Transgenics in ornamental crops: creating novelties in economically important cut flowers

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Development of transgenics is the need of the modern era of plant breeding, as they possess the potential to incorporate those characters in crop varieties which are either difficult or impossible through conventional breeding approaches. In case of ornamental crops, the progress made in transgenic breeding is not that impressive like in cereals, pulses and vegetables, but the initiatives taken and advancements made have implicated the bright future of this technology in ornamental crops. Improved morphology, flower colour, resistance and fragrance are some of the desired novel traits in ornamental crops where transgenic approaches need to intervene. Transgenic breeding in major cut-flower crops like rose, chrysanthemum, gladiolus and carnation has provided avenues for incorporation of novel traits in other ornamental crops as well and has made such crops an ideal target for application of other advanced technologies.

Keywords: Cut flowers, ornamental crops, novel traits, transgenics.

ORNAMENTAL plants represent an important sector of horticulture industry, and play a fundamental part in human life because of their aesthetic and economic importance. Cultivation of flower crops has been considered as a lucrative and income-generating venture. This sector plays a major role in economic strengthening of several poor African countries, as flower crops are flourishing as major income-providing commodities in Costa Rica, Colombia, Kenya, Ethiopia and Eucador[1]. This huge industry comprises cut flowers, loose flowers, pot plants, flowering and foliage, ornamental grasses, trees, shrubs, annuals and other plants of ornamental value, which together fulfil the aesthetic needs of humans and also form an integral part of ecological sustenance. For the continuous development of ornamental plant industry, novelty is the major driving force as it thrives on consumers' preference, which is always for something new, whether it is flower colour, flower form, fragrance or a new creation flower crop. Moreover, due to continuous quest for something new in ornamentals, the industry has become more competitive, which requires improvement in existing products in innovative and refined ways with strengthened research and development.

F_1 hybrid breeding has been a popular method in many flowers crops and breeders have developed promising hybrids in ornamental crops, including cut foliage plants and lawn grasses. However, through the continuous adoption of conventional breeding strategies in improvement programmes, the available gene pool for incorporation of new traits is becoming narrower, which limits their use in further improvement programmes. Particularly in some sterile flower crops such as orchids, hybridization or other conventional methods are not an option to create novelty and in some flowers, mutations and hybrid breeding are quite lengthy or difficult to develop new varieties[2]. In such cases, alternatives need to be searched for variety improvement. Many floricultural crops are vegetatively propagated and are highly heterozygous, which causes a complex inheritance of genetic factors as well as the polyploidy, making it difficult to improve through conventional breeding[3]. Moreover, it is either difficult or impossible to find the gene of interest in natural gene pool; it contains unfavourable genes along with the favourable ones, making it unrealistic to rely upon classical breeding tools like hybridization and selection for improvement of ornamental crops where we need a specific change or gene for a specific trait. Also, in ornamental crops the sources of resistance for pests and diseases are limiting and there is a need to transfer the resistant genes from external sources like other species, genera or from unrelated organisms, which is not possible through conventional breeding[4]. The present constraints of classical breeding specifically pertaining to ornamental crops necessitate the search for alternative breeding strategies which can be applied suitably for creation of novelty. Transgenic breeding which has created wonders in many agricultural crops like maize, soybean, canola and cotton through incorporation of herbicides and insect resistance, has much to offer in resolving the constraints associated with classical breeding approaches in ornamental crops. With transgenic breeding novel genes like those encoding for resistance (against harmful diseases and pests), unique colours, and peculiar forms can be incorporated into the plants with precision and without much alteration of exiting elite genotype. Primary advantages are its precision and improvement of a trait without altering the genetic

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constitution of an elite genotype. Ornamental crops are ideal candidates for molecular breeding for the production of novel transgenics, as they serve an aesthetic purpose and may be more acceptable to the public, unlike the other genetically modified crops having food value where public concerns are more restrictive regarding their adoption. However, many public and private research organizations are in favour of development of transgenic crop varieties due to expected benefits in terms of increased profitability and ecological stability[5], which further provides an avenue for novel improvements in ornamental crops where efforts are still underway.

Scope of the review

Among the different categories of ornamental crops, maximum interest has always been towards cut flowers and in terms of their sales value, the dominant players in the international flower markets are rose, tulip, chrysanthemum, carnation, gerbera, lily and orchids[6], whereas under Indian conditions, gladiolus makes significant contributions for fulfiling the demand in local markets. According to Casanova[7], a prominent place as cut flowers worldwide has been occupied by rose, carnation, chrysanthemum and gladiolus. Keeping in view the importance of these cut-flower crops in the international market, this article covers the transgenic research undertaken in rose, chrysanthemum, gladiolus and carnation for quality improvement and incorporation of novel traits, which also present potential opportunities for other ornamental crops as well.

Protocols for transgenic development in ornamental crops

Genetic manipulation or transgenic breeding possesses great potential in the breeding of novel ornamental plants for changing market standards and can only be achieved with the development of an efficient protocol for transformation. Although the choice of method for introducing exogenous genes is determined according to the plant species, methods based on *Agrobacterium tumefaciens* are the most simple and efficient. However, this method of transformation is practically limited to dicots as majority of the monocots are resistant to infection by *Agrobacterium* species. The genetic manipulation of monocots is based upon the direct transfer of genes through micro bombardment and electroporation, as done in transgenic corn, cane sugar and rice[8]. The challenges associated with transformation of ornamental plants are the same as those faced in any plant species such as resistance to infection of monocotyledons ornamental crops by *Agrobacterium* species, transformation efficiency and difficulties associated with the regeneration of plant tissue[9]. However, majority of transformation protocols used in ornamental species are based on gene transfer mediated by *Agrobacterium* and particle bombardment (biolistic process)[10]. Any transformation method ultimately thrives upon the ability of plant tissue to regenerate into a complete viable plant with stable expression of the introduced gene. With the successful transformation of petunia in 1987, ornamental crops came into limelight for genetic transformation research and many species were transformed successfully, including commercial flower crops like chrysanthemum, gladiolus, rose, iris, gerbera, poinsettia, etc. Till date, 30 ornamental species have been transformed[7]; in this study we cover only the major cut flower crops – rose, chrysanthemum, gladiolus and carnation due to limited scope of this review.

Rose (*Rosa hybrida*)

In *Rosa hybrida* plant architecture, thorns (number and shape), onset of senescence, floral scent, and biotic and abiotic stress resistance are a few peculiar characters which are economically important according to the export market standards. For improvement of these traits in existing rose cultivars and species, genetic transformation is an important tool as it is well suited for specific improvement of individual traits without losing the existing rose characters[11]. Working on the transformation protocols, Firoozabady *et al.*[12] first reported transformation based upon infection by *A. tumefaciens* in hybrid tea rose cultivar Royality. Later, many researchers have reported this method as useful for rose transformation – based upon co-cultivation of friable embryogenic tissues with *A. tumefaciens* or *A. rhizogenes* for genes controlling flower colour[13]; based on PEG-mediation using *GFP* as reporter gene[14]; to enhance flower yield, resistance and essential oil[15]; using embryogenic calluses and green florescent protein 5, *gfp* gene in rose cultivar 'Tineke'[16] and using somatic embryos in *Rosa chinensis* cv. Old Bush[11]. According to Merchant[17], electrofusion was effective for the production of rose heterokaryons from protoplasts of four English rose cultivars, with a maximum fusion frequency of 1.62%. Later, Merchant *et al.*[18] demonstrated biolistic transformation in rose cv. Glad Tidings using embryogenic callus for regeneration of transgenic plants. Rose is highly susceptible to black spot which spreads under warm, humid conditions and has been considered as most widespread and devastating disease in rose. Other diseases of economic importance are powdery mildew, cercospora leaf spot and grey mould[19]. Black spot susceptible *Rosa hybrida* L. cv. Glad Tidings was transformed with a rice gene encoding a basic chitinase, which upon expression effectively reduced disease development in transgenic plants[20]. Later, resistance to black spot in susceptible cultivars was incorporated through somatic hybridization with resistant wild rose cultivars using their non-embryogenic cell suspension cultures[14]. Partial

resistance to important rose diseases like black spot, powdery mildew, downy mildew and rust was incorporated in cultivars Heckenzauber and Pariser Charme with genes that encode for antifungal proteins (class II chitinase, class II β-1,3-glucanase, type I ribosome inhibiting protein) and antibacterial T4-lysozyme gene through *Agrobacterium*-mediated gene transfer and somatic embryogenic regeneration[21]. Enhanced resistance to powdery mildew was observed by Li *et al.*[22] in transgenic lines of *Rosa hybrida* cv. Carefree Beauty, which harbours *Ace-AMP1* gene (an antimicrobial protein gene). Out of 500 putative transgenic plants, 62% was found positive for the transgenes *Ace-AMP1* and *nptII* (neomycin phosphotransferase). Chen *et al.*[23] developed transgenic lines with enhanced freezing tolerance in China rose with the successful introduction of *MtDREB1C* gene, isolated form *Medicago trancatula*, without any abnormalities in existing plant characters. For alteration of plant morphology, studies[24,25] advocated the role of *Rosa hybrida rolA*, *rolB* and *rolC* genes, where insertion of *rolC* gene led to the production of dwarf plants in roses with small-sized, less fertile flowers of varying colours and numerous thorns, whereas insertion of *rolA* + *B* + *C* genes enhanced the rooting in cuttings with accompanying effects like reduced shoot length and apical dominance. Ito *et al.*[26] demonstrated that *Apple latent spherical virus* (ALSV) vector infects roses without adversely affecting plant health and could be important for endogenous gene silencing in rose. This virus-induced gene silencing (VIGS)-inducing system in rose would be further helpful in functional validation of genes governing flower morphology, presence of thorns and other important horticultural traits.

Since rose species lack delphinidin-based anthocyanins, development of blue or violet coloured varieties through classical breeding was an impossible task for the breeders. Breaking this barrier, Katsumoto *et al.*[27] developed the first true blue roses, i.e. with increased delphinidin by down regulation of endogenous dihydroflavanol reductase gene (*DFR*) via RNAi-mediated silencing and overexpressing the *Iris* × *hollandica DFR* gene, viola *F3′5′H* gene in rose cultivars having higher vacuolar pH, large amount of flavonols (co-pigments) with weak or no F3′H activity. *A. tumefaciens* containing pSPB130 transformation was carried out in embryogenic calluses of selected cultivars and the functioning of the introduced *F3′5′H* gene in transgenic roses was confirmed by the production of delphinidin and myricetin.

Ethylene resistance is the desired trait for better post-harvest longevity of cut roses. Specific upregulation of *ipt* gene under conditions favouring senescence in roses transformed with fusion gene, *PSAG12-ipt* resulted in better post-harvest life as transgenic plants showed resistance to early leaf senescence and ethylene production[28]. Progressing towards the advancement in transgenic breeding, several novel technologies like next-generation sequencing methods (NGS), parallel detection of SNPs via chip technologies, targeted mutagenesis via designer nucleases and related methods have boosted research in many model as well as non-model plants[19], and have also provided possible avenues for advancement in rose breeding. In roses, NGS was applied to isolate and characterize the *Rdr1* gene governing black spot resistance. After a contig of overlapping BAC clones from a *R. multiflora* hybrid spanning the *Rdr1* locus had been established, 454 sequencing of four overlapping bacterial artificial chromosomes (BACs) revealed the sequence of nine *TIR–NBS–LRR* genes within a region of less than 200 kb. One of these genes was characterized as *Rdr1*[29,30]. SNP analysis in cut and garden roses revealed more than 60,000 SNPs that are currently used to genotype biparental tetraploid rose populations as well as association panels for resistance-related quantitative trait loci (QTLs)[31].

Chrysanthemum (Dendranthema grandiflora)

The prime objectives in chrysanthemum breeding, viz. resistance to biotic and abiotic stresses, leaf shape or architecture, longer post-harvest life and novel flower colours[32,33] need to be accomplished through transgenic technologies. Many researchers have developed efficient transformation protocols for the introduction of novel genes attributed to desired traits in florist chrysanthemum among these *Agrobacterium*-mediated transformation has been suggested by many workers[34–37], whereas biolistic-mediated transformation is not frequently used in chrysanthemum as direct induction of shoots or formation of callus is often difficult from the cells into which foreign genes have been introduced via particle bombardment[38]. Annadana *et al.*[34] suggested that *cauliflower mosaic virus* (CaMV)-based promoters are not preferred for transgenic breeding of chrysanthemum, especially where high levels of transgene expression are desired, as *Lhca3.St.1* promoter was found more active than dCaMV promoter on quantitative evaluation of GUS activity in 127 transformants, contrary to earlier studies where use of various cauliflower mosaic 35S (CaMV) promoter variants has been suggested to drive the transgene in chrysanthemum[39–41]. Aida *et al.*[42] also suggested the use of Tobacco *EF1* α promoter as a substitute for 35S promoter of CaMV in chrysanthemum for enhanced transgene expression. Plants with unique morphology like dwarfism and altered branching patterns occupy a prominent place in chrysanthemum breeding programmes and many such desired morphological alterations in chrysanthemum have been achieved through transgenic breeding. Lee *et al.*[35] generated transgenic plants using *A. tumefaciens* C58C1 that showed significantly lower lateral branching than nontransgenic plants (43% versus 5% nodes without axillary buds) following transformation with the *LeLs* (late

embryonic, lateral suppressor) antisense gene, providing a practical way to manipulate plant architecture. Huh *et al.*[36] confirmed the role of *Ls* genes in axillary meristem initiation. They obtained five transgenic 'Jinba' plants using *A. tumefaciens* C58C1 carrying antisense *Ls* cDNA which showed decreased axillary branching, although this was strongly dependent on the season (highest percentage of viable axillary buds when planted in April, followed by planting in August and then June), and changes in floral structure were also shown by selected transformants. Insertion of *35S-rolC* gene of *Agrobacterium rhizogenes* in *Chrysanthemum morifolium* led to the induction of dwarfism in chrysanthemum plants with altered plant morphology[43]. Susceptibility to viral, fungal and bacterial diseases is the main constraint that several researchers have tried to combat through transgenic approaches. Sherman *et al.*[44] generated three spotted wilt virus (TSWV)-resistant transgenic lines in chryasnthemum cv. Polaris through *Agrobacterium*-mediated transformation employing nucleocapsid (*N*) gene constructs (containing either a full-length *N* gene (pTSWVN+), a full-length *N* gene encoding a truncated N protein (pTSWVNt), or an antisense version of the full-length *N* gene (pTSWVN–), all derived from a dahlia isolate of TSWV (TSWV-D)). Highly TSWV-resistant pTSWVNt line had no detectable levels of N protein, and all three resistant lines had low levels of *N* gene transcript and at least three transgene insertion sites within their genomes, which were confirmed by molecular analysis. This was the first time a major ornamental crop had been genetically engineered for disease resistance. Mitiouchkina *et al.*[45] also attempted *Agrobacterium*-mediated transformation of *C. morifolium* 'White Snowdon', for the introduction of single and double copies of the gene encoding for the virus B coat protein but were not successful in obtaining virus-resistant plants, which indicated that technologies still need to be refined in order to obtain true virus-resistant transformants in chrysanthemum. Takatsu *et al.*[46] used the rice chitinase gene (cDNA clone named: *RCC2*) for conferring resistance against grey mould (*Botrytis cinerea*) in spray chrysanthemum. Transgenic lines obtained through *A. tumefaciens* strain C58 and MP90 showed enhanced resistance to grey mould. Through the introduction of rice chitinase gene into the internodes of cultivar 'Snow Ball', Sen *et al.*[47] obtained four putative transformants on hygromycin-supplemented medium using *Agrobacterium*-mediated transformation. Even though they failed to obtain resistant transformants to *Septoria obese* (which causes leaf spot disease), they achieved 2.2% transformation efficiency with reduced symptoms on transformed plants. Xu *et al.*[48] were successful in obtaining transgenic plants expressing *hpaGXoo* gene from *Xanthomonas oryzae* pv. *oryzae*, which showed resistance to alternaria leaf spot through leaf disc-mediated *A. tumefaciens* (EHA105) transformation. Valizadeh *et al.*[49] suggested that *SAE* (Sea Anemone Equistatin) gene could be a promising agent for the control of some aphid species in transgenic plants, as chrysanthemum genotype 1581 transformed with the *SAE* gene showed resistance against the pea aphid, *Acyrthosiphon pisum* and the cotton aphid, *Aphis gossypii* infesting chrysanthemum. After seven days, *M. persicae* populations on specific transgenic lines were up to 69% smaller relative to control populations in a whole plant bioassay and the mortality of cotton aphids was 11% on control lines and up to 32% on transgenic lines after five days. As plant resistance to herbivores like aphids and moths can also be increased by the overexpression of linalool, the main compound of floral scent[50,51], linalool synthase gene *FaNES1* was introduced into chrysanthemum plants for imparting resistance against Western Flower thrips (WFT). Expression of this gene in the plastids of chrysanthemum plants resulted in linalool emissions and accumulation of several forms of linalool glycosides. During the first 15 min, WFT significantly preferred these FaNES1 plants, but in the next 24 h gradually changed their preference to the wild type[52]. Recently, Shinoyama *et al.*[53] developed genetically modified chrysanthemums by introducing a modified *cry1Ab* gene of *Bacillus thuringiensis* var. *kurstaki* HD-1 (*mcbt*), which showed strong resistance against four species of lepidopteran larvae (*Helicoverpa armigera* and others), and a modified *sarcotoxin IA* gene of *Sarcophaga peregrine* (*msar*) with or without the 5′-untranslated region of the alcohol dehydrogenase gene of *Arabidopsis thaliana* (*AtADH*-5′UTR, as *ADH*), which showed strong resistance against white rust. Transformation efficiency achieved was 6.8%. Response of chrysanthemum (*Chrysanthemum zawadskii* Herbich) to hydric stress caused by hypoxia during waterlogging has been elucidated by Yin *et al.*[54] through isolation of a full-length cDNA of the alcohol dehydrogenase gene (*CgADH*) from chrysanthemum. Presence of ethylene suppressed the *CgADH* induction and expression, which can be countered by the formation of aerenchyma and adventitious roots or by adding 1-MCP, an inhibitor of ethylene action. In chrysanthemum photoperiodic manipulation of flowering is a common practice, where transgenics with altered flowering time are needed to maintain year-round flower supply and for which study of genes governing photoperiodic response in chrysanthemum is imperative. Higuchi *et al.*[55] identified an antiflorigen gene, *Anti-florigenic FT/TFL1 family protein* (*AFT*) from a wild chrysanthemum (*Chrysanthemum seticuspe*), whose expression is mainly induced in leaves under non-inductive conditions. A transient gene expression assay indicated that *CsAFT* inhibits flowering by directly antagonizing the flower-inductive activity of CsFTL3, a *C. seticuspe* ortholog of FT (*FLOWERING LOCUS T*), through interaction with CsFDL1, a basic leucine zipper (bZIP) transcription factor FD homolog of *Arabidopsis*. This antiflorigen production system prevents precocious flowering and enables the year-round supply of marketable flowers by

manipulation of day length. In response to temperature-induced dormancy and inhibition of flowering, Sumitomo *et al.*[56] assessed transgenic lines of chrysanthemums cv. 'Sei-marine' having mutated ethylene receptor genes (generated from the chrysanthemum ethylene receptor gene *DG-ERS1*) for ethylene-stimulated, temperature-induced dormancy in chrysanthemum and reported reduced ethylene sensitivity in transgenic lines. Leaf yellowing was observed in wild-type chrysanthemums, but leaves remained green in the transgenic lines on exposure to ethylene. At 20°C, the transgenic lines showed the same stem elongation and flowering as the wild type, while at cooler temperatures the wild type formed rosettes with an inability to flower and entered dormancy. However, some transgenic lines continued to elongate and flower which supported the involvement of the ethylene response pathway in temperature-induced dormancy of chrysanthemum and implicated the role of mutated ethylene receptor gene in the production of transgenic varieties with altered flowering behaviour. Shao *et al.*[57] obtained eight transgenic lines in *C. morifolium* through transformation of plant expression vector with CaMV 35S promoter for LFY cDNA. According to their reported results, three lines flowered 65, 67 and 70 days earlier and two lines delayed flowering by 78 and 90 days respectively. Xu *et al.*[48] further confirmed the role of *hpaGXoo* gene in acceleration of chrysanthemum development, as trangenic chrysanthemeum expressing *hpaGXoo* gene flowered early in comparison to wild types. Working on the production of transgenic chrysanthemum with novel colours, Ohmiya *et al.*[58] reported that using RNAi technology white petals could be converted into yellow petals by suppressing the expression of *carotenoid cleavage dioxygenase* (*CmCCD4a*). Later, using this technique Ohmiya *et al.*[59] produced 'Yellow Jimba' variant of white flowered cultivar 'Jimba'. Out of the 50 double-transformed plants obtained, more than half showed yellow coloured petals. Violet/blue-coloured chrysanthemum flowers cannot be generated by classical breeding practices due to the lack of a *F3′5′H* activity. The first report on the production of anthocyanins derived from delphinidin in chrysanthemum petals leading to novel flower colour was by Brugliera *et al.*[60]. They have successfully utilized *F3′5′H* genes to produce transgenic bluish chrysanthemums that accumulate delphinidin-based anthocyanins. A pansy *F3′5′H* gene under the control of a chalcone synthase promoter fragment from rose resulted in the effective diversion of the anthocyanin pathway to produce delphinidin in transgenic chrysanthemum flower petals. The resultant petal colour was bluish, with 40% of total anthocyanidins attributed to delphinidin. Increased delphinidin levels (up to 80%) were further achieved by hairpin RNA interference-mediated silencing of the endogenous *F3′H* gene and the resulting petal colours were novel bluish hues. Blue pigmentation in petals of chrysanthemum has also been reported by Noda *et al.*[61] through generation of delphinidin-based anthocyanins by expression of the flavonoid 3′,5′-hydroxylase gene. Huang *et al.*[62] also used RNAi technology to induce red and blue flowers in *C. morifolium* by downregulating *CmF3′H* (flavanone 3-hydroxylase gene hydroxylated at the 3′-position) and overexpressing the *Senecio cruentus F3′5′H* (*PCFH*) (flavanone 3-hydroxylase gene hydroxylated at the 3′- and 5′-positions) gene in chrysanthemum. Brighter red flowers with higher cyaniding content were obtained as a result of the *CmF3′H* gene, but F3′5′H only exhibited F3′H activity and could not result in blue flowers. Ethylene receptor gene from melon (*CmETR1/H69A*) was introduced into chrysanthemum to induce male sterility and prevent transgene flow via pollen using a disarmed strain of *A. tumefaciens*, EHA105, carrying the binary vector pBIK102H69A. Three GM lines were identified with complete absence of pollen grains at 20°–35°C. However, it was temperature-dependent as mature pollen grains were formed in these lines at 15°C due to the suppression of *CmETR1/H69A* at lower temperature. Female fertility was also observed to be less in GM lines, which indicated that mutated ethylene receptor is able to reduce both male and female fertility significantly in transgenic chrysanthemum[63].

Gladiolus (Gladiolus grandiflorus L.)

Among the different cut-flower crops, this monocotyledonous, bulbous ornamental crop occupies an important position. Numerous hybrids have been developed with attractive flower colours, longer spikes, better post-harvest longevity, but succeptibilty to diseases surpasses the superiority of these varieties in other characters. So, transgenic breeding in gladiolus is mostly done with the objective to develop resistance to major diseases. Graves and Goldman[64] successfully demonstrated *Agrobacterium*-mediated transformation in gladiolus for the first time through infection of corm discs. Biolistic transformation or particle bombardment had also been attempted in gladiolus using suspension cells and callus tissues[65], and cormel slices[66]. Regarding the use of effective promoters in gladiolus, Kamo *et al.*[67] suggested that for normal growth of transgenic gladiolus promoters such as *GUBQ2* and *GUBQ4* should be used, although their expression level is less in comparison to commonly used promoters such as *CaMV 35S*. Kamo *et al.*[68] observed that expression of *gusA* is highly tissue-specific in gladiolus with the use of different promoters – under *mas*2 promoter, it expressed throughout the roots and under *rolD* promoter, it expressed in leaves as well as in root tips, whereas under EF-1a promoter *gusA* expressed strongly in root tips and regenerating callus. As gladiolus propagates asexually and three years are required for corm to develop properly for production of flowering spike, expression of transgene will take a longer time. For

this, transgene stability of *UidA* gene which was incorporated previously under control of CaMV 35S, Act1, Ubi3 and Ubi7, UBQ3, *rolD*, translation elongation factor 1 subunit a, and *mas2* promoters[69] was studied over several growing seasons. UidA silencing did not occur in transformed gladiolus plants carrying the *bar–uidA* fusion gene under the control of CaMV 35S, rolD, *mas2*, or UBQ3 promoters following three seasons of dormancy. However, the highest expression level of GUS (beta-glucuronidase) was observed under the control of CaMV 35S promoter in the callus, shoots and roots of plants carrying the *bar–uidA* fusion gene, whereas with *rolD* promoter the expression was more in shoots and roots of indoor grown plants[70]. Later, Kamo[71] verified long-term gene expression which continued for two years, rather than silencing, while studying the differences in transgene expression for gladiolus plants grown under protected environment or in open field for several years. More variability in transgene expression as well as the higher expression was achieved for plants grown outdoor than in the greenhouse.

Kamo *et al.*[72] transformed gladiolus plants with BYMV coat protein gene either in its sense or anti-sense orientation to confer resistance against Bean yellow mosaic virus, and observed delayed infection in the transgenic plants containing the viral genes in either orientation. Later, resistance to Cucumber mosaic virus (CMV) was incorporated in gladiolus using CMV coat protein and CMV replicase genes[73], or using genes encoding for single chain variable fragment antibodies (scFv) to CMV groups I and II (ref. 74). For conferring resistance to *Fusarium oxysporum* f. sp. *gladioli* causing fusarium wilt, which is the major devastating disease in gladiolus, Kamo *et al.*[75] transformed gladiolus cv. Peter Pears with three antifungal genes, a non-heme chloroperoxidase from *Pseudomonas pyrrocinia*, and an exochitinase and endochitinase from *Fusarium venetanum* under CaMV 35S promoter. Cell extracts of transformed lines showed restrictive effect on growth of germinated *F. oxysporum* spores. Transformed plant lines also developed lower density of hyphae on roots as well as less necrotic lesions on shoots than non-transformed lines.

Carnation (Dianthus caryophyllus)

In carnation, classical flower breeding is limited and being a vegetatively propagated crop, it further limits the available gene pool. This makes it an ideal target for gene transfer technologies that have the potential to hasten the production of new genotypes and broaden the available gene pool[76]. Carnations are the world's first genetically engineered commercial flowers, having appeared some 10 years after the first report of success in the genetic manipulation of flower colour through plant transformation[77]. In the genus *Dianthus*, carnation has been mostly used for genetic transformation. The first successful transformation was achieved through *Agrobacterium*-mediated methods with stem explants, where transgenic shoots were directly induced explants[78]. Cell suspension cultures were also used for genetic transformation in the genus *Dianthus*[79]. In case of carnation, the main objectives to be achieved through transformation methods include different plant forms, colour; specifically the lacking blue, resistance to fusarium wilt and insects and reduced ethylene sensitivity. Meng *et al.*[80] isolated some novel lines of carnation with altered morphology for the first time, which is a highly desired character in carnation like other ornamental crops. *35S:PttKN1* (a novel member of *KNOX* gene family) was transformed to carnation via *A. tumefaciens* to obtain a total of 32 T0 progeny with aberrant phenotypes, which include tricussate whorled, multiple-cussate whorled phyllotaxis versus typical opposite phyllotaxis of wild type, thicker and flatter stems versus round stems of wild type and dwarfness. Insertion of *35S-rolC* gene of *Agrobacterium rhizogenes* in carnation produced slightly dwarf plants with increased number of lateral shoots, better rooting ability of cuttings with increased number of flowering stems and smaller flowers in one line[81,82]. Casanova *et al.*[83] also observed enhanced ratio of petal and leaf blade in 35 : rolC carnation plants compared to that of control plants.

Engineering novel colours in carantion was based upon the gene encoding F3′5′-hydroxylase, which was isolated from petunia[84]. In 1997, the first genetically modified blue carnation, Moon series was introduced to the market which demonstrated the success of genetic manipulation of flower colour. Transgenic violet carnations have been successfully developed by the introduction of a *F3′5′H* gene together with a petunia *DFR* gene into a *DFR*-deficient white carnation. The petals of the engineered carnations contain predominantly delphinidin, that native carnations do not produce. Such a bluish hue in the transgenic flowers has never been achieved by traditional breeding of carnation. This blue colour of carnation is stable following repeated vegetative propagation[77]. Zuker *et al.*[85] observed the colour change from orange-red to white through introduction of the antisense of flavonoid biosynthetic gene, flavanone 3-hydroxylase (*F3H*). Along with the colour modification, transgenic plants emitted higher levels of methyl benzoate and so were more fragrant than control plants. Unlike the other novel traits, floral scent was never a target for carnation breeders. So, modern carnation varieties, with a few exceptions, lack distinct fragrance[86]. Lavy *et al.*[87] transformed a carnation variety lacking detectable levels of monoterpenes with the *Clarkia breweri lis* gene. Molecular and detailed fragrance analyses revealed that ectopic expression of *lis* leads to the production of linalool and its derivatives' *cis*- and *trans*-linalool oxide in the transgenic plants. Though the level of floral scent was too low to be detected by human senses, study generated possibilities for scent

engineering in carnation cultivars. *Dianthus caryophyllus* L. cv. 'Tempo' was transformed using disarmed *A. tumefaciens* strain EHA 105 harbouring binary vector pBin BtI containing insect resistance (*cry1Ab*) and kanamycin resistance (*npt*-II) genes. Highest transformation frequency (17.67%) was achieved using callus regeneration system. All the transformed plants showed strong expression of insect resistance gene, inferred from lesser feeding of the leaves by the larvae compared to control, and phenotypes were also found normal[88].

Carnation is highly sensitive to ethylene and during senescence, autocatalytic production of ethylene leads to deterioration of petals[89]. Regarding control of senescence in carnation flowers by regulating ethylene production, two major contributing genes have been identified, viz. *DC-ACS1* (encoding ACC synthase) and *DC-ACO1* (encoding ACC oxidase)[90,91]. To enhance post-harvest longevity of carnation, regulation of ethylene-induced senescence is of utmost significance economically, as transgenic carnation with reduced ethylene production will lead to the complete elimination of costly and harmful chemicals used to lengthen the vase life[92]. Many researchers have made efforts in this direction and developed transgenic lines of carnation with reduced ethylene sensitivity. Savin *et al.*[93] generated the carnation lines transformed with a carnation ACC oxidase cDNA in antisense orientation with longer vase life, whereas Kosugi *et al.*[94] genetically transformed the flower with carnation ACC oxidase cDNA in sense orientation, which showed reduced ethylene sensitivity and longer vase life. Iwazaki *et al.*[92] transformed the carnation cv. Nora with carnation ACC synthase (*DC-ACS1*) cDNA in sense orientation having *sACS* transgene or antisense orientation having *aACS* transgene, and observed reduced ethylene production in transformed lines. Bovy *et al.*[95] introgressed an *Arabidopsis thaliana etr1-1* allele into carnation gemone to generate transgenic lines, where its heterologous expression reduced ethylene production and enhanced flower longevity. Kinouchi *et al.*[96] generated transgenic lines in potted carnation harbouring ACC oxidase (*DCACO1*, *s/aACO*) or ACC synthase (*DC-ACS1*, *s/aACS*) in sense or antisense orientation, or mutated carnation ethylene receptor cDNA (*DC-ERS2'*). Inokuma *et al.*[97] observed reduced ethylene production and longer vase life in the transgenic lines of 'Lillipot' carnation harbouring an *sACO* transgene. These studies showed that by down-regulating the action of genes responsible for ethylene production through transformation, longevity of cut carnations can be enhanced.

Conclusion

Transgenic approaches developed in cut flowers present their wide applicability to other sectors of ornamental plant industry having high preference. Many aspects of the cut-flower crops have been studied and improved through transgenic breeding; however, with respect to adoption and commercialization, we could not find many examples like the transgenic Moon series of carnation which has been commercialized only in a few countries despite the novel blue colour. If we compare the progress with cereal crops and other agricultural crops, transgenic research in ornamental crops is lagging far behind due to their less rewarding share in the agriculture sector. A major hindrance for breeders from developing transgenics in ornamental crops is the incurring cost for securing regulatory approval[98]. As it is not possible to overcome these regulatory hurdles readily, so, in order to widen the scope of transgenic varieties, each country must frame a less rigid system taking into account all the risks associated up to an acceptable level. This can only be accomplished through international cooperation and harmony.

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Received 8 August 2016; revised accepted 30 November 2016

doi: 10.18520/cs/v113/i01/43-52