# From Lab to Bouquet: The Biotechnological Frontier in Modern Floriculture for sustainable and resilient flower farming


## Abstract

Modern floriculture faces unprecedented challenges in meeting the rising global demand for flowers while addressing environmental concerns and the need for sustainability. This paper explores the transformative role of biotechnology in revolutionizing flower farming practices, offering solutions for sustainable and resilient cultivation. The integration of biotechnological tools such as genetic engineering, tissue culture, and molecular breeding techniques has opened new avenues for enhancing the efficiency and resilience of flower crops. Genetic modification allows the development of varieties with improved traits, such as disease resistance, longer vase life, and enhanced stress tolerance. Tissue culture facilitates the rapid propagation of elite plant material, ensuring consistent and high-quality flower production. Furthermore, molecular breeding enables the precise selection of desirable traits, accelerating the traditional breeding process. The use of molecular markers for disease resistance, flower colour, and fragrance can streamline the development of new flower varieties tailored to market preferences. Additionally, advanced omics technologies, including genomics, transcriptomic, and metabolomics, provide valuable insights into the molecular mechanisms underlying flower development and responses to environmental stress. Biotechnological interventions extend beyond genetic improvements, encompassing sustainable cultivation practices. Precision agriculture, enabled by sensor technologies and data analytics, optimizes resource utilization, minimizing environmental impact. Smart irrigation systems, nutrient management, and pest control strategies contribute to the efficient use of resources and reduced ecological footprint. The biotechnological frontier in modern floriculture not only addresses current challenges but also positions the industry to adapt to future uncertainties. Climate change, emerging pests, and shifting market demands necessitate resilient flower farming systems. Biotechnology offers the tools to develop adaptable flower varieties and sustainable production methods, promoting long-term ecological and economic viability.



Keywords: resilience, molecular, genomics, flower, management


## Introduction

Biotechnology is a multidisciplinary field that involves the application of biological systems, organisms, or derivatives to develop, improve, or create products and processes for various purposes**[1]**. It encompasses a wide range of scientific techniques, including genetic

engineering, molecular biology, microbiology, and bioinformatics. The fundamental principle of biotechnology revolves around harnessing the inherent capabilities of living organisms at the molecular and cellular levels to address practical challenges and innovate in diverse sectors**[2]**. This can include applications in agriculture, medicine, environmental management, and industry. Biotechnology has played a pivotal role in revolutionizing various industries by offering solutions such as genetically modified crops, therapeutic proteins produced through biopharmaceuticals, and environmental remediation using bioengineering approaches**[3]**. The dynamic nature of biotechnology ensures its continual evolution, contributing to advancements that benefit society by improving efficiency, sustainability, and overall well-being.

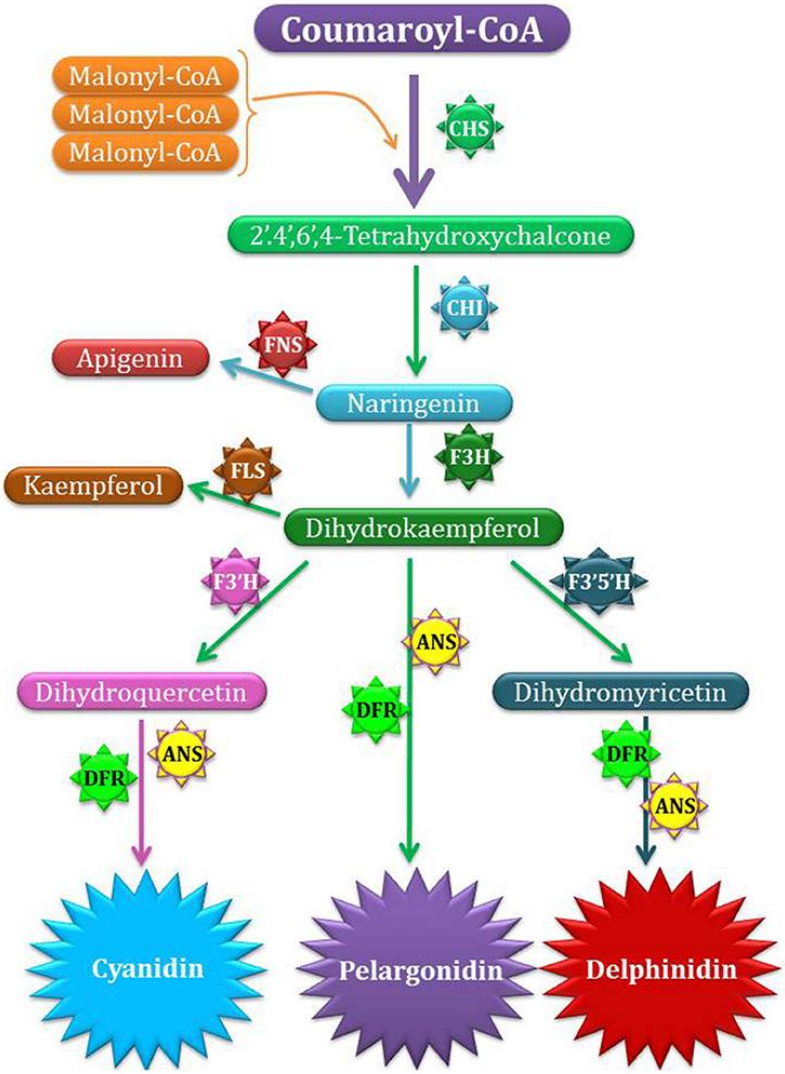


Fig 1. Environmental remediation pathway

Because ornamental plants are utilized in a broad variety of applications, including home gardening, professional landscaping, and the production of cut flowers, the horticultural sector has undergone a substantial transformation**[4]**. The distinctive characteristics of these

plants, which include increased anatomical qualities, floral colour, pigments, stress tolerance, and disease resistance, have led to their transformation into commodities that are traded on a global scale**[5]**. The traditional methods of breeding have been utilized extensively for the purpose of establishing new plant lines; however, these methods have a number of restrictions and downsides, such as a high level of heterozygosity. Genetic engineering (GE) and genome editing are two examples of techniques that have gained widespread acceptance in recent years as more practical approaches to overcoming the inherent challenges that are inherent to traditional methodologies**[6]**. The cultivation of genetically modified crops has reached its highest point, with the total area of GM crop production reaching 181.5 million hectares in 2014. Food and feed, increases in herbicide and pesticide tolerance, and improvements and enhancements to quality features for the industry are the primary aims of the growing contributions that the business sector and the government are making to the fields of biotechnology and genetic engineering**[7]**. The most significant advantage of utilizing genetic engineering is that it enables the introduction of genes from other species into ornamental plants. This opens the door for the introduction of genes that confer resistance to disease and tolerance to stress in ornamental plant species. In addition, genetic engineering can be used to modify plant traits such as flower architecture, colour, aroma, resilience to abiotic stress, and post-harvest life**[8]**. The current century is often regarded as the period of the bio-economy, which is driven by bioscience and biotechnology and is closely connected to the development of sustainable practices in fundamental aspects of agriculture, the environment, and the economy. Transgenic ornamental plants have the potential to become prospective benefits to growers and consumers due to their altered floral look, innovative colours, and increased smell**[9]**. Currently, work is being done to generate genetically modified flowers with a broad colour range and other characteristics. Plants that are grown for ornamental purposes are susceptible to a variety of challenges, including problematic sexual hybridization, high heterozygosity, high chromosome number, insufficient gene pool, and heightened sterility**[10]**. It is possible that genome-based alterations for flowers could result in the greatest possible benefits in a variety of circumstances. Today, efforts are being made to develop genetically modified flowers that have a variety of colours and other characteristics.

**Plants pigments and flowering pattern due to genetic engineering**

Traditional methods of plant breeding have been utilized to improve the attractiveness and efficiency of ornamental plants; however, these methods have limitations in terms of the gene pool and other features that have been documented in species that are sexually similar to one

another**[11]**. Over the course of the past two decades, biotechnology has been responsible for the creation of novel and unique characteristics in ornamentals through the adoption of genes from a variety of plant species. Floriculturists and other entrepreneurs of a similar nature are anxious to create new colour combinations for flowers, which are the primary factor that determines their beautiful appearance. Anthocyanins, flavonoids, carotenoids, and betalains are the primary pigments that are responsible for the hues of flowers respectively**[12]**.

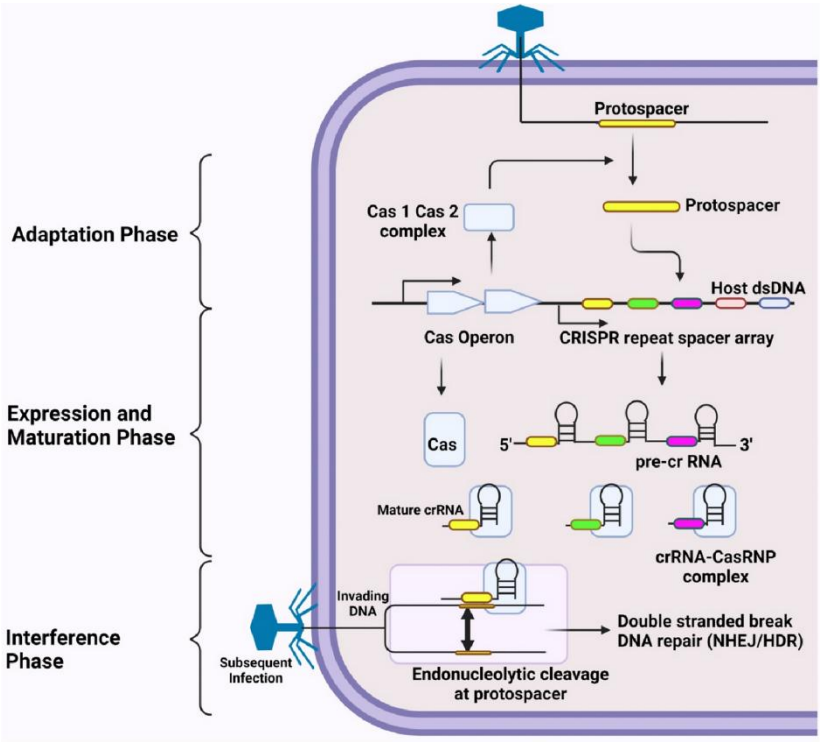


Fig 2. Plants pigments and flowering pattern due to genetic engineering

There have been documented instances of a number of different types of anthocyanins, the majority of which are based on six different types of anthocyanidin: cyanidin, delphinidin, peonidin, petunidin, malvidin, and pelargonan**[13]**. Among the anthocyanidins that have been described, three are considered to be main kinds. Delphinidin and its derivatives are typically found in large concentrations in blue flowers, but pelargonidin, which functions as an anthocyanidin base, is responsible for the vivid red hue of the blooms**[14]**. When it comes to the study of floral colour alterations that are produced by genetic engineering, ornamental plants such as petunia and torenia are believed to be significantly more suited. As a starting point for the generation of changed floral hues, changes in gene expression were searched out. One of the most effective methods for achieving differences in flower colour is to use mutations in genes that are responsible for distinct biosynthetic pathways**[15]**. A wide variety of flower hues, including orange, yellow, red, white, and pink, can be produced by

ornamental plants through the process of cross-breeding and mutation breeding. These alterations in hue are directly connected to the regulation of specific genes that are responsible for directing the synthesis of pigment precursors**[16]**. White flowers have been obtained from a variety of transgenic plants through the process of down-regulating genes that are responsible for the production of anthocyanin. For the purpose of determining the most appropriate promoter for the expression of the chrysanthemum gene, the EF1α promoter, which includes the elongation factor 1α protein, was coupled with the GUS gene and introduced into the *C. morifolium* cv. Ramat strain**[17]**. Chrysanthemum plants that had been transgenic displayed high levels of GUS expression as well as petal-based transgene expression, which was mediated by the 35S CaMV promoter.


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Fig 3. genetic engineering

The carotenoids and/or red malonylated cyanidin glucosides that are responsible for the various colours of chrysanthemum are the primary producers of these colours. There is a gene called CmCCD4a that is only expressed in the ray petals of the white chrysanthemum**[18]**. This gene is important for inhibiting the production and accumulation of carotenoids in the petals. When it comes to the research of flower colour variations, it has been observed that suppression technologies such as RNA interference (RNAi), co-suppression, and antisense mediated silencing are more useful**[19]**.

The lack of flavonoid 3′,5′-hydroxylase (F3′5′H) in chrysanthemum is the primary cause of the absence of anthocyanins that are based on delphinidin. Continued progress was achieved with the introduction of F3′5′H genes under the control of several promoters. Under the influence of rose chalcone synthase promoter, there was a significant increase in the accumulation of delphinidin cells**[20]**. An increase in the amount of delphinidin-based

anthocyanins in transgenic plants was demonstrated by the combination of many promoters and the F3′5′H gene.

The sepals of Lilly flowers may contain either anthocyanins, carotenoids, or both of these pigments. Following the transfer of several critical genes for the carotenoid biosynthesis pathway under 35S CaMV, Azadi et al. (2010) demonstrated the presence of a large number of pigments in transgenic Lilium calli and leaves for the first time**[21]**. An increase in the expression of Ph F3′5′H led to a change in color from pink to a barely noticeable purple. In contrast to expression alone, the synchronized expression of Ph F3′5′H and HyDfr resulted in the generation of a dark purple colour. Because gerberas do not contain any anthocyanins that are created from delphinidin, there has been a revival of interest in the cultivation of blue flowers through the application of genetic engineering**[22]**.

During the day, plants express their genes in a cyclical manner, which enables them to commence photoassimilation when the sun is shining or to produce odours in the evening when pollination agents are active**[23]**. For instance, the development of Petunia circadia makes it possible for an internal circadian clock to control the colour of the flower, which results in the colour of the flower typically changing around once every twelve hours. In order to achieve phenotypes that are significantly altered, genome editing with zinc finger nucleases and CRISPR-Cas systems can be of assistance**[24]**. In order to accomplish the desired colour change, it is not only necessary to over-express a certain gene that is involved in the production of important enzymes, but it is also necessary to pick an appropriate host that has a genetic background that is suitable. The selection process helps to reduce the antagonism of native routes with the enzyme that is being introduced, or it allows for the down-regulation of another pathway that is competing with the enzyme**[25]**.

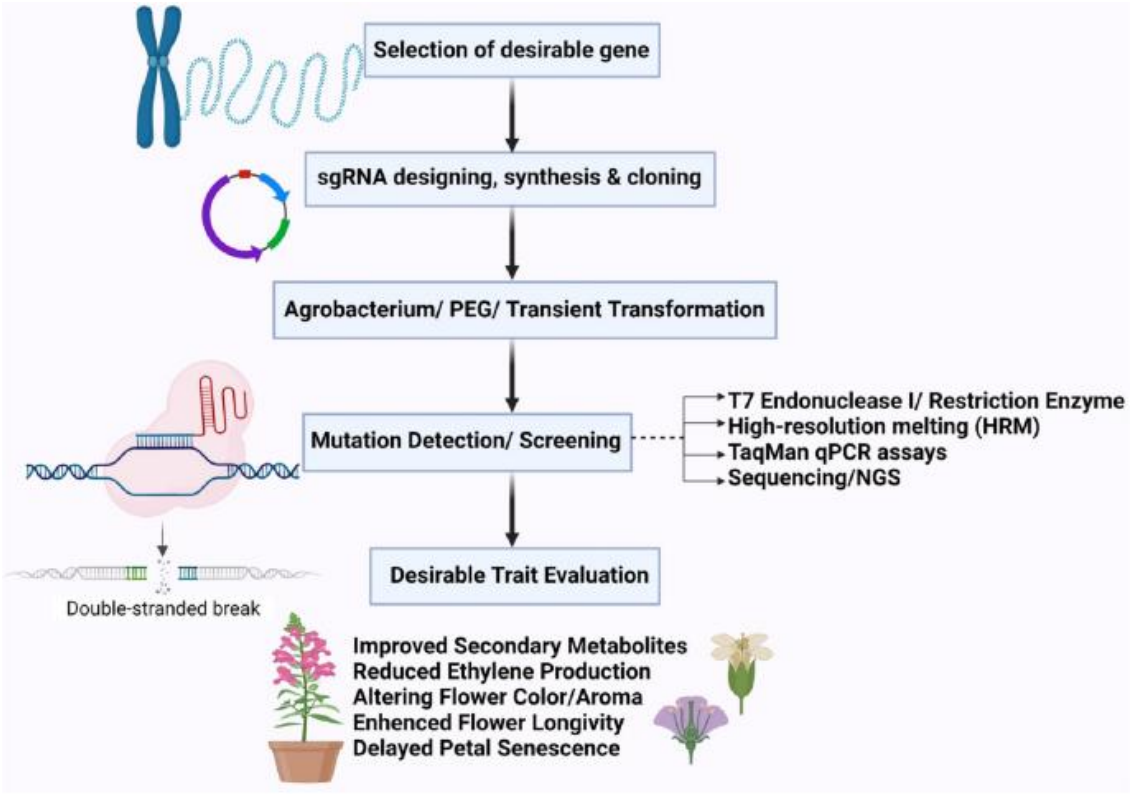


Fig 4. Genetic pathway

With the use of synthetic biology, flowers that have the potential to change their colour features have been produced. This demonstrates the ability to outline our world in a way that is appealing to everyone**[26]**. Flowering time is believed to be the most important factor in determining the performance of commercial plants. Inducing early flowering in plants allows them to better meet the requirements of humans by producing a greater quantity of flowers and fruits. Beneficial enhancements for the operation of floral characteristics can be achieved by the transformation of essential flowering-associated genes through genetic changes**[27]**. There are significant breeding goals in the field of ornamental plant breeding, including the reduction of blooming time through the development of early flowering cultivars or plants that are able to produce blooms for an extended period of time**[28]**. Because of their low production costs, they are extremely feasible for both producers and customers, such as the Chrysanthemum industry. There have been reports that provide a complete description of the effective introduction of genes to generate blooms in a relatively short amount of time. One such example is the MADS box gene family member known as AP1, which plays a role in the process of blooming growth**[29]**.

Within Siningia sp., transformation that was mediated by Agrobacterium provided support for the hypothesis that exogenous LFY over-expression encourages early flowering. The

development of flowers in transgenic Gloxinia plants that had either an overexpression of miRNA159 or a suppression of it resulted in either a late or an early flowering**[30]**. In addition, the expression of GAMYB, which is regulated by MiR159, plays a significant part in the regulation of the flowering time period in ornamentals. The CsFTL3 gene, also known as the Flowering Locus T like paralog, has been discovered to function as a photoperiodic flowering regulator in Chrysanthemum seticuspe blooming systems**[31]**. When Chrysanthemum is subjected to conditions of lengthy day length, the overexpression of this gene can cause blooming to occur. In order to facilitate gene expression in meristems and illustrate quantitative impacts of bulb vernalisation on flowering, RNA sequencing has been employed as a method. Flowering locus T, often known as FT, is the primary integrator of a number of flowering genes that allow plants to react to a variety of cues, including light, temperature, and others. Through the upregulation of flowering genes like AP1, Lfy, and SOC1, it lends support to the process of flowering**[32]**. When VcFT-Aurora was overexpressed in transgenic blueberry plants, it resulted in early and continuous flowering in both in vitro shoots and greenhouse plants. The final conclusion of the VcFT-OX experiment is the differential expression of 110 pathway genes for five phytohormones**[33]**. This finding suggests that phytohormones may play a function in the regulation of plant growth and development as signals. Despite the progress that has been made in the management of flowering time in crops through the over-expression of genes or the inhibition of gene activity, there is still a great deal of work to be done in order to obtain a wide variety of transgenic flowers that are available for purchase**[34]**.

**Engineering, as well as the anatomy and morphology of flowers**

When it comes to the production of innovative cultivars for ornamental plants, which are grown for the purpose of beautifying, embellishing, or improving human habitats, floral anatomy and morphology, as well as engineering, are extremely important tools**[35]**. On the other hand, due to the large inflorescence variations that exist between plant species, the molecular mechanisms that are responsible for creating flower patterns in the meristem have remained a secret. Cross-breeding techniques that are considered conventional cannot be utilized in order to determine the genes that are responsible for flowering patterns**[36]**.

The numerous actions of genes and the different ways in which they are expressed reflect the range of responses that plants have. An example of this would be the overexpression of the tobacco phytochrome b1 gene in chrysanthemum, which resulted in the creation of plants that were smaller in size and had bigger branch angles**[37]**. Subsequently, the introduction of the Arabidopsis GA insensitive gene led to a reduction in the height of the chrysanthemum plant.

By transferring the CAG gene into C. morifolium in an antisense orientation, it was shown that the repression of the CAG gene causes the gynoecium and androecium to transform into tissues similar to those of the corolla. However, the rate of transformation was very slow, and it only resulted in a change in the phenotypic for flower shape**[38]**.

A potential technique for the creation of dwarf chrysanthemum variations is the collective silence of the DmCPD and DmGA20ox genes. GRAS TF is responsible for limiting the formation of lateral branches at the same time**[39]**. The D27 gene, which is involved in the production of strigolactone, has been shown to exhibit significant tillering while also exhibiting a dwarf phenotypic appearance. Cloned DgD27, which was derived from the expression of D. grandiflorum in Arabidopsis, presented a novel strategy for the research and production of chrysanthemum cultivars that have a lower number of tillers**[40]**. When compared to natural kinds, transgenic plants that included the 35S::L MADS1-M gene from the lily also produced a greater number of flowers from leafy branches. Additionally, transgenic Lisianthus flowers exhibited a change in floral structure, which included the transformation of the second whorl of the petals into structures similar to sepals and the deformation of the third whorl of the stamens**[41]**.

In addition to having a significant economic impact, floral smells play an important role in the reproductive process of plants. It is essentially the case that they enhance the visual qualities of ornamental plants. Terpenoids, phenylpropanoid/benzenoid combinations, and aromatic amino acids are the three categories that contain a significant number of floral fragrance volatiles**[42]**. Flowers create a large variety of particular metabolites, such as hormones to trigger or suppress signalling cascades, fragrant volatiles to guard against herbivores or diseases, and fragrant volatiles to attract pollinators. Flowers also produce a wide range of metabolites**[43]**.

It is possible that an increase in the production of floral-specific metabolites could perhaps contribute to the detection, isolation, and identification of chemicals, as well as the enhancement of flower attributes such as scent and coloration. Researchers have succeeded in locating a number of genes that influence scent, despite the fact that the biochemistry of floral scents is still relatively new**[44]**. Enzymes that directly catalyse the synthesis of volatile chemicals are encoded by just a small number of smell genes, but not all of them. The use of genetic engineering to manipulate fragrance genes has shown that it is possible to successfully implement this technology for the purpose of increasing the potential of floral scents being produced**[45]**. There are a number of transcriptional factors (TFs) that play a significant role in the production of specialized metabolites for smell biosynthesis. These

metabolites are not exclusively dependent on interactions between enzymes. In recent years, there has been a significant amount of research conducted on the transcriptional regulation of fragrance biosynthesis pathways**[46]**. This research has led to the discovery that many transcriptional factors play important roles in the regulation of scent emission. Although just a small number of TFs that are involved in the regulation of scent emission have been identified, master regulators that govern the generation of a wide variety of volatile compounds and the metabolic pathways that are linked with them have not yet been found**[47]**. As a result of recent developments in the identification of genes and the enzymes that they produce, which are involved in the manufacture of volatile substances, metabolic engineering has been stated to be exceedingly accessible. With regard to the enhancement of plant defence and the enhancement of the scent and perfume value of flowers and fruits, there have been notable accomplishments recorded**[48]**. In conclusion, genetic engineering (GE) for the purpose of altering flower smells has a large amount of promise; nevertheless, the findings of the research also reveal the difficulties that are the result of our inadequate knowledge of the metabolic processes that are responsible for the odours and how they are regulated**[49]**.

**Shelf life and stress management through biotechnology**

There is a considerable relationship between the biotic and abiotic stresses that plants experience and their growth and output. The absence of sufficient resistance genes makes it difficult to breed ornamental plants that are resistant to the effects of stress. Recently, biotechnological strategies have been developed to confer resistance against various challenges, including as drought and disease attack. Various tactics have been produced using biotechnology**[50]**. When compared to natural plants, transgenic ornamental plants have demonstrated a substantially higher level of resilience to biotic stressors. The growth and yields of plants can be greatly hampered by infections such as fungi, viruses, or bacteria, which can ultimately result in a drop in the quality of ornamental goods**[51]**. In order to strengthen resistance to powdery mildew illness, it has been discovered that transgenic roses have antifungal genes. These genes include class II chitinase and type I RIP. A decrease in the disease index and the occurrence of Alternaria leaf spot can be observed in chrysanthemum when the PGIP gene from *Prunus mumei* is transferred to the plant. The environmental circumstances are an extremely important factor in the survival of plants**[52]**. The conditions that are now present in the environment have a direct correlation with the prevalence of pathogens and the spread of their infections. Several genes derived from a wide variety of sources have been investigated and found to play an important function in plant life

when it is subjected to stressful situations**[53]**. The fact that the targets of these genes that are related to biotic or abiotic stress also participate in a number of cellular responses and metabolic processes is indicative of the wide range of gene functions that are involved in the fight against abiotic stress. The expression of the cry1Ab gene in chrysanthemum has been demonstrated to have a substantial correlation with insect resistance. On the other hand, the transformation of the LLA gene in transgenic chrysanthemum plants by Agrobacterium has been shown to increase resistance to aphids**[54]**. The stable expression of many genes in ornamental plants such as lilies has demonstrated encouraging outcomes whether the plants are grown in either an outdoor or greenhouse environment. Transgenic Lily plants have been shown to exhibit high levels of expression of genes such as bar and nptII, regardless of the environmental circumstances they are exposed to. In conclusion, the application of biotechnological methods in the field of ornamental plants has demonstrated encouraging outcomes in terms of conferring resistance against biotic and abiotic challenges**[55]**.

A significant number of factors, including nutrient acidity, nutrient imbalance, water scarcity, and saline soil, are fundamental restrictions that significantly restrict plant output and other characteristics. In order to generate abiotic stress-tolerant cultivars, conventional plant breeding and genetic engineering techniques are utilized**[56]**. These techniques include the utilization of transcription factors such as ZIP, WRKY, and NAC, which play an essential role in the overall response of plants to stress conditions such as drought and high temperature. In addition, ornamental plants display a variety of reactions when subjected to abiotic challenges, such as the ability to tolerate cold stress, frost stress, and salt stress. The field of genetic engineering presents an unparalleled opportunity for the enhancement of plant characteristics, notably in the realm of decorative plants**[57]**. This is because characteristic traits such as resistance to environmental stress and disease can be preserved in transgenic cultivars, which also result in an expanded product variety. One of the most difficult challenges that researchers face is the prospect of extending the shelf life of ornamental plant products while preserving their features, which may include aroma, taste, and other attributes**[58]**. There is a possibility that transgenic ornamental plants could improve the lifetime of their leaves and flowers. Additionally, in order to extend the amount of time that cut flowers can be stored, they are typically treated with a variety of chemicals. The goal of achieving an increased vase life has been accomplished through the utilization of a variety of biotechnological approaches**[59]**. In order to enhance the vase life of fruit, it has been discovered that reducing the amount of autocatalytic ethylene that is produced by suppressing genes that are involved in the ethylene production pathway, such as ACO (ACC oxidase) or

ACS (1-aminocyclopropane-1-carboxylic acid synthase), is particularly beneficial**[60]**. Carnation plants that have been transgenic and display delayed petal senescence have been created by inhibiting the ACO gene, which is responsible for downregulating the production of ethylene. Enhancing the shelf life of a product can be accomplished by either preserving its resistance to ethylene or by suppressing the genes responsible for ethylene biosynthesis**[61]**. The presence of success in Oncidium and Odontoglossum has been observed by the mutation of the ethylene receptor gene. Experiments employing ACS or ACO genes have been carried on by a variety of research groups in a counterintuitive manner with the purpose of extending the shelf life of items derived from ornamental plants**[62]**. Ethylene-induced fruit ripening can be modulated by transcriptional regulators such as ERFs, which stand for ethylene response factors of the plant. In the future, it is hoped that transgenic plants with lower ethylene sensitivity, like chrysanthemum, will have increased vase life**[63]**. The reduced leaf senescence that has been observed in transgenic D. grandiflorum has been shown to be of great benefit. This deficiency, on the other hand, brings to light the necessity of a tissue-specific expression gene of genes that regulate certain essential processes. Moreover, the endogenous production and distribution of plant growth regulators including IAA, iPA, and ABA during the process of floral induction and initiation may make it easier to better plan the harvest schedule in order to achieve maximum bloom uniformity and improvement in the quality of ornamentals**[64]**.

**Case study of coloured carnation and roses**

Rose plants cannot produce true blue colour flowers due to the lack of flavonoid 3', 5'-hydroxylase enzyme and delphinidin involved in flavonoid biosynthesis pathways. In 1991, Florigene Company isolated the blue gene from petunia and patented it in 1992. Incorporating the petunia gene into a carnation plant produced blue flowers, but this technique did not yield good results**[65]**. In 1996, Florigene Company developed the Mauve-coloured carnation variety'moondust', the first genetically modified carnation variety in the commercial market. Another purple variety,'moonshadow', was developed by Florigene Company. They also incorporated both F3'5'h and Dfr genes into Dfr deficient white carnation plants for transgenic violet colour carnation flowers**[66]**. The genetically modified rose 'Applause' was commercially released and marketed in 2009 in Japan. The demand for blue roses was high, with prices ranging from $22 to $33 per stem. Black roses, found in Turkey's village of Halfeti, have been discovered due to their unique nutritional content in the soil from the Euphrates River. These flowers bloom twice a year in spring or spring, survive within 15 days, and cost 320 rupees**[67]**.

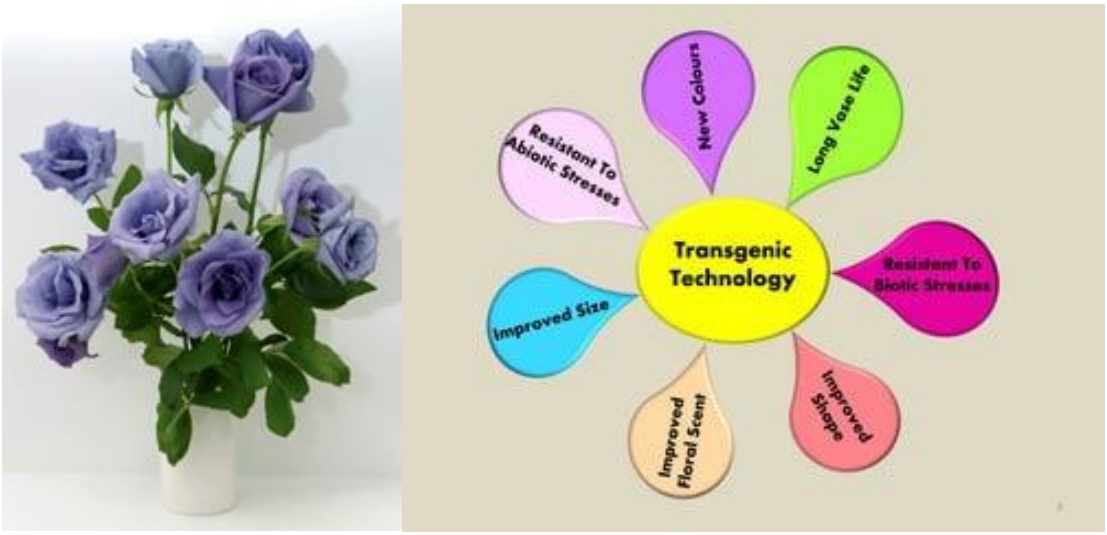


Fig 5. Transgenic technology

**Yellow and red orange colours flowers**

The presence of flavonoids and anthocyanins is essential for defining the colour of flowers, and the application of genetic engineering in floriculture has the potential to produce novel flower hues. Flower pigments known as aurone flavonoids are responsible for the vivid yellow colour of blooms produced by Antirrhinum majus and Dahlia variables**[68]**. The overexpression of the AmAS1 gene, on the other hand, may result in the failure to produce aurones, which are the pigments that give transgenic plants their characteristic yellow hue. It is possible for transgenic plants to generate yellow blooms through the co-expression of the chalcone 4'-O-glucosyltransferase (4'CGT) and AmAS1 genes, as well as through the down-regulation of the anthocyanin biosynthesis pathway through the use of RNA interference**[69]**. Petunia is capable of producing cyanidin and delphinidin derivatives, however it is unable to make pigment pelargonidin compounds because the Dfr gene expressed in petunia is substrate specific. The production of purple to white or purple to red flowers in commercial varieties of Petunia hybrids was successfully accomplished by suppressing endogenous flavonoid biosynthetic genes and expression of heterologous genes or combinations of both genes**[70]**. Flavonoids are responsible for the colour of the buds and flowers of the petunia plant. The production of a large number of self-sufficient transgenic lines, the selection of stable phenotypes, and the maintenance of tissue culture for the purpose of future multiplication are all necessary steps for commercialization**[71]**.

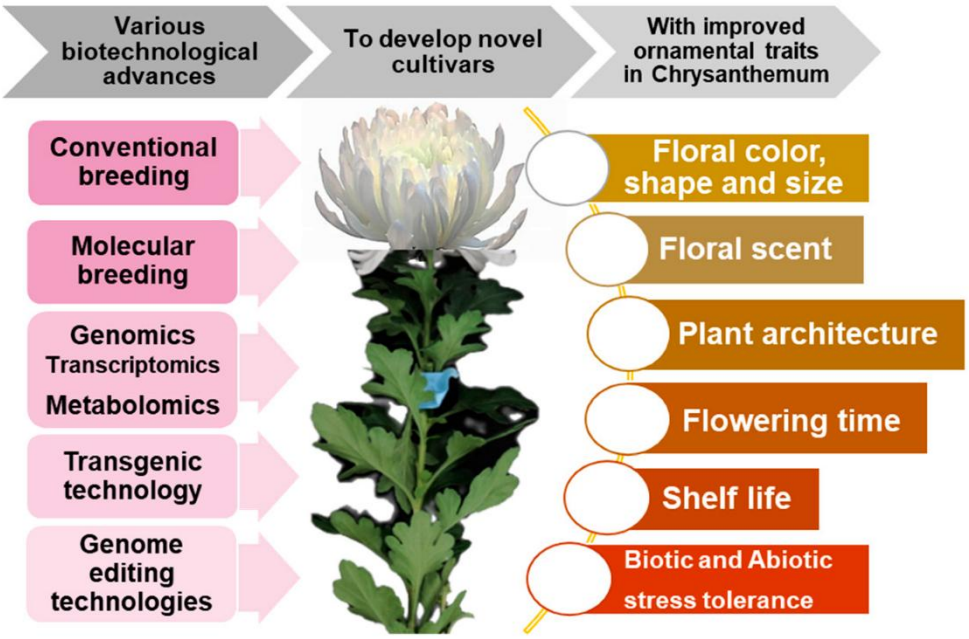


Fig 6. Biotechnological advances

**CRISPR/Cas9 Genome Editing in floriculture**

Over the course of the last three decades, there has been a significant amount of progress made in the field of specifically modifying the genomes of plant organisms. Prior to the deployment of the CRISPR/Cas9 technology in 2013, sequence-specific nucleases like as zinc finger nucleases (ZENs) and transcription activator-like effector nucleases (TALENs) were applied in the process of plant genome editing**[72]**. In the realm of genome editing technologies, there are two distinct categories: RNA-guided systems, which encompass CRISPR/Cas9 and targetrons, and DNA-based-guided systems, which encompass structure-guided endonucleases (SGNs), peptide nucleic acids (PNAs), and triplex-forming oligonucleotides (TFOs)**[73]**. Ribonucleoproteins (RNPs) that have been pre-configured with the CRISPR/Cas9 system make it possible to change the genome of plants without using DNA. Because of this, there is no longer a need for codon optimization or highly specialized regulators in order to achieve expression in host cells. A targeted gene editing technique is made possible by the direct delivery of CRISPR/Cas9 RNPs to the protoplast system. This technique paves the way for the production of a genome that has been edited in plants but does not contain any DNA**[74]**. It is possible for Cas9 RNPs to break the target DNAs not long after the transfection has been performed, and the DNAs are then swiftly eliminated within the cells. This leads to a significant decrease in the number of unintended modifications that take place in locations that are not the intended targets. Because they do

not involve the insertion of transgenes, preassembled CRISPR/Cas9 ribonucleoproteins can also be utilized to circumvent the regulations governing the distribution of genetically modified organisms as cultivars**[75]**.

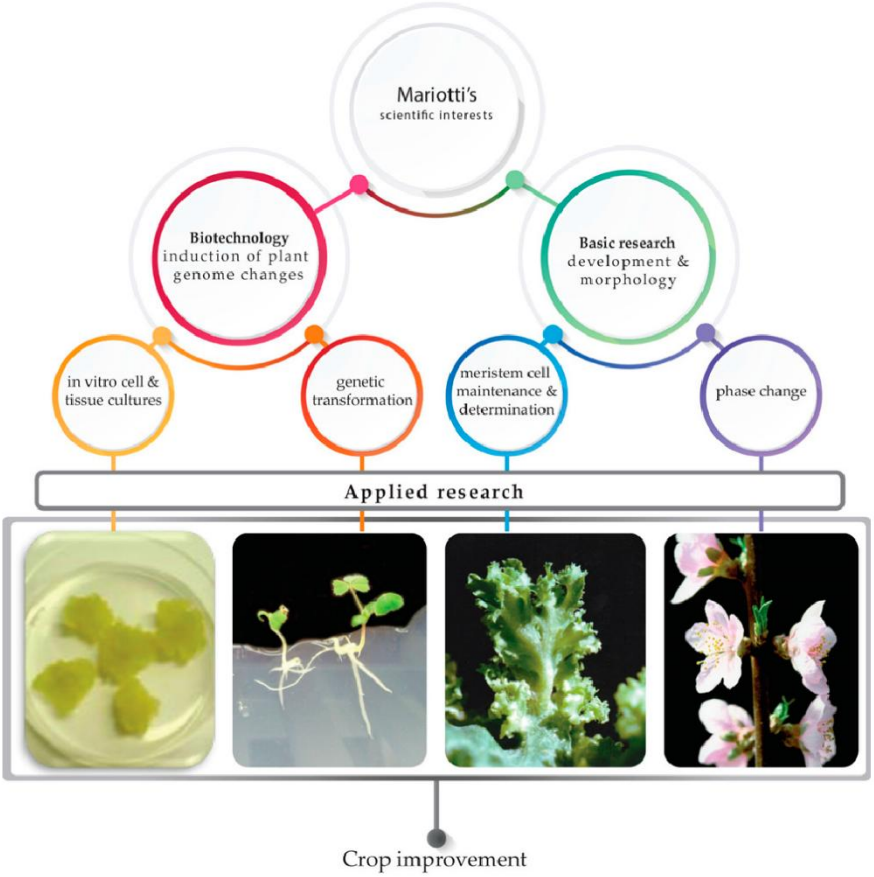


Fig 7. Crop improvement

When it comes to the design of gRNA, one of the most significant concerns is the potential for off-target effects. These effects are caused by unanticipated breakage at genomic areas that are similar to the sequences that are being targeted. In order to anticipate potential off-target sites, there are a few online off-target prediction tools that can be utilized**[76]**. The Burrows-Wheeler Alignment Tool (BWA), Bowtie, CCTop, and Cas-OFFinder are a few different examples of the applications that fall under this category. The most prevalent application of CRISPR/Cas9 technology in plants has been to remove genes, particularly in plants that are produced for their aesthetic value**[77]**. This has been the case up to this point. It is possible that the corrected alteration that was formed via the process of Mendelian segregation will be accurate and will be able to be handed down to following generations. On the other hand, the database of the International Service for the Acquisition of Agri-biotech Applications (ISAAA) only has information about three species of transformed and genetically modified (GM) ornamental plants that have been approved as biotech or GM

crops. Consequently, this suggests that there have been a relatively limited number of regulatory permits issued for plants of this kind**[78]**. Numerous genetically modified crops, both edible and inedible, are still in the research phase or in restricted field trials and are ready for commercialization. This is despite the fact that there is significant opposition from farmers and non-governmental organizations (NGOs) who are concerned about the safety of genetically modified crops and their effects on biodiversity. A defensive mechanism against phages that infect the organism, including plasmid DNA, has been shown to be the CRISPR/Cas9 system**[79]**. This system is a component of the immune system in bacteria and archaea, and it has been discovered that it is utilized by these organisms. This mechanism is responsible for the acquisition of phage-derived spacer sequences, which give protection against re-infection. In addition to functioning as a repository for previous infections, this mechanism is also responsible for the acquisition of these sequences. A collection of genes that are situated in close proximity to the CRISPR array are responsible for encoding the Cas9 proteins. Interference, adaptation, and the generation of CRISPR RNA (crRNA) are the three steps of the process that are controlled by these genes. They have the responsibility for controlling all three processes**[80]**.

During the process of adaptation, it is possible for bacteria to incorporate fragments of external DNA into their own genomes in order to create a memory of the infection. This is done in order to enable the bacteria to remember the infection. Through a process that involves translating the CRISPR array into two short RNAs, known as crRNA and trans-activating CRISPR RNA (sometimes referred to as "tracrRNA"), memory can be kept. The interference apparatus is able to recognize a particular place within the target nucleic acids after infection**[81]**. This is accomplished by the utilization of complementary base pairing, which ultimately leads to target cleavage that is catalysed by the Cas9 enzyme. Specifically, the CRISPR/Cas9 defensive mechanism is comprised of three separate phases. The stages that are being discussed here are known as interference, adaptation, and the creation of CRISPR RNA. During the development phase of the adaptation cycle, the Cas1–Cas2 complex is the one that is accountable for inserting a protospacer that is derived from viral DNA that has invaded the CRISPR array of the host. Conversion of the CRISPR array into a long pre-crRNA is accomplished by the process of transcription. Afterwards, Cas9 proteins or cellular RNases are responsible for transforming this pre-crRNA into mature crRNA expression. During the interference phase, mature crRNAs make use of complementary base pairing in order to recognize the foreign DNA that is invading the cell. This enables them to

drive Cas9 nucleases to the foreign DNA that is pertinent to the process that is being carried out**[82]**.

Endonuclease is the enzyme that is responsible for cleaving DNA in a specific spot within the cell. This cleavage can lead to homology-directed repair (HDR), non-homologous end joining (NHEJ), or microhomology-mediated end joining (MEEJ). HDR, which stands for high dynamic range, is a genetic modification that enables repair templates to be modified with a higher degree of precision**[83]**. The MMEJ method of repair is a technique that involves the combination of insertions and deletions, as well as the implantation of micro homologous sequences into damaged ends prior to joining. This technique is prone to mistakes. It would appear that non-homologous end joining (NHEJ) is the most prevalent kind of double-strand break (DSB) repair, and it is primarily responsible for DSB repair in somatic cells. A technique known as non-homologous end joining (NHEJ) is used to repair double-strand breaks (DSBs), which frequently leads to the loss of genes or the degradation of protein function. In addition, the utilization of chemically stabilized double-stranded oligodeoxy-nucleotide donors (dsODNs) with 5-phosphorylated ends is an additional technique that can be utilized to promote targeted insertion through the utilization of NHEJ**[84]**.

**Applications and approaches in CRISPAR technology in floriculture**

Betalains, carotenoids, and flavonoids are the primary components that are responsible for the colour of flowers, which is an essential aspect of the commercial flower production process. The suppression of floral pigmentation has been accomplished by a variety of strategies for genetic alteration, such as CRISPR Cas9-mediated mutagenesis, which has resulted in the generation of flower hues that are desired**[85]**. Watanabe et al. employed CRISPR Cas9-mediated mutagenesis to adjust colour in higher plants. They did this by changing the carotenoid cleavage dioxygenase (CCD) gene in Ipomea nil, which resulted in plants with pale yellow petals (55.5% of the total). Because of a genetic difference in flavone 3-hydrolase (F3H), which encodes the essential enzyme for flavonoid production, Nishihara et al. were able to employ the CRISPR/Cas9 system to identify colour alterations in *Torenia fournieri*. These modifications ranged from blue to white and were roughly eighty percent significant**[86]**. Su et al. made the observation that the aberrant expression of TfCYC2 or TfRAD1 disrupted the asymmetric corolla pigmentation pattern, which led to flowers that were severely dorsal. Through the use of a CRISPR/Cas9 construct that targeted PDS, Zhang et al. were able to modify petunias and produce an albino phenotype that ranged from 55–87%. The researchers Tasaki et al. focused their attention on genes in the Japanese gentian, such as anthocyanin 5/3'-aromatic acyltransferase (Gt5/3'AT), anthocyanin 5-O-

glycosyltransferase (Gt5GT), and anthocyanin 3'-O-glycosyltransferase (Gt3GT), and came to the conclusion that glycosylation is necessary for the development of blue blooms**[87]**. Petunias with a pale purple-pink flower hue were generated by Yu et al. by the creation of a mutant line of petunias that contained mutations across both F3H genes. A CRISPR/Cas9-based system was developed by Chib et al. for the purpose of facilitating future research improvements in the saffron (*Crocus sativus L.*) plant. Zhang et al. presented evidence for redefining the role of DPL in Petunia plants. They demonstrated that the disappearance of the vein-associated anthocyanin pattern above the abaxial surface of the flower bud was caused by a mutation at DPL that was mediated by CRISPR/Cas9, but not at the corolla tube venation**[88]**. This suggests that DPL did not have any influence over the development of the corolla tube venation.

In order to eliminate flavonoid 3'-hydroxylase (F3'H) from red blooming poinsettias (*Euphorbia pulcherrima*) cultivar 'Christmas Eve,' Nitarska et al. applied the CRISPR/Cas9 system. They anticipated that the plants would have orange bracts and a significant concentration of pelargonidin. It has been suggested that the enzyme F3'H, which encodes a key enzyme in the flavonoid/anthocyanin production pathway, could be a possible target in floral colour engineering**[89]**.

Many flowering plants exhibit a decrease in floral lifetime as a result of increased ethylene production, which is a fundamental trait of flowering plants. Flower longevity is an essential characteristic. Numerous studies have demonstrated that reducing the expression of the EIL1 and EIL2 genes can lengthen the lifespan of flowers; however, this has not been associated with any phenotypic alterations. In petunia, the enzyme known as 1-aminocyclopropane-1-carboxylate oxidase (ACO) has been the focus of researchers' efforts to cut down on the manufacture of ethylene**[90]**. In addition to having a longer blossom lifespan, PhACO1-edited mutant lines produced a much lower amount of ethylene. Additionally, it is believed that ethylene plays a significant role in the regulation of seed germination. This is supported by the fact that three unique petunia mutants exhibited a reduced germination percentage, a delayed germination time, and seedling growth in comparison to wild-type plants**[91]**. It is essential to reevaluate the ethylene production method whenever there is a modification made in order to preserve the quality of the flowers after harvest. Genome editing using the CRISPR/Cas9 system has the potential to modify a variety of floral properties for financial gain. On a global scale, orchids, which belong to the family Orchidaceae, hold a large amount of commercial significance. On the other hand, there is a paucity of genome sequencing data available for orchid species species. Chrysanthemum, the yellowish-green fluorescent protein

gene (CpYGFP) discovered in *Chiridiuspoppei*, and the protoplast system of *Petunia hybrida* have all been subjected to the use of CRISPR/Cas9 technology**[92]**.

Scientists are now able to manufacture desired modifications in plants willingly thanks to the CRISPR/Cas9 system, which has developed into a flexible and cutting-edge tool. This enables the creation of plant mutants. In contrast to conventional genetic methods, which necessitate intensive breeding cycles, CRISPR allows for the modification of a desired characteristic in a manner that is site-specific within a few generations. It may be concluded that the CRISPR/Cas9 system has developed into a versatile and cutting-edge technology that enables scientists to manufacture desirable modifications in plants freely, hence enabling the creation of plant mutants**[93]**.

Fig 8. Plant mutants

**Futuristic scope**

Plants that are grown for ornamental purposes and have huge genomes, polyploidy, and a high number of chromosomes provide difficulties for traditional breeding techniques. A greater number of cultivars that possess outstanding qualities, such as flowering promotion, floral longevity, colour spectrum, scents, and innovation in flower structure, are required by the floriculture business**[94]**. The CRISPR/Cas9 technique, which was initially presented in 2013, has gained appeal because to the fact that it is easy to use, economical, and has a wide range of applications. Increasing essential oil characteristics with scents that are utilized in the cosmetics business is not the subject of any report that is currently available. An analysis of the nuclear genomes of hybrid rose plants and wild roses has been carried out, which enables future utilization of the information. It is possible that the CRISPR/Cas9 mutation technology will be necessary for the technological advancement of functional studies on key genes involved in flower smell and essential oils. CRISPR/Cas9-based genome editing research needs to be conducted in order to address the gap for flower crops that are based on

essential oils and scents. Gaining an in-depth understanding of the tools and techniques utilized by CRISPR/Cas9 can lead to improved qualitative characteristics as well as innovative ideas for the development of flowers that are both competitive and sustainable. New kinds with enhanced characteristics could be created through the use of non-transgenic gene editing technology, which would satisfy the requirements of modern society while also benefiting investors and producers.

## Conclusion

It is possible to produce desirable qualities in flowering crops with the use of CRISPR/Cas9 technology, which is effective in genome editing. These features include flowering promotion, colour spectrum, fragrances, and innovation in flower structure. Existing floriculture plants can have their traits improved with the use of this technology, which can also contribute to increased worldwide competitiveness. On the other hand, this cutting-edge technology has a number of limitations, one of which is the requirement for gene transformation based on plant tissue culture. This is the most effective method for producing genome editing events at the moment, but it is only applicable to a limited number of plant species. In order to make progress with CRISPR/Cas9 genome editing, it is essential to develop a breakthrough transformation approach that does not involve plant regeneration. There are several plant species that are resistant to transformation by Agrobacterium, which is the most frequent technique of providing gene-editing chemicals. Agrobacterium has a limited host range, and thus is the most popular method. The use of A. rhizogenes can reduce the amount of time that passes between the administration of the reagent and the evaluation of the mutation, as well as broaden the range of species that are altered.

In the case of CRISPR/Cas9 genome editing, off-target effects are a key cause for concern since they have the potential to influence precise breeding by altering other essential agricultural characteristics. The Cas9/sgRNA genes should not be introduced into the plant genome and should only exist in the target cells for a brief period of time. This will help reduce the chance of off-target effects occurring. It is also possible to lessen the impact of off-target effects by developing high-fidelity single-guide RNA (sgRNA) and employing the appropriate Cas9 enzymes and genome editing tools. By combining the proofreading enzyme with the Cas9 enzyme, it is possible to correct any error that was caused by an event that was not intended.

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