**GENETIC MODIFICATION IN FLORICULTURAL PLANTS**

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**ABSTRACT**

Conventional breeding methods like *in-vitro* inter-specific hybridisation, micro-propagation, embryo rescue technique, mutagenesis by physical or chemical means, pedigree selection have been used by breeders in the floriculture industry for many years. In recent years, breeders in the sector of floriculture are engaging their efforts to provide elite breeding material in a range of flower colours, enhanced fragrances with biotic and abiotic resistance, improved vase life by using recently developed genetic modification techniques. Until recent time, many genes which are of potential utility to the floricultural industry have been identified and utilized and efforts are made towards improving genetic factors and molecular mechanisms underlying phenotypic traits which are of great importance to the industry, however, only a few genetically modified floricultural varieties has been released in the market and very limited work has been done especially in the sector of floriculture. This chapter primarily discusses the possible applications of various techniques of genetic modification for improving floricultural traits along with recent works done in particular areas in last few years.

*Key words*: Breeding methods, floriculture, flower colour, genetic modification, phenotype.

**Introduction**

Floriculture is an integral part of horticulture as well as agriculture sector. Owing to steady increase in the demand of floricultural products in both national and international market, floriculture has become one of the important commercial commodity in agriculture. Development of novel plants and flowers with diversified colours and illustrious fragrance with improved vase life has become paramount as well as vital driving force for growth of floriculture industry. Floriculture industry is mainly consisting of flowering plants, non-flowering (foliage) plants, pot plants, dry flowers, perfume industry, etc. Flowering plants has major contribution in floriculture industry, whereby plants are either used as cut/loose flowers, in which case the harvested flowers are utilised as the finished product or as pot plants in interior decoration or urban landscape.

 There is a long history of the floricultural industry by using different biotechnology approaches in the advancement of both propagation and breeding. Induced mutations, polyploidy and hybridization techniques have been used in the genetic improvement of genetics of flowering plants, however, these methods are crucial for increasing variability (Singh, 2014). Tissue culture techniques such as micro-propagation and meristem culture are found to be effective in generating virus-free and high-quality planting material. In addition to this, anther culture and embryo rescue methods of tissue culture strengthens the breeding programs in floriculture (Davies, 1981). Recent advancement in molecular markers and molecular biology techniques revolutionised the field of floriculture. Flower breeders have successfully utilised marker-assisted breeding programs as an aid to conventional breeding programmes by using Restriction Fragment Length Polymorphism (RFLP) analysis to generate gene linkage maps (Scovel *et al*., 1998). Recently, genome-wide association studies (GWAS) approach is being used for detecting potential candidate genes which are responsible for variations in phenotypic traits in floriculture (Sun *et al*., 2017; Fu *et al*., 2018).

Recent advances in biotechnological interventions such as proteomics, PCR, microarray, genomics and gene mapping methods are also being applied in the sector of floriculture. Percentage of various genetically modified floricultural plants and traits on which genetic engineering were conducted enlisted below in Table 1 and Table 2. Newer fields of biotechnology may be used to boost national income by creating new floricultural varieties that have better, superior, and desirable traits to suit consumer preferences. This is due to the rapid increase in research and development on genetic modification in floriculture and the growing global market for genetically modified floricultural plants. As a result, this chapter concentrated on potential uses of various genetic modification approaches in enhancing floricultural sector advancement.

**Table 1:** An enumeration of genetically modified floricultural plants (Boutigny *et al*., 2020).

|  |  |
| --- | --- |
| **Plants** | **Percentage** |
| Anthurium | 0.6 |
| Campanula  | 1.2 |
| Celosia  | 0.6 |
| Chrysanthemum  | 26.7 |
| Cyclamen  | 0.6 |
| Dianthus  | 5.5 |
| *Euphorbia pulcherrima* | 0.6 |
| Eustoma | 4.2 |
| *Ficus lyrata* | 1.2 |
| Forsythia  | 0.6 |
| Gentiana | 3.0 |
| Gerbera  | 1.2 |
| Gladiolus  | 2.4 |
| Gypsophila  | 0.6 |
| Hemerocallis | 0.6 |
| Hibiscus  | 0.6 |
| Ipomoea  | 1.8 |
| Kalanchoe | 3.6 |
| Lavatera | 0.6 |
| Lilium | 4.8 |
| Orchidaceae | 6.7 |
| Ornithogalum  | 0.6 |
| Osteospermum | 0.6 |
| Pelargonium  | 2.4 |
| Petunia  | 15.2 |
| *Rosa*  | 6.7 |
| *Sinningia speciosa* | 0.6 |
| Torenia | 5.5 |
| Tricyrtis | 0.6 |

**Table 2:** A list of traits on which genetic modification is made till date (Boutigny *et al*., 2020).

|  |  |
| --- | --- |
| **Genetically modified traits** | **Percentage** |
| Flower colour  | 29.1 |
| Morphology  | 12.7 |
| Longevity  | 12.1 |
| Early flowering  | 8.5 |
| Fungi resistance  | 7.9 |
| Virus resistance  | 7.9 |
| Flower fragrance | 5.5 |
| Flower anatomy  | 4.8 |
| Cold resistance  | 4.8 |
| Drought resistance  | 4.2 |
| Bacteria resistance  | 3.0 |
| Salinity resistance  | 3.0 |
| Herbicides  | 2.4 |
| Heat  | 1.2 |
| Others traits  | 10.3 |

**Applications of genetic modification in floricultural traits**

Recent progress in molecular biology has opened up a world of possibilities. Helps the breeder and breeding of floricultural plants in general in improving their effectiveness and quickness the process of varietal development. Applications such as mapping, gene modifications labelling, gene tagging and  gene pyramiding, amplification-based cloning, and marker-assisted selection (MAS/ MARS), applications for fingerprinting, such as varietal identification, assuring early elimination or selection, phylogeny and evolution research, diversity analysis  duplication of germplasms (Rout *et al*., 2020) also assisted in genetic engineering technique for selection of transgenes by using molecular markers. The first commercially available genetically modified flower in the global flower market was the "Moondust" carnation, developed by the Australian biotechnology enterprise Florigene (Singh and Sisodia, 2017), and the "Applause" rose, developed and marketed by the Japanese corporation Suntory (Chandler and Brugliera, 2011). Additionally, new genetically modified varieties for floriculture are being generated continually on a global scale using a range of approaches. In floriculture, there are various applications for which various genetic modification techniques are being used.

**Improvement of floral traits**

*Flower colour*

The genetic manipulation of flower colour has been the most widely used strategy in floriculture and this is where the majority of research on genetic modification of flower crops is concentrated. Three forms of pigment, flavonoids, carotenoids and betalains are primarily responsible for flower colour. These three forms of pigment provide a wide spectrum of hues from yellow to red to blue. The anthocyanin family of flavonoids, which are all O-glycosides, is one that significantly influences floral colour. Carotenoids, on the other hand, are phytonutrients that are found in the plastid and help to contribute yellow, orange, red and violet colours (Forkmann, 1991). Since flower colour is an essential characteristic of ornamental plants and has a significant impact on flower market value. Colours of petunia are controlled by genes that function in the favonoid/anthocyanin biosynthesis pathway, which includes the essential enzyme favanone 3′-hydroxylase (F3H). Therefore, it is thought that the F3H gene could be a possible target in floral colour engineering. Inhibiting or over-expressing the F3H gene by stable genetic alterations has already been used to alter the bloom colour of ornamentals belonging to the genera of torenia, dianthus, and nierembergia as well as petunia in past years (Suzuki *et al*. 2000; Tsuda *et al*. 2004; Ueyama *et al*. 2006; Zuker *et al*. 2002).

 Genetic engineering by inserting transgenes to improve or alter anthocyanin accumulation in plant cell remains one of the most prevalent strategies to modify flower colour (Sadhukhan and Huo, 2020). Biosynthetic pathways can be altered to produce new or different flower colours by adding new genes, upregulating target genes or silencing target genes (Parmar *et al*., 2017). Anthocyanins, which are derivatives of flavonoids to exhibit colour, give flowers their vibrant hues of magenta, blue and purple as a result of the differing pH levels. Carotenoids, on the other hand, give flowers their hues of orange, red, and pink (Jeknic *et al*., 2014). In Petunia hybrida, the first use of gene technology to alter flower colour was observed, leading to the development of the orange-pelargonidin flower (Meyer *et al*., 1987). To achieve this, the appropriate strain of petunia was transfected with the A1 gene, dihydro-flavanol-3-reductase (dfr), from maize. Since then, modification of the flavonoid biosynthetic pathway has revealed the molecular underpinnings of the biosynthesis of flavonoids, including anthocyanins, flavones, flavonols, and aurones (Mol *et al*., 1995; Davies and Schwinn, 2007). Recent research on model flowering plants has revealed that the complex of basic helix-loop-helix (bHLH), R2R3-MYB and WD40 repeats (WDR) transcription factors affects the expression of the flavonoid genes (Grotewold, 2005; Quattrocchio *et al*., 2006). It has been observed that these triplet-complex transcription factors control the pigmentation of the petals of flowering plants like petunia, snapdragon, morning glory and gerbera (Morita *et al*., 2006; Nakatsuka *et al*., 2008). Another study extracted the *Sadenosylmethionine:anthocyanin* 3,5-O-*methyltransferase* gene from the petals of *Torenia hybrida*, which accumulates malvidin type anthocyanins. This gene was subsequently inserted into roses, which do not contain methylated anthocyanins. Due to an increase in the accumulation of malvidin and other methylated anthocyanins, brilliant magenta petals were expressed in GM rose and cupflower (*Nierembergia* sp.) petals (Nakamura *et al*. 2015). Another cutting-edge technology is the CRISPR-Cas system, which allows for the development of plant mutants by allowing plants to undergo a variety of desirable mutations within a few generations, CRISPR modifies an intended trait in a site-specific manner (Brooks *et al*., 2014; Feng *et al*., 2014; Zhang *et al*. 2014; Ma *et al*., 2016; Pan *et al*., 2016; Subburaj *et al*., 2016). Utilizing an *Agrobacterium* *tumefaciens*-mediated transformation system to transfer DNA encoding the necessary components is the most widely used technique for CRISPR-mediated genome editing in plants. In order to create certain desired mutations in plants, researchers and breeders can now use the CRISPR-Cas system, which is transforming the agriculture industry. Since it does not require codon optimization or particular promoters for expression in plant cells, DNA-free genome editing by Cas9-ribonucleoproteins (RNPs) delivery offers several benefits in plants. In a different study, the introduction of delivery resulted in mutations in both F3H genes of petunia, which exhibited a pale purplish pink coloured flowers (Yu *et al*., 2021).

*Fragrance*

Due to the commercial value of flower volatiles in perfumery, the chemical composition of floral smells has been intensively investigated for hundreds of years. Recent ecological research on the biology of plants have looked at the functions of flower fragrance. Due to its sensual connotation, fragrance is of utmost importance after floral colour in terms of market attraction. Another key factor in luring pollinators is fragrance. Purely made up of fatty acids, fragrance or flowery perfume is formed from a variety of substances such benzenoids, phenylpropanoids and terpenoids (monoterpenes and sesquiterpenes). Numerous of these scent compounds have had their structures examined. Though the number of cloned genes involved in the biosynthesis of different floral scent compounds is rapidly rising, biochemical and molecular biological knowledge of the biosynthesis of fragrance compounds is still restricted (Dudareva and Pichersky, 2000). Even fewer studies have been conducted till date on the genetic engineering for alteration of flower scents. According to a study on petunias, the secondary metabolic pathways are intriguingly connected via modulating the anthocyanin biosynthetic process. Transcriptional regulators may have a greater impact on scent production (Zvi *et al*., 2012). In another study, the shikimate pathway's 3-deoxy-diarabino-heptulosonate 7-phosphate synthase enzyme was introduced into *Petunia hybrida* plants in order to enhance their aroma. The phenylalanine content of the flower petals from these genetically engineered plants was high. In a particular study, transformed flowers released higher phenylalanine which is a precursor to aromatic volatile benzenoid-phenyl propanoids. Even the transgene's phenylalanine, which was expressed in some leaves, was carried to the floral portions to improve scent (Oliva *et al*., 2015). The treatment with phenylalanine boosted the formation of volatile phenylpropanoid-benzenoid compounds in the chrysanthemum blooms, which also produced fragrance (Kumar *et al*., 2021). The insertion of the transcription factor PAP1 in rose led to a 20-fold increase in the accumulation of the phenyl propanoid molecule eugenol in transgenic plants compared to control plants. In transgenic flowers, concentrations of the main sesquiterpene volatile Germacrene D were up to 8.5 times greater. The emission of the norisoprenoid compound b-ionone was also up to six times higher in PAP1-transgenic flowers. When compared to control plants, the total amount of volatile chemicals released by PAP1-transgenic lines' flowers was up to about four times higher overall (Zvi *et al*., 2012).

*Flower anatomy*

Flowers have been engineered to develop novel flower designs that can boost their ornamental worth, as well as their commercial value. Sepal, petal, stamen and pistil development during flower creation is controlled by many genes involved in flower organ identity (Thiruvengadam and Yang, 2009). Overexpressing tobacco *phytochrome b1* in chrysanthemum resulted in little plants with pronounced branch angles (Zheng *et al*., 2001). *Arabidopsis* GA insensitive gene insertion also resulted in decreased plant height (Petty *et al*., 2003). Gynoecium and androecium are transformed into corolla-like tissues when the CAG gene was suppressed, which alters the phenotypic for flower morphology (Aida *et al*., 2008). However, *DgLsL* gene transformation in chrysanthemum using vector pCAMBIA1301-sense and antisense *DgLsL* led to extensive branching. By using GRAS TF, lateral branching was restrained in plants (Jiang *et al*., 2010). The amount of flowers and the length of petal ligules significantly increased as a result of overexpression of CmCYC2c gene in Chrysanthemum lavandulifolium (Huang *et al*., 2016). It was also possible to create dwarf chrysanthemum cultivars by collectively silencing the *DmCPD* and *DmGA20ox* genes (Xie *et al*., 2015). *D. grandiflora* cloned gene DgD27 expression in Arabidopsis resulted in fewer tillers (Wen et al., 2016). In chrysanthemum, overexpression of CmTCP20, a gene belonging to the teosinte branched1/cycloidea/proliferating cell factors (TCPs) gene family, resulted in longer petals and larger flower inflorescences (Wang *et al*., 2019). Drop axillary branching was caused by the antisense expression of the Ls-like gene in transgenic *Dendranthema grandiflorum* (Han *et al*., 2007). Transgenic *Eustoma grandiflorum* plants that were ectopically expressing the MADS box gene LMADS1-M from lily (*Lilium longiflorum*) showed altered flower transition and development (Thiruvengadam and Yang, 2009). *PttKN1ectopic* expression in *D. caryophyllus* was seen in pleiotropic morphological changes that included adjustments to phyllotaxis (Meng *et al*., 2009). By overexpressing the *GSQUA2* transgenic gene in gerbera, petite inflorescence, restricted root production and elongation of the vegetative axis were achieved (Ruokolainen *et al*., 2010). Other studies have demonstrated the alteration of flower characteristics in transgenic torenia employing TCP3 or chimeric repressors of Arabidopsis AGSRDX (Sasaki *et al*., 2016). Transgenic lisianthus flowers showed altered floral structure, including malformed third-whole stamens and second whorls of petals that resembled sepals. The transgenic carnations with the rol C gene and the 35S CaMV promoter showed higher flowering stems (Noman *et al*., 2017). Thus, changes in floral structures and shapes can be linked to a variety of gene expressions, which enables scientists and researchers to support the use of genetic engineering as a breeding method for ornamental plants.

*Male sterility*

In some cultivated crops, such as floricultural plants, male sterility is a desired characteristic in order to produce hybrids or to prevent horizontal gene transfer from transgenic plants to related or other wild species via pollen. Inducing male sterility in desirable species through genetic manipulation is sometimes advantageous. According to some recent research in this area, the overexpression of the ethylene receptor genes CmETR1/H69A from melon resulted in temperature-sensitive male sterility in *Chrysanthemum morifolium* (Shinoyama et al., 2012). In one study, the transgenic lines failed to produce pollen between the temperature range of 20 and 35°C, whereas, normal pollen was effectively produced between the temperatures of 10 and 15°C due to the suppression of the transgene at the low temperatures. In order to minimise the negative effects of temperature on male sterility, more research in this area is required. As a result, there is a chance that GM chrysanthemums could cross with their wild counterparts, introducing transgenes into the ecosystem. However, this likelihood is probably modest.

**Improvement of morphological traits**

Many candidate genes that regulates the flower and plant morphology pathways has been cloned. Alteration in plant size and shape is regulated by phyto-harmones. By expressing oncogenes from the Ti plasmid of *Agrobacterium tumefaciens* or the Ri plasmid of *Agrobacterium rhizogenes*, transgenic plants can have their ratio of cytokinin to auxin altered. For instance, if bushy and short plant height are required, constitutive expression of the gene Rol-C, which encodes a cytokinin-p-glucosidase, from the Ri plasmid releases active cytokinin-b-glucosidase from inactive sugar conjugates. One of the best methods for creating interesting, new plants is branch control. In a study, the overexpression of Lateral-shoot Inducing Factor (LIF), a petunia zinc-finger type transcription factor, in petunia under the control of a CaMV35S promoter led to a significant increase in the number of lateral shoots. In the process of transforming flowering plants by genetic engineering, additional genes that are responsible for altering plant morphology and architecture are listed in Table 3.

**Table 3:** Gene responsible for plant morphology in ornamentals (Singh, 2014)

|  |  |
| --- | --- |
| **Gene** | **Action** |
| Lazy, TAC1 | Branching angles of tillers |
| GA insensitive (gai) | Stem elongation and plant height |
| Brassinosteroid | Plant height |
| Phytochrome | Harvest index and shading response |
| Rol C | Plant branching and architecture |
| Clavata, Wuschel | Establishment and maintenance of shoot apical meristem |
| MAX | More axillary branching |

**Transformation**

Several breeding and biotechnological techniques, including molecular and transgenic breeding, protoplast and biolistics-based techniques, viral induced gene silencing, somatic embryogenesis using *Agrobacterium*-mediated protocols and particle bombardment have been used to modify a large number of floricultural plants (Shibata, 2008). The most common method of producing transgenic plants in floriculture involves direct shoot regeneration from various plant tissues (leaf, cotyledon, petiole, petal, root, stem, and protocorm) that have previously been co-cultivated with modified *Agrobacterium*-mediated strains (Kishi-Kaboshi *et al*., 2018; Boutigny *et al*., 2020). Numerous dicotyledonous floricultural crops, including tulip, rose, jasmine, carnation, geranium, and chrysanthemum are also made possible by this technology. Although it is also reported that monocotyledonous species of flowers are often more difficult to transform as compared to dicotyledonous species, transformation is claimed to be unlikely to be a barrier for utilising genetic alteration for any flower species of interest.

Disarmed strains of *Agrobacterium tumefaciens* that carry transformation vectors are frequently used to transform dicotyledonous flowering plants (Tzfira and Citovsky, 2006), whereas, many of the major floricultural crops, including rose, carnation, chrysanthemum and gerbera are found to be sensitive to infection with disarmed *A. tumefaciens*. The method of gene delivery most frequently utilised for monocotyledonous plants, including lily, tuberose, and tulip, is micro-projectile bombardment (Watad *et al*., 1998; Men *et al*., 2003). In order to increase the effectiveness of *Agrobacterium*-mediated transformation in plants, the micro-projectile bombardment technique has also been discovered to be used (Zuker et al., 1999). In floriculture, some monocotyledonous plants have also been reported to have been transformed using *Agrobacterium tumefaciens* (Suzuki *et al*., 2001; Akutsu *et al*., 2004).

**Improvement of vase life**

Many cut flowers lose their quality quickly after being harvested, often even before they reach the consumer. This issue of short vase life represents a significant challenge for the floriculture sector. Vase life of many flowers ends when their petals begin to senesce after being harvested. One of the most important and effective methods for extending the life of flowers is genetic regulation of the biochemical process of senescence, which is primarily regulated by the phytohormone ethylene. The carnation has long been utilised as a model system for research into the mechanisms behind floral senescence. Senescence of the petals in a carnation flower occurs actively and involves numerous physiologic and biochemical alterations.

As a result of short ethylene biosynthesis pathway, it is possible to suppress ethylene biosynthesis by down-regulating the ACC oxidase or synthase. Cut flowers from such genetically modified plants engineered in this way showed delayed senescence. Whereas, these flowers remain sensitive to ethylene from outside source (Stead *et al*., 2006; Chandler, 2007). Over-expression of mutant ethylene receptor genes has been discovered to display delayed senescence as well as to overcome the sensitivity to external ethylene, hence prolonging vase life, in earlier investigations by Sriskandarajah *et al*. (2007), Milbus *et al*. (2009) and Raffeiner *et al*. (2009). In carnations, both technologies have been applied and resulted prolonged vase life has been successfully demonstrated to be stable in field tests and intercontinental transit studies. However, because of obtaining regulatory permission is highly expensive, genetically engineered carnations with extended vase lives have not yet been commercialised. In a different study, Milbus *et al*. (2009) found that some significant pot plants had increased ethylene resistance. However, they also noted that because ethylene is a crucial phytohormone that affects disease sensitivity and vegetative propagation, it is crucial to ensure that transgene expression is strictly restricted to the floral organs. This is a significant issue as majority of floricultural crops are vegetatively propagated (Shibata, 2008; Kumar *et al*., 2021). Similar to this, Wilkinson *et al*. (1997) created ethylene-insensitive petunia by introducing *etr1-1* under the control of the constitutive CaMV35S promoter. However, following the transfection of petunia with *etr1-1* under the control of floral-specific promoters FBP1 (floral binding protein) and AP3, a vase life of up to 5 times was seen in transformed petunia (involved in floral organ development). Insensitive to ethylene, newly transgenic converted petunia plants bear larger flowers with a longer vase life than non-transformed plants (Netam, 2018).

**Table 4.** Genes for improving vase life in ornamental flowers (Aswath and Hanur, 2009).

|  |  |
| --- | --- |
| **Traits**  | **Candidate gene and its pathway** |
| ACC synthase | Inhibition with reduced ethylene production |
| ACC oxidase | Inhibition with reduced ethylene production |
| ACC deaminase | Over expression with rediuced ethylene production |
| Etr 1 | Expression of a defective gene with reduced ethylene production |
| ERS | Expression of a mutated gene with reduced ethylene production |

**Improvement of flowering time**

Flowering time is also an important trait of flowering plants which can be modified through genetic modification methods. In recent years, several reports have described successful attempts towards gene introduction to produce flowers in short time and allowing their production at a lower cost. MADS box genes constitute an example as they can control both flowering time as well as floral organ development at a time (Noman *et al*., 2017). In a research, early flowering in transgenic chrysanthemum plants were induced by overexpression of AP1*-*like genes (member of MADS box gene family) from *Asteraceae*. Moreover, transgenic flowers of chrysanthemum displayed prior colour development and complete inflorescence opening in comparison with non-transgenic plants (Shulga *et al*., 2011). Transgenic Dendrobium orchids overexpressing DOAP1(an AP1 ortholog) resulted earlier flowering and earlier termination of inflorescence meristems into floral meristems than wild-type or non-transgenic orchids (Sawettalake, 2017). Flowering in transgenic lisianthus plants, transformed with the MADS box gene OMADS1 from orchid found to be significantly earlier than non-transgenic plants (Thiruvengadam and Yang, 2009). Similarly, overexpression of MADS box genes (OMADS1 and DOSOC1), promoted early flowering in transgenic orchids (Thiruvengadam *et al*., 2012; Ding *et al*., 2013). Budding and blooming of the transgenic chrysanthemum cultivar ZJL and the scion HSJQ grafted onto transgenic rootstock flowered in significantly shorter time than those of the non-transgenic chrysanthemums due to the effects of the silencing of *CmMET1* by RNA interference (Li *et al*., 2019).

**Improvement of stress tolerance**

*Tolerance to abiotic stress*

Studies conducted by An *et al*. (2014) and Song *et al*. (2014) proved constitutive overexpression of Salt Overly Sensitive 1 (SOS1) encoding a plasma membrane Na+/K+ antiporter from *Chrysanthemum crassum* and the TF gene Inducer of CBF Expression 1 (ICE1) from *Chrysanthemum dichrum* in the cultivar ‘Jinba’ found to increase tolerance to salt, drought and cold in the transgenic plants. These genes provide protection to the transgenic plants via harmful Na+ extrusion and beneﬁcial K+ ion retention, osmolyte accumulation and reactive oxygen species management under stress. In another study conducted by Li *et al*. (2015) cis-genic chrysanthemums overexpressing CmWRKY17 transcriptional repressor from *C. morifolium* proved to increase the sensitivity of transgenic plants to salt stress. This happened due to the suppression of several stress related genes including SOS pathway genes and ion transporters.

Instead, silencing such repressors might prove to be a methodical and effective way to improve salt resistance in floricultural plants. By overexpressing the Arabidopsis Ca2+/H+ antiporter CAX1, it was possible to genetically modify gerbera plants to be cold-tolerant (Olsen et al. 2015). In floriculture, phosphorus (P) deficient soils have an impact on plant development and yield, and specific P transporters control P uptake in deficient environments. When the same species of C. morifolium's root-expressed Phosphate Transporter 1 (CmPT1) gene was overexpressed, it significantly increased phosphorus accumulation and plant biomass in P-deficient environments (Liu *et al*., 2014).

*Tolerance to biotic stress*

Insects and diseases causes a consequential cost to producers of floricultural industry, whereas, flower breeders continually put efforts to improve abiotic resistance in plants. Tolerance to some devastating insects and diseases like aphids, mites, thrips, bacterial wilt, rusts, *Botrytis* blight, black spot and anthracnose are major targets to breeders as these are copious and intractable to manage. In a study performed by Vieira *et al*. (2015) a modiﬁed cystatin transgene from the rice was overexpressed in the cultivar of lily (*Lilium longiﬂorum*) Nellie White. The delivery of transgene was done from rice with the help of gene gun to confer resistance to *Pratylenchus penetrans*. The cystatins present in genetically modified plants of lilies up taken by the pests which inhibited nematode digestive proteases preventing their growth. In another study, resistance to *Botrytis cinereal* which is a major fungal pathogen in lilium was achieved in its cultivar Star Gazer by overexpression of the rice RCH10 chitinase gene (Núñez de Cáceres González *et al*., 2015) where gene transfer was carried out by *Agrobacterium*-mediated transformation. Biocontrol of *Fusarium oxysporum* in gladiolus was conducted by biolistic-mediated delivery to suspension cells of a gene encoding a synthetic antimicrobial peptide D4E1. This antimicrobial peptide effectively kills the fungus due to the formation of an ion-leaking channel inside its membrane. The gene causing more resistance to root infection by *Fusarium* disease was expressed under the constitutive CaMV 35S promoter in order to regenerate genetically engineered disease resistant gladioli (Kamo *et al*., 2015). A deadly powdery mildew disease in floricultural plants caused by fungus *Podosphaera* causes great reduction in quality and yield of flowers. Membrane transporter was encoded by mildew resistance locus 1 (MLO1) which is necessary for the fungus to enter the plant. Resistance to powdery mildew in *Rosa multiflora* (Qiu *et al*., 2015) and *Petunia hybrida* (Jiang *et al*., 2016)was increased by RNA interference-based knockdown of homologs of MLO1.

In *Chrysanthemum morifolium*, joint overexpression of modiﬁed sarcotoxin IA gene and *Bacillus thuringiensis* cry1Ac gene from the ﬂy *Sarcophaga peregrina* resulted in tolerance to both lepidopteran insect pest and a disease, *e.g*., lepidopteran larvae *Helicoverpa armigera* and white rust-causing pathogen *Puccinia horiana*, respectively. It was found that sarcotoxin IA transcripts were more abundant and genetically modified chrysanthemums were more pathogen resistant when attached to Arabidopsis alcohol dehydrogenase 5’-untranslated region and heat shock protein 18.2 gene terminator, the indicating importance of enhancer elements in transgene expression (Shinoyama *et al*., 2015). A wide range of aphid resistance was found in chrysanthemum, by overexpression and association mapping by the promotion of lignin synthesis (Wang *et al*., 2017; Fu *et al*., 2018).

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