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REVIEW ARTICLE

Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review

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Abstract Genetic engineering technique offers myriads of applications in improvement of horticultural crops for biotic and abiotic stress tolerance, and produce quality enhancement. During last two decades, a large number of transgenic horticultural crops has been developed and more are underway. A number of genes including natural and synthetic *Cry* genes, *protease inhibitors*, *trypsin inhibitors* and *cystatin* genes have been used to incorporate insect and nematode resistance. For providing protection against fungal and bacterial diseases, various genes like *chitinase*, *glucanase*, *osmotin*, *defensin* and *pathogenesis-related* genes are being transferred to many horticultural crops world over. *RNAi* technique has been found quite successful in inducing virus resistance in horticultural crops in addition to *coat protein* genes. Abiotic stresses such as drought, heat and salinity adversely affect production and productivity of horticultural crops and a number of genes encoding for biosynthesis of stress protecting compounds including mannitol, glycine betaine and heat shock proteins have been employed for abiotic stress tolerance besides various transcription factors like *DREB1*, *MAPK*, *WRKY*, etc. Antisense gene and *RNAi* technologies have revolutionized the pace of improvement of horticultural crops, particularly ornamentals for color modification, increasing shelf-life and reducing post-harvest losses. Precise genome editing tools, particularly CRISPR/Cas9, have been efficiently applied in tomato, petunia, citrus, grape, potato and apple for gene mutation, repression, activation and epigenome editing. This review provides comprehensive overview to draw the attention of researchers for better understanding of genetic engineering advancements in imparting biotic and abiotic stress tolerance as well as on improving various traits related to quality, texture, plant architecture modification, increasing shelf-life, etc. in different horticultural crops.

Keywords Genetic engineering · Horticultural crops · Abiotic and biotic stresses · Quality improvement · Genome editing

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Introduction

Biotechnology has offered tremendous scope and potential to conventional methods of crop improvement, crop protection, crop quality management and improving other horticultural traits. It extends remarkable opportunities in fruit production enhancement 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 made possible in some horticultural crops. 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. Transgenic technology has been rated as the fastest growing technology in agriculture (ISAAA 2017). It refers to a set of techniques used for transferring desirable gene(s) from any source (plants, animals, microorganisms or even artificially synthesized genes) across taxonomic boundaries into a certain plant by non-conventional methods. In contrast to conventional breeding which involves the random mixing of tens of thousands of genes present both in 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 only to closely related plant species (Lurquin 2002). Combating various types of biotic and abiotic stresses is the foundation and crux of sustainable agriculture. Although conventional breeding and marker-assisted breeding nowadays are being used to develop more promising cultivars, however, in case of biennials or perennial horticultural crops, particularly fruit trees, such techniques are not feasible due to long sexual generation periods. The major advantages of transgenic technology lie in that the genes governing for various agronomically important traits can be sourced from any organism—plants or microorganisms, etc. and can be employed for plant transformation. Thus, novel traits from any background can be incorporated into the target plant with ease. However, for single gene transfer into elite backgrounds, the development and standardization of a high frequency, efficient plant regeneration and genetic transformation protocol is the utmost pre-requisite. A number of studies had been carried out in the past to develop suitable regeneration and genetic transformation protocol in many horticultural species including apple (Rustaee et al. 2007), pomegranate (Parmar et al. 2012, 2013, 2015), chilli (Sharma et al. 2006; Khan et al. 2011a), cucumber (Vasudevan et al. 2007), lily (Kathryn and Han 2008), sweet orange (Singh and Rajam 2010), broccoli (Kumar and Srivastava 2015), datepalm (Aslam et al. 2015), chrysanthemum (Naing et al. 2016), etc.

Horticultural biotechnology has been a leading example in many 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 first field trials of transgenic horticultural plants had been carried out in France and USA in 1986 (James and Krattiger 1996). Transgenic Flavr Savr tomato is the first successful example of genetically modified food crop and was approved for commercialization in USA in 1994. The main resistant traits introduced into horticultural plants and already commercialized are insect-pest resistance (*Bt*. toxin gene) and herbicide tolerance while other important studies concern virus resistance, male sterility, etc. Among various genetically modified (GM) 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 (ISAAA 2017). Also, the RNA interference (*RNAi*) technology has gained popularity in plant genetics and system biology during these days due to its stable transgene expression. The applications of this technology cover wide range from insect resistance, viral and disease resisance, drought, heat, salinity tolerance; developing designer flower colors by knocking down the expression of certain endogenous genes, increasing shelf-life, plant architecture modification etc. 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. The most studied crop so far is tomato, but research activities had already been carried out in various horticultural crops such as fruits, vegetables and flowers. With the advancement of regeneration and genetic transformation protocols, extensive research efforts have been made to incorporate genes for various biotic and abiotic stress tolerance/resistance, enhancing shelf-life, modification of plant architecture and color and texture modification traits in a number of horticultural crops, which have been summarized and discussed in this review paper. An overview of various transgenic strategies targeting horticultural crop improvement is depicted in Fig. 1.

Biotic stress management through transgenic approach

Insect-pest resistance

At present, insect-pest resistance is lacking generally in many crop plants. The use of chemical control measures is proving hazardous to the consumers and also not environmentally sustainable. From a grower's perspective, any genetic improvement that could reduce the cost of chemical application to combat pests would be of significant benefit. *Bt* (*Cry*) gene isolated from a soil bacteria *Bacillus thuringiensis* has proven highly effective in controlling various lepidopteran insects in a number of crops successfully. 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

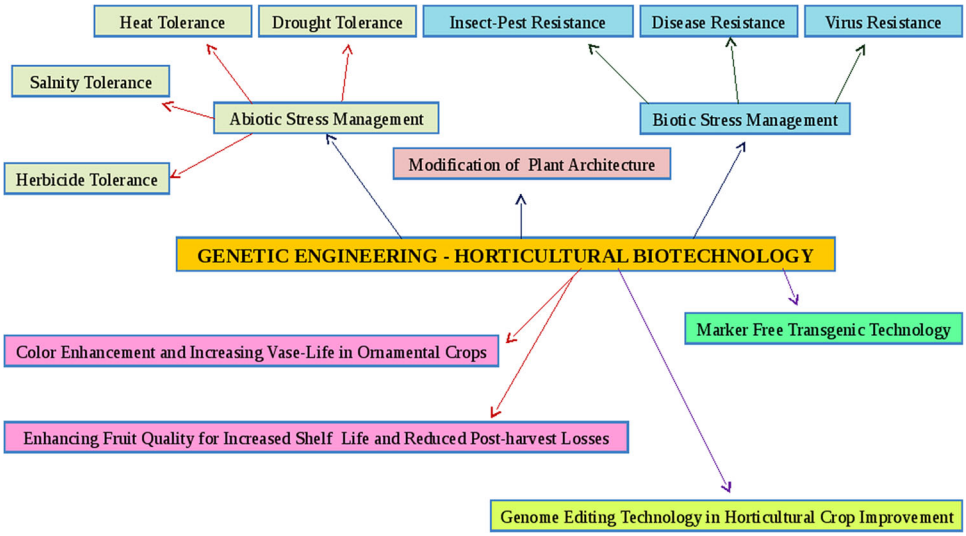

Fig. 1 Overview of various transgenic strategies targeting horticultural crop improvement

transgenic persimmon carrying *cryI* gene were found resistant to *Plodia interpunctata* and *Monema flavescens* (Tao et al. 1997). Brinjal is among the highly consumed vegetables in Asia and specifically in Indian subcontinent. However, it is extremely damaged by a lepidopteran insect, i.e., *Leucinodes orbonalis*. Kumar et al. (1998) transformed a synthetic *cry1Ab* gene coding for an insecticidal crystal protein (*ICP*) to brinjal (*Solanum melongena* cv. Pusa Purple Long) by co-cultivating cotyledons with *A. tumefaciens*. Gene expression was evaluated by double-antibody sandwich ELISA analysis. The transgenic lines displayed significant differences in the insect mortality in fruit bioassays. It was suggested to express a very high level of insecticidal crystal protein to confer complete protection against *Leucinodes orbonalis* by employing some fruit-specific promoter for better inducible expression. 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 *cryIA*(*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*) line DTC 507 with a synthetic fusion gene of *B. thuringiensis* encoding a translational fusion product of *cry1B* and *cry1Ab* δ-endotoxins to confer resistant against diamondback moth (*Plutella xylostella*), the most destructive pest of cruciferous plants across the globe. *Bt* cabbage plants expressing the fusion protein in mature leaves caused 100% mortality to all the four larval stages of diamondback moth. Complete mortality of the neonate larvae had been observed within 24 h and within a period of 48 h in case of other three stages of larvae. *Bt* gene (*Cry1Ac*) has been successfully transformed and expressed in okra (*Abelmoschus esculentus*) for incorporating resistance against fruit and short borer (*Earias vittella*), which is the most serious insect-pest of this crop in Asia (Narendran et al. 2013). Okra is severely affected by *Earias vittella* and its larvae bore into pods and shoots of the plant and eat the internal tissues leading to withering of the plant and reduction in the market value of the pods. In insect bioassays, fruits from transgenic lines showed 100% larval mortality. Natural as well as synthetic insect resistance genes had been transferred into a number of horticultural crops for imparting resistance against various insect-pests. Zhang et al. (2015) transformed kiwifruit plant (*Actinidia chinensis*) with a synthetic chimeric gene *SbtCry1Ac* encoding for protein *btCry1Ac*, When the transgenic plants were screened for insect resistance in insect bioassays, an average of 75.2% *Oraesia excavate* inhibition rate was reported at 10 days of post-infection. This technology could be highly useful to protect yield losses of kiwifruit due to insect attack, which is an economically as well as nutritionally important fruit crop, offering a remarkably high vitamin C content.

Other genes such as protease inhibitors, trypsin inhibitors, lectins, etc. have also been employed for incorporation of insect-pest resistance in many crop species. Ding et al. (1998) developed insect-resistant transgenic Taiwan cauliflower *Brassica oleracea* var. *botrytis* cvs. Known You Early no. 2, Snow Lady and Beauty Lady expressing the *trypsin inhibitor* gene, isolated from local sweet potato. The transgenic plants expressed resistance to *Spodoptera*

litura and *Plutella xylostella* in *in-planta* bioassays. Transgenic strawberry expressing cowpea (*Vigna unguiculata*) *protease trypsin inhibitor* (*CpTi*) gene under a constitutive promoter developed resistance against vine weevil (*Otiorhynchus salcatus*). The *CpTi* transgenic lines reduced the frequency of survival of weevil larvae and pupae during insect bioassays (Graham et al. 2002). Gessler and Patocchi (2007) developed transgenic apple lines with *trypsin inhibitor* encoding *CpTI* gene from cowpea and *cry1A*(*c*) gene of *B. thuringiensis* for incorporation of resistance against codling moth pest. Almost all the genotypes of chrysanthemum are infested by two aphids, namely *Myzus persicae* and *Aphis gossypii*, lowering the flower quality and also transmitting viruses. Valizadeh et al. (2013) transformed chrysanthemum genotype 1581 by *Agrobacterium tumefaciens*-mediated technique with *SAE* gene, under the control of chrysanthemum *RbcS* promoter to incorporate aphid resistance. The *protease inhibitor* sea anemone equistatin (*SAE*) has three domains for inhibition of both cysteine and aspartic proteases. In another study, *Chrysanthemum morifolium WRKY48* (*CmWRKY48*) transcription factor over-expressing transgenic chrysanthemum plants were found to inhibit the population growth of aphids (Li et al. 2015).

Another category of plant pests, root-knot nematode (*Meloidogyne incognita*) causes severe yield losses in many horticultural crops. Genetic transformation of various *proteinase inhibitor* genes from plants is considered as the most potential strategy to prevent such yield losses. *Cysteine proteinases* are involved in the digestion process of root-knot nematodes and binding of various *cystatins* to the active sites of *proteinases* inhibits their activity by proteolytic digestion (Shingles et al. 2007). 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 defense. In another study, Papolu et al. (2016) developed transgenic brinjal plants expressing a modified rice *cystatin* (*OC-IΔD86*) gene under a root-specific promoter, *TUB-1* for inducing resistance against root-knot nematode (RKN). Transgenic plants were confirmed for gene integration and expression using PCR, Southern and Western blotting, ELISA and qPCR assays. When one transgenic line (single copy event) was challenged with root-knot nematode, 78.3% inhibition rate in reproduction of root-knot nematode had been reported. In an earlier study, transgenic banana plants expressing the same *cystatin* gene (*OC-IΔD86*) exhibited 70% resistance to the migratory endoparasite, *R. similis* (Atkinson et al. 2004). Lilley et al. (2004) had also reported a partial resistance (67%) against *M. incognita* in transgenic potato roots expressing same gene. Root lesion nematode (RLN), *Pratylenchus penetrans*, is one of the main pests of lily producers, particularly in USA, where lily (*Lilium longiflorum*) cv. 'Nellie white' assumes a great economic importance as cut flowers and constitutes one of the most valuable species. Vieira et al. (2015) developed transgenic lilies over-expressing the *OC-IΔD86* gene which displayed an enhanced resistance to root lesion nematode infection by means of nematode reduction up to 75%. The transgenic lily plants also exhibited an increased biomass and better growth performance additionally as compared to non-transformed plants.

Disease resistance

The next major constraint limiting the production of horticultural crops is different diseases caused by pathogenic 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. One of the main targets of genetic transformation is to improve tolerance or to incorporate resistance in plants against different pathogens. Genetic engineering of disease resistance in crops has become popular and valuable in terms of cost and efficacy. For imparting resistance against bacterial and fungal diseases, various genes like *chitinase*, *glucanase*, *osmotin*, *defensin,* etc. are being transferred into various horticultural crops world over. Various glycolytic enzymes encoded by genes like *chitinase, glucanase*, *PR proteins,* etc. inside the plant cells have cell wall degrading capabilities, which attract their use for developing transgenic plants for incorporation of resistance against fungal pathogens (Ceasar and Ignacimuthu 2012).

Among different strategies used for genetic engineering for disease resistance, the employment of systemic acquired resistance (SAR)-related genes is of paramount importance. SAR is long lasting and often associated with local and systemic accumulation of salicylic acid (SA) and induced expression of many genes including pathogenesis-related (PR) genes (Ryals et al. 1996). A gene for a 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). Lin et al. (2004) introduced *Arabidopsis thaliana*-derived *NPR-1* gene into tomato. Transgenic tomato plants developed enhanced heat tolerance and resistance against tomato mosaic virus (*ToMV*). The transgenic lines also conferred significant level of resistance to bacterial wilt (BW) and Fusarium wilt (FW) along with moderate degree of enhanced resistance to gray leaf spot (GLS) and bacterial spot (BS). Malnoy and Aldwinckle (2007) developed transgenic apple lines over-expressing *MpNPR1-1* (ortholog of *AtNPR1*), which exhibited broad spectrum resistance

against *V. inaequalis*, *Gymnosporangium juniper-virginianae*, a causative agent of cedar apple rust and *Erwinia amylovora*, which causes fire blight. The Fuji apple, the most popular and most widely cultivated variety of apple in China, is highly susceptible to powdery mildew disease. Apple powdery mildew, which is caused by *Podosphaera leucotricha,* damages leaves and young fruits, thus leading to huge yield losses (Qu et al. 2009). Chen et al. (2012) introduced *Malus hupehensis* derived *NPR* (*MhNPR1*) gene into 'Fuji' cultivar of apple for development of resistance against powdery mildew disease. *NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated SAR in plants. *MhNPR1* gene induced the expression of *MdPRs* and *MdMLO* genes which interact with powdery mildew as revealed by RT-PCR and the transgenic apple plants expressed enhanced resistance to powdery mildew disease. Over-expression of *AtNPR1* gene in tomato and carrot plants also exhibited resistance to bacterial and fungal pathogens (Lin et al. 2004; Wally et al. 2009). Commercial sweet orange cultivars are suffering from this deadly disease. Similar findings were reported by over-expression of a *Vitis vinifera NPR1,1* (*Vv NPR1,1*) gene which conferred to enhance resistance against powdery mildew in grapevine (Le et al. 2011). In the United States, Huanglongbing (HLB) is a very serious disease of citrus, which is associated with a phloem-limited bacterium *Candidatus liberibacter asiaticus* (CLas) (Duan et al. 2009). Dutt et al. (2015) over-expressed an *Arabidopsis thaliana NPR1* gene under a constitutive promoter *CaMV35S* and also under a phloem-specific *Arabidopsis SUC2* (*AtSUC2*) promoter in sweet orange cultivar 'Hamlin' and 'Valencia'. *NPR1* gene is involved in the induction of expression of several native genes involved in the plant defense signaling pathways. The transgenic plants exhibited reduced disease severity and a few lines remained disease-free even after three years of planting in a high-disease pressure field site.

Another category of genes imparting disease resistance is *Chitinases,* which are glycosyl hydrolases that catalyze the degradation of chitin, an insoluble, linear β-1,4-linked polymer of *N*-acetyl glucosamine, a cell wall component of various bacteria and fungi, and thus code for pathogen resistance. *Chitinase* gene has been transferred to a number of crops for harboring fungal resistance. In carrot, the tobacco class I *ChiC* gene has shown resistance against *Botrytis cinerea* (Punja and Raharjo, 1996), *RCC2* gene, a rice *chitinase* displayed enhanced resistance to *Sphaerotheca humuli* in transgenic strawberry plants (Asao et al. 1997). In another study, Yamamoto et al. (2000) transformed a rice *chitinase* gene (*RCC2*) into the somatic embryos of grapevine cv. Neo Muscat and reported an increased resistance level against powdery mildew fungus, *V. necator*. Schestibratov and Dolgov (2005) also developed transgenic strawberry plants expressing *thaumatin II* gene from *Thaumatococcus danielli* and reported some level of resistance to *B. cinerea* during in vitro assays. Vellice et al. (2006) expressed a *chitinase* gene from *Phaseolus vulgaris*, a *glucanase* or a *thaumatin-like protein*, both from *Nicotiana tabacum* and a combination of both in strawberry cv. 'Pajaro'. Two transgenic lines expressing *chitinase* gene showed enhanced tolerance to *Botrytis cinerea*. Khan et al. (2008) attempted to confer resistance to early blight of potato, caused by *Alternaria solani*, transformed a *chitinase* gene, *ChiC*, isolated from *Streptomyces griseus* strain HUT 6037, along with a bialaphos resistance (*bar*) gene into potato. The herbicide-resistant transgenic potato plants demonstrated enhanced resistance against *Alternaria solani* in in vitro bioassays. However, in another study, Moravcikova et al. (2004) reported that the high level of expression of cucumber class III *ChiC* gene in potato could not enhance resistance against the phytopathogenic fungus, *Rhizoctonia solani* (causing black scurf disease in potato) to any considerable level. Das and Rahman (2010) had also expressed bacterial *chitinase* (*chi B*) gene in litchi cv. Bedana, which, however, showed low level of *chitinase* activity and only partial resistance against *Phomopsis* sp. pathogen had been reported in transgenic plants. Various endo-chitinases genes such as *CHIT42* and *CHIT33* from *Trichoderma harzianum* had also been successfully transformed and expressed to impart increased fungal tolerance in potato (Lorito et al. 1998), apple (Bolar et al. 2001), broccoli (Mora and Earle 2001), carrot (Baranski et al. 2008) and lemon (Distefano et al. 2008). Girhepuje and Shinde (2011) developed transgenic tomato plants over-expressing a wheat *chitinase* gene, *chi194*, under the control of maize *ubiquitin 1* promoter. The transgenic tomato lines showing higher expression of *chitinase* activity were found to be highly resistant to Fusarium wilt disease of tomato caused by *Fusarium oxysporum f.* sp. *Lycopersici*. In another study, transgenic litchi (*Litchi chinensis*) plants containing a rice *chitinase* gene were developed to increase the anti-fungal response. The transgenic lines exhibited higher *chitinase* activity and disease resistance than the non-transformed plants (Das et al. 2012). Guava wilt disease caused by a soil borne fungus *Fusarium oxysporum f.* sp. *psidii* is emanating as a serious threat to guava growers throughout the entire globe. To control this disease, Mishra et al. (2016) transferred a *Trichoderma-endochitinase* gene into guava (*Psidium guajava*). *In vitro* pathogen inhibition assay and spore germination assay revealed that the crude extract of the transformed plants inhibited the germination of fungal conidia and were resistance to wilt disease.

A number of defense mechanisms were evolved in plants over thousands of years to overcome pathogen attack/infection and the role of many genes or various pathways has been investigated and identified (Islam

2006). The disease resistance conferred by *glucanase* gene may be attributed to solubilizing elicitors from the fungal cell walls which induce production of antifungal phytoalexins (Keen and Yoshikawa 1993). Yoshikawa et al. (1993) also proposed the role of *glucanase* in the induction of the transcription of a plant defense gene, *phenylalanine ammonia lyase* in response to fungal attack. Further, *glucanase* is a hydrolytic enzyme, which breaks down the cell wall component, glucan of many necrotrophic fungal pathogens. An increased level of resistance in transgenic potato plants expressing soybean *glucanase* gene against *Phytopthora infestans* has been reported due to the increased *glucanase* activity (Borkowoska et al. 1998). Transgenic kiwifruit over-expressing soybean *β-1,3 glucanase* gene exhibited a six fold increased enzyme activity leading to a decrease in the disease lesion area caused by the gray mold fungus, *Botrytis cinerea* (Nakamura et al. 1999). Almost all the cultivated varieties of brinjal are susceptible to wilt disease caused by *Verticillum dahliae* and *Fusarium oxysporum*, which cause considerable yield losses annually (Najar et al. 2011). To generate wilt disease resistance in brinjal, Singh et al. (2014) transformed alfalfa *glucanase* gene coding for an acidic *glucanase* into brinjal cv. Pusa Purple Long. The selected transgenic lines, confirmed with DNA and protein blotting techniques, showed enhanced level of resistance against these wilt causing fungi with a delay of 5–7 days in disease development as compared to the non-transgenic plants. Sometimes, it has been found that the transgenes were capable of inducing disease resistance trait, but have altered the plant growth processes due to the use of a constitutive promoter. In a study, Mercado et al. (2015) expressed *β-1,3-glucanase* gene *bgn13,1*, isolated from *Trichoderma harzianum* in strawberry under the control of *CaMV 35S* promoter. The transgenic lines showed reduced anthracnose symptoms (from 61.0 to 16.5%) in leaf and crown than control plants after inoculation with *Colletotrichum acutatum*. However, most of the transgenic lines displayed stunted phenotype and reduced yield due to the reduction in number of fruits per plant and a reduced fruit size.

The use of various antimicrobial proteins coding genes like *defensins* had been advocated for combating a large class of fungal and bacterial pathogens (Collinge et al. 2010). Defensins represent a class of antimicrobial peptides which play an important defensive role against fungi, bacteria and protozoa, but are non-toxic to mammalian cells and plants. Defensin genes encoded proteins react by creating certain pores in the fungal hyphal membrane and, thus, disturb the ion-influx–outflux and kill the fungal pathogens. Zainal et al. (2009) reported an enhanced level of resistance in transgenic tomato expressing *Capsicum annum defensin* gene against various fungal pathogens. A bell pepper *J1 defensin* gene was also reported to confer resistance against anthracnose disease of mango, which is caused by *Colletotrichum gloeosporioides* (Rivera-Dominguez et al. 2011). Protein extract from processed embryos of transgenic mango cv. 'Ataulfo' inhibited the growth of *C. gloeosporioides, Aspergillus niger* and *Fusarium* spp. Transgenic banana plants over-expressing two defensin genes—*PhDef1* and *PhDef2* had been found resistant against *Fusarium oxysporum* f. sp. *cubense* (Ghag et al. 2012). In addition to that, genetic transformation of many non-plant antimicrobial compounds like cercopin, attacin, phytoalexins had been reported to enhance resistance level in plants expressing them (Mondal et al. 2012; Ahuja et al. 2012). Transgenic apple expressing maize Leaf color (*Lc*) gene exhibited resistance to fire blight and scab diseases (Flachowsky et al. 2010). In another study, transformation of a biotin binding protein (Markwick et al. 2003) and a *proteinase inhibitor* gene from *Nicotiana alata* exhibited resistance against light brown apple moth disease. Constitutive expression of a fungus-inducible *carboxy esterase* gene (*PepEST*) under *CaMV35S* promoter was reported to increase the anthracnose disease resistance in transgenic pepper (*Capsicum annum*) (Ko et al. 2016). *PepEST* gene expression in fruits leads to disease resistance development by generation of H_2O_2 and expression of pathogenesis-related (*PR*) genes, which encode for a number of small proteins having antimicrobial activity. On infection of the anthracnose fungus, *Colletotrichum gloeosporioides* on the transgenic fruits of pepper cv. Nokkang, a higher level of expression of *PR* genes, namely *PR3*, *PR5*, *PR10* and *PepThi* was reported than the non-transgenic plants. Further, a lower rate of disease occurrence (30%) was reported in the transgenic fruits than in the wild-type plants.

Various types of polyamines including putrescine, spermidine and spermine play a key role in imparting tolerance/resistance to both biotic and abiotic stresses. Hazarika and Rajam (2011) transformed tomato cv. Pusa Ruby with a human *S-adenosyl methionine decarboxylase* (*samdc*) gene, which is involved in the biosynthesis of polyamines viz. spermidine and spermine. The transgenic tomato plants synthesized higher level of polyamines and also expressed enhanced level of resistance against *Fusarium oxysporum* causing wilt disease and *Alternaria solani*, the early blight causing fungus. In addition to that, the transgenic lines also expressed better tolerance to a variety of abiotic stresses including high temperature, drought, salinity and chilling stress. Shin et al. (2002) developed transgenic chilli pepper plants (*Capsicum annum* cv. Nockwang) with *Tsi1* (tobacco stress-induced 1) gene via *Agrobacterium tumefaciens*-mediated gene transfer technique using cotyledon and hypocotyl explants. The protein product of *Tsi1* gene has got some role in regulating stress-responsive genes and pathogenesis-related (*PR*) genes. The transgenic chilli plants expressed enhanced

resistance to pepper mild mottle virus, cucumber mosaic virus, a bacterial pathogen—*Xanthomonas campestris* pv. *Vesicatoria* and a fungal pathogen—*Phytophthora capsici*. Genetic transformation of *Vf* gene, imparting scab disease resistance caused by *Venturia inaequalis* under *CaMV 35S* promoter in apple, had been found to impart scab resistance in susceptible cultivars of apple in a number of studies (Malnoy et al. 2008; Szankowski et al. 2009; Joshi et al. 2009). Banana (*Musa* sps.) is one of the most important fruit crops being cultivated in about 120 countries across the globe. India is the largest global producer of banana. Banana Xanthomonas wilt (BXW), which is caused by *Xanthomonas campestris* pv. *musacearum*, is considered as one of the most destructive diseases of this fruit crop, particularly in East and Central Africa (Tripathi et al. 2009). In a study, Namukwaya et al. (2012) expressed *plant ferredoxin like protein* (*Pf1p*) gene under the control of *CaMV35S* promoter in transgenic banana cv. 'Sukali Ndizi' and 'Nakinyika' to develop resistance against BXW disease. In bioassay studies, 67% of the transgenic lines were found resistant to BXW and did not show any disease symptoms, while the wild-type plants expressed severe symptoms of wilting. In another study, grapevine rootstock of *V. berlandieri* × *V. rupestris* cv. Richter 110 had been transformed with an *Agrobacterium* oncogene-silencing gene to develop crown gall-resistant lines (Galambos et al. 2013). Lindow et al. (2014) reported a reduced severity of Pierce's disease and pathogen mobility in transgenic grape cv. Freedom by the over-expression of an *rpfF* gene (from *Xylella fastidiosa*), which codes for the *synthase* for diffusible signal factors (*DSFs*). Cheng et al. (2016) transformed *Vitis vinifera* Thompson Seedless grape with a *stilbene synthase* gene, *VqSTS6*, isolated from Chinese wild-type *V. quinquangularis* accession Danfeng-2 under a fruit-specific promoter to develop resistance against powdery mildew disease, caused by *Uncinula necator*. The transgenic plants synthesized enhanced quantity of trans-resveratrol and other stilbene compounds as compared to the control plants and expressed enhanced resistance to powdery mildew fungus. It has been found that *VqSTS6* gene is involved in resveratrol biosynthetic pathway in grapes and, thus, plays a key role in imparting protection against invading pathogens. Jiwan et al. (2012) reported antisense expression of the peach *MLO* gene in strawberry (*Fragaria* × *ananasa*) conferred cross-species resistance to *Fragaria*-specific powdery mildew. RNA-interference (*RNAi*) technology being used recently is quite successful in controlling various bacterial and fungal diseases in plants by switching off the expression of certain endogenous genes. In one such study, transgenic tomato plants expressing *hpRNA* constructs against *Agrobacterium iaaM* and *ipt* oncogenes were found to be resistant to crown gall disease (Escobar et al. 2001). Recently, Pessina et al. (2016) reported an increased level of resistance in grapevine to powdery mildew by *RNAi*-mediated silencing of the susceptible (*S*-gene) *MLO-7*.

Virus resistance

In fruit crops, the *coat protein*-mediated approach to engineer virus resistance has been in application to introduce resistance against various viral diseases. Papaya is 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. Strawberry is susceptible to various devastating fungi, bacteria and viruses. Finstad and Martin (1995) developed transgenic strawberry plants expressing a *coat protein* (*cp*) gene from strawberry mild yellow edge potexvirus (*SMYELV-CP*) and these lines conferred resistance to the virus. In another study, Lee et al. (2009) developed transgenic chilli pepper plants with a *coat protein* gene (*CMVP0-CP*). Three independent transgenic events, which were earlier highly tolerant to CMVP1 pathogen, were also found to be tolerant to CMVP0 pathogen. The production and productivity of watermelon (*Citrullus lanatus*) have been affected considerably by two viruses, namely Zucchini yellow mosaic virus (ZYMV) and papaya ring spot virus type W (PRSV W) worldwide. In an attempt to get rid of these two viruses altogether, Yu et al. (2011) transformed three watermelon cultivars, namely 'Feeling', 'China rose', and 'Quality' with chimeric construct containing truncated *ZYMV coat protein* (*CP*) and *PRSV W CP* genes via *Agrobacterium tumefaciens*-mediated gene transfer technique. Two completely immune transgenic lines of 'Feeling' cultivar had been obtained during greenhouse bioassays where these two lines showed complete resistance to *ZYMV* and *PRSV W* and no virus accumulation was detected by Western blotting from these transgenic lines.

Also, transgenic papaya plants with the mutated *replicase* (*RP*) gene from PRSV showed high resistance or immunity against PRSV in the field (Xiangdong et al. 2007). Borth et al. (2011) developed transgenic banana (cv. Dwarf Brazilian) plants resistant to banana bunchy top virus (BBTV) by transforming four gene construct derived from the *replicase associated protein* (*Rep*) gene of the Hawaiian isolate of BBTV. The transgenic plants showed no bunchy top symptoms, while the non-transgenic plants expressed bunchy top symptoms. Azadi et al. (2011)

transformed lily cv. 'Acapulco' plants with a defective cucumber mosaic virus *replicase* gene and four transgenic lines were found to show enhanced level of virus resistance.

RNAi technology has been found successful to impart resistance to various viral diseases in plants. 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). 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. Transgenic banana plants expressing *siRNA* targeted against viral replication initiation (*Rep*) gene were developed by Shekhawat et al. (2012), which showed high level of resistance to BBTV infection. Transgenic development work carried out in various horticultural crops for imparting biotic stress resistance has been summarized in Table 1.

Abiotic stress management through transgenic approach

Abiotic stresses such as heat, drought and salinity are the major environmental constraints affecting production and productivity of almost all horticultural crops. Conventional plant breeding has not been proved that much successful in addressing abiotic stress mitigation so far. The reason might be that the traits are controlled by a number of genes present at a quantitative trait locus (QTL). To combat the negative effects of various abiotic stresses, it is pre-requisite to identify potential candidate genes or QTLs (gene networks) associated with broad-spectrum multiple abiotic stress tolerance. Various abiotic stresses including drought, high temperature, salinity, frost and flood, etc. adversely affect overall crop growth and productivity by affecting the vegetative and reproductive stages of growth and development. These stresses generally trigger a series of physiological, biochemical and molecular changes in the plants which often result in damage to the cellular machinery (Rai et al. 2011). These changes include the disruption of cellular osmotic balance leading to dysfunctional homeostasis, ion distribution and oxidative stresses which cause denaturation of integral proteins of plants. Plants respond to such stresses in a variety of mechanisms which trigger the cell signaling process, transcriptional controls and production of a number of stress conditions related tolerant proteins, antioxidants and osmotic solutes to maintain homeostasis and to protect and repair the damaged integral proteins. Generally, plants which are stress sensitive are unable to synthesize such compounds under stress conditions and, thus, are rendered liable to various stresses which hamper their overall growth. A number of genes have been identified in a number of plants/organisms, closely or distantly related, which code for the synthesis of these stress protecting compounds and thus can be targeted for genetic transformation into sensitive genotypes. Such genes have been classified into three categories as: (a) genes which code for the synthesis of various osmolytes such as mannitol, glycine betain, proline, and heat shock proteins, (b) genes responsible for ion and water uptake and transport like aquaporins and ion transporter, etc. and (c) genes regulating transcriptional controls and signal transduction mechanism, examples *MAPK*, *DREBI,* etc. Research on genetic modification of various horticultural crops for improved abiotic stress tolerance has been explored.

Drought tolerance

Various genes controlling signaling and gene regulatory pathways offer certain key targets for genetic engineering for abiotic stress tolerance. Transcription factors (*TFs*) that regulate or switch on the expression of a number of genes involved in imparting abiotic stress tolerance in plants have been proposed as the most efficient targets for genetic transformation (Bhatnagar-Mathur et al. 2008). These transcription factors include *DREB1* gene family, *Myb* gene family, etc. 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 water deficit stress than the wild-type plants. Pasquali et al. (2008) reported improved tolerance to cold and drought stress in transgenic apple by the over-expression of a cold-inducible *Osmyb 4* gene from rice, which codes for a *TF* belonging to *Myb* family. The over-expression of *DREB1b TF* gene had also been reported to induce cold tolerance and drought tolerance in transgenic grapevine (Jin et al. 2009). Chrysanthemum is one of the leading ornamental cut flowers across the globe and its production is severely hampered by various environmental conditions (Gao et al. 2012). Drought stress harms this crop to the maximum extent by retarding its growth. *WRKY* transcription factors (*TFs*) work as positive or sometimes negative regulators in various abiotic stress responses in plants. Fan et al. (2016) transformed a *CmWRKY1 TF* derived from *Chrysanthemum morifolium* and over-expressed it in chrysanthemum cultivar 'Jinba'. It was found that *CmWRKY1* regulates an ABA-mediated

Table 1 Transgenic horticultural crops for biotic stress resistance/management

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
(A) Insect-pest resistance						
1.	Brinjal (egg plant) cv. Pusa Purple Long	Synthetic *cry 1Ab* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Insect mid-gut dissolution	Resistance against fruit and shoot borer, *Leucinodes orbonalis*	The transgenic lines displayed significant differences in the insect mortality in fruit bioassays	Kumar et al. (1998)
	Brinjal	Modified rice *cystatin* gene (*OC-IΔD86*); *Agrobacterium tumefaciens*-mediated gene transfer	Binding of cystatin to the active sites of proteinases inhibit their activity, thus affecting their proteolytic digestion	Resistance against root-knot nematode (*Meloidogyne incognita*)	78.3% inhibition rate in reproduction of root-knot nematode in transgenic plants	Papolu et al. (2016)
2.	Banana cv. Gonja manjaya	Maize *cystatin* gene (*CC-II*); *Agrobacterium tumefaciens*-mediated gene transfer	Binding of cystatins to the active sites of proteinases inhibit their activity, thus affecting their proteolytic digestion	Resistance against reniform nematode (*Potylenchulus reniformis*) and *Helicotylenchus multicinctus*	Reduced infection of *P. reniformis* and *H. multicinctus* in transgenic plants	Roderick et al. (2012)
	Banana	*Cystatin* gene (*OC-IΔD86*); *Agrobacterium tumefaciens*-mediated gene transfer	Binding of cystatins to the active sites of proteinases inhibit their activity, thus affecting their proteolytic digestion	Resistance against *Radopholus similis*	Transgenic banana plants exhibited 70% resistance to the migratory endoparasite, *R. similis*	Atkinson et al. (2004)
3.	Strawberry (*Fragaria* × *ananasa*)	*CpTi* (*Cowpea protease trypsin inhibitor*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	*CpTi* protein inhibits the internal proteases within the plant cell on pest attack and thus protect the integral protein functions	Resistance against vine weevil (*Otiorhynchus sulcatus*)	*CpTi* transgenic lines reduced the frequency of survival of weevil larvae and pupae during insect bioassays	Graham et al. (2002)
4.	Cabbage (*Brassica oleracea* var. *capitata*)	*Cry1B-cry1Ab* fusion gene; *Agrobacterium tumefaciens*-mediated gene transfer	Insect mid-gut dissolution	Resistance against Diamond back moth (*Plutella xylostella*)	Reduced infestation of *P. xylostella* in transgenic plants	Paul et al. (2005)
5.	Okra (*Abelmoschus esculentus*), an inbred line of Maharashtra Hybrid Seeds Company Ltd., Jalna, India	*Cry1Ac* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Insect mid-gut dissolution	Insect resistance (fruit and shoot borer)	In insect bioassays, fruits from transgenic lines caused 100% larval mortality	Narendran et al. (2013)
6.	Kiwifruit (*Actinidia chinensis*)	Synthetic chimeric gene *sbtCryIAc*; *Agrobacterium tumefaciens*-mediated gene transfer	Insect mid-gut dissolution	Insect resistance against *Oraesia excavate*	An average of 75.2% *O. excavate* inhibition rate at 10 days of post-infection during in vitro insect bioassay	Zhang et al. (2015)
7.	Potato	*Cystatin* gene (*OC-IΔD86*); *Agrobacterium tumefaciens*-mediated gene transfer	Binding of cystatins to the active sites of proteinases inhibit their activity, thus affecting their proteolytic digestion	Resistance against *M. incognita*	A partial resistance (67%) against *M. incognita* in transgenic potato roots	Lilley et al. (2004)
8.	Lily (*Lilium longiflorum*) cv. 'Nellie white'	Rice *cystatin* gene, *OC-IΔD86; Agrobacterium rhizogenes*-mediated gene transfer	Binding of cystatins to the active sites of proteinases inhibit their activity, thus affecting their proteolytic digestion	Root lesion nematode (*Patrylenchus penetrans*) resistance	Enhanced resistance to root lesion nematode infection by means of nematode reduction up to 75% and an increased biomass and better growth performance as compared to non-transformed plants	Vieira et al. (2015)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
9.	*Chrysanthemum morifolium*	*CmWRKY48* transcription factor; *Agrobacterium tumefaciens*-mediated gene transfer	*WRKY48 TF* works as positive regulator in various biotic stress responses in plants	Aphid resistance	Inhibition in the population growth of aphids in transgenic chrysanthemum plants	Li et al. (2015)
	C. morifolium genotype 1581	Protease inhibitor sea anemone equistatin (*SAE*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	*SAE* has three domains for inhibition of both cysteine and aspartic proteases of aphids	Aphid resistance	Enhanced resistance against *Myzus persicae* and *Aphis gossypi*	Valizadeh et al. (2013)
10.	Taiwan cauliflower (*Brassica oleracea* var. *botrytis*) cvs. Known You Early no. 2, Snow Lady and Beauty Lady	*Trypsin inhibitor* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Inhibits trypsin	Insect resistance	Enhanced resistance to *Spodoptera litura* and *Plutella xylostella* in transgenic plants in *in-planta* bioassays	Ding et al. (1998)
(B) Fungal resistance						
1.	Strawberry (*Fragaria* × *ananassa*)	A rice *chitinase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance against *Sphaerotheca humuli*	Enhanced resistance to *Sphaerotheca humuli* in transgenic strawberry plants	Asao et al. (1997)
	Strawberry	*Osmotin; Agrobacterium tumefaciens*-mediated gene transfer	Increase the level of proline accumulation	Resistance against gray mold disease caused by *Botrytis cinerea*	The transgenic plants showed higher levels of antifungal activity and significantly increased resistance to wilt and gray mold diseases	Martinelli et al. (1997)
	Strawberry	*Thaumatin II* gene (from *Thaumatococcus danielli; Agrobacterium tumefaciens*-mediated gene transfer	Some role in fungal resistance	Resistance to *Botrytis cinerea*	Enhanced resistance to *B. cinerea*	Schestibratov and Dolgov (2005)
	Strawberry cv. Pajaro	*Chitinase* and a *glucanse* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* and *glucanase* degrades chitin and glucan, cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance to *Botrytis cinerea*	Two transgenic lines expressing *chitinase* gene showed enhanced tolerance to *Botrytis cinerea*	Vellice et al. (2006)
	Strawberry	*β-1,3-glucanase; Agrobacterium tumefaciens*-mediated gene transfer	Dissolves glucan component of cell wall of fungi, thus imparting fungal resistance	Resistance to anthracnose crown rot caused by *Colletotrichum acutatum*	Reduced crown rot lesions in transgenic events as compared to the non-transgenic lines	Mercado et al. (2007)
	Strawberry cv. Camarosa	*β-1,3-glucanase* gene (*bgn13,1* from *Trichoderma harzianum*); *Agrobacterium tumefaciens*-mediated gene transfer	Dissolves glucan component of cell wall of fungi, thus imparting fungal resistance	Resistance to crown gall disease	The transgenic lines showed reduced anthracnose symptoms (from 61 to 16.5%) in leaf and crown than control plants	Mercado et al. (2015)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
2.	Carrot (*Daucus carota*)	Tobacco *class I ChiC* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3-60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance against *Botrytis cinerea*	Enhanced resistance against *Botrytis cinerea* in transgenic carrot plants	Punja and Raharjo (1996)
	Carrot	*AtNPR1* gene (derived from *Arabidopsis thaliana*); *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated systemic acquired resistance (SAR) in plants	Resistance against bacterial and fungal pathogen	Enhanced resistance to bacterial and fungal pathogens	Wally et al. (2009)
3.	Potato	Soybean *glucanase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Dissolves glucan component of cell wall of fungi, thus imparting fungal resistance	Resistance against *Phytopthora infestans*	An increased level of resistance in transgenic potato against *Phytopthora infestans*	Borkowoska et al. (1998)
	Potato	A cucumber *class III ChiC* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3-60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance against *Rhizoctonia solani* causing black scurf disease in potato	No resistance against the phytopathogenic fungus, *Rhizoctonia solani*	Moravcikova et al. (2004)
	Potato cvs. Waseshiro, Benimaru and May Queen	A *chitinase* gene, *ChiC* from *Streptomyces griseus* and *bar* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinases* are glycosyl hydrolases which catalyze the degradation of chitin, an insoluble, linear β-1,4-linked polymer of *N*-acetyl glucosamine, a cell wall component of various bacteria and fungi, and thus codes for pathogen resistance	Resistance against early blight of potato caused by *Alternaria solani*	The herbicide resistant transgenic potato plants demonstrated enhanced resistance against *A. solani* in in vitro bioassays	Khan et al. (2008)
4.	Kiwifruit	Soybean *β-1,3-glucanase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Dissolves glucan component of cell wall of fungi, thus imparting fungal resistance	Resistance against gray mold fungus *Botrytis cinerea*	Transgenic kiwifruit exhibited a six fold increased enzyme activity leading to a decrease in the disease lesion area caused by the gray mold fungus, *Botrytis cinerea*	Nakamura et al. (1999)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
5.	Grapevine (*Vitis vinifera*)	Rice *chitinase* gene (*RCC2*); *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Powdery mildew resistance	Increased resistance level against powdery mildew fungus, *V. necator*	Yamamoto et al. (2000)
	Grapevine	*Vv NPR1,1* gene (derived from *Vitis vinifera*); *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated systemic acquired resistance (SAR) in plants	Resistance against powdery mildew	Enhanced resistance to powdery mildew disease	Le et al. (2011)
	Grapevine rootstock of *V. berlandieri* × *V. rupestris* cv. Richter 110	*Agrobacterium* oncogene-silencing gene; *Agrobacterium tumefaciens*-mediated gene transfer	Oncogenes when silenced lead to no crown gall formation	Resistance against crown gall disease	Enhanced resistance to crown gall-disease	Galambos et al. (2013)
	Grapevine cv. Freedom	*rpfF* gene (from *Xylella fastidiosa*); *Agrobacterium tumefaciens*-mediated gene transfer	*rpfF* gene codes for the *synthase* for diffusible signal factors (*DSFs*) imparting resistance against Pierce's disease	Resistance against Pierce's disease	A reduced severity of Pierce's disease and pathogen mobility in transgenic grape	Lindow et al. (2014)
	Grapevine cv. Thompson Seedless grape	A *stilbene synthase* gene, *VqSTS6* from wild grapevine, *V. quinquangularis; Agrobacterium tumefaciens*-mediated gene transfer	*VqSTS6* gene is involved in resveratrol biosynthetic pathway and plays a key role in imparting protection against invading pathogens	Resistance against powdery mildew disease caused by *Uncinula necator*	The transgenic plants synthesized enhanced quantity of trans-resveratrol and other stilbene compounds and expressed enhanced resistance to powdery mildew fungus	Cheng et al. (2016)
	Grapevine	*RNAi*-mediated silencing of *MLO-7* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*MLO-7* is a powdery mildew susceptible gene, its silencing by *RNAi* imparts resistance trait	Resistance against powdery mildew disease	Enhanced resistance against powdery mildew disease in transgenic plants	Pessina et al. (2016)
	Grape cv. Chardonnay	*MLO-7* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Genome editing to bring targeted mutagenesis	Resistance against powdery mildew	Enhanced resistance against powdery mildew in transgenic plants	Malnoy et al. (2016)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
6.	Apple (*Malus domestica*)	*Malus pumila NPR1* (*MpNPR1*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated systemic acquired resistance (SAR) in plants	Fungal and bacterial resistance	Resistance to two fungal pathogens, *Venturia inaequalis* and *Gymnosporangium juniperi-verginianae*, and a bacteria, *Erwinia amylovora*	Malnoy et al. (2007)
	Fuji Apple (Fuji Naga-fu no. 6)	*Mh-NPR1* gene (derived from *Malus hupehensis*); *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated systemic acquired resistance (SAR) in plants	Resistance against apple powdery mildew, caused by *Podosphaera leucotricha*	*MhNPR1* gene induced the expression of *MdPRs* and *MdMLO* genes which interact with powdery mildew and the transgenic apple plants expressed enhanced resistance to powdery mildew disease	Chen et al. (2012)
	Apple cv. Golden delicious	*DIPM-1*, *DIPM-2* and *DIPM-4* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Genome editing to bring targeted mutagenesis	Fire blight disease resistance	Enhanced resistance to fire blight disease in transgenic plants	Malnoy et al. (2016)
7.	Litchi (*Litchi chinensis*) cv. Bedana	Bacterial *chitinase* gene (*ChiB*); *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance against *Phomopsis sp.*	Transgenic plants showed low level of *chitinase* activity and only partial resistance against *Phomopsis* sp. pathogen	Das and Rahman (2010)
	Litchi cv. Bedana	*Chitinase* gene (rice-derived); *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Fungal resistance	The transgenic lines exhibited higher *chitinase* activity and disease resistance than the non-transformed plants	Das et al. (2012)
8.	Mango (*Mangifera indica*) cv. Ataulfo	Defensin *J1* gene from bell pepper; Biolistic-mediated DNA delivery method	*defensin* gene acts as proteinase inhibitor, also creates pores in cell membrane of fungal hyphae	Resistance against anthracnose disease caused by *Colletotrichum gloeosporioides*	Protein extract from processed embryos of transgenic mango cv. 'Ataulfo' inhibited the growth of *C. gloeosporioides, Aspergillus niger* and *Fusarium* sps.	Rivera- Dominguez et al. (2011)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
9.	Tomato	*Defensin* gene form chilli; *Agrobacterium tumefaciens*-mediated gene transfer	*Defensin* gene acts as proteinase inhibitor, also creates pores in cell membrane of fungal hyphae	Various fungal pathogens	Enhanced level of resistance in transgenic tomato plants	Zainal et al. (2009)
	Tomato cv. Pusa Ruby	*Samdc* (a human derived *S-adenosyl methionine carboxylase gene); Agrobacterium tumefaciens*-mediated gene transfer	*Samdc* is a key gene involved in polyamine biosynthesis, imparting tolerance to a variety of biotic and abiotic stresses	Biotic and abiotic stress resistance/tolerance	The transgenic tomato plants expressed enhanced level of resistance against *Fusarium oxysporum* and *Alternaria solani* and also expressed better tolerance high temperature, drought, salinity and chilling stress	Hazarika and Rajam (2011)
	Tomato cv. Pusa Ruby	*chi194,* a wheat endochitinase gene; *Agrobacterium tumefaciens*-mediated gene transfer	Chitinase degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Resistance against tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*	The transgenic tomato lines found to be highly resistant to Fusarium wilt	Girhepuje and Shinde (2011)
	Tomato	*At NPR1* gene (derived from *Arabidopsis thaliana*); *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene plays a key role in regulating salicylic acid (SA)-mediated systemic acquired resistance (SAR) in plants	Resistance against bacterial and fungal pathogen	Enhanced resistance to bacterial and fungal pathogens	Lin et al. (2004)
	Tomato	Wasabi *defensin* (*WD*) gene from *Wasabia japonica; Agrobacterium tumefaciens*-mediated gene transfer	Defensins are antimicrobial proteins, inhibit proteases and create pores in fungal hyphal membrane	Fungal resistance	The transgenic tomato plants exhibited enhanced resistance against a number of fungi including *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Erysiphe lycopersic*.	Khan et al. (2011b)
10.	Brinjal (*Solanum melongena*) cv. Pusa Purple Long	*Glucanase* gene from alfalfa; *Agrobacterium tumefaciens*-mediated gene transfer	Dissolves glucan component of cell wall of fungi, thus imparting fungal resistance	Resistance against *Verticillium dahliae* and *Fusarium oxysporum*	The transgenic lines showed enhanced level of resistance against these wilt causing fungi with a delay of 5–7 days in disease development as compared to the non-transgenic plants	Singh et al. (2014)
11.	Guava (*Psidium guajava*)	*Trichoderma-endochitinase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Chitinase* degrades chitin, which constitutes 3–60% of cell wall composition of fungi, by hydrolysis and thus codes for disease resistance	Wilt disease resistance	Crude extract of the transformed plants inhibited the germination of fungal conidia	Mishra et al. (2016)

Table 1 continued

S. no.	Crop species/cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement (transgenic vs normal plants)	References
12.	Pepper cv. Nokkang	A pepper *esterase* gene (*PepEST*); *Agrobacterium tumefaciens*-mediated gene transfer	*PepEST* gene expression leads to generation of H_2O_2 and expression of pathogenesis-related (*PR*) genes, which encode for a number of small proteins having antimicrobial activity	Anthracnose disease resistance (caused by *Colletotrichum gloeosporioides*)	A lower rate of disease occurrence in the transgenic fruits, approximately 30% of than in the wild-type plants	Ko et al. (2016)
13.	Citrus (sweet orange) cv. 'Hamlin' and 'Valencia'	*AtNPR1* gene (from *Arabidopsis thaliana*); *Agrobacterium tumefaciens*-mediated gene transfer	*NPR1* gene is involved in the induction of expression of several native genes involved in the plant defense signaling pathways	Resistance against Huanglongbing (HLB) disease, caused by a bacterium *Candidatus Liberibacter asiaticus* (CLas)	The transgenic plants exhibited reduced disease severity and a few lines remained disease-free even after 3 years of planting in a high-disease pressure field site	Duan et al. (2009)
(C) Virus resistance						
1.	Potato cv. 'spuncta'	ds RNA of *coat protein* gene of *PVY; Agrobacterium tumefaciens*-mediated gene transfer	Antisense gene inhibition	Resistance against Potato virus Y (*PVY*)	Transgenic plants were found highly resistant to three strains of *PVY*, each belonging to three different subtypes of the virus (PVY^N, PVY^O and PVY^{NTN})	Missiou et al. (2004)
2.	Poinsettia	*RNAi; Agrobacterium tumefaciens*-mediated gene transfer	*siRNA*-mediated gene silencing	Poinsettia mosaic virus resistance	Enhanced resistance to Poinsettia mosaic virus	Clarke et al. (2008)
3.	Papaya	*PRSV* Coat protein (*CP*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Coat protein*-mediated virus resistance	Papaya ring spot virus	Enhanced resistance against PRSV infection	Suzuki et al. (2008)
4.	Watermelon (*Citrullus lanatus*), three cvs. namely 'Feeling', 'China rose' and 'Quality'	A chimeric gene construct containing truncated *ZYMV-cp* and *PRSV W cp* genes; *Agrobacterium tumefaciens*-mediated gene transfer	RNA mediated post-transcriptional gene silencing (PTGS)	Resistance against two viruses namely, ZYMV and PRSV W	Two completely immune transgenic line of 'Feeling' cultivar showed complete resistance to ZYMV and PRSV W	Yu et al. (2011)
5.	Banana cv. Rasthali	*ihp-RNA-Rep* and *ihp-RNA-ProRep; Agrobacterium tumefaciens*-mediated gene transfer	siRNA, derived from the transgene sequence coded for resistance against BBTV by establishing RNAi mechanism to silence *Rep* gene transcript	Resistance against banana bunchy top virus (BBTV)	Transgenic plants were found resistant to BBTV	Shekhawat et al. (2012)
6.	Strawberry	*CP* gene from strawberry mild yellow edge potexvirus; *Agrobacterium tumefaciens*-mediated gene transfer	*Coat protein*-mediated virus resistance	Resistance against strawberry mild yellow edge potex virus	Transgenic lines were found resistant to SMYEPV	Finstad and Martin (1995)
7.	Lily cv. 'Acapulco'	A defective *cucumber mosaic virus replicase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Defective-viral genome-mediated resistance	*CMV* virus resistance	Four transgenic lines showed enhanced level of virus resistance	Azadi et al. (2011)

pathway by suppressing the expression levels of various genes including *PP2C*, *ABI1* and *ABI2*, and activating the expression levels of genes like *PYL2*, *SnRK2-2*, *ABF4*, *MYB2*, *RAB18* and *DREB1A* in a positive regulation. The transgenic plants displayed increased drought tolerance in polyethylene glycol (PEG) stress as compared to control plants. Also, multiple abiotic stress tolerance in banana had been reported by the over-expression of *MusaWRKY71* gene, which is a very potential abiotic stress-responsive *WRKY TF* gene, cloned from *Musa species* cv. Karibale Monthan (Shekhawat and Ganapathi 2013).

A bacterial *mannitol-1-phosphate dehydrogenase* (*mtlD*) gene driven by the constitutive cauliflower mosaic virus (*CaMV*) *35S* promoter was transferred into tomato plants which provides improved 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. To impart tolerance to various abiotic stresses in potato, Gangadhar et al. (2016) transformed a potato-derived gene *StnsLTP1* into potato (*Solanum tuberosum* cv. Desiree) using *Agrobacterium tumefaciens*-mediated genetic transformation method. Under stress conditions, transgenic potato lines displayed enhanced cell membrane integrity by reduced membrane lipid peroxidation activity and H_2O_2 content, comparatively. Also an increased level of antioxidant enzyme activity with enhanced accumulation of ascorbates and upregulation of various stress-related genes including *StAPX, StCAT, StSOD*, etc. was reported in transgenic potato plants. In an attempt to improve abiotic stress tolerance in mulberry, a very important plant of silk industry, *Hva1* gene encoding for late embryogenesis abundant protein from barley, was transformed by *Agrobacterium tumefaciens*-mediated method (Checker et al. 2012). LEA proteins comprise a group of hydrophillins, which are induced as a response to dessication in seeds and are also stimulated under various abiotic stress conditions like dehydration, salinity, chilling or high temperature stress in vegetative tissues of plants (Khurana et al. 2008). The transgenic lines displayed an enhanced level of tolerance to drought, salinity and cold conditions than normal plants as quantified by free proline, membrane stability index (MSI) and PS II activity. Glycine betaine plays an important role in drought stress tolerance by scavenging oxidative stress-inducing molecule (free radicals) and it also protects the photosynthetic system in plants. Cheng et al. (2013) transformed *choline oxidase* gene (*CodA*) isolated from *Arthrobacter globiformis*, which is involved in the biosynthesis of glycine betaine, into potato cv. 'Superior' under an oxidative stress-inducible *SWAP2* promoter for inducing drought stress tolerance trait. Under water-stress conditions, transgenic potato plants showed expression of *codA* gene and an accumulation of glycine betaine with a higher leaf water potential as compared to the non-transformed plants. In the stress-recovery treatment, transgenic potato plants displayed a stronger antioxidant activity, higher chlorophyll content, more efficient photosynthesis and better recovery, comparatively. Plant micro RNA (*miRNA*) regulates several developmental and physiological phenomena inside the plants including drought responses. Zhang et al. (2011a, b) transformed tomato with an *miR 169* family member, *Sly-miR169c*, which can effectively down-regulate the transcripts of the target genes—three nuclear factor Y subunit genes (*SINF-YA1/2/3*) and one multidrug resistance-associated protein gene (*SIMRP1*), which are down-regulated under drought stress. The transgenic tomato plants over-expressing *Sly-miR169c* displayed reduced stomatal opening, reduced leaf water loss and transcription rate with enhanced drought tolerance traits.

Heat tolerance

Under heat stress, many reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide are produced inside the plant cells, leading to various kinds of physiological disorders in plants which affect crop growth and productivity. These ROS denature enzymes and damage various cellular components inside the plant cells. Tolerance to heat stress is straightway correlated with the increased capacity of plants to scavenge ROS (Chaitanya et al. 2002). Thus, it is very important to scavenge ROS to maintain normal growth and metabolism of plants. Plants have developed a variety of mechanisms to combat ROS by the production of various enzymatic systems like superoxide dismutase (SOD) to remove superoxide ions, glutathione reductase (GR) and peroxidase to scavenge peroxide ions (H_2O_2), etc. (Noctor and Foyer 1998). Thus, over-expression of ROS scavenging enzymes in plants via genetic transformation offers a much potential strategy to overcome heat stress. Wisniewski et al. (2002) reported over-expression of cytosolic ascorbate peroxidase (*cAPX*) gene improved tolerance to heat stress in transgenic apple. Wang et al. (2006) developed transgenic tomato plants which over-expressed *cAPX* gene with enhanced tolerance to heat (40 °C), In field tests, detached fruits from field grown transgenic tomato plants showed enhanced resistance with the exposure to direct sunlight as compared to the fruits from wild-type (non-transgenic) plants. Over-expression of *Cu/Zn superoxide dismutase* (*Cu/Zn SOD*) gene (derived from *Manihot esculenta*) under an oxidative stress inducible promoter *SWPA2* in potato led to enhanced heat stress tolerance (Tang et al. 2006). *Cu/Zn SOD* is an ROS scavenging enzyme and, thus, helps in quenching of free radicals released under heat stress in plants. Transgenic plants expressed enhanced tolerance to 250 µM

methyl viologen and the visible damage due to heat stress was around 25% in the transgenic plants as compared to the non-transgenic/wild-types, which were destroyed completely under heat stress.

The non-enzymatic methods involve the production of a variety of chemical compounds including polyamines, carotenoids, ascorbic acid, tocopherol, etc., which directly react with ROS, scavenge them and thus provide protection to the plants against heat stress. Polyamines play an important role in imparting thermal stress tolerance in plants. *S-adenosyl-I-methionine decarboxylase* (*SAMDC*) is one of the key regulatory target enzymes in polyamines biosynthesis. Cheng et al. (2009) over-expressed *SAMDC* cDNA, isolated from *Saccharomyces cerevisae*, in tomato plants for enhanced polyamines production. Transgenic lines produced 1.7–2.4-fold higher levels of spermidine and spermine with enhanced antioxidant enzyme activity and better protection of membrane lipid peroxidation as compared to wild-type plants, leading to enhanced tolerance to high temperature stress (38 °C). Over-expression of heat shock proteins in plants has been proposed as one of the potential strategies to combat heat stress. HSPs function as molecular chaperons, who are involved in correct protein folding, assembly, translocation, degradation and they also provide stability to integral proteins and cell membranes under heat stress (Boston et al. 1996). Song et al. (2014) over-expressed *CgHSP70* gene conferring for heat tolerance in chrysanthemum. The transgenic lines exhibited an increased peroxidase (POD) activity, higher proline content and reduced malondialdehyde (MDA) content. Proline is an important osmoprotectant that protects cells from damage under heat stress and transgenic plants were better able to tolerate heat stress than wild-type plants.

Salinity tolerance

Salinity or salt stress is one of the most prevalent abiotic stresses that severely affect the quality and quantity of different horticultural produce. Around 20% of the world irrigated agricultural land is affected with salinity problem (Rengasamy 2006). Salinity tolerance is a complex mechanism governed by many genes (Bojorquez-Quintal et al. 2014). Plants which are exposed to abiotic stress conditions produce several pathogenesis-related proteins to compensate the adverse effect of stress conditions. *Osmotin* is one of the important pathogenesis-related proteins, which is produced by the plants to combat various biotic and abiotic stresses. Husaini and Abdin (2008) over-expressed tobacco *osmotin* gene in strawberry (*Fragaria* × *ananasa* Duch.) and found that the transgenic strawberry plants exhibited tolerance to salt stress. Chilli plants are not easily amenable to tissue culture and genetic transformation, thus limiting the scope of genetic improvement for various biotic and abiotic stresses (Kothari et al. 2010). Subramanyam et al. (2011) could successfully improve the tolerance of chilli pepper (*Capsicum annum* L. cv. Aiswarya 2103) plants by the ectopic expression of tobacco *osmotin* gene via *Agrobacterium tumefaciens*-mediated gene transfer technique. T_2 generation of transgenic pepper plants revealed enhanced levels of chlorophyll, proline, glycine betaine, ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione reductase (GR) and relative water content (RWC) in biochemical analysis and survived in salinity level up to 300 mM NaCl concentration. In comparison to other horticultural crops, citrus species are the most sensitive to soil salinity, which greatly limit growth and productivity of citrus crops across the globe. Cervera et al. (2000) transformed Carrizo citrange, an excellent rootstock of citrus with a yeast-derived halotolerance gene, *HAL 2*, which is involved in salt tolerance mechanism. *HAL2* gene is involved in the methionine biosynthetic pathway and confers tolerance to lithium and sodium ions. It encodes for a salt-sensitive *biphosphate nucleotidase*, which is required for sulfate accumulation. The transgenic lines expressing *HAL2* protein showed improved tolerance to salinity than the wild-type plants. Tomato is considered as one of the most important vegetable crops worldwide for the commercial value it offers. Wang et al. (2005) developed transgenic tomato plants expressing tolerance to chilling and salt stress by incorporation of *cytosolic ascorbate peroxidase* (cAPX) gene, derived from pea (*Pisum sativum* L.). *Ascorbate peroxidase* plays a key role in quenching hydrogen peroxide (H_2O_2) in plant cells, thus providing protection against oxidative injury induced by chilling and salt stress. The transgenic plants showed better seed germination rate (26–37%) than the wild type (3%) when the seeds were placed at 9 °C for 5 weeks. *APX* activity was found 10–25 folds higher in transgenic plants under salinity stress (200–250 mM) conditions, thus ensuring minimum damage to the leaves comparatively.

Various abiotic stresses including salinity, chilling and oxidative stresses are the critical factors limiting the cultivation and productivity of sweet potato (*Ipomoea batatas*), a root vegetable crop. It has been observed that the increased production of glycine betaine in plant cells improves their tolerance level towards these stresses. Fan et al. (2012) transformed sweet potato cv. Sushu-2 with a chloroplastic *betaine aldehyde dehydrogenase* (*SoBADH*) gene from *Spinacia oleracea*, which is involved in the biosynthesis of glycine betaine. The over-expression of *SoBADH* gene in transgenic sweet potato improved tolerance towards salinity, oxidative stress and low temperature by providing protection against cell damage by maintaining cell membrane integrity, stronger photosynthetic activity, reduced ROS production and activation of ROS scavenging mechanism. To enhance the tolerance of tomato plants to

salinity stress, Lim et al. (2016) transformed a strawberry *D-galacturonic acid reductase* (*GalUR*) gene into cherry tomato (*Solanum lycopersicum*) lines to increase the ascorbic acid content. Transgenic tomato plants enriched with high fruit ascorbic acid contents had been found more tolerant to abiotic stress induced by viologen, NaCl and mannitol as compared to the wild-type plants. The transgenic events could survive at a salt stress up to 200 mM and also showed higher expression levels of antioxidant genes including *APX* and *CAT*, responsible for imparting additional capabilities to the transgenic plants for salt tolerance. Under high salinity stress conditions, ion-homeostasis within the plant cells get disturbed altering the overall metabolism. Bulle et al. (2016) developed transgenic chilli pepper (*Capsicum annuum*) plants expressing wheat Na^+/H^+ antiporter gene (*TaNHX2*) to develop tolerance towards salinity stress. Transgene integration and expression were confirmed by PCR, Southern hybridization and RT-PCR in T_1 generation. In biochemical assays, transgenic lines gave enhanced levels of proline, chlorophyll, superoxide dismutase, ascorbate peroxidase, relative water content and reduced level of H_2O_2 and malondialdehyde as compared to the non-transformed plants under salt stress conditions. Over-expression of *TaNHX2* gene has already been evaluated in tomato to combat salinity stress (Yarra et al. 2012). To improve salt tolerance in bottle gourd, Han et al. (2015) transformed a bottle gourd line 'G5' with *Arabidopsis thaliana*-derived *H+-pyrophosphatase AVP* gene. The *AVP1*-expressing transgenic lines exhibited an improved salt tolerance and maintained higher relative water content under salt stress regime in glasshouse. When watermelon plants were grafted onto the transgenic bottle gourd root stock, they also exhibited greater salt tolerance generating higher biomass and photosystem II quantum yields.

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 found 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. Transgenic development work in various horticultural crops for imparting abiotic stress tolerance/resistance has been summarized in Table 2.

Enhancing fruit quality for increased shelf-life and reduced post-harvest losses

Excessive softening is the main factor limiting fruit shelf-life and storage. Transgenic plants modified for 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 had enhanced shelf-life for approximately 2 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 biosynthesis (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

Table 2 Transgenic horticultural crops for abiotic stress tolerance/management

S. no.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
(A) Drought tolerance						
1.	Apple	*Osmyb4; Agrobacterium tumefaciens*-mediated gene transfer	A *Myb* family transcription factor, leads to accumulation of various solutes compatible to abiotic stress tolerance	Drought and cold tolerance	Enhanced tolerance to drought and low temperature stress in transgenic plants	Pasquali et al. (2008)
		MdC1PK61; Agrobacterium tumefaciens-mediated gene transfer	Synthesizes a CBL-interacting protein kinase (*C1PK*)	Salt, drought and chilling tolerance	Enhanced tolerance to drought and low temperature and salt stress in transgenic plants	Wang et al. (2012)
2.	Banana	*MusaWRKY71; Agrobacterium tumefaciens*-mediated gene transfer	Encodes a *WRKY* transcription factor	Multiple abiotic stress tolerance	Enhanced tolerance to drought, salinity and high temperature	Shekhawat and Ganapathi (2013)
		MusaSAP1; Agrobacterium tumefaciens-mediated gene transfer	Encodes for stress associated proteins (SAPs)	Multiple abiotic stress tolerance	Enhanced tolerance to drought, salinity and high temperature	Sreedharan et al. (2012)
3.	Citrus	*P5CS* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Encodes for the biosynthesis of proline	Drought tolerance	Enhanced tolerance to drought and low temperature stress in transgenic plants	De Carvalho et al. (2013)
		P5CSF129A; Agrobacterium tumefaciens-mediated gene transfer	Provides protection against reactive oxygen species(ROS) by altering enzymes activity	Drought tolerance	Enhanced tolerance to drought and low temperature stress in transgenic plants	De Campos et al. (2011)
4.	Strawberry	*Osmotin; Agrobacterium tumefaciens*-mediated gene transfer	Increase the level of proline accumulation	Salinity tolerance	Enhanced tolerance to salinity stress in transgenic plants	Husaini and Abdin (2008)
5.	Mulberry cv. K2	*Osmotin; Agrobacterium tumefaciens*-mediated gene transfer	Increase the level of proline accumulation	Salinity and drought tolerance	Enhanced tolerance to drought and salinity stress in transgenic plants	Das et al. (2011)
6.	Chrysanthemum (*Chrysanthemum morifolium*) cv. 'Jinba'	*CmWRKY1* transcription factor (derived from *C. morifolium*); *Agrobacterium tumefaciens*-mediated gene transfer	*CmWRKY1* works as positive regulator in drought stress	Drought stress tolerance	The transgenic plants displayed increased drought tolerance in PEG stress as compared to control plants	Fan et al. (2016)
7.	Tomato (*Solanum lycopersicum* cv. Aika Craig)	*Sly-miR169c*, an miR169 family member; *Agrobacterium tumefaciens*-mediated gene transfer	Down regulates the transcripts of target genes namely; three nuclear factor Y subunit genes (*S1NF-YA1/2/3*) and one multidrug resistance-associated protein (*slMRP1*) gene	Drought tolerance	Transgenic plants exhibited reduced stomatal opening, decreased transpiration rate, lowered leaf water loss and enhanced drought tolerance	Zhang et al. (2011a, 2011b)

Table 2 continued

S. no.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
8.	Potato cv. Superior	*Cod A* gene (from *Arthrobacter globiformis); Agrobacterium tumefaciens*-mediated gene transfer	*Cod A* gene codes for glycine betaine, which scavenges oxidative stress-inducing molecule (free radicals) and it also protects the photosynthetic system in plants	Drought tolerance	The transgenic potato plants displayed a stronger antioxidant activity, higher chlorophyll content, more efficient photosynthesis and better recovery, comparatively	Cheng et al. (2013)
9.	China rose (*Rosa chinensis*)	*RcXET* and *MtDREBIC* genes; *Agrobacterium tumefaciens*-mediated gene transfer	*XET and DREBIC* genes are up-regulated in response to various abiotic stresses and imparts tolerance to the plant cells	Freezing and drought tolerance	Increased EC%, proline content, soluble sugar, photosynthesis rate, negative water potential and turgor loss point in transgenic plants leading to a better tolerance towards drought and freezing	Chen et al. (2016)
(B) Heat tolerance						
1.	Tomato (*Solanum lycopersicum*)	Yeast *SAMDC* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*SAMDC* (*S-adenosyl-methionine decarboxylase*) is one of the key enzymes involved in the biosynthesis of polyamines, which protect the plants against heat stress	Heat stress	Transgenic plants produced 1.7–2.4 times, higher level of spermidine and spermine than the wild-type plants and expressed tolerance to heat stress with enhanced antioxidant enzyme activity and protection of membrane lipid peroxidation	Cheng et al. (2009)
	Tomato cv. Zhongshu No. 5	*cAPX* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*APX* encodes for antioxidant enzyme which removes H_2O_2, a reactive oxygen species (*ROS*) produced under heat stress	UV-B and heat stress tolerance	Transgenic plants and fruits expressed enhanced tolerance to heat (40 °C) and UV-B stress as compared to wild-type plants	Wang et al. (2006)
2.	*Chrysanthemum morifolium* cv. 'Zhongshanzigui'	*CgHSP70* gene (from *C. morifolium*); *Agrobacterium tumefaciens*-mediated gene transfer	*HSPs* function as molecular chaperons, which are involved in correct protein folding, assembly, translocation, degradation and they also provide stability to integral proteins and cell membranes under heat stress	Heat tolerance	The transgenic lines exhibited an increased peroxidase (*POD*) activity, higher proline content and reduced malondialdehyde (*MDA*) content and were better able to tolerate heat stress than wild-type plants	Song et al. (2014)
3.	Potato (*Solanum tuberosum*) cv. Desiree	*StnsLTP1* gene (from potato); *Agrobacterium tumefaciens*-mediated gene transfer	*StnsLTPI* gene imparts tolerance to various abiotic stresses as a result of enhanced activation of antioxidative defense mechanisms via cyclic scavenging of reactive oxygen species (*ROS*) and co-coordinating the expression of stress related genes	Heat, drought and salinity tolerance	Transgenic potato lines displayed enhanced cell membrane integrity and an increased level of antioxidant enzyme activity with enhanced accumulation of ascorbates and upregulation of various stress related genes including *StAPX, StCAT, StSOD* etc. under stress conditions	Gangadhar et al. (2016)
	Potato	Cu/Zn *SOD* (from *Manihot esculenta*); *Agrobacterium tumefaciens*-mediated gene transfer	*ROS* scavenging enzyme and thus helps in quenching of free radicals released under heat stress in plants	Heat stress	Transgenic plant expressed enhanced tolerance to 250 µM methyl viologen and reduced visible damage due to heat stress	Tang et al. (2006)

Table 2 continued

S. no.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
(C) Salinity tolerance						
1.	Apple	*MdNHX1* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Acts as Na^+/H^+ antiporter	Salinity tolerance	Enhanced tolerance to salinity stress in transgenic plants	Li et al. (2010)
2.	Banana	*MusaDHN-I* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Over-expression of *dehydrin* gene and a *LEA* protein	Drought and salinity	Enhanced tolerance to drought and salinity stress	Shekhawat et al. (2011)
3.	Kiwi	*AtNHX1* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Keeps K^+/Na^+ ratio high during salinity stress conditions	Salinity	Enhanced tolerance to salinity stress in transgenic plants	Tian et al. (2011)
4.	Pear	*SAMDC2* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Encodes for the biosynthesis of polyamines	Salinity	Enhanced tolerance to salinity stress in transgenic plants	He et al. (2013)
	Pear	*SPDS* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Encodes for the biosynthesis of polyamines	Salinity	Enhanced tolerance to salinity stress in transgenic plants	Wen et al. (2010)
5.	Tomato (*Solanum lycopersicum*) cv. Zhongshu No. 5	*cAPX gene; Agrobacterium tumefaciens*-mediated gene transfer	*Ascorbate peroxidase* plays a key role in quenching hydrogen peroxide (H_2O_2) in plant cells, thus providing protection against oxidative injury induced by chilling and salt stress	Chilling and salinity	The transgenic plants showed better seed germination rate (26–37%) than the wild type (3%) when the seeds were placed at 9 °C for 5 weeks and higher *APX* activity under salinity stress (200–250 mM) conditions	Wang et al. (2005)
	Cherry tomato—C, H and F lines	*GalUR gene* (*D-galacturonic acid reductase gene*), derived from strawberry; *Agrobacterium tumefaciens*-mediated gene transfer	*GalUR* gene codes for higher level of ascorbic acid biosynthesis, which imparts tolerance to salinity stress	Salinity stress	Transgenic tomato plants were found more tolerant to abiotic stress-induced viologen, Nacl and mannitol and with higher expression levels of *APX* and *CAT*, responsible for imparting additional capabilities for salt tolerance	Lim et al. (2016)
	Tomato	Na^+/H^+ antiporter gene (*TaNHX2*) from wheat; *Agrobacterium tumefaciens*-mediated gene transfer	*TaNHX2* gene leads to the production of osmolytes to maintain the cell membrane stability	Salinity tolerance	Better salinity tolerance in transgenic plants comparatively	Yarra et al. (2012)
6.	Citrus (Carrizo citrange root stock)	Yeast *HAL2* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*HAL-2* gene is involved in the pathway of methionine biosynthesis and confers tolerance to Lithium and Sodium.	Salinity tolerance	Transgenic plants showed better tolerance to salt stress than non-transgenic plants	Cervera et al. (2000)

Table 2 continued

S. no.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
7.	Sweet potato (*Ipomoea batatas*) cv. Sushu-2	*SoBADH* (*Spinacia oleracea* derived *betaine aldehyde dehydrogenase* gene); *Agrobacterium tumefaciens*-mediated gene transfer	Glycine betaine protects the plant cells from abiotic stress by providing protection against cell damage by maintaining cell membrane integrity, stronger photosynthetic activity, reduced *ROS* production and activation of *ROS* scavenging mechanism	Salinity, oxidative stress and chilling/cold stress	The transgenic plants improved tolerance towards salinity, oxidative stress and low temperature	Fan et al. (2012)
8.	Chilli pepper (*Capsicum annum* L.) cv. Aiswarya 2103	*Osmotin* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Over-expression of *osmotin* gene induces biosynthesis of proline and confers tolerance to osmotic stress	Salinity tolerance	Transgenic pepper plants could survive salinity level up to 300 mM Nacl concentration	Subramanyam et al. (2011)
	Chilli pepper cv. Q4	Na^+/H^+ antiporter gene (*TaNHX2*) from wheat; *Agrobacterium tumefaciens*-mediated gene transfer	*TaNHX2* gene leads to the production of osmolytes to maintain the cell membrane stability	Salinity tolerance	Transgenic lines revealed enhanced levels of proline, chlorophyll, superoxide dismutase, ascorbate peroxidase, relative water content and reduced level of H_2O_2 and malondialdehyde as compared to the non-transformed plants under salt stress conditions	Bulle et al. (2016)
9.	China rose (*Rosa chinensis*)	*AtDREB2A-CA* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Over-expression of *AtDREB2A-CA* gene enhances salinity stress tolerance in Chinese rose by altering the leaf ultra structure in response to salt stress	Salinity stress	Enhanced salinity tolerance in transgenic plants	Josine et al. (2015)
10.	*Chrysanthemum morifolium*	*CmWRKY17* transcription factor; *Agrobacterium tumefaciens*-mediated gene transfer	*WRKY* transcription factors work sometime as positive and sometime as negative regulators in a variety of abiotic stress responses in plants	Salinity stress	Over-expression of *CmWRKY17* TF in chrysanthemum increased plants sensitivity to salinity stress	Li et al. (2015)
11.	Mulberry (*Morus indica*) cv. K-2	*HvaI* gene (from barley); *Agrobacterium tumefaciens*-mediated gene transfer	*LEA* proteins (encoded by *HvaI* gene) protect cells against abiotic stresses by sequestering ions, stabilization of macro molecules and membranes, and act as antioxidants	Salinity, drought and cold tolerance	The transgenic lines displayed an enhanced level of tolerance to drought, salinity and cold conditions than normal plants as quantified by free proline, membrane stability index (MSI) and PSII activity	Checker et al. (2012)
12.	Bottle gourd line 'G5′	*AVP1* gene derived from *Arabidopsis thaliana; Agrobacterium tumefaciens*-mediated gene transfer	*AVP1* gene is involved in imparting salt tolerance	Salinity tolerance	The transgenic lines exhibited an improved salt tolerance and maintained higher relative water content under salt stress regime in glasshouse	Han et al. (2015)
(D) Herbicide tolerance						

Table 2 continued

S. no.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
1.	*Petunia hybrida*	*EPSP synthase* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Over-production of the enzyme renders inhibition by glyphosate ineffective	Glyphosate tolerance	Transformed petunia cells as well as regenerated transgenic plants were found tolerant to glyphosate	Shah et al. (1986)
2.	Pineapple	*Bar* gene; Particle bombardment -mediated gene transfer	*Bar* gene codes for enzyme *PAT*, which acetylates bialaphos rendering it inactive	Bialaphos resistance/tolerance	Transgenic 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	Sripaoraya et al. (2006)
3.	Potato	*Bar* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*Bar* gene codes for enzyme *PAT*, which acetylates bialaphos rendering it inactive	Bialaphos resistance/tolerance	Transgenic potato expresses tolerance to bialaphos	Khan et al. (2008)

be possible to create super strawberries that taste sweeter using transgenic approaches. Blackheart is a fruit defect caused by exposure of pineapples to higher temperatures which stimulates *polyphenol oxidase* (PPO) activity. 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 has 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*) promoter and fruit-ripening specific (*E8*) promoter 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. Sajeevan et al. (2017) over-expressed *AtSHN1*, a transcription factor associated with epicuticular wax biosynthesis to increase leaf surface wax load in mulberry. It has been proposed that leaf surface waxes contribute for cuticular resistance, protect mesophyll cells from dessication and, thus, reduce post-harvest water losses. Transgenic mulberry plants expressing *AtSHN1* gene displayed dark green shiny appearance with increased leaf surface wax content and showed significant improvement in leaf moisture retention capacity even 5 h after the harvest as compared to wild-type plants.

Recombinant-DNA technology also finds its multi-faceted applications in improvement of the nutritional quality of fruits. To improve the vitamin C accumulation in fruits and make them more health supportive to the consumers, Zhang et al. (2011a, b) used *RNAi* technology to silence the expression of a mitochondrial APX gene in tomato fruit. *Ascorbate peroxidase* (APX) oxidizes the reduced L-ascorbic acid in ascorbate metabolic pathway and, thus, reduces vitamin C content. The mitochondrial *APX* enzyme activity in transgenic lines was reduced by 0.4–0.8 times than the non-transformed plants and this led to 1.4–2.2 times increase in ascorbic acid content in tomato fruits. Altering the fruit texture leading to softening of fruits is a desirable trait from the processing point of view. Kaur et al. (2010) transformed tomato cv. Pusa Uphar with a fruit-specific *expansion* gene, *LeEXP1* under the control of a fruit-specific promoter *LeACS4*. The over-expression of *LeEXP1* gene resulted in enhanced fruit softening, as determined by force required to rupture the fruit pericarp than the non-transgenic plants with increased red

coloration of fruits at different stages of ripening. This trait may find usefulness in tomato processing industry. Behboodian et al. (2012) silenced the expression of *ACC oxidase* (*ACO1*) gene in tomato using *RNAi* approach to lower down ethylene production, which is involved in regulating fruit ripening and flower senescence. The transgenic tomato lines harboring the *hpRNA-ACO1* gene exhibited lower ethylene production and a longer fruit shelf-life of 32 days as compared to 10 days for wild-type fruits. Further, fruits of transgenic tomato demonstrated reduced level of firmness loss as a result of decrease in *pectin methylesterase* (PME) and *polygalacturonase* (PG) activities.

Color enhancement and increasing vase life in various ornamental crops

Genetic engineering techniques had so far limited impact in ornamental horticulture field. However, ornamental horticulture and particularly floriculture are 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. Ornamental plants generally have eye-catching attraction and aesthetic importance. Altering flower color or plant architecture has been an important area of work for all the floriculturists. Several ornamental plants, including carnation, rose and gerbera, have been engineered for modified flower color. The primary color imparting pigments present in flowers are a wide class of flavonoids, carotenoids and betains, which are the targets of genetic engineering for color alterations/modifications. Flower color modification venture includes modifying the flavonoid biosynthetic pathway through the introduction of new genes, over-expression of certain key regulatory genes or by silencing the expression of those target genes by co-suppression strategy, antisense gene technology or by *RNAi* technique. 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 *dihydroflavonol-4 reductase* (*dfr*) gene from maize in a petunia line (Meyer et al. 1987). Chalcone synthase (*Chs*) is another gene which had 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 with the introduction of *F3′5′H* gene from *Petunia hybrida* which encodes 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 had been produced and exhibited longer vase life. Flowers of the transgenic carnation plants exhibited low climacteric ethylene production and a markedly delayed petal senescence (Savin et al. 1995).

Till date, the only genetically modified product 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 so far achieved by hybridization breeding. Worldwide, chrysanthemum is considered as one of the most economically valuable flowers. Research efforts have been focused on improving various traits in chrysanthemum including flower color and architectural variants. Chrysanthemums are typically used as cut flowers or potted plants. Obtaining bright red and blue colored flowers has always been the charm of chrysanthemum growers. In an attempt to develop red colored flowers, He et al. (2013) down-regulated *CmF3′H* gene using *RNAi* approach and over-expressed the *Senecio cruentus F3′5′H* (*PCFH*) gene in chrysanthemum. The transgenic chrysanthemum plants developed bright red flowers and exhibited a significantly increased cyanidin content.

Modification of plant architecture

Genetic engineering has succeeded in relevance to modifying plant architecture to satisfy the instinct of beauty of mankind. Ornamental plants are being grown for the purpose

of beautifying, embellishing or improving human environments. Differential expression of a number of diverse genes in different backgrounds has led to diversification in plant forms. Reduction in height is a major consideration during commercial production of chrysanthemum. In an attempt to reduce plant height, Zheng et al. (2001) ectopically expressed a tobacco *phytochrome B1* (*PHY-B1*) gene in 'Iridon' chrysanthemum under the control of *CaMV35S* promoter. The transgenic plants developed a short stature as compared to the wild-type plants looking similar to that caused by the application of a commercial growth retardant. The leaves of transgenic plants developed more intense color inferring the biosynthesis of higher levels of chlorophyll pigment. Later in 2003, Petty et al. reported decrease in chrysanthemum plant height by introducing *Arabidopsis GA insensitive* (*gai*) gene. In another study, Aida et al. (2008) transferred *CAG* gene in antisense orientation into *Chrysanthemum morifolium* and observed alteration in gynoecium and androecium to corolla-like tissues. Khodakovskaya et al. (2009) developed transgenic chrysanthemum by transforming *ipt* (*isopentyl transferase*) gene, a cytokinin biosynthetic gene, in an attempt to change the plant architecture. The transgenic plants were found shorter in height with more number of flowers, but with smaller size and late flowering period than the wild-type plants. In another study, Jiang et al. (2010) reported lessened branches formation in transgenic chrysanthemum transformed with *DgLsL* gene in antisense orientation. Dwarfed transgenic plants of petunia had also been developed by transforming *gai* (gibberellic acid insensitive) gene from *Arabidopsis thaliana* by Tanaka et al. (2005). Further, Han et al. (2007) reported that *Ls*-like sense gene expression in transgenic carnation resulted in lack of axillary branches formation. Cultivars of many crops having shorter stems generally exhibit higher harvest index, offering additional commercial advantage to the growers. In another study, the ectopic expression of a *PHYA* gene in potato plants significantly inhibited stem elongation and increased the harvest index through hypersensitivity to far red (FR) light (Robson et al. 1996). The potential applications of transgenic technology for improving quality, color, texture, shelf-life and plant architecture of horticultural crops have been compiled in Table 3.

Though at present, transgenic crops are being cultivated over an acreage of 179.7 m ha by 70–80 million farmers in 28 countries across the globe (ISAAA, 2017), still people have many more apprehensions in their minds regarding biosafety, health and environmental risks posed by the consumption and commercialization of genetically modified crops. This has led to the development of new technologies to address such concerns, referred to as marker-free (Clean-gene) transgenic technology and genome editing technology.

Marker-free transgenic technology

Generally, the methods of genetic transformation employ selection markers such as antibiotic resistance genes or herbicide tolerance genes for the selection of desirable transgene expression in transformed cells (Bevan et al. 1983; Akama et al. 1995). However, except for the role as a selectable marker, these genes do not have any relevant function inside the plant cell and, thus, they exert an extra burden on the plant genome. Also, the constitutive expression of these genes encoded proteins affects the plant metabolism in a negative way. Further, use of marker genes, particularly those coding for antibiotic resistance, has been facing a strong criticism and opposition, particularly in edible crops including fruits and vegetables. Developing marker-free plants or finding out suitable alternatives of antibiotic or herbicide tolerance genes has been proposed with the hope of increasing consumers' acceptance for genetically modified crops. A set of new technologies has been developed which involve the elimination of marker genes during transgenic plants development, which come under the recent molecular advent as 'Marker-free transgenic technology' or 'Clean-gene technology'. Such technologies would be helpful to minimize biosafety concerns during biosafety research trials and the transgenic products would fetch wider consumer acceptance. Site-specific recombination has been suggested by many workers as a potential strategy (Dale and David 1991; Gleave et al. 1999; Puchta 2000). Hare and Chua (2002) proposed chemically inducible site-specific recombination systems as valuable tools for excision of transgenes when their expressions are not required. De Vetten et al. (2003) suggested the use of marker-free gene construct for genetic transformation of potato followed by polymerase chain reaction (PCR)-based selection of transformed cells for identification of transformants. Co-transformation with two gene constructs followed by segregation of marker gene and gene of interest in segregating generation has been explored. Sun et al. (2009) devised a strategy to eliminate public concerns regarding proliferation of antibiotic and herbicide resistance genes into the environment by constructing a super binary vector having two T-DNA systems to generate marker-free transgenic chrysanthemum plants. The vector system was designed having two T-DNA regions—one having *hygromycin phosphotransferase* (*hpt*) selectable marker gene and the other T-DNA containing *β-glucuronidase* (*uidA*) gene, placed adjacent to each other with no intervening region. A total of 17 *hpt*-resistant/*gus* positive T_o chrysanthemum plants were evaluated for segregation in T_1 generation and among

Table 3 Transgenic horticultural crops for improvement of other desirable traits

S. No.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
(A) Modification of fruit quality, increasing shelf-life and reducing post-harvest losses						
1.	Tomato	*Polygalacturonase* gene in antisense orientation; *Agrobacterium tumefaciens*-mediated gene transfer	*PG* gene encodes for polygalacturonase enzyme which degrades pectin, the major component of fruit cell wall, its antisense expression inhibits that activity	To increase shelf-life of tomato fruit	The fruits of transgenic tomato got shelf for approx. 2 weeks longer without softening and developed improved flavor and total soluble solids (TSS)	Smith et al. (1988)
	Tomato	*Arabidopsis thaliana* H^+/cation exchanger (*CAX*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	Increased expression of *CAX* gene in plants causes dramatic increase in Ca^{2+} ions leading to increased shelf-life	Prolonged shelf-life	Fruit from transgenic tomato plants had more calcium (Ca^{2+}) and prolonged shelf-life when compared to controls	Park et al. (2005)
	Tomato	*Yeast spermidine synthase* (*ySpdSyn*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	Polyamines, particularly *Spd* aids in increasing fruit shelf-life probably by reducing post-harvest senescence and decay	Increased shelf-life	The *ySpdSyn* transgenic fruits had a longer shelf-life, reduced shriveling and delayed decay symptom development in comparison to the wild-type	Nambeesan et al. (2010)
	Tomato	*RNAi* based silencing of mitochondrial *APX* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Ascorbate peroxidase (*APX*) oxidizes the reduced L-ascorbic acid in ascorbate metabolic pathway and thus reduces vitamin C content	Increased vitamin content	The mitochondrial *APX* enzyme activity in transgenic lines was reduced by 0.4–0.8 times than the non-transformed plants and this led to 1.4–2.2 times increase in ascorbic acid content in tomato fruits	Zhang et al. (2011a, b)
	Tomato cv. Pusa Uphar	*LeEXP1* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Over-expression of *LeEXP1* gene influence fruit texture and juice viscosity in tomato and thus assists in fruit softening	Enhanced fruit softening	The over-expression of *LeEXP1* gene resulted in enhanced fruit softening. This trait may find usefulness in tomato processing industry	Kaur et al. (2010)
	Tomato	*RNAi*-mediated suppression of *DET1* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*DETI* is a phytomorphogenesis regulatory gene	To improve carotenoid and flavonoid content in fruits	Enhanced carotenoid and flavonoid content in transgenic tomato fruits	Davuluri et al. (2005)
	Tomato cv. MI1	*RNAi* gene construct of *ACO1* gene; *Agrobacterium tumefaciens*-mediated gene transfer	To inhibit ethylene production	To increase shelf-life	The transgenic tomato lines exhibited lower ethylene production and a longer fruit shelf-life of 32 days as compared to 10 days for wild-type fruits	Behboodian et al. (2012)
	Tomato cv. Micro-Tom and Ailsa Craig	*SlIAA-9* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Genome editing to bring targeted mutagenesis	To introduce parthenocarpy	T_o mutants showed morphological changes in leaf shape and seedless fruit, which is a characteristic of parthenocarpic tomato	Ueta et al. (2017)
2.	Watermelon (*Citrullus lanatus*)	*CIPDS* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Genome editing to bring targeted mutagenesis in *CIPDS* gene by CRISPR/Cas9	To develop *CIPDS* gene knockouts for albino phenotype	All the transgenic watermelon plants harbored *CIPDS* mutations and developed clear or mosaic albino phenotype	Tian et al. (2017)
(B) Color enhancement and increasing vase life in various ornamental crops						
1.	Petunia	*acc* and *aco* genes in antisense orientation; *Agrobacterium tumefaciens*-mediated gene transfer	Inhibition of ethylene	Delayed leaf senescence	Delayed leaf senescence in transgenic petunia than wild-type	Clark et al. (2003)
	Petunia	*dfr* gene from maize; *Agrobacterium tumefaciens*-mediated gene transfer	*dfr* gene from maize is capable of synthesizing pelargonidin pigment, thus giving brick red colored flowers	Color modification	Transgenic orange pelargonidin-producing Petunia variety, which produced flowers with pale brick color	Meyer et al. (1987)

Table 3 continued

S. No.	Crop species/ cultivar	Gene and genetic transformation method used	Mechanism of action	Target trait	Trait improvement	References
2.	Petunia, Carnation, Gerbera and Rose	*chs* gene in sense and antisense orientation; *Agrobacterium tumefaciens*-mediated gene transfer	Alteration in flavonoid biosynthetic pathway	Color modification	Development of pink, white and variegated flowers in transgenic petunia, chrysanthemum, gerbera and rose	van der Krol et al. (1988)
3.	Carnation	*F3′5′H* gene from *Petunia hybrida; Agrobacterium tumefaciens*-mediated gene transfer	*F3′5′H* gene encodes a flavonoid required for the biosynthesis of delphinidin	Color modification	Transgenic violet carnations	Holton et al. (1993)
	Carnation	Antisense *aco* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*aco* gene is involved in ethylene biosynthesis	Increased vase life	Flowers of the transgenic plants exhibited low climacteric ethylene production and a markedly delayed petal senescence	Savin et al. (1995)
4.	Red rose	A package of 3 gene viz, *DFR* gene in *RNAi* vector, *delphinidin* gene from blue pansy and a *DFR* gene from iris; *Agrobacterium tumefaciens*-mediated gene transfer	Synthetic *RNAi* gene switches off the red rose dihydroflavonol reductase (*DFR*) gene, *delphinidin* gene and *DFR* gene have an affinity for producing delphinidin	Modified flower color	Transgenic roses exclusively accumulated delphinidin in the petals, and the flowers had blue hues	Katsumoto et al. (2007)
5.	*Chrysanthemum morifolium*	*RNAi* suppression of *CmF3′H* gene and over-expression of *Senecio cruentus F3′5′H* gene; *Agrobacterium tumefaciens*-mediated gene transfer	Delphinidin biosynthetic pathway modification leading to increased synthesis of cyanidin and thus flower color modification	Flower color modification	The transgenic chrysanthemum plants developed bright red flowers and exhibited a significantly increased cyanidin content	He et al. (2013)
(C) Modification of plant architecture						
1.	Chrysanthemum	*ipt* gene; *Agrobacterium tumefaciens*-mediated gene transfer	*ipt* (*isopentyl transferase*) gene is a cytokinin biosynthetic gene	Modified plant architecture	The transgenic plants were found shorter in height with more number of flowers, but with smaller size and late flowering period than the wild-type plants	Khodakovskaya et al. (2009)
	Chrysanthemum	*gai* gene from *Arabidopsis thaliana; Agrobacterium tumefaciens*-mediated gene transfer	*gai* gene inhibits the effect of gibberellic acid in plants and thus results in dwarfism	Modified plant architecture	Decrease in transgenic chrysanthemum plant height	Petty et al. (2003)
	Chrysanthemum cv. Iridon	Tobacco *phytochrome B1* (*PHYB1*) gene; *Agrobacterium tumefaciens*-mediated gene transfer	The ectopic expression of the tobacco *PHYB1* gene increases the sensitivity of plants to red wavelengths of light, resulting in the inhibition of stem elongation	Reduction in plant height	The transgenic plants developed a short stature as compared to the wild-type plants	Zheng et al. (2001)
2.	Petunia	*gai* gene from *Arabidopsis thaliana; Agrobacterium tumefaciens*-mediated gene transfer	*gai* gene inhibits the effect of gibberellic acid in plants and thus results in dwarfism	Modified plant architecture	Decrease in transgenic petunia plant height	Tanaka et al. (2005)
3.	Potato	*PHYA* gene; *Agrobacterium tumefaciens*-mediated gene transfer	The ectopic expression of *PHYA* gene increases the sensitivity of plants to far-red wavelengths of light, resulting in the inhibition of stem elongation	Reduction in plant height	Dwarfism in transgenic potato plants with increased harvest index through hypersensitivity to far red (FR) light	Robson et al. (1996)

those, approximately 15.7% carried the transgene. Suitability of a twin T-DNA system for generating marker-free transgenic plants has been established.

For the development of selectable marker-free transgenic plants, Multi-Auto-Transformation (MAT) vector system (Ebinuma et al. 1997), which combines positive selection using *isopentenyl transferase* (*ipt*) gene, the first enzyme of cyokinin biosynthesis, with a site-specific recombination (Barry et al. 1984) and removal system offers a very potential tool (Sugita et al. 1999). Marker gene is removed from the transformed cells by the mechanism of homologous recombination. The MAT vector backbone is composed of yeast site-specific recombination R/RS system to excise the DNA fragment and the *ipt* gene cloned between two directly oriented recombination sites (Araki et al. 1987). The MAT vector system has been employed in a number of crops to develop marker-free transgenic plants including *Antirrhinum majus* (Minlong et al. 2000), citrus (Ballester et al. 2007), *Kalanchoe blossfeldiana* (Thirukkumaran et al. 2009) and *Petunia hybrida* (Khan et al. 2010). Khan et al. (2011b) used MAT vector in which *ipt* gene was used as a selection marker and *Wasabi defensin* (WD) gene, isolated from *Wasabia japonica* as a target gene, to transform tomato plants. The marker-free transgenic tomato plants exhibited enhanced resistance against a number of fungi including *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Erysiphe lycopersici*. Also, *phosphomannose isomerase* (PMI) gene derived from *E. coli* had been developed as an efficient positive selection marker for apple transformation, which induced the capability to grow on mannose supplemented medium in the transformed cells (Degenhardt and Szankowski 2006; Degenhardt et al. 2007). Further, plastid engineering has also been advocated as one of the most viable techniques to avoid transgene spread to other related crop (non-target species).

Cisgenic crops represent a step towards a new generation of genetically modified crops. Development of genetically modified crops, which do not possess any selectable marker (e.g., antibiotic resistance or herbicide tolerance) gene in the end product and also, if the inserted gene is derived from the same organism/plant, would be a welcome step to increase consumers' acceptance for that product and to minimize the environmental risks associated with genetically modified crops. In this direction, Vanblaere et al. (2011) developed cisgenic apple plants by inserting the endogenous scab resistance gene *HcrVf2* under the control of its own regulatory sequences into the scab susceptible apple cultivar 'Gala' using R/RS vector system to develop marker-free transgenic plants. Dhekney et al. (2011) also used cisgenic approach to develop disease-resistant apple.

Genome editing technology in horticultural crop improvement

Recent technology relies on certain engineered endonucleases (EEN) that cleave DNA in sequence-specific manner due to the presence of a sequence-specific DNA-binding domain. These *endonucleases* recognize specific DNA sequence and, thus, efficiently and precisely cleave the target genes. The double-strands breaks (DSBs) of DNA result in cellular DNA repair mechanisms, including homology-directed repair (HDR) and non-homologous end joining breaks (NHEJ), leading to gene modification at the target sites in the genome of plants. Generally, this technology employs three types of engineered endonucleases viz. Zinc finger nucleases (ZFNs), TALENs and CRISPR/CaS for site-specific cleavage. The CRISPR/Cas9 system has originated from bacteria and archae (Wiedenheft et al. 2012). CRIPSR/Cas9 is comparatively easy to prepare, affordable and can be better upscaled than ZFNs and TALENs. Cas9-induced double strand breaks in the plant genome are repaired by non-homologous end joining (NHEJ) method (Li et al. 2013). During this repair, small insertions or deletions may occur disturbing the open reading frame of a protein or introduces a stop codon (Belhaj et al. 2013). Zinc finger nucleases (ZFNs) and transcription activator-like effector (TALENs) technologies of genome editing affected from the disadvantages of high technical complexity and low efficiency. On the other hand, the clustered regularly interspersed short palindromic repeats (CRISPR)-associated protein 9 (CRISPR/Cas9) technology has revolutionized genome editing by overcoming the disadvantages of ZFNs and TALENs due to a high efficiency, low cost involvement, simplicity and versatility (Cardi and Stewart 2016). CRIPSR/Cas9 genome editing technology will probably avoid the current GM regulations mechanisms as the Cas9 protein-guide RNA complexes get rapidly decomposed in the regenerating cell cultures and thus will broaden the utility of this technology with greater global acceptance levels in comparison to the transgenic technology. A gene-edited crop does not necessarily contain any transgene and, thus, does not require very stringent regulation and thus such crops may find quick acceptance among consumers (Jones 2015). Precise genome editing offers a wonderful technology to decipher plant gene functions and in improvement of crop plants. CRISPR/Cas9 editing tools have been efficiently applied in a number of horticultural crops including tomato, petunia, citrus, grape, potato and apple for gene mutation, repression, activation and epigenome editing (Nishitani et al. 2016; Ren et al. 2016; Song et al. 2016).

Genome editing in plants has been revolutionized with the development of CRISPR/Cas9 technology. Recently, this technology has found application in developing resistance

against many viruses (Ali et al. 2015; Baltes et al. 2015). Chandrasekaran et al. (2016) could successfully develop virus resistance in cucumber using Cas9/subgenomic RNA (sgRNA technology) to disrupt the function of recessive *eIF4E* (eukaryotic translation initiation factor 4E) gene. Homozygous T_3 transgenic cucumber plant which was targeted to both eIF4E sites developed immunity to cucumber vein yellowing virus (Ipomovirus) infection and resistance to the potyviruses—Zucchini yellow mosaic virus and Papaya ring spot mosaic virus-w. In a recent study, Malnoy et al. (2016) reported the direct delivery of purified CRISPR/Cas9 ribonucleoproteins (RNPs) targeting to bring mutagenesis in *MLO-7*, a susceptible gene in order to increase resistance to powdery mildew in grape cultivar Chardonnay and *DIPM-1*, *DIPM-2* and *DIPM-4* genes in apple cultivar Golden delicious to increase resistance to fire blight disease. Tian et al. (2017) demonstrated the usefulness of genome editing technology, CRIPSR/Cas9—as a powerful tool to effectively create knockout mutations in watermelon. They targeted *CIPDS* (*phytoene desaturase*) gene for mutagenesis for developing albino phenotype. All the transgenic watermelon plants harbored *CIPDS* mutations and developed clear or mosaic albino phenotype, indicating that CRIPSR/Cas9 system has 100% genome editing efficiency in transgenic watermelon lines to introduce new functions. Induction of parthenocarpy has always been desired in horticultural crop plants for various industrial purposes and for eating quality. Ueta et al. (2017) demonstrated a CRISPR/Cas9 system-based breeding strategy to generate parthenocarpic tomato plants. Using CRISPR/Cas9 system, they could effectively introduce 100% somatic mutations into *SlIAA9*—a key gene controlling parthenocarpy in T_o tomato plants. Regenerated T_o mutants showed morphological changes in leaf shape and seedless fruit, which is a characteristic of parthenocarpic tomato. In a very recent study, Kishi-Kaboshi et al. (2017) reported for the first time gene editing in chrysanthemum using CRISPR/Cas9 system and developed transgenic chrysanthemum plants expressing the yellowish-green fluorescent protein (*CpYGFP*) gene from *Chiridius poppei*. Two sgRNAs were selected to target different positions in the *CpYGFP* gene and they obtained transgenic calli containing mutated *CpYGFP* genes (CRISPR-*CpYGFP*-chrysanthemum). Finally, the CRISPR-*CpYGFP*-chrysanthemum shoot containing a mutation in the *CpYGFP* gene was obtained.

Conclusion

The applications of recombinant-DNA technology or genetic engineering in crop improvement are immense to solve the problem of global hunger as population is increasing day by day with depriving sustainable intensification. However, horticultural crops have got less attention in this area so far. In contrast to the increasing global adoption of biotech field crops, biotechnology has limited commercial success to date in horticultural crops including fruits, vegetables, flowers and landscape plants. 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. Transgenic technology provides a potential technique for genetic enhancement using desirable trait of interest in plants. There is a need to address various regulatory obstacles for commercial release of various transgenic crops so that the real benefit of this wonderful technology may reach the consumers, the end users. After the advent of Next Generation Sequencing (NGS) technologies, many horticultural crops including strawberry, papaya, grapevine, sweet orange, mango, etc. have been sequenced, which has now solved the problem of lack of genomic information and thus facilitated the target gene/site to be modified using genome editing technology. This has also improved the breeding efficiency as various genes/QTLs coding for various horticulturally important traits have been identified. In addition to that, transcriptome sequences of a number of horticultural crops are now available in public databases. This vast information will assist in identifying various genes governing various important traits and will help in identifying the target sites for genome editing and genetic transformation.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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