**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 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 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 demand of flower in both national and international market floriculture has become one of the important commercial trades in agriculture. An important driving force for the floriculture industry is a development of novel plants and flowers with variety of colours and fragrance with improved vase life. Floriculture industry is mainly composed of flowering plants, non-flowering (foliage) plants, pot plants, dry flowers, perfume industry, etc. Flowering plants used in floriculture either as cut/loose flowers where the harvested flowers are the final product or as pot plants where flowering pots are used in the decoration of home or urban landscape.

The use of numerous techniques of biotechnology in floricultural industry for both propagation and breeding has an old history. Genetic improvement in the flowering plants has been accomplished through induced mutations, polyploidy and hybridization technique which play an important role in creating larger variability (Singh, 2014). Conventional techniques like meristem culture and micro-propagation are found to be used for generating virus-free and high-quality planting material. Breeders commonly use other tissue culture techniques such as anther culture and embryo rescue to supplement breeding programs (Davies, 1981).Those have also used marker-assisted breeding programs using Restriction Fragment Length Polymorphism (RFLP) analysis in order to generate gene linkage maps, as an aid to conventional breeding techniques (Scovel *et al*., 1998). In recent years, genome-wide association studies (GWAS) is being used for detecting potential targeted loci and candidate genes responsible for variations in phenotypic traits (Sun *et al*., 2017; Fu *et al*., 2018).

Newer areas of biotechnology such as PCR, microarray, genomics, proteomics, and gene mapping have also been applied in the sector of floriculture. Enumeration of percentage of various genetically modified floricultural plants and traits on which genetic engineering were conducted and published in scientific publications are given in Table 1 and Table 2. Given the rapid increase of research and development on genetic modification in floriculture and the growing global market for genetically modified ornamental plants, biotechnology may be used to increase national income by developing new ornamental varieties that have superior and desirable characteristics that match consumer preferences. Therefore, this chapter focussed on possible applications of various techniques of genetic modification in improving floricultural traits as well as recent literatures where the work has been done in similar areas.

**Table 1:** Enumeration of genetically modified floricultural plants in scientific publications (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:** Enumeration of traits in scientific publications in which genetic modification is conducted (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 |

**Possible applications of genetic modification in floricultural traits**

In the global flower market, the first genetically modified variety on commercial level was released in carnation called “Moondust” which was developed by the Australian biotechnology company Florigene (Singh and Sisodia, 2017) and later in rose called “Applause” which was developed and commercialized by the Japanese company Suntory (Chandler and Brugliera, 2011). Furthermore, new genetically modified varieties in floriculture have been developed and being continuously developed on global level through various techniques. There are many areas for which various genetic modification methods can be applied in floriculture.

**Improvement of floral traits**

*Flower colour*

The alteration of flower colour has been the most widely used tool of genetic modification in floriculture and this is where the bulk of research on genetic modification of flower crops is focussed. Flower colour is predominantly due to three types of pigment, *viz*., flavonoids, carotenoids and betalains. The flavonoids are the most common among these three types of pigment which contribute to a wide range of colours from yellow to red to blue. The flavonoid molecules localized in the vacuoles of petal epidermal cells. Among flavonoids, one which makes the major contribution to flower colour are the anthocyanin which are all O-glycosides. Whereas, carotenoids are phytonutrients which is located in the plastid which contribute to impart majority of yellow hues in a number of flowers. On the other hand, betalains are the least abundant accessory pigment found in flowers which imparts various hues of ivory, yellow, orange, red and violet (Forkmann, 1991).Since, in ornamental plants flower colour is an important trait that has strong influence on the flower market value. In petunia, colours are determined by genes that are involved in the favonoid/anthocyanin biosynthesis pathway, in which favanone 3′-hydroxylase (F3H) is one of the key enzymes. Thus, F3H gene is supposed to be a potential target in flower colour engineering. Modification in flower colour by inhibiting or over-expressing the F3H gene through stable genetic transformations in ornamentals of the genera of torenia, dianthus and nierembergia as well as petunia had been already done in previous years (Suzuki *et al*. 2000; Tsuda *et al*. 2004; Ueyama *et al*. 2006; Zuker *et al*. 2002).

 Genetic modification by introducing transgenes to enhance or modify anthocyanin accumulation in plant cell remains one of the most prevalent strategies to modify ﬂower colour (Sadhukhan and Huo, 2020). To produce new or different flower colour, biosynthetic pathways can be modified through the introduction of new genes, over expression or silencing of target genes (Parmar *et al*., 2017). Flavonoids and their derivatives that exhibit colour, anthocyanins imparts various bright colours due to different pH in flowers such as magenta, blue and purple, whereas, carotenoids imparts orange, red and pink colour to flowers (Jeknic *et al*., 2014). The first application of gene technology in the modification of flower colour was found in *Petunia hybrida* which lead to creation of orange-pelargonidin flower (Meyer *et al*., 1987). This was achieved by expressing the A1 gene- dihydro-flavanol-3-reductase (*dfr*) from maize to the suitable line of petunia. Since then, the molecular basis of the flavonoids biosynthesis including anthocyanins, flavones and flavonols and aurones has been elucidated (Mol *et al*., 1995; Davies and Schwinn, 2007) *via* manipulation of the flavonoid biosynthetic pathway. In recent studies of model flowering plants, expression of the flavonoid genes is found to be manipulated by the complex of basic helix-loop-helix (bHLH), R2R3-MYB and WD40 repeats (WDR) transcription factors(Grotewold, 2005; Quattrocchio *et al*., 2006). Such triplet-complex transcription factors are found to regulate the petal pigmentation of flowering plants such as petunia, snapdragon, morning glory and gerbera (Morita *et al*., 2006; Nakatsuka *et al*., 2008).In other study, from *Torenia hybrida* petals, *Sadenosylmethionine:anthocyanin 3,5-O-methyltransferase* gene was isolated that accumulate malvidin type anthocyanins which was then introduced into rose that doesn’t contain methylated anthocyanins. Brilliant magenta petal colour in GM rose and cupflower (*Nierembergia* sp.) petals were expressed due to increased accumulation of malvidin and other methylated anthocyanins (Nakamura *et al*. 2015). Another technology named CRISPR-Cas system is a versatile and cutting-edge mechanization to generate various desired mutations in plants, enabling to develop plant mutants. CRISPR modifies a target trait of interest within a few generations in a site-specific manner (Brooks *et al*. [2014](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR5); Feng *et al*. [2014](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR7); Ma *et al*. [2016](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR11); Pan *et al*. [2016](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR15); Subburaj *et al*. [2016](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR19); Zhang *et al*. [2014](https://link.springer.com/article/10.1007/s00299-020-02593-1#ref-CR28)). The most popular method for CRISPR-mediated genome editing in plants is to use an *Agrobacterium tumefaciens*-mediated transformation system to deliver DNA encoding the components. The CRISPR-Cas system is now revolutionizing agriculture sector by allowing researchers and breeders to generate various desired mutations in plants. In particular, DNA-free genome editing by Cas9-ribonucleoproteins (RNPs) delivery has many advantages in plants as it does not require codon optimization or specific promoters for expression in plant cells. In another study, Cas9-ribonucleoproteins delivery resulted in mutations in both F3H genes of petunia, which exhibited a pale purplish pink coloured flowers (Yu *et al*., 2021).

*Fragrance*

For hundreds of years chemical composition of floral scents has been extensively investigated due to the commercial value of floral volatiles in perfumery. In recent, several ecological studies regarding the biology of the plant have examined the roles of floral scent.Fragrance is one of the most appealing flower trait to consumer after flower colour due to its sensual association. Fragrance also plays an important role in attracting pollinators. Fragrance or floral scent is purely fatty acid which is derived from various compounds such as benzenoids, phenylpropanoids and terpenoids (monoterpenes and sesquiterpenes). The structures of hundreds of these fragrance compounds have been studied. Biochemical and molecular biological knowledge of the biosynthesis of fragrance compounds is still limited though the number of cloned genes involved in the biosynthesis of various floral fragrance compounds is steadily increasing (Dudareva and Pichersky, 2000). Studies on the modification of floral fragrances using genetic engineering are even rarer until now. A study conducted in petunia revealed that fragrance affected by modulation of anthocyanin biosysntheis that related to an intriguing link between the secondary metabolic pathways. The fragrance production can be more likely affected by transcriptional regulators (Zvi et al., 2012). In another study, plants of *Petunia hybrida* were transformed with a bacterial gene encoding 3-deoxy-diarabino-heptulosonate 7-phosphate synthase enzyme of the shikimate pathway in order to increase the fragrance of petunia flowers by the formation of aromatic amino acids. These genetically modified plants produced high phenylalanine levels in ﬂower petals. Phenylalanine is a precursor of fragrant volatile benzenoid-phenyl propanoids and in a given study it emitted increasingly from the modified ﬂowers. Even phenylalanine produced by the transgene expressed in the leaves in some lines were transported to the ﬂoral parts to produce increased fragrance (Oliva *et al*., 2015). Phenylalanine treatment also generated scent in the flowers of chrysanthemum by increased production of phenylpropanoid-benzenoid volatiles (Kumar *et al*., 2021).

In rose, Arabidopsis Production of Anthocyanin Pigment1 (PAP1) transcription factor was introduced, resulting the accumulation of phenyl propanoid compound eugenol in transgenic upto 20 folds higher than the control plants. The major volatile compound sesquiterpene Germacrene D were upto 8.5 fold higher in transgenic flowers. Norisoprenoid compound b-ionone emission was also increased upto six-fold in PAP1-transgenic flowers. Taken as a whole, the total emitted volatile compounds was upto approximately fourfold higher in flowers of PAP1-transgenic lines as compared to the control plants (Zvi *et al*., 2012).

*Flower anatomy*

Flowers to increases their ornamental and commercial value have been engineered to create new flower shapes that can increase their ornamental value. Flower formation involves the development of sepals, petals, stamens, and pistils, regulated by several genes involved in flower organ identity (Thiruvengadam and Yang, 2009). In chrysanthemum, overexpression of tobacco *phytochrome b1* produced small sized plants with larger branch angles (Zheng *et al*., 2001). Further, decreased plant height was achieved by introduction of *Arabidopsis* GA insensitive gene (Petty *et al*., 2003). Suppression of CAG gene modifies gynoecium and androecium to corolla like tissues which change the phenotype for flower shape (Aida *et al*., 2008). However, abundant branching was achieved with transformation of *DgLsL* gene with vector pCAMBIA1301-sense and antisense *DgLsL* in chrysanthemum. Lateral branching was restrains by GRAS TF (Jiang *et al*., 2010). Overexpression of CmCYC2c in *Chrysanthemum lavandulifolium* led to significant increase in flower numbers and petal ligule length of ray florets (Huang *et al*., 2016). Dwarf chrysanthemum cultivars were also accomplished by collective silencing of *DmCPD* and *DmGA20ox* genes (Xie *et al*., 2015). Cloned gene DgD27 expression in Arabidopsis from *D*. *grandiflora* produced reduced number of tillers (Wen *et al*., 2016). The overexpression of CmTCP20, a member of teosinte branched1/cycloidea/proliferating cell factors (TCPs) gene family, led to larger flower inflorescences and longer petals in chrysanthemum (Wang *et al*., 2019).

 The antisense expression of *Ls*-like gene in transgenic *Dendranthema grandiflorum* resulted in drop axillary branching (Han *et al*., 2007). Alteration of flower transition and formation was observed in transgenic *Eustoma grandiflorum* plants ectopically expressing the MADS box gene LMADS1-M from lily (*Lilium longiflorum*) (Thiruvengadam and Yang, 2009). In *D*. *caryophyllus*, the expression of *PttKN1ectopic* expressed in pleiotropic morphological wavering inclusive of phyllotaxis modifications (Meng *et al*., 2009). In gerbera, tiny inflorescence, limited root formation with elongation of vegetative axis were performed by overexpression of *GSQUA2* transgenic gene (Ruokolainen *et al*., 2010). Other studies have reported the modification of flower traits in transgenic torenia using chimeric repressors of Arabidopsis AGSRDX (Narumi *et al*., 2008) or TCP3 (Sasaki *et al*., 2016). A change in floral structure i.e., alteration of second whorl in petal into sepal like structures and deformed third whorl stamen was observed in transgenic lisianthus flowers. Transgenic carnations porting rol C gene with promoter 35S CaMV demonstrated more number of flowering stems (Noman *et al*., 2017). Thus, modification in floral shapes and structures can be attributed by several gene expressions and help researchers as well as scientific communities to endorse genetic engineering as a platform in ornamental plant breeding.

*Male sterility*

Male sterility is a desired trait in some cultivated crops including floricultural plants for the production of hybrids or to arrest horizontal gene transfer from transgenic plants *via* pollen to related or other wild species. Genetic modification is sometimes useful to induce male sterility in desired species. Some recent work in this field reveals that the overexpression of ethylene receptor genes CmETR1/H69A from melon caused male sterility (temperature-sensitive) in *Chrysanthemum morifolium* (Shinoyama *et al*., 2012). The transgenic lines did not produce pollen at 20°-35°C in a given study, whereas, in temperature ranging between 10°-15°C normal pollen were successfully produced due to suppression of the transgene at the low temperatures. Some more research in this field is necessary to optimise the unwanted temperature effects on male sterility.Hence, there is the possibility for crosses to occur between GM chrysanthemums and wild relatives, resulting in transgene flow into the environment, however, this possibility is likely to be low.

**Improvement of morphological traits**

Many potential genes that are involved in the pathways associated with flower and plant morphology have been cloned. Modification in plant size and shape is controlled by phyto-hormones. The ratio of cytokinin to auxin can be modified in transgenic plants by expressing oncogenes from Ti plasmid *Agrobacterium tumefaciens* or Ri plasmid from *Agrobacterium rhizogenes*. For instance, constitutive expression of gene named Rol-C, encoding a cytokinin-p-glucosidase, from the Ri plasmid releases active cytokinin-b-glucosidase from inactive sugar conjugates (Estruch *et al*., 1991) which lead to bushy and short plant height if desired. Control of branching is considered to be one of best way to generate attractive novel plants. In a study, the overexpression of a petunia zinc-finger type transcription factor, Lateral-shoot Inducing Factor (LIF), in petunia under the control of a CaMV35S promoter resulted in a considerable increase in the number of lateral shoots. Some other genes responsible for the modification of plant morphology and architecture are given in Table 3 which is being used in the process of transformation of flowering plants through genetic engineering.

**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**

Many floricultural plants have been transformed using various techniques of breeding and biotechnology like molecular and transgenic breeding, protoplast and biolistics based methods, viral induced gene silencing, somatic embryogenesis using *Agrobacterium*-mediated protocols or particle bombardment (Shibata, 2008). In fact, direct shoot regeneration from different plant tissues (leaf, cotyledon, petiole, petal, root, stem, protocorm) previously co-cultivated with engineered *Agrobacterium*-mediated strains, is the most widespread way to obtain transgenic plants in floriculture (Kishi-Kaboshi *et al*., 2018; Boutigny *et al*., 2020) This enabling technologies extends to many floricultural crops such as rose, jasmine, carnation, geranium and chrysanthemum (dicotyledonous) as well as tuberose, lily and tulip (monocotyledonous). For any flower species of interest transformation is said to be unlikely a barrier for employing genetic modification, though it is also said that monocotyledonous species of flowers are generally more difficult to transform when compared to dicotyledonous species.

Dicotyledonous flowering plants are most commonly transformed by using disarmed strains of *Agrobacterium tumefaciens* carrying transformation vectors (Tzfira and Citovsky, 2006), whereas, many of the major floricultural crops like rose, carnation, chrysanthemum and gerbera are found to be sensitive to infection with disarmed *A. tumefaciens*. For monocotyledonous plants, such as lily, tuberose and tulip, micro-projectile bombardment method has most often been used to deliver gene (Watad *et al*., 1998; Men *et al*., 2003). Micro-projectile bombardment method has also been found to use as a wounding method to increase efficacy of *Agrobacterium*-mediated transformation in plants (Zuker *et al*., 1999).In floriculture, dome 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**

After harvesting, many cut flowers deteriorate rapidly even before reaching to the consumer and this problem of short vase life constitutes as major problem in floriculture market. The end of vase life in many flowers is determined by the onset of senescence of petals after harvesting. To achieve delayed withering and longevity of flowers, one of the major and successful strategies is the genetic control of the biochemical process of senescence which is mostly controlled by phyto-hormone ethylene. Since many years, carnation has been used as a model system for studying the mechanism of senescence of flowers. Petal senescence in carnation flower is an active process which involves so many physiological and biochemical changes. The petal exhibits characteristic “in-rolling” behaviour as senescence commences and also in response to exogenous supply of ethylene. This behaviour can be prevented or delayed by some effective treatments such as application of silver ions or gibberellins which works as anti-ethylene and ultimately prevents the biosynthesis of ethylene.

As the ethylene biosynthesis pathway is quite short, down-regulation of the pathway to suppress ethylene biosynthesis is also possible by down-regulation of ACC oxidase or ACC synthase. Cut flowers from genetically modified plants modified in this way truly exhibit delayed senescence. Whereas, this flowers remain sensitive to external source of ethylene (Stead *et al*., 2006; Chandler, 2007). In previous studies conducted by Sriskandarajah *et al*. (2007), Milbus *et al*. (2009) and Raffeiner *et al*. (2009), over-expression of mutant ethylene receptor genes has been found to show delayed senescence as well as to overcome the sensitivity to environmental ethylene thereby improving vase life. In carnation both technologies have been used and the subsequent longer vase life have been successfully evinced to be stable in conducted field trials as well as in inter-continental transport studies. However, genetically modified carnation with modified vase life has not been commercialised due to the high cost of regulatory approval. In another study conducted by Milbus *et al*. (2009) enhanced ethylene resistance in a number of important pot plants has shown, but also noted the need to ensure expression of the transgene is strictly confined to the floral organs, as ethylene is an important phyto-hormone involved in regulating vegetative propagation and disease sensitivity. This is a significant issue as most of the floricultural crops are primarily vegetatively propagated (Shibata, 2008; Kumar *et al*., 2021). Similarly, Wilkinson *et al*. (1997) developed ethylene-insensitive petunia where they introduced etr1-1 under the control of the constitutive CaMV35S promoter. However, in transformed petunia a vase life of upto 5 times was observed during the transfection of petunia with etr1-1 placed under the control of floral-specific promoters FBP1 (floral binding protein) and AP3 (involved in floral organ development). Newly transgenic transformed petunia plants were insensitive towards ethylene, and bearslarger flowers with long 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.

On the other hand, silencing of such repressors may prove to be systematic and efﬁcient strategy to enhance salt resistance in floricultural plants. Cold-tolerant gerbera plants were genetically modified by overexpressing Arabidopsis Ca2+/H+ antiporter CAX1 (Olsen *et al*. 2015). In floriculture, plant growth and yield are affected by phosphorous (P) deﬁcient soils and certain P transporters regulate P uptake under deﬁcient conditions. A root-expressed Phosphate transporter 1 (CmPT1) gene which was isolated from *C. morifolium* then overexpressed in the same species with a considerable increase in P accumulation as well as plant biomass under P-deﬁciency (Liu *et al*., 2014).

*Tolerance to biotic stress*

Insects and diseases are a significant cost to producers of floricultural industry, whereas, flower breeders continually try to improve abiotic resistance in plants. Tolerance to some devastating insects and diseases like aphids, mites, thrips, *Fusarium*, *Botrytis*, rusts and black spot are major targets to breeders as these are prolific and difficult to control. In a study performed by Vieira *et al*. (2015) a modiﬁed cystatin transgene from the rice was overexpressed in *Lilium longiﬂorum* cv. Nellie White. The transgene from rice was delivered by gene gun in order to confer resistance to the root nematode (*Pratylenchus penetrans*). The cystatins up taken by the pests from the genetically modified lilies inhibited nematode digestive proteases preventing their growth. In another study, resistance to a major fungal pathogen *Botrytis cinereal* was achieved in lilium cv. Star Gazer by overexpression of the rice RCH10 chitinase gene (Núñez de Cáceres González *et al*., 2015). In this study, gene transfer was done by *Agrobacterium*-mediated transformation. Biocontrol of a deadly fungal pathogen of gladiolus, *viz*., *Fusarium oxysporum*, was conducted by biolistic-mediated delivery to suspension cells of a gene encoding a synthetic antimicrobial peptide D4E1. This antimicrobial peptide kills the fungus by the process of making an ion-leaking channel in its membrane. The gene was expressed under the constitutive CaMV 35S promoter and regenerated genetically engineered gladioli which was more resistant to root infection by *Fusarium* disease (Kamo *et al*., 2015). A deadly disease powdery mildew caused by fungus *Podosphaera*, affects many floricultural plants. Mildew resistance locus 1 (MLO1) encodes a membrane transporter necessary for the pathogen to enter the plant. RNA interference-based knockdown of homologs of MLO1 has successfully increased resistance to powdery mildew in *Rosa multiflora* (Qiu *et al*., 2015) and *Petunia hybrida* (Jiang *et al*., 2016).

In *C. 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). In a studies of Wang *et al*. (2017) and Fu *et al*. (2018), a wide range of aphid resistance were found in the chrysanthemums, by overexpression (which promoted lignin synthesis) and association mapping, respectively.

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