

Chapter 6

Conventional and Molecular Interventions for Biotic Stress Resistance in Floricultural Crops



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Abstract Biotic stresses are one of the major problems for ornamental crop loss worldwide. In general, the spreading of biotic stresses is controlled by the spray of chemicals or by other means. But the residue of some chemical is retained in the soil and hence cause groundwater contaminations. Also, the chemicals used for disease control adversely affect pollinators and humans. Despite this conventional and molecular breeding approaches have been applied for disease improvement in several ornamental crops. Genetic engineering also offers an attractive approach for creating biotic stress resistance in ornamentals. But the limited availability of the transformation and tissue culture protocol, genome complexity, lack of gene pool information, high heterozygosity or genetic variability level and limited genomic information of the ornamental crops restricts the development of biotic stress resistant varieties. Nevertheless, the currently available sequencing technology along with newly emerging genome editing tools will definitely help in unravelling the molecular and genetic basis of disease development and herbivory. This understanding will ultimately assist for the improvement of ornamentals against disease and insect pest attack. This book chapter gives a comprehensive look over the diseases and pests affecting the economically important ornamental crops and also highlight the recent progress in developing biotic stress resistance in ornamental crops through conventional breeding, molecular breeding, genetic engineering, RNAi, and genome editing tools.

Keywords Breeding · Floriculture · Pathogen attack · Insect pest · CRISPR Cas9

6.1 Introduction

Floriculture deals with the cultivation of ornamental crops with the objective of development of new varieties, cultivation, marketing, and value-added products. The business of this industry is growing at a very high pace globally with the growth rate

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of 6–10% per annum. In floriculture, the estimated revenue is approximately 300 billion USD. The cut flower market individually holds a promising global market (Chandler and Sanchez 2012). India has a total of 0.61% share in global floriculture trade, thus ranks 18th in floriculture trade (Vahoniya et al. 2018). Cultivation of flowers is usually carried out in both open and protected conditions. Ranges of important cut flowers like rose, gerbera, bird of paradise, carnation, tuberose, gladiolus, etc. have been cultivated for their use in flower arrangements, bouquet making, garden display and in landscaping. However, loose flowers like marigold are used in making garlands, rangoli decoration, temple offerings, etc. Due to global warming, and climate change abnormalities, like any other crop, floriculture crops are also encountering number of abiotic and biotic stresses that directly and indirectly affect the growth and development of crops, thus result in bad quality flower, poor yield, and low income. Both yield and flower quality get affected by abiotic and biotic stresses which subsequently cause huge loss to farmers.

6.2 Biotic Stress in Floriculture Crops

Market value of cut flowers depends upon their phenotype and quality. Therefore, any visible symptoms of disease on floriculture crop can lead to major impact on its quality, market value and productivity. Most common diseases in flower crops is generally caused by fungi followed by virus and bacteria (Matthews 2019). Among fungal pathogens *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Sclerotinia* are the common enemies of ornamental production. Several control methods based on chemical use have been used for controlling fungal diseases, but compared to fungal diseases, it is more challenging task to control diseases associated with viruses and bacteria. Upon the assessment of losses that occur due to diseases caused by virus and bacteria, it was found that about 10–100% loss usually occurs during growth and distribution of floriculture crops. The major obstacle in the cultivation and export of ornamental crops is their susceptibility to multiple viruses (Loebenstein et al. 1995). It is therefore become very important to use quality planting material (disease free/virus free) for the control of diseases among floriculture crops. Looking at the importance of quality and market value of ornamental crops, it is must to develop resistance against various insect, pest and various diseases for effective biotic stress management using conventional and molecular approaches (Tables 6.2 and 6.3).

Table 6.1 Biotic stress in floricultural crops

S. No	Crop	Major diseases	Major pests
1	Rose	Black spot, powdery mildew, botrytis blight, crown gall other disease like stem canker, cercospora leaf spot, and rust etc.	Aphids, two spotted spider mite, scale, thrips, beetles, and leaf cutter
2	Gerbera	Root rot, crown rot, powdery mildew, and botrytis blight	White fly, leaf miner, thrips, red scale, and leaf eating caterpillar
3	Carnation	Fusarium wilt, foot rot, basal rot, leaf spot, and botrytis blight	Thrips, mites, and bud caterpillar
4	Chrysanthemum	Septoria leaf spot, powdery mildew, and blossom blight	Aphids, thrips, mites, and leaf miner
5	Gladiolus	Fusarium wilt, and cucumber mosaic virus	Thrips, and spike caterpillar
6	Orchid	Crown rot, anthracnose, petal blight, and bacterial soft rot	Scale, aphids, thrips, shoot borer, mealy bug, red spider mite, and snail
7	Anthurium	Anthracnose, bacterial blight, and root rot	White fly and thrips
8	Lily	Bulb and scalar rot, foot rot, and gray rot	Tulip breaking virus, tobacco rattle virus, leaf bulb mites and aphids

6.3 Different Approaches to Alleviate Biotic Stress in Ornamentals

The floriculture trade is in full boom globally. Therefore, the major challenge in commercial floriculture is to produce quality planting material of international standards. Disease and pests' management strategies aim to prevent the establishment of diseases and pests in greenhouse, as well as to minimize the development and spread of the same. Control measures against virus and bacteria include detection at earlier stage, making production areas free from infected planting material and other precautionary methods to control the insect vectors are being used presently in floriculture (Powell and Lindquist 1992). Moreover, to improve disease management, other measures which are urgently needed include, preliminary screening of greenhouses, selection of Disease free (virus and bacteria) planting stock from the cultivation areas, weed removal, appropriate surveillance of insect-pest inhabitants and sensible use of pesticides. Moreover, the use of resistant varieties can be used as effective strategy for the alleviating biotic stress in floricultural crops. Conventional breeding strategies also play pivotal role in providing resistance against diseases. However, looking at the diversity of ornamentals and their susceptibility to variety of pathogens, the most challenging task is the identification of sources of disease resistance genes. Above that, it is very rare to find germplasm in ornamental plants with disease resistance. In case a germplasm with disease resistance gets identified

then it may require back-crossing generations for multiple times for the gene flow or transfer of a single gene into the desired selected floricultural crop. This practice is not very feasible and may also cause reduction of the resistance to disease as compared to the source material. Therefore, to achieve biotic stress resistance effectively, genetic engineering and recently emerging tools like gene editing can be used to modify plant defence against diseases. Moreover, this will minimize the use of pesticides/insecticides and other agro-chemicals for the insect pest and disease management and will improve both quality and quantity of flower crops in an eco-friendly manner.

6.3.1 Conventional Breeding

Most of the floricultural crops are domesticated and the most desired wish of ornamental plant breeders is improved disease resistance. As the cultivation of ornamental crops rely on regular breeding programs for biotic stress resistance, and such breeding programmes involves monocultures at large scale which ultimately make crop susceptible towards several diseases (Hayes et al. 1955). For improving crop against diseases, breeding has a vital role in selection of parents with desirable characteristics and producing offspring with desirable combinations. General steps in crop improvement involves creation of variation, selection, evaluation and release of new cultivar. In conventional breeding, the selection of methods and approaches for improving crop against biotic stresses primarily relies on the presence of sources that carry resistance to diseases. And more importantly it also depends upon reproduction system of crops (self-pollinating and cross pollinating). For self-pollinated species, breeding methods involves mass selection, pure line selection, population bulking, pedigree analysis, backcrossing, single seed descent, multiline, composite and heterosis, while cross pollinating species involves mass selection, recurrent selection and heterosis etc. As the whole marketability of ornamental crops depends upon their cosmetic appearance, so it is very important to control disease and pests in ornamental crops Mutation breeding as one of the conventional breeding method can also lead to the development of biotic stress resistance. This technique can be exploited when gene of interest/variability is not present in existing population. As chemical control is not always economical and long lasting, therefore breeding resistant varieties offer durable economical way out. But for the successful breeding program genetic variation in the form of wild germplasm or variation at species level should be available, so that it can be its successfully transferred to the next generation.

6.3.1.1 Heterosis

Heterosis is a common phenomenon among plants. It is the superiority of F_1 over its parents for growth, yield and others characters yield, earliness, growth vigor and stress tolerance in many plants. This term was first coined by Shull (1914). Nowadays

heterosis studies in ornamental crops are mainly resolute on yield and stress tolerance. Hydrangea is a temperate ornamental plant known for its large flower heads. The main reason for its yield loss is powdery mildew (PM) disease. However, resistance to PM disease was achieved in F1 hybrid resulted from cross between *Hydrangea angustifolia* and *Hydrangea macrophylla* (Kardos et al. 2009). Anthurium is a well-known cut flower valued for its foliage and coloured spathe. The major hindrance in its cultivation is bacterial blight disease which is caused by *Xanthomonas axonopodis* pv. *Dieffenbachiae* (Anais et al. 1998). But an interspecific cross between *A. andreaeanum* and *A. antioquiense*. resulted in germplasm of anthurium with improved resistance against bacterial blight (Anais et al. 1998; Elibox and Umaharan 2010). Likewise, Fusarium wilt is a major disease in carnation caused by *Fusarium oxysporum* f.sp. *dianthi*. Around 500 varieties were collected and crosses were made to finally obtain wilt resistant carnation plants (Mitteau 1987). Chrysanthemum, (*Chrysanthemum* × *morifolium*) as an important cut flower also suffers losses in the form of quality and yield due the disease named white rust, caused by *Puccinia horiana*. In a study, few resistant cultivars of chrysanthemum cultivars showed no macroscopic lesions upon infection with white rust. The resistance in such cultivars was regulated by a single dominant gene (De Jong and Rademaker 1986). So finding and identifying genes that are sources of resistance to pathogens can solve the problem to some extent. Gladiolus corm production is majorly hampered by Fusarium wilt. A resistant variety Dheeraj against Fusarium was developed by crossing between Watermelon Pink × Lady John. Similarly, Arka Shringar developed by cross between Mexican Single × 'Pearl' Double is found resistant against root-knot nematode which is a devastating pest.

6.3.1.2 Mutation Breeding

Conventional breeding involves developing resistant varieties against various stresses by simply crossing different varieties. But this is only possible when resistance is present in the germplasm but in the absence only way out to create variability is going for mutation. Use of mutation to create new variability in a population for a particular cause like against biotic stress is known as mutation breeding. Mutation breeding can be more advantageous in floricultural crops as it may change other important phenotypic floral attributes like flower color, shape and size, flower structure etc. (Ibrahim et al. 2018). Mutation is sudden heritable changes in phenotype of an individual or change in the DNA base pair. This term was first time used by Hugo de vries in 1900 by seeing changes in primrose. Individual showing such changes are known as mutant. Generally mutant genes are generally recessive to their wild or normal type. Mutations are generally lethal and very few are beneficial i.e. frequency is as low as 0.1% (Patil and Patil 2009). For mutation breeding, two types of mutagenic agents are used, that includes physical mutagens (gamma radiation, UV ray and X-ray irradiation) and chemical mutagens (colchicine, EMS, MMS, etc.). For physical mutagens LD₅₀ (lethal dose 50) is calculated or standardize beforehand, it is the dose that cause 50% lethality. Therefore, in order to get more desirable results less than

LD₅₀ dose should be used. Mutagens are used on seed, seedling, cutting or any other plant or in vitro plantlets. X-rays is most common mutagen used in case of ornamental plants. Mutation caused by using different types of agent is called as induced mutation and mutations occurring naturally in are known as spontaneous mutations. Mutation can occur in cell, tissue or organism. The frequency and types of mutations depends upon the amount of mutagen and duration of exposure to the mutagen rather than its type. The key in mutation breeding is to identify offspring with desirable changes. Mutant confirmation is a process of re-evaluation of putative mutants under controlled and replicated trails (Oladosu et al. 2016). Mutation breeding offers time saving in breeding program and it is mainly used to create new flower color and shape. In liliium, Fusarium rot occurs at early bulblet stage or in scales. Resistance against Fusarium has been reported in oriental lily with higher ploidy level using colchicine and mutant with high saponin content (Liu et al. 2011). In camation, two mutants named ‘Maiella-lonchabi’ and ‘Galatee-lonvego’ generated after gamma-ray irradiation showed high level of resistance to Fusarium wilt. Similarly, another gamma ray mutant Loncerda verified to be tolerant against Fusarium (Mitteau and Silvy 1983). Moreover, disease resistance against Alternaria was found in X-ray induced mutant lines of carnation cv. Mystere (Cassells et al. 1993). Another successful example of mutation breeding involves improved resistance to mildew in a mutant named “Herloom” of *Begonia elatior* hybrids cv. Schwabenland Pink, generated through fast neutron induced mutation (Mikkelsen 1975). James (1983) has reported a gamma ray mutant ‘Pink Hat’, in Floribunda rose which was found to be resistant to mildew. Nambisan et al. (1980) has also developed leafspot-resistant mutants of *Jasminum grandiflorum*.

6.3.1.3 Recurrent Selection

In a perspective of huge biotic stresses, there is pressing demand for plant cultivars with favourable gene frequency that are adapted to such conditions. To achieve this demand recurrent selection serves its role. As defined by its name recurrent selection involves re-selection generation after generation. This has significant role in maintaining genetic variability in the population. This is a cyclic selection for enhancing the chances of desirable/favourable genes’ frequency in a breeding population (Ramalho et al. 2005) or it is a variant of backcross where selection for better type is exercised in consecutive segregating progeny. This method is most suited to cross pollinated crops. This method was first time used in maize (Bolanos and Edmeades 1993). The concept of recurrent selection was first introduced by Hayes and Garber in 1919, and East and Jones in 1920 and in 1940, Jenkins named scientist described the method of recurrent selection. Later on, the term recurrent selection was coined by Hull in 1945. This method can be used for both Intra and inter-populations to advance the combining ability. Recurrent selection can be utilized to greater extent in case of heterozygous base population and cytoplasmic male sterile (CMS) population. Crosses between accession of *Lilium dauricum* (resistant) and *Lilium longiflorum* (susceptible) resulted in resistant offspring (Löffler et al. 1994). *Magnolia* ×

brooklynensis var ‘Yellow Bird’ has been developed by cross between *M. acuminata* var. *subcordata* and *M. × brooklyensis* var. *vamaria* found resistant against the pest known as Japanese long scale (<https://planthealthportal.defra.gov.uk/>). Similarly this method has been used in many other agricultural crops viz., for developing bacterial disease resistant lines in alfalfa (Barnes et al. 1971). Recurrent selection is most suitable for quantitative traits, or controlled by many genes where each gene has its own little effect on the expression of a particular character. Therefore, such breeding approach can be used against devastating disease like bacterial wilt whose resistance is usually governed by multiple genes.

6.3.1.4 Multiple Lines Breeding Approaches

Multiline varieties are the mixture of several isogenic lines having similar agronomic characteristics but having different genes for disease resistance. To make a variety commercially successful disease resistant variety is alone not sufficient along with resistance characters all other agronomical features (high yield, agronomic performance, and vase life) should also be present. Therefore, multiline breeding is a useful tool to develop composite varieties. But this method is still unutilized in case of ornamental plants (Table 6.2).

6.3.2 Molecular Approaches

6.3.2.1 Molecular Breeding

Ornamental plants are one of the important for commercial purposes as well as for improving human living environment. Previously these crops have been improved for various traits like color, flower size, scent, biotic and abiotic resistance. Biotic stresses (virus, insects, fungi and bacteria) are one of the main problems leading to the losses in ornamental crops globally. Therefore, different breeding strategies have been applied to develop new disease resistant cultivars of economically important ornamental crops. However, complex genome, lack of gene pools and limited genetic variation within gene pool are the major drawbacks which limits the application of conventional breeding for creating biotic stress resistance in floriculture crops. Being an economically important flower crop, Rose is used widely for various purposes such as medicinal, and cosmetics industry (Debener and Byrne 2014). In One of the most devastating disease of Rose is Powdery mildew which is caused by *Podosphaera pannosa*, spreads most commonly in the green houses. It has been found that the loss of the function of the *MLO* gene (susceptible Mildew Locus) can confer disease resistance against powdery mildew (Buschges et al. 1997) In rose, upon analysis of the allelic variants of *RhMLO* genes, about 10 alleles per gene were found. This analysis further demonstrates that out of 19 *RhMLO* genes, *RhMLO1* has the maximum number of sequences (23) whereas *RhMLO4* has lowermost number

Table 6.2 Status of biotic stress management through conventional approaches

Crop	Disease/Pest	Resistant/Tolerant variety	References
Rose	Black Spot	Hybrid Tea: Pride N Joy Floribunda: Sexy Remy, Rainbow Sorbet, Julia Child Grandiflora: Prima Donna	Hutabarat (2012)
	Cercospora leaf Spot	<i>Rosa wichuriana</i> , carefree Wonder Nozomi, Hansa	Hutabarat (2012)
	Spider Mites	Parfait, Lady Rose and Hildalgo	Grzeszkiewicz and Wiltaszek (1994)
		Rajhans, Apsara and Rose evening	Hole and Solunkhe (2005)
		Tinike, Skyline, Confittee, Grand Gala and First Red	Dhananjaya Kumar (2007)
Thrips	Landora	Gahukar (2003)	
Carnation	Bacterial Wilt	Super Gold × <i>Dianthus capitatus</i> line 91BO4-2	Onozaki et al. (1998)
	Fusarium Wilt	Arbel and Scarlette	Ben et al. (1997)
	Stem Rot	William and Vermillion Protuding	Guba and Ames (1953)
Gerbera	Foot Rot	H229/3, H134/8 and H147/5	Maria and Chis (2006)
	Powdery Mildew	Figaro, Marinila and Palmira	Kumar et al. (2013)
	Mites	Sirtaki, Rondona, fame and Blanca	Krips et al. (2001)
Gladiolus	Fusarium Wilt	Novalux and White prosperity	Dallavalle et al. (2002)
China Aster	Fusarium Wilt	Heart of France, Crego, Ostrich, Feather, stardust, American Branching, Roment, Powderpuff and Lapplator	Kratka and Duskova (1991)
	Nematode	IIHR-2 (highly resistant), Shashank, AST-5, IIHR-17 and IIHR-21 (resistant), Poornima (Moderately resistant)	Nagesh et al. (1999)
Marigold	Alternaria leaf spot	Golden Guardian and Doubloon	Beckerman and Lopez (2009)

(continued)

Table 6.2 (continued)

Crop	Disease/Pest	Resistant/Tolerant variety	References
Pansy	Cercospora leaf spot resistance	Bingo Red, Bingo Yellow, Crown Blue, Crown Golden, Crystal Bowl Supreme Yellow, Crystal Bowl True Blue, Dynamite Red, Dynamite Yellow, Majestic Giants Yellow, Sorbet Blackberry Cream	Beckerman and Lopez (2009)

of sequences (6). Moreover, in *RhMLO1*, *RhMLO2* and *RhMLO3* around 271, 161 and 337 single nucleotide polymorphisms (SNPs) were detected respectively. Thus, this study concluded that *RhMLO1* and *RhMLO2* are the potential susceptible targets for creating resistance against powdery mildew disease (Fang et al. 2021). Another important candidate gene (*Rpp1*) has been identified in rose which exhibits race specific resistance against powdery mildew. The molecular mapping of this dominant resistance gene (*Rpp1*) has been performed by using sequence characterized amplified region (SCAR) markers (Linde et al. 2004). Similarly, in roses, quantitative trait loci (QTLs) in genomic regions involved in the pathotype specific resistance were identified by utilizing combinations of 20 amplified fragment length polymorphism (AFLP) and 43 simple sequence repeat (SSR) primers. This may help in the understanding of the underlying molecular mechanisms involved in disease development and resistance in roses (Moghaddam et al. 2009, 2012). Gerbera is another commercially important and most popular cut flower among the ornamental plants. Due to its high demand, this crop need to be transported at long distances. High humidity during transportation at distant places leads to high chances of infection with fungus *Botrytis cinerea* in gerbera, ultimately result in the loss of quality of cutflower (Elad et al. 2016). However, in Gerbera, a genetic map using SNP markers developed from the expressed sequence tags was constructed. A total of 20 QTLs were observed in the parental maps which help in the understanding of the complex molecular mechanism of *Botrytis cinerea* (Fu et al. 2017). Another study confirmed that root-rot disease in gerbera lead to high expression of the tyrosine metabolism pathway genes such as *GhTAT*, *GhAAT*, *GhHPD*, *GhHGD* and *GhFAH*, thus suggested their possible role in infection (Munir et al. 2019). Among bulbous crops, lilies hold an important place. However, *Fusarium oxysporum* and *Lily mottle virus* (LMoV) are two major problems faced during lily's bulb production. However, some of the Asiatic lily hybrids were found to be resistant against the *Fusarium oxysporum* and *Lily mottle virus*. Using three different molecular marker systems (DArT, AFLP and NBS profiling) in an intraspecific Asiatic lily backcross population, a genetic map was constructed. QTL mapping using AFLP Markers showed four linkage groups for *Fusarium oxysporum* and one locus for LMoV. Thus such loci can be used for molecular breeding of lilies against fusarium wilt and viral disease (Van et al. 2001; Shahin et al. 2009). Similarly, about six putative QTLs in the genetic map of AA

population of liliium was identified, which conferred resistance against the *Fusarium oxysporum* (Shahin et al. 2011). Most of the pathogenesis related genes (PR) get upregulated by different stimuli such as fungal/ bacteria/virus. It has been found that the plant hormones such as salicylic acid (SA), methyl jasmonate (MeJA), gibberellic acid (GA₃), abscisic acid (ABA), and ethylene (ET) could induce the expression of the *PR10* (Pathogenesis related 10) gene family (Borsics and Lados 2002; Liu et al. 2003). In addition, compared with the susceptible variety of liliium, the expression of the *LrPR10* gene family including *LrPR10-2*, *LrPR10-4*, *LrPR10-5*, *LrPR10-6*, *LrPR10-7*, and *LrPR10-9* was strongly increased by *Fusarium oxysporum* in resistant liliium variety (He et al. 2014). In carnation, a genetic linkage map using RAPD and SSR markers was constructed and a quantitative trait loci (QTL) for resistance against bacterial wilt caused by *Burkholderia caryophylli* was mapped This will further help in improvement in carnation breeding program (Yagi et al. 2013).

6.3.2.2 Genetic Engineering

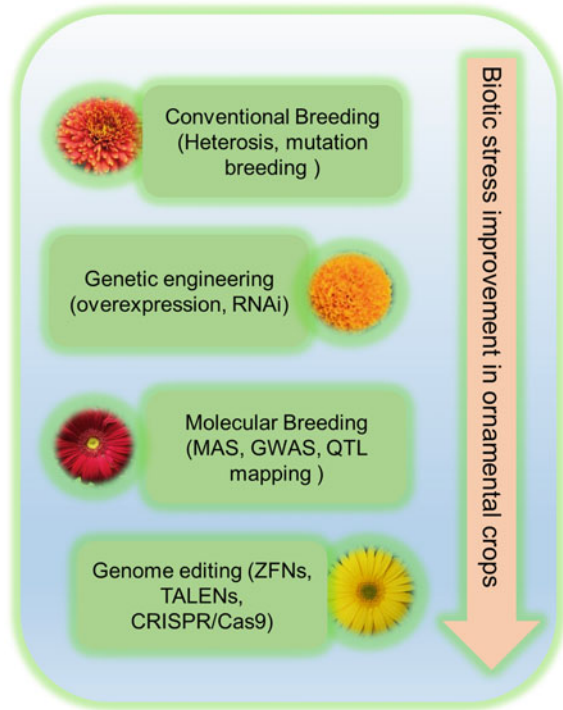
Genetic mapping for biotic stress resistance is relatively unusual due to the heterozygosity, complex genome and high ploidy level present in the ornamental plants. Nevertheless, recent advancements in the genome sequencing have provided information for further molecular breeding program for creating biotic stress resistance in the floriculture crops. Genetic engineering is a powerful tool that enables the improvement of the ornamental plants in response to various biotic and abiotic stresses. Moreover, introduction of gene of interest which are not existing naturally in the target plant is a key advantage of genetic engineering over conventional breeding (Azadi et al. 2016). Among floriculture crops, chrysanthemum is one of the economically important floriculture crops used as cut flower, pot flower, garden plant, also used as medicinal tea and cosmetic industry (Mekapogu et al. 2020). Chrysanthemums are severely affected by wide variety of biotic stresses such as fungal (leaf spots, gray mold, rusts, and powdery mildew) and insect (lepidopteran larvae). However, the use of large quantity of chemicals to control these diseases results in environment pollution, health problem and increase in the cost of production. Several efforts have been paid to make disease resistant varieties of chrysanthemum through genetic engineering. Previously a transgenic chrysanthemum was developed by transferring the *cryIAb* gene from *Bacillus thuringiensis* var. *kurstaki HD-1* (mcbt) and a modified gene sarcotoxin 1A from sarcophagi peregrine (*msar*) accumulate *cryIAb* soluble protein which confer strong resistance against the white rust and four species of lepidopteran larvae (Ichikawa et al. 2015). Similarly, *Agrobacterium* mediated transformed heterogeneously expressed *hpaGXoo* gene from *Xanthomonas oryzae* pv. *oryzae* confer resistance to *Alternaria* leaf spot and also accelerate the chrysanthemum development (Xu et al. 2010). In another case overexpression of rice chitinase gene in chrysanthemum exhibited increased resistance against *Septoria obesa* and *Botrytis cinerea* (Takatsu et al. 1999; Sen et al. 2013). The N-methyltransferase involved in the biosynthesis of caffeine stimulates the production of salicylates which results in the activation of several defense mechanisms in biotic stresses. In chrysanthemum,

overexpression of the N-methyltransferase genes such as *CaXMT1*, *CaMXMT1*, and *CaDXMT1* results in enhanced resistance against grey mold (*B. cinerea*) (Kim et al. 2011). Beside this overexpression of the linalool synthase gene *FaNES1* enhanced resistance against western flower thrips (WFT) in transgenic chrysanthemum (Yang et al. 2013). In case of rose, petals are main source of essential oil used in cosmetics, medicine, rose tea and perfume industry. However, its cultivation is affected by various pathogens and insect pests such as powdery mildew, black spots, botrytis blight, downy mildew, rust, *Rose mosaic virus*, Cercospora leaf spot, aphids, spider mites and thrips occurring widely and adversely affecting the yield and quality (Feng et al. 2010). In *Rosa hybrida* cv. carefree beauty, transformation of an antimicrobial protein gene *Ace-AMPI* by *Agrobacterium* showed increased resistance to powdery mildew (Li et al. 2003). Similarly the overexpression of rice chitinase gene in rose confer resistance against blackspot disease (Marchant et al. 1998). In another case overexpression of genes of particular antifungal proteins results in increased resistance to blackspot in transgenic rose (Dohm et al. 2001). In case of carnation, transformation of jasmonate methyl transferase gene through *Agrobacterium* the transgenic lines show the increase in resistance against Fusarium wilt (Ahn et al. 2004). Besides, the overexpression of the rice chitinase gene also confers the resistance against Fusarium wilt (Brugliera et al. 2000). In case gladiolous, overexpression of the chloroperoxidase gene from *Pseudomonas pyrrocinia*, and an exochitinase and endochitinase from *Fusarium venetanum* results in the resistance to *Fusarium oxysporum* f. sp. *gladioli* (Kamo et al. 2016). Transformation of a defective replicase or coat protein subgroup II gene from *Cucumber mosaic virus* through *Agrobacterium* in gladiolus shows enhanced resistance against *Cucumber mosaic virus* in transgenic lines (Kamo et al. 2010). Similarly, overexpression of the bacterial chloroperoxidase or fungal chitinase genes in gladiolus confers increased resistance against *Fusarium oxysporum* f. sp. *gladioli* (Kamo et al. 2015). In another study transgenic gladiolus containing synthetic antimicrobial peptide, *D4E1* gene exhibited enhanced resistance to *Fusarium oxysporum* f. sp. *gladioli* (Kamo et al. 2015). Genetic manipulation in the ornamental plants is challenged by the transformation protocol, transformation system, and regeneration of plant tissue. Although introducing foreign gene into ornamental plants through *Agrobacterium* is simple and efficient.

6.3.2.3 RNAi/Antisense

RNA interference (RNAi) is a sequence specific gene silencing which involves utilization of RNA for the sequence-specific suppression or degradation of the gene expression by double stranded RNA. The mechanism of RNA-silencing initiated with the cleavage of dsRNA by RNaseIII-like enzyme or dicer into short 21–24 nucleotides referred as small interfering (siRNA) or microRNA (miRNA) duplex. Then this double stranded siRNA binds with RNA-induced silencing complex (RISC) containing argonaute (AGO) protein that has siRNA binding domain and have endonuclease activity for cleavage of target RNA strand. The unwinding of RISC from the siRNA results in the formation of sense and antisense strand in an ATP

Fig. 6.1 Different approaches used for biotic stress improvement in ornamental crops



dependent reaction. While sense strand undergoes degradation, RISC containing the antisense strand undergoes binding by complementary base pairing with mRNA strand therefore degrade the mRNA and disrupt protein biosynthesis or translation (Vaucheret 2006; Liu and Paroo 2010). In chrysanthemum (*Chrysanthemum morifolium* Ramat cv. *White Snowdon*) transformation of *Chrysanthemum virus B* (CVB) coat protein gene sequence in sense, antisense and double sense orientation was conducted in transformation vector for induction of RNA interference. The resulted transgenic chrysanthemum plants obtained through *Agrobacterium* mediated transformation confer the resistance against *Chrysanthemum virus B* (Mitiouchkina et al. 2018) (Fig. 6.1).

6.3.2.4 Gene Editing

Due to ethical issues raised against the genetically modified (GM) crops such as horizontal gene transfer, allergic, carcinogenic, infertility in cattle etc. the crops modified through genetic engineering are not widely accepted. Genome editing is one of the emerging tools in modern biology which is rapidly expanding its possibilities and opening wide opportunities to introduce biotic stress resistance in ornamental crops. Due to high efficiency, high specificity and low off targeting makes it's globally

acceptance over the existing available transgenic technologies. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated 9 (Cas9) is a gRNA dependent DNA cleavage through restriction endonuclease (Cas9) mechanism in which cleave at target site in DNA by recognizing palindromic sequence (PAM) (Doudna and Charpentier 2014). The cleavage in the double strand of DNA undergoes repair mechanism through homologous and non-homologous manner, which results in the introduction of insertion or deletion at target site (Li et al. 2013). The first genome editing in chrysanthemum using CRISPR/Cas9 system was utilized to introduce mutation in the transgenic plant expressing the yellow–green fluorescence protein gene from *Chiridius poppei* (*CpYGFP*) (Kaboshi et al. 2017). Previously genome wide transcriptome analysis of *Lilium longiflorum* showed upregulation of about 38 *WRKY* genes, which suggested its role in providing resistance against biotic stress under both in vivo and in vitro conditions. Beside this, five genes *LIWRKY* such as *LIWRKY3*, *LIWRKY4*, *LIWRKY5*, *LIWRKY10*, and *LIWRKY12* have been identified which can offer strong resistance against leaf blight caused by *Botrytis elliptica*. This study set a background that *LIWRKY* genes can be potential target to create resistance to *Botrytis elliptica* in *Lilium longiflorum* through CRISPR Cas9 mediated gene editing (Kumari et al. 2021). Similarly, in case of rose RNA-Seq of rose petal analysis revealed that 188 transcription factor (TFs) such as *ERF*, *WRKY*, *bHLH*, *MYB*, *NAC*, *bZIP*, *TGA*, *HSF*, *GTE*, *MADS-box*, *MYC*, *zinc-finger*, and *NFYC* family members are upregulated in response to *B. cinerea*. Out of 188 TFs the *ERF* family, genes show highly upregulation in response to *B. cinerea*. This molecular knowledge about ERF gene family will also help to create target mutagenesis to develop resistance mechanism against *B. cinerea* (Li et al. 2020). Similarly in other ornamental crops such as carnation (Jo et al. 2015), *Lagerstroemia indica* (Wang et al. 2015), Tulip (Miao et al. 2016; He et al. 2019), orchid (Niu et al. 2016), rose (Moghaddam et al. 2014; Diaz-Lara et al. 2020; Chandran et al. 2021), gerbera (Fu et al. 2016; Bhattarai et al. 2020) etc. the availability of genomic and transcriptomic information provide base for the genome editing through CRISPR/Cas9 to create disease resistance varieties. Although availability of few tissue culture protocols for ornamental crops and regeneration protocol remain drawbacks for the CRISPR/Cas9 mediated gene editing (Table 6.3).

6.4 Conclusion

Commercial floriculture has immense significance in the diversification of agriculture and national economy. With the global boom in floriculture trade, production of quality flowers of international standards has become a major challenge in commercial floriculture. Biotic stresses (fungal, bacterial, insects and viruses) are one of the main factors for ornamental crop loss. It is therefore, need to develop such new varieties which are resistant to biotic stresses. However, development of new varieties through convention breeding is time consuming and are not genetically stable. Recent advancement in the next generation sequencing technology help us to obtain whole

Table 6.3 Status of biotic stress management through molecular approaches

S. No	Crop name	Targeted resistance	Target gene (s)	Molecular approach	References
1	Chrysanthemum	White rust and lepidopteran larvae	<i>cryIAb</i>	Overexpression	Ichikawa et al. (2015)
		<i>Puccinia horiana</i>	<i>CmWRKY15-1</i>	Overexpression and RNAi	Bi et al. (2021)
		<i>Chrysanthemum virus B</i>	<i>CVB</i>	RNAi	Mitiouchkina et al. (2018)
		<i>Septoria obesa</i> and <i>Botrytis cinerea</i>	<i>Rice chitinase</i> gene	Overexpression	Sen et al. (2013)
		Western flower thrips	<i>FaNES1</i>	Overexpression	Yang et al. (2013)
2	Rose	Powdery mildew (<i>Sphaerotheca pannosa</i>)	<i>Ace-AMP1</i>	Expression	Li et al. (2003)
		<i>Botrytis cinerea</i>	<i>RcERF099</i>	RNAi	Li et al. (2020)
		Powdery mildew	<i>RhMLO1</i>	RNAi	Qiu et al. (2015)
3	Petunia	Powdery mildew	<i>MLO1</i>	Overexpression	Jiang et al. (2016)
			<i>chitinase gene</i>	Overexpression	Khan et al. (2012)
		<i>Cucumber mosaic virus (CMV)</i>	<i>PhERF2</i>	RNAi	Sun et al. (2016)
		<i>Pseudomonas syringae</i>	<i>MKS1</i>	VIGS	Gargul et al. (2015)
4	Lilium	<i>Botrytis cinerea</i>	<i>RCH10 chitinase gene</i>	Overexpression	Gonzalez et al. (2015)
		<i>Botrytis elliptica</i>	<i>lre-miR159a</i>	Overexpression	Gao et al. (2020)
5	Sunflower	<i>Sclerotinia sclerotiorum</i>	<i>Oxalate oxidase (OXO)</i>	Overexpression	Hu et al. (2003)
		<i>Verticillium dahliae</i> and <i>Sclerotinia sclerotiorum</i>	<i>glucanase</i> and <i>chitinase</i>	Overexpression	Radonic et al. (2008)
6	Geranium	<i>Botrytis cinerea</i>	<i>Mannitol dehydrogenase (MTD)</i>	Overexpression	Williamson et al. (2013)
7	Carnation	Bacterial Wilt	<i>Oat Thionin Gene (Asthi 1)</i>	Expression	Seo et al. (2002)

genome sequence information which can be further utilized for the identification of genes and understanding of molecular mechanism related to biotic stresses among floriculture crops. Moreover, the identified genes involved in biotic stress can be used to produce transgenic varieties through genetic engineering. Recently, genome editing technologies have progressed rapidly and become one of the most important genetic tools in the implementation of pathogen resistance in plants. Therefore, this genome editing tool has potential to create desirable mutation at a particular location to create disease resistance ornamental crop varieties.

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