlled the systemic disease spread caused by plum pox virus without preventing local infection (Pandolfini et al., 2003). Using a hairpin RNA gene silencing strategy, transgenic poinsettia plants resistant to Poinsettia Mosaic Virus have been developed (Clarke et al., 2008). RNAi technology has been found to impart resistance to various bacterial plant diseases.

For imparting bacterial and fungal resistance, various genes like Chitinase, Glucanase, Attacin, Osmotin, Cercopin, defensin etc. are being transferred into various horticultural crops world over. A gene for a pathogenesis-related (PR) protein from tomato (PR-5) had been expressed in transgenic sweet orange and regenerants showed increased tolerance to Phytophthora citrophthora (Fagoaga et al., 2001). The HcrVf2 gene from a wild apple conferred scab resistance to a transgenic cultivated variety of apple (Belfanti et al., 2004). Faize et al. (2004) developed transgenic apple plants with a wheat puroindoline-b (pin B) gene under a CaMV35S promoter and observed that the expression of pin-B gene reduced the scab susceptibility in transgenic apple plants. In another study, the Arabidopsis NPR1 (non-expressor of PR genes) gene was introduced into a tomato cultivar, which possesses heat-tolerance and resistance to tomato mosaic virus (ToMV). The transgenic lines expressing NPR1 were normal with regards to overall morphology and horticultural traits for at least four generations. Disease screening against eight important tropical diseases revealed that in addition to the innate ToMV-resistance, the tested transgenic lines conferred significant level of enhanced resistance to bacterial wilt (BW) and Fusarium wilt (FW), and moderate degree of enhanced resistance to gray leaf spot (GLS) and bacterial spot (BS) (Lin et al., 2004). Rosa x hybrida had been genetically modified for mildew resistance (Lin et al., 2003) and also, caffeine production in transgenic chrysanthemum (Dendranthema grandiflorum) was shown to confer resistance to grey mould (Kim et al., 2011). Girhepuje et al. (2011) developed transgenic tomato plants expressing a wheat endochitinase gene and during disease screening, the transgenic plants exhibited enhanced resistance to Fusarium oxysporum. Rivera-Dominguez et al. (2011) carried out genetic transformation of mango (Mangifera indica) cv. Ataulfo embryos with defensin J1 gene. In vitro tests showed that protein extracts from processed somatic embryos inhibited the growth of Colletotrichum gloesporiodes, Aspergillus niger and Fusarium sps.

Herbicide tolerance: Herbicide tolerance in bedding plants can be expected to significantly reduce the cost of weeding in a landscape environment. The herbicide glyphosate is a potent inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) in higher plants. A complementary DNA (cDNA) clone encoding EPSP synthase was isolated from a complementary DNA library of a glyphosate-tolerant Petunia hybrida cell line (MP4-G) that overproduces the enzyme. This cell line was shown to overproduce EPSP synthase messenger RNA as a result of a 20-fold amplification
of the gene. A chimeric EPSP synthase gene was constructed with the use of the cauliflower mosaic virus 35S promoter to attain high level expression of EPSP synthase and introduced into petunia cells. Transformed petunia cells as well as regenerated transgenic plants were tolerant to glyphosate (Shah et al., 1986).

Transgenic pineapple plants transformed with the bar gene for bialaphos resistance were developed (Sripaoraya et al., 2006) and evaluated for tolerance to herbicide Basta. Seven months after transfer to the field, plants were found tolerant to 1600 ml/rai of the herbicide Basta® X (stock concentration 15% w/v glufosinate ammonium), this being twice the dose recommended for field application of the herbicide. Transgenic plants tolerant to glufosinate ammonium should facilitate more effective weed control in pineapple plantations without damage to the crop.

**Abiotic stress tolerance:** High temperature, high light intensity, humidity, drought, frost and salinity are the major abiotic stresses that reduce the yield and quality of fruit by affecting the vegetative and reproductive stages of growth and development. Nevertheless, abiotic stresses remain the greatest constraint to crop production. Research on genetic modification of various horticultural crops for improved abiotic stress tolerance has been explored. Tsai-Hung et al. (2002) transformed tomato plants with a DNA cassette containing an Arabidopsis C repeat/dehydration-responsive element binding factor 1 (CBF1) cDNA and a nos terminator, driven by a cauliflower mosaic virus 35S promoter. These transgenic tomato plants were more resistant to deficit water stress than the wild type plants. Plants, when exposed to abiotic stress conditions produce several pathogenesis-related proteins to compensate the effect of stress conditions. Among those proteins, osmotin is one of the important one released during abiotic stress conditions. Husaini and Abdin (2008) over-expressed tobacco osmotin gene in strawberry (Fragaria x ananasa Duch.) and found that the transgenic strawberry plants exhibited tolerance to salt stress. Also, Subramanyam et al. (2011) expressed tobacco osmotin gene in Capsicum annum and the transgenic chilli plants exhibited improved salt tolerance. Cheng et al. (2009) developed transgenic tomato plants expressing yeast SAMDC gene, which improved the efficiency of CO₂ assimilation and protected the plants from high temperature stress (38 °C) as compared to the wild-type plants. A bacterial mannitol-1-phosphate dehydrogenase (mtlD) gene driven by the constitutive cauliflower mosaic virus (CaMV) 35S promoter was transferred into tomato plants in an attempt to improve abiotic stress tolerance in the transformed plants (Khare et al., 2010). Drought (polyethylene glycol in medium) and salinity (sodium chloride in medium) tolerance tests revealed that transgenic lines exhibited a higher tolerance for abiotic stresses than non-transformed plants.

**Modification of fruit quality for increasing shelf life and reducing post-harvest losses:** Excessive softening is the main factor limiting fruit shelf life and storage. Transgenic plants modified in the expression of cell wall modifying enzymes have been used to investigate the role of particular activities in fruit softening during ripening. Fruit ripening has been modified by altering the activity of cell wall enzymes such as polygalacturonases that are involved in tissue softening and deterioration. The biosynthesis of ethylene - the fruit ripening hormone, has also been blocked in several ways to delay fruit ripening. Calgene Inc., USA (1994) developed the first commercialized transgenic plant, a long shelf life tomato by the suppression of polygalacturonase (PG) gene by antisense strategy (Smith et al., 1988). PG gene encodes for polygalacturonase enzyme which degrades pectin, the major component of fruit cell wall. Calgene Inc. has given the brand name ‘Mac Gregor’ to its transgenic tomato and the fruits of this plant can stay on the market shelf for approx. two weeks longer without softening. The Flavr Savr tomatoes have improved flavor and total soluble solids (TSS), in addition to the enhanced shelf-life. However, this Flavr Savr variety was withdrawn from the market three years later because of its disease susceptibility and lack of productivity.

The plant hormone ethylene is involved in senescence in many flowers and fruits and their vase life can be extended by either blocking ethylene biosynthesis or ethylene reception (Bovy et al., 1999). Later on, other tomato varieties with increased shelf life were developed through antisense RNA inhibition of ACC synthase or ACC oxidase, two ethylene precursors. Delayed leaf senescence
has been achieved in tobacco and petunia by manipulation of cytokinin synthesis (Clark et al., 2003). Researchers at the Horticultural Research International, the United Kingdom, have identified the genes which control the taste, smell and color of strawberries. As a result, it would now be possible to create super strawberries that will taste sweeter using transgenic approaches. Stewart et al. (2001) cloned a PPO gene from pineapple fruits under conditions that produce blackheart. The PPO gene has been silenced in transformed plants and transgenic plants are under field evaluation. Also, Park et al. (2005) demonstrated that fruit from tomato plants expressing Arabidopsis thaliana H+/cation exchanger (CAX) gene have more calcium (Ca^{2+}) and prolonged shelf life when compared to controls. Nambeesan et al. (2010) expressed a yeast spermidine synthase (ySpdSyn) gene under constitutive (CaMV35S) and fruit-ripening specific (E8) promoters in Solanum lycopersicum (tomato). The ySpdSyn transgenic fruits had a longer shelf life, reduced shriveling and delayed decay symptom development in comparison with the wild-type (WT) fruits. Crop maturity indicated by the percentage of ripening fruits on the vine was delayed in a CaMV35S-ySpdSyn genotype, with fruits accumulating higher levels of the antioxidant lycopene. Notably, whole-plant senescence in the transgenic plants was also delayed compared with wild-type plants. Blackheart is a fruit defect caused by exposure of pineapples to higher temperatures which stimulates polyphenol oxidase (PPO) activity. Recombinant-DNA technology also finds its multifaceted applications in improvement of the nutritional quality of fruits. Zhang et al. (2011) developed transgenic tomato plants by silencing the expression of mitochondrial APX gene by RNAi mechanism and observed increased vitamin C content in the transgenic tomato fruits.

Color enhancement and increasing vase-life in various ornamental crops: Genetic engineering technique has so far had a limited impact in the field of ornamental horticulture. However, ornamental horticulture and particularly floriculture, is very well suited to the approach of genetic engineering technology. The primary focus on color modification is important in cut flowers because flower color is an important driver of new variety development. Several ornamental plants, including carnation, rose and gerbera have been engineered for modified flower color. Research has been 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 (Lu et al., 2003). The first application of genetic engineering to modify flower color led to the production of an orange pelargonidin-producing Petunia variety, which produced flowers with pale brick color. This was achieved by the expression of dihydroflavonal-4 reductase (dfr) gene from maize in a petunia line (Meyer et al., 1987). Chalcone synthase (Chs) is another gene which has been used for production of pink, white and variegated flowers (sense and antisense genes were used) in petunias, chrysanthemum, gerbera and roses (van der Krol et al., 1988). Transgenic violet carnations have been successfully produced by the introduction of F3'5'H gene from Petunia hybrida which is encoding a flavonoid required for the biosynthesis of delphinidin (Holton et al., 1993).

The vase life of flowers can be altered by manipulating the biosynthesis of ethylene. The enzymes ACC synthase and ACC oxidase are encoded by genes acs and aco respectively and both have been cloned from many species including carnation. Transgenic carnation having antisense aco gene have been produced and exhibited longer vase-life. Flowers of the transgenic plants exhibited low climacteric ethylene production and a markedly delayed petal senescence (Savin et al., 1995). To date, the only genetically modified products commercialized on a significant scale are the color modified carnations developed in a joint venture by Suntory Ltd. and Florigene Ltd. Florigene is selling transgenic Moon series carnations engineered for dark violet-purple color around the world. The varieties are developed in Australia and flowers are produced primarily in South America for marketing in the United States and Japan. In another study, fruit specific RNAi-mediated suppression of a photomorphogenesis regulatory gene (DETI) was reported to enhance the carotenoid and flavonoid content in tomatoes (Davuluri et al., 2005). Recently, in 2009, transgenic blue roses had been developed by two companies namely Florigen Ltd. and Suntory Ltd. in Australia. A package of three genes was
transferred to red rose plants as; a synthetic RNAi gene that switches off the red rose *dihydroflavonol reductase (DFR)* gene, a *delphinidin* gene from blue pansy and a *DFR* gene from iris that had an affinity for producing delphinidin (Katsumoto et al., 2007). The resultant roses exclusively accumulated delphinidin in the petals, and the flowers had blue hues, not achieved by hybridization breeding.

**CONCLUSION**

The applications of recombinant-DNA technology or genetic engineering in crop improvement are immense. However, horticultural crops have got less attention in this area so far. At this juncture of time, we cannot ignore the potential of this technology for the genetic enhancement of our horticultural crops to combat various production constraints like biotic or abiotic stresses and fruit quality improvement. Also, we must have a single window regulatory mechanism for commercialization of such genetically modified crops in our country so that the real benefits of this technology can be harvested to the maximum.

**REFERENCES**


