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BREEDING FOR DIFFERENT FLOWER FORMS IN ORNAMENTAL CROPS: A REVIEW

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ABSTRACT

Flowering plants are extremely important in our daily lives due to their aesthetic value. The distinctiveness of various flower types is greatly valued, with double blossoms having more ornamental value than their single counterparts. Researchers have presented a novel ABCDE model that builds on the classic ABC model and discovered critical transcriptional variables to identify floral organs. In this new model, A+E designate sepals, A+B+E denote petals, B+C+E represent stamens, C+E indicate carpels, and D+E symbolize ovules. To breed cultivars with novel flower forms, a range of technologies are used, including hybridization, mutation, polyploidy, and genetic engineering. The genetic control of single, semi-double and double flower forms can be attributed to either a single gene or numerous genes. It is possible to successfully develop double flowers by carefully choosing the right hybridization techniques. The selection of mutants with modified apparent characteristics, such as altered flower color, shape, size, leaf form and growth habit, also becomes feasible through induced mutagenesis. By doubling the number of chromosomes, polyploidy breeding doubles plant size, leaf size, branch development and flower components. Genetic engineering has made it possible to manipulate a variety of features though biotechnological developments like RNAi, CRES-T, CRISPR/Cas9 and miRNA. These traits include flower color, fragrance, resistance to abiotic stress, disease and pest resistance, alteration of plant and flower form and architecture, flowering time and post-harvest longevity. The shapes of plants like torenia, chrysanthemum, morning glory, petunia, orchids, gentian, cyclamen and rose plants, among others have been successfully altered using these techniques. In spite of the abundance of these techniques, only a small number of cultivars have been created for commercial purpose.

Key words : Ornamental crops, ABCDE flower model, Flower form, Mutation, Polyploidy, Genetic engineering.

Introduction

Our daily lives depend heavily on ornamental plants, both for their aesthetic value and for their capacity to improve the environment in which we used to grow. Their value lies in their striking morphology, captivating flower colors, and unique shapes. Among these characteristics, floral form is particularly important for attractive plants. The development of novel flower forms in the phonotype is regularly given priority in breeding programs, whether it takes the form of semi-double or double flowers, which have greater ornamental value than single flowers, or changes in specific floral parts like petals, sepals, or serrations. Such alterations carry substantial commercial value due to their distinctiveness. Engineered traits are valuable to either the consumer or the producer.

Advancements in our understanding of floral development have been achieved through the study of model plants such as Arabidopsis and Antirrhinum majus, which have elucidated key transcriptional factors responsible for identifying floral organs (Bowman *et al.*,

1989; Coen and Meyerowitz, 1991). Phenotypes with unusual flower forms or double flowers are typically prized for their higher ornamental value than their single counterparts. A key feature of beautiful blooming species is the presence of double flowers, which are distinguished by the presence of double whorls of petals as a result of excessive development or the conversion of other floral organs into petals. The broad diversity of cultivated double-flowered species that exist today are the result of human selection for aesthetically pleasing features, which has been a major factor in the development of double flowers (Abbo *et al.*, 2014; Ross-Ibarra *et al.*, 2007). It's interesting to note that many double-flowered ornamental crop types have single-flowering wild forebears as their ancestors (Liu *et al.*, 2013). This historical journey serves to highlight the double blossoms' ongoing relevance and attractiveness in decorative horticulture.

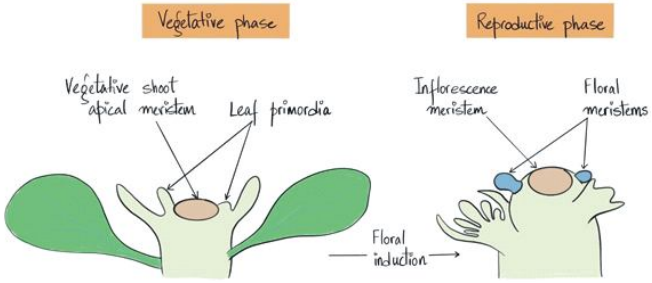

Fig. 1 : Phases of flower development.

The apical meristem changes its program from generating leaves to flowers to flower tissue at a certain stage of plant development. At this stage the apical meristem (AM) develops into an inflorescence meristem (IM). Numerous primordia's are formed by the IM, and these later give rise to sepal, petal, carpel and stamen.

The Molecular and Developmental Basis of Flower Development as Illustrated by the ABC Model. The ABC model of flower development provides a theoretical framework for knowing how blooming plants control the patterns of gene expression in their meristems, which ultimately result in the creation of a reproductive organ called a flower. Three significant physiological shifts are involved in this complicated process. First, the beginning of flowering is marked by the plant's transformation from sexual immaturity to sexual maturity. Second, the apical meristem's function changes from a vegetative meristem to that of an inflorescence. Finally, the flower's individual organs start to grow and differentiate. The ABC model delves into the molecular and developmental genetics underpinning the latter phase, providing insights into the biological mechanisms responsible for the formation of flower organs.

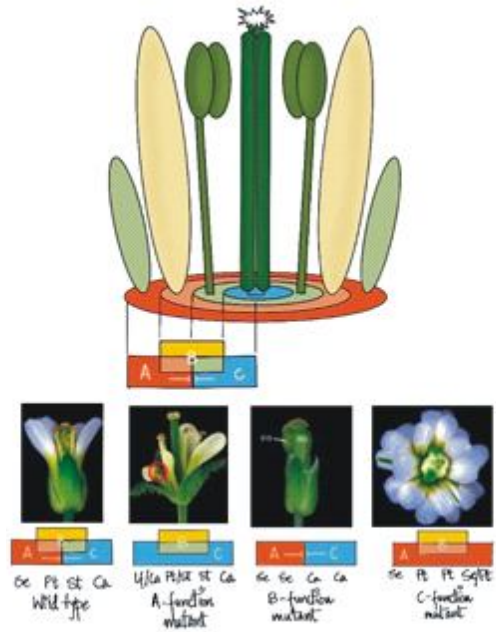

Fig. 2 : Mutation in Floral organ identify genes.

The distinctive identity of organs within the four floral verticils results from the intricate interplay of at least three distinct types of gene products, each with its unique functions. The roles of functions A and C are crucial for defining the identities of the perianth verticils and the reproductive verticils, respectively, in the ABC model. These roles are mutually exclusive, so when one is absent, the other takes over and determines the identification of every floral verticil. The differentiation between stamens and carpels inside the tertiary verticil is similarly guided by the B function, as it plays an important role in separation between petals and sepals within the secondary verticil.

The transition from the vegetative phase to the reproductive phase marks a significant change in the life cycle of the plant, maybe one of the most important ones because successful reproduction depends on its proper execution. The inflorescence meristem, which will eventually give rise to a cluster of flowers or, in some cases, a single flower, begins to grow during this phase. This morphogenetic change is affected by both internal and environmental variables. Specific prerequisites must be met, for example, the plant must have a certain number of leaves and reach a certain level of total biomass. Environmental conditions, such as a particular photoperiod, also play a vital role in triggering this transformation. Plant hormones are essential in coordinating this process, with gibberellins playing an essential part.

The ABCDE Model: Understanding Flower Development Stages

The ABCDE Model is a framework for the specification of floral organs in flower development.

According to the ABCDE model of flower formation (Coen and Meyerowitz, 1991; Rijpkema *et al*., 2010), floral organ identity is controlled by five different classes of homeotic genes, which are designated as A, B, C, D and E. The interaction of A and E-class proteins results in the development of sepals as the fundamental floral organs in the first whorl, according to the quartet models for floral organ specification (Coen and Meyerowitz, 1991; Smaczniak *et al*., 2012). The A, B and E-class proteins work together to determine petals in the second whorl. While the third whorl sees the participation of B, C, and E-class proteins in the determination of stamens and the fourth whorl relies on C and E-class proteins for carpel specification.

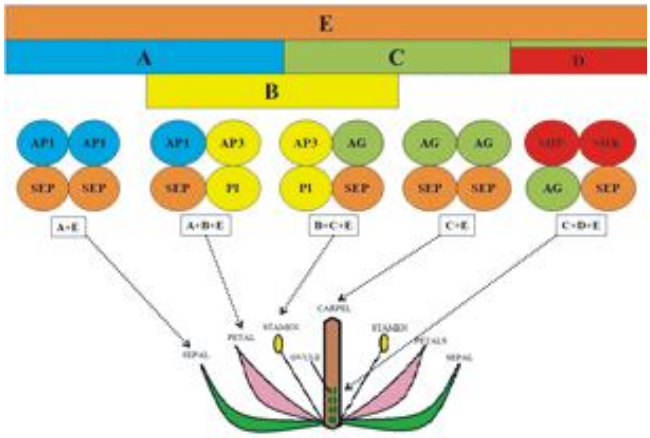

Fig. 3 : ABCDE Model of flower Development (Dornelas and Dornelas, 2005).

With the exception of the class A gene APETALA2 (AP2), the cloning of ABCDE homeotic genes in Arabidopsis has shown that these genes contain MADS-box transcription factors (Jofuku *et al*., 1994). The class A MADS-box gene in Arabidopsis is AP1, class B genes include AP3, PISTILLATA (PI), class C gene is AGAMOUS (AG), class D gene comprise SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHP2 and class E genes in the plant are SEPALLATA1 (SEP1), SEP2, SEP3, and SEP4. According to research, class E genes play partially overlapping roles in determining the identities of sepals, petals, stamens and carpels, with D-class proteins and E-class proteins dictating the ovule identity (Mandel *et al*., 1992; Jack *et al*., 1992; Goto and Meyerowitz, 1994; Yanofsky *et al*., 1990; Favaro *et al*., 2003; Pinyopich *et al*., 2003; Pelaz *et al*., 2000; Ditta *et al*., 2004). The amazing diversity of floral morphologies seen in angiosperms has been produced through the diversification of MADS-box genes over evolutionary time (Litt and Kramer, 2010). And the MADS-Box Genes are the MADS box is a conserved sequence motif found in genes which comprise the MADS-box gene family. The DNA-binding MADS domain is encoded by the MADS-Box genes. The MADS-Box has a length between 168 and 180 base pairs. A class of MAD-Box genes includes homeotic genes. The MADS-Box gene family got its name as an acronym referring to the four founding members:

- MCM1 from the budding yeast, *Saccharomyces cerevisiae*
- AGAMOUS from the thale cress *Arabidopsis thaliana*
- DEFICIENS from the snapdragon *Antirrhinum majus*
- SRF from the human *Homo sapiens*

The development of male and female gametophytes, floral organ identification, flowering time determination, embryo and seed development, root, flower, and fruit development are all the key components of plant development that are regulated by MADS-Box genes.

Origins and Applications the ABC model of flower development was initially conceptualized by George Haughn and Chris Somerville in 1988. It became an innovative framework to clarify the complex genetic pathways bringing about the establishment of floral organ identity in two different plant groups: the Rosids, illustrated by Arabidopsis thaliana, and the Asterids, illustrated by Antirrhinum majus. Sepals, petals, stamens and carpels are all arranged in four separate whorls in each of these species. These floral organs' identities are determined by the distinctive expression patterns of particular homeotic genes within each whorl. According to this model, sepals are identified by the expression of the A gene alone, whereas the identity of petals is determined by the co-expression of the A and B genes. While carpels only need the activation of the C genes, the B and C genes each play crucial roles in defining the identity of stamens. Notably, the regulatory network is further complicated by the reciprocal antagonistic behavior of the A and C genes (Haughn George W., Somerville, Chris R., 1988).

When a particular gene, like the A gene, is not expressed, it is clear how important homeotic genes are in determining the identity of an organ. According to Bowman *et al.* (1991), a flower in Arabidopsis lacking the expression of the A gene consists of three verticils: one with carpels, another with stamens, and a third with carpels. Researchers use a variety of strategies to investigate how genes operate, such as reverse genetics procedures that involve the creation of transgenic plants

with mechanisms for gene silencing through RNA interference. Alternatively, to identify and clone the desired gene, forward genetics methods like genetic mapping are employed to investigate the phenotypes of flowers displaying structural defects. These aberrant flowers might have alleles of the gene under inquiry that are overexpressed or inactive (Somerville and Somerville, 1999).

Two additional functions, D and E have also been proposed in addition to the previously stated A, B and C functions. Function D is responsible for specifying the identity of the ovule, a distinct reproductive function that occurs separately from the determination of carpels, which takes place prior to ovule development. On the other hand, Function E is linked to a physiological need that all floral verticils must meet. Although initially described as essential for the development of the three innermost verticils, its broader definition suggests its necessity in all four verticils. As a result, the loss of Function E causes the floral organs of the three outermost verticils to change into sepals, while the loss of Function D results in ovule structures that resemble leaves It should be noted that the gene products in charge of functions D and E are also MADS-box genes (Hong, 2005).

Genetic analysis of floral development using homeotic mutants

Homeotic mutations are the changes that take place in homeotic genes. These genes encode transcription factors that help to create the overall body plan by regulating how body components are identified and organizing development. The remarkable conversion of one body part into another displayed by homeotic mutants, offers important new information about how genes regulate development. Homeotic mutations have proved crucial in unraveling the floral development model in the model plant *Arabidopsis thaliana*.

Arabidopsis produces homeotic mutant flowers when the mutation occur in ABC genes. Notably, type A and C genes have reciprocal antagonistic behavior, whereby the expression of the C gene's activity results from the loss of function in the A gene (Bowman *et al.*, 1991). The calyx and corolla are the primary targets of A gene mutations, which result in the formation of carpels in place of sepals and stamens in place of petals. One example of this transformation is seen in the *A. thaliana* APETALA2 (AP2) mutant. The corolla and stamens, on the other hand, are affected by mutations in the B gene, resulting in the formation of sepals rather than petals and carpels rather than stamens. In A. thaliana, mutants like APETALA3 and PISTILLATA exhibit this behavior.

Stamens and carpels, which are reproductive organs, are directly impacted by mutations in the C gene, leading to the formation of petals in place of stamens and sepals in place of carpels. This change is best illustrated by the *A. thaliana* AGAMOUS (AG) mutant (Bowman *et al*., 1989 and Bowman *et al*., 1991). Therefore, the loss or alteration of C gene activity is crucial for producing an excess of petals and, as a result, the development of double blooms. The phyllody phenotype in Rosa chinensis cv. Viridiflora, according to Yan *et al*. (2016), is connected to the up-regulation and ectopic expression of RcSOC1 and A-class genes, together with the down-regulation of B, C and E class genes involved for floral organ identity. The complex regulatory networks that control floral growth and the emergence of distinctive flower shapes are clarified by this research.

Techniques for detecting differential expression

Cloning investigations have been conducted on the DNA within genes associated with the altered homeotic functions observed in the aforementioned mutants. These research used serial analysis to examine the patterns of gene expression at different phases of floral development, and the results show that the ABC model predicts many of these patterns.

Surprisingly, these genes exhibit traits of transcription factors, which is compatible with their function in controlling gene expression. As was expected, a collection of factors found in yeasts and mammalian cells and these transcription factors have structural similarities. The term MADS, which stands for the variety of elements included in this category is used to refer to this group as a whole. Although it is still conceivable that additional components are involved in the complex control of gene expression, MADS factors have been found in every plant species tested, which is significant (Taiz and Zeiger, 2002).

Genes exhibiting type-A function

Function A is predominantly represented in two important genes in Arabidopsis thaliana: APETALA1 (AP1) and APETALA2 (AP2). While, AP2 is a member of the exclusive AP2 family of genes that are found only in plants, AP1 is categorized as a MADS-box type gene (Bowman, 1989). Surprisingly, it has been shown that AP2 and the co-repressor TOPLESS (TPL) work together to suppress the C-class gene AGAMOUS (AG) in developing floral buds. The shoot apical meristem (SAM), which shelters the latent stem cell population throughout the adult life of Arabidopsis, is notable because it does not express AP2. This raises the possibility that TPL works in concert with another A-class gene in the SAM to suppress AG. As a type A gene, AP1 is essential

for identifying the identity of sepals and petals as well as for the growth of the floral meristem. On the other hand, AP2 participates in the growth of ovules and even leaves, acting not only in the first two whorls of floral organs but also exerting its influence on the remaining two whorls.

Genes exhibiting type-B function

Two MADS-box genes, APETALA3 (AP3) and PISTILLATA (PI), give rise to type-B function of *A. thaliana*. The homeotic transformation of petals into sepals and of stamens into carpels is brought on by a mutation in one of these genes. This also occurs in its orthologs in *A. majus*, which are DEFICIENS (DEF) and GLOBOSA (GLO) respectively (Schwarz-Sommer, 1990). Eudicotyledonous angiosperms have four separate whorls of floral parts that include sepals, petals, stamens, and carpels. According to the ABC model, the identity of these organs is governed by the action of homeotic genes A, A+B, B+C and C, respectively.

However, in contrast to the typical arrangement of sepals and petals in eudicots, many plants within the Liliaceae family exhibit a unique floral morphology with two nearly identical external petal-like whorls known as tepals. A modified ABC model was brought up in 1993 by van Tunen *et al*. to clarify the floral structure of the Liliaceae family. According to this revised hypothesis, class B genes are expressed in whorls 1 as well as whorls 2 and 3, not just those two. Because of the expression of class A and class B genes, whorls 1 and 2 have petaloid features.

The cloning and characterization of homologs of the Antirrhinum genes GLOBOSA and DEFICIENS in a *Liliaceae* species, the tulip *Tulipa gesneriana*, allowed for the experimental validation of this theoretical paradigm. These genes were found to be expressed in whorls 1, 2 and 3. Homologs GLOBOSA and DEFICIENS were also identified and characterized in *Agapanthus praecox* ssp. orientalis (Agapanthaceae), a phylogenetically distant species from the model organisms. Both of these genes, ApGLO and ApDEF, are encoded proteins of 210 to 214 amino acids each. These sequences were connected to the monocotyledon B gene family through phylogenetic analysis. Studies using in situ hybridization further demonstrated that whorls 1, 2, and 3 express both ApGLO and ApDEF. Collectively, these data offer strong support for the modified ABC model's alignment with the floral development mechanism in Agapanthus species, which has been reported in Liliaceae species. This information sheds insight on the remarkable diversity of floral structures among different plant families.

Genes exhibiting type-C function

The C function in Arabidopsis thaliana is controlled by the single MADS-box type gene AGAMOUS (AG). In additional to contributing to form the floral meristem, AG is essential for determining the identities of both stamen and carpels (Bowman, 1989). As a result, androecium and gynoecium are absent in the phenotype of AG mutants, which is instead defined by the development of petals and sepals in place of these structures. Additionally, the flower's center is still poorly differentiated, which causes the whorls of petals and sepals to repeatedly grow.

Genes exhibiting type-D and E functions

The identification of D function genes in 1995 signaled a significant advancement in the study of flowers. Despite some similarities with C function genes, these genes, which are members of the MADS-box protein family, have a different function than the ones previously mentioned. These two genes are referred to as FLORAL BINDING PROTEIN7 (FBP7) and FLORAL BINDING PROTEIN1L (FBP1l). They were discovered to be extremely important for ovule formation in Petunia. Subsequently, equivalent genes were discovered in Arabidopsis, where they are likewise responsible for controlling the growth of the carpel, ovule, and structures related to seed dispersal.

In 1994, a novel function in the floral development model was discovered as a result of intriguing phenotypes discovered in Petunia RNA interference investigations. The three innermost whorls of floral organs were once thought to grow primarily as a result of the E function. However, later studies enhanced our knowledge of its function in floral development by showing that its expression is necessary in each floral whorl.

Strategies for improving flower shape include

Hybridization : It defined as the process of crossing two or more plants with distinct genetic backgrounds, plays a pivotal role in the creation of new crop varieties. Combining desired features into a single variety is an efficient way to increase genetic diversity and to take the advantage of hybrid vigor. The best strategy for achieving distinctive flower shapes is to cross two diverse types. Depending on the genetic makeups involved, the results of such crossings can produce a diverse spectrum of morphologies, including single, double and semi-double. A single gene or a number of genes may control the genetic makeup of the many flower forms found in ornamental crops. Breeders can create new cultivars with the appropriate floral kinds by carefully choosing compatible

parent genotypes.

For instance, as shown by Debener in 1999, a dominant allele governs the inheritance of the double flower shape in roses. Therefore, it is important to cross cultivars having double-type flowers with suitable single-flower cultivars in order to produce new double-type cultivars. According to Chen *et al*. (2012), a recessive gene governs the double flowering trait in *Catharanthus roseus*. Furthermore, they suggested that single flowers, whether in a homozygous or heterozygous state, are controlled by a dominant allele. These genetic discoveries direct the breeding efforts to produce unique flower shapes in ornamental crops. Crossing two diverse forms is the best approach to create a unique shape. The outcome may take on a variety of shapes, including single, double, and semi-double, depending on genetic make-up. For example: Tuberose. In order to create new cultivars of the double flower form in roses, cultivars with double flower forms must be crossed with suitable single flower form cultivars since the inheritance of the double flower form is regulated by a dominant allele. Hybridization between single and double cultivars was conducted by Debener et al., 1999 and Shen *et al*. in 1987. Numerous single and few double plants were produced in the progeny of reciprocal crosses between single and double cultivars.

Hybridization techniques are essential in plant breeding to create new and improved cultivars. Two common methods of hybridization are intervarietal hybridization (intraspecific) and interspecific hybridization (intrageneric):

Intervarietal Hybridization (Intraspecific): In this method, crosses are made between plants from two different varieties of the same species. It is an effective method for enhancing both cross-pollinated and self-pollinated crops. This approach is frequently used to create cultivars for a variety of flowering plants, such as Chrysanthemum, Gladiolus, Rose, Bougainvillea, Hibiscus, and Camellia. "April Blush," "April Dawn," "April Rose," and "April Snow" are a few cultivars that were created through intervarietal hybridization.

Interspecific Hybridization (Intrageneric): In this approach, plants from two different species within the same genus are crossed. In order to create new cultivars of plants like verbena, petunia, orchid, bougainvillea, lilium and amaryllis, interspecific hybridization is used. Interspecific hybridization is commonly seen in species crosses as those between the lilium, orchid, Hemerocallis, *Victoria amazonica* and *Bougainvillea spectabilis* and *Bougainvillea glabra*.

These methods of hybridization enables plant breeders to introduce genetic diversity and create new cultivars with desirable traits, ultimately contributing to the enhancement of ornamental crops and their aesthetic appeal. Chrysanthemum: Huang *et al*. (2011) conducted interspecific hybridization experiments involving *Chrysanthemum morifolium* varieties 'Hongxinju' and 'Xinbaiju' as females and *Chrysanthemum indicum* and *Chrysanthemum nankingense* as males. Bud pollination was found to enhance heterozygosity in these interspecific hybrids, resulting in aneuploid chromosomes and significant character segregation. Cheng *et al*. (2010) employed ovary rescue techniques to create six interspecific hybrids by crossing *Dendranthema morifolium* 'rm20-12' (2n-54) with its wild diploid relative, *Dendranthema nankingense* (2n=18)

Comparing these hybrids to their *D. morifolium* parent, the cold tolerance of these offspring was much higher. Chrysanthemum cultivars can be improved by interspecific hybridization. Gerbera: The history of gerbera breeding dates back to the late 19th century in Cambridge, England, when R.I. Lynch crossed two South African species, *G. jamesonii* and *G. viridifolia*, resulting in the creation of *G. cantabrigiensis*, known today as *G. hybrida*. The descendants of these two species are the source of the majority of commercially grown gerbera cultivars. Gerbera cultivars come in a wide range of sizes, colors, and shapes, including white, yellow, orange, red, and pink. *Dianthus caryophyllus*, or the carnation, has the potential to be crossed with a number of different wild species, according to research. Between *D. silvestris, D. knappi, D. sequierii, D. carthusianorum*, and *D. caryophyllus*, successful crossings were seen that produced seeds and hybrid plants.

Mutation breeding : involves sudden heritable changes that occur in an organism, deviating from Mendelian principles of segregation and recombination. Mutants are those individuals that display these heritable alterations. De Vries first proposed the idea of mutation in the year 1900. Gene, chromosomal, or cytoplasmic alterations can induced mutations or cause them to occur spontaneously.

Mutagens are agents, either physical or chemical, that artificially induce mutations. Physical mutagens like as alpha rays, beta rays, X-rays, gamma rays, neutrons, and UV rays can all result in these mutations. Chemical mutagens encompass a range of substances such as 5-bromouracil, 5-chlorouracil, mustard gas, sulphur mustard, nitrogen mustard, ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), ethylene oxide, ethylene imine, azasorine, mitomycin C and streptonigrin,

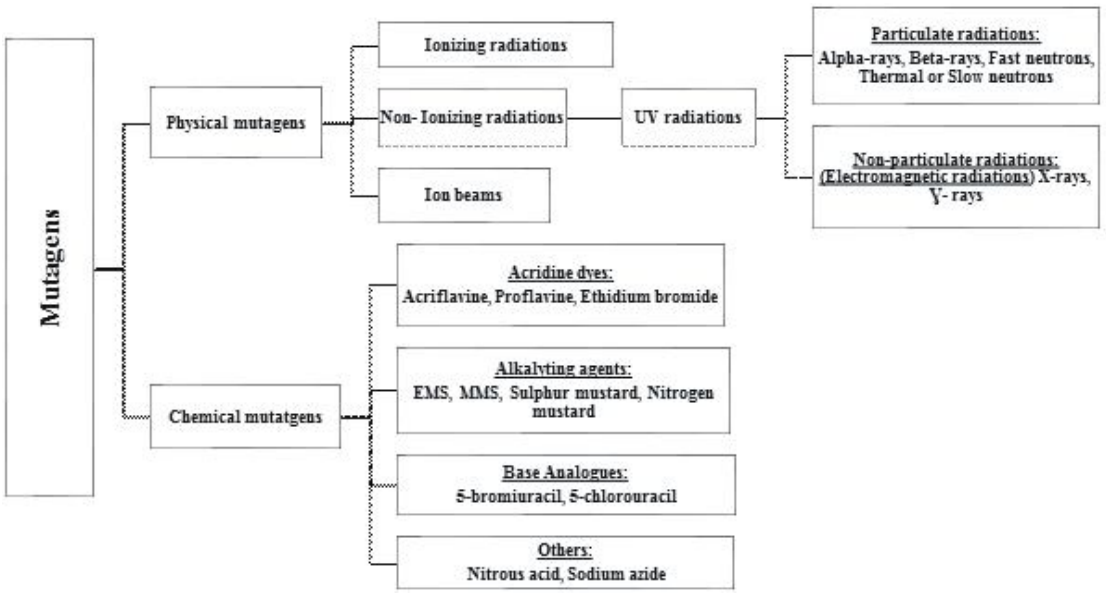

Fig. 4 : Common Mutagens used in plant mutation induction.

among others. These substances are used to purposefully cause mutations for various breeding and research goals.

Induced mutation

Mutation breeding in plants : Mutation induction techniques find a particularly favorable application in ornamental plants due to the ease of monitoring economically important traits such as flower characteristics and growth habits after mutagenic treatment. Numerous kinds of ornamental plants are heterozygous and reproduce vegetatively, which makes it easier to find, pick out, and preserve mutants in the M1 generation. For instance, the moss rose originated as a mutant of *Rosa centifolia* and approximately 5,819 rose cultivars have been developed through bud mutations. The 50% of rhododendron and chrysanthemum cultivars are the result of spontaneous or artificial mutations. The cultivar 'Faraday' of tulip contained the first known floral mutation. Due to their variety of economically valuable features, which can be easily tested and defined after treatment, ornamental plants are very advantageous systems for mutagenic treatments. The identification, selection, and retention of mutants in the M1 generation are also made possible by the heterozygous nature of many ornamental plants and their frequent vegetative propagation (Schum and Preil, 1998). Induced mutations observed in ornamental plants encompass a wide range of features, including flower characteristics (such as color, size, morphology, and fragrance), leaf traits, growth habits, and physiological attributes such as alterations in photoperiodic responses, early flowering, increased flowering, improved post-harvest longevity, and enhanced tolerance to biotic and abiotic stress factors (Schum and Preil, 1998).

The morphology of flowers and inflorescences can also be significantly affected by the mutation induction. In some cases, it has resulted in an increase in flower size, although more commonly, mutagenic treatments have led to undesired reductions in flower size. Additionally, it has been observed that mutation induction can alter petal form, which can occasionally lead to ornamental advancements. Additionally, mutagenic treatments have been shown to change petal counts in both the upward and downward direction. There have been reports of larger whorls of ligulate florets and changes from ligulate to tubular florets in the Compositae family as a result of mutagenic treatments.

Polyploidy breeding in ornamental crops : Crop species with genetic chromosomal number 'n' are referred to as haploid, while those with somatic chromosome number (2n) are referred to as diploid. Euploid crop species are those that have a somatic chromosomal number that is a direct multiple of the basic number. Aneuploid crop species, on the other hand, are those whose somatic number is not an exact multiple of the basic number. According to the multiplicity of the basic number, euploids can be further divided into monoploid (x), diploid (2x), triploid (3x), tetraploid (4x), hexaploid (6x), octaploid (8x), and so on. Polyploid refers to species that are more complex than diploids. Autopolyploids are polyploids with the same genome number and allopolyploids are those with differing genome numbers.

Haploids are typically weaker and infertile, whereas other polyploids show traits including larger plant parts,

Table 1 : Transformation of Flower Morphology in Flower crops through hybridization.

Crop name	Ploidy Level	Species/ Cultivars
Amaranthus	Tetraploid	Amar Tetra
Amaryllis	Triploid	Kiran
	Tetraploid	Samrat, Tetra Apricot, Tetra Starzynski
	Hexaploid	*A. belladona*
	Heptaploid	*A. blumenvia*
Antirrhinum	Tetraploid	Tetra Giant, Tetra Guilt, Velvet Beauty, Red Shades
Anthurium	Diploid	*A. Andreanum, A. hookerii, A. magnificum*
	Triploid	*A. Scandens*
	Tetraploid	*A. Digitatum, A. wallisii*
Bougainvillea	Triploid	Cypheri, Temple Fire, Lateritia, Perfection, Poultoni Special
	Tetraploid	Crimson King, Princess, Mahara, Magnifica, Shubhra, Mrs. McClean, President Roosevelt, Lady Mary Baring, Thimma, Zakariana
	Aneuploid	Begum Sikander, Wajid Ali Shah, Chitra
Carnation	Tetraploid	*D. chinensis*
Dahlia	Tetraploid	*D. imperialis*
	Octaploid	*D. variabilis, D. coccinea, D. rosea*
Day Lily	Diploid	Barbara Mitchell Ruffled, Master Piece, Ruffled Perfection
	Tetraploids	Tetra Apricot, Tetra Peach, Crestwood Series, Wedding Band
Gladiolus	Triploid	Manmohan, Monohar, Manhar, Mukta, Manisha, Mohini
	Pentaploid	*G. psittacinus*
	Aneuploid	Archana, Arun
Jasmine	Triploid	*J. primulinum, J. sambac, J. grandiflorum*
	Tetraploid	*J. flexile, J. angustifolium*
Lily	Triploid	*Lilium tigrinum*
Marigold	Diploid	*T. erecta, T. tenuifolia*
	Tetraploid	*T. patula, T. minuta, T. biflora, T. remotiflora*
	Triploid	Seven Star, Showboat, Nugget
Narcissus	Triploid	*N. pseudonarcissus hispanicus*
	Hexaploid	*N. bulbocodium*
	Diploid	*N. pseudonarcissus, N. poeticus*
Orchid (Dendrobium)	Amphidiploids	Jacquelin Thomas Y 166
Orchid (Phalaenopsis)	Tetraploid	Riverbend
Orchid (Oncidium)	Tetraploid	Popcorn
Orchid (Spathoglottis)	Tetraploid	Lion
Orchid (Vanda)	Tetraploid	Atherton, Juliet, Hula Girl, Wood Lawn
Petunia	Double Haploid	Mitchel
	Autotetraploid	State Fair, Old Mexico

Table 1 continued...

Table 1 continued...

Primula	Diploid	*P. frondose*
	Tetraploid	*P. farinosa*
	Hexaploid	*P. scotica*
	Octaploid	*P. Scandinavica*
Rose	Diploid	*R. gigantea, R. multiflora, R. wichuriana, R. chinensis, R. moschata*
	Triploid	*R. bourboniana,* Prema, Surekha, Surya
	Tetraploid	*R. gallica, R. damascena, R. foetida*
	Trisomic	Mohini
Stock	Aneuploid	Snow Flake
Tulip	Triploid	*T. lanata, T. stellata*
	Tetraploid	*T. clusiana, T. stellata*
	Pentaploid	*T. clusiana*

larger cells and slower growth rates as compared to diploids. In diploid species, monosomics hardly persist, whereas nullisomics rarely flourish in polyploid species. A form of allopolyploid called amphidiploid has two copies of each genome within. Tetraploid plants are distinguished by their vigor, strong vegetative growth, thicker leaves, and larger blooms. Triploids exhibit a combination of both hybrid vigor and polyploidy-induced vigor.

Increasing a species' chromosome count by polyploidy breeding is a highly successful way to add genetic variations, especially when there are few natural variants. Chromosome doubling produces genetic diversity, which can be used to improve breeding strategies. This strategy has been extensively used in a variety of crops to improve plant characteristics and produce novel forms with better plant architecture, hence supplying useful data for additional breeding efforts and cultivar improvement (Mata, 2009).

Induced polyploidy often results in an increase in the size and dimensions of numerous plant parts, such as leaves, branches, floral components, fruits, and seeds (Chopra, 2008). Particularly tetraploids exhibit increased vigor and greater stature. Colchicine and oryzalin are the main drugs used to induce polyploidy, and the dosage and length of the treatment are very important. Numerous studies have investigated this aspect, with Gantait *et al*. (2011) found that tetraploid *Gerbera jamesonii* Bolus cv. Sciella had strong plant growth, longer stem length and larger blossom diameter. Similar to this, Hanzelka and Kobza (2001) also noted a bigger bloom of diameter (4.20 cm) after colchicine-induced polyploidy.

The production of polyploids involves several methods and factors, resulting in tetraploids and higher levels of polyploidy:

Regeneration Methods : Polyploids, including tetraploids, can be induced through regeneration techniques. This process often includes heat and cold treatments applied to germinating seeds.

Chemical Induction : Polyploidy can also be induced chemically using substances such as colchicine, nitrous oxide, oryzaline, trifluralin and phosphoric amide.

Somatic Mutation : Some instances of polyploidy are the result of somatic mutations, where disruptions in mitosis lead to chromosome doubling. For example, this phenomenon has been observed in Primula kewensis.

Unreduced Gametes : Polyploidy can occur when unreduced gametes (eggs and sperm) unite. These gametes have not undergone normal meiosis and still maintain a 2n chromosome constitution.

Environmental Factors : Polyploidy tends to be more frequent at high altitudes, high latitudes, and in wet soils and meadows.

***In vitro* Polyploidization :** Recently, *in vitro* polyploidization methods have been developed and employed to expedite heterosis breeding in ornamental plants. This method speeds up the process of inducing polyploidy and decreases the number of aberrant plants.

Polyploidy in ornamental plants has made significant advances. The evolution of numerous decorative species and cultivars, such as tulips, dahlias, anthuriums, bougainvilleas, lilies, cactus, primulas, narcissus and roses, has been significantly influenced by polyploidy. Various ornamental crops, including marigolds, petunias, snapdragons, portulacas, chrysanthemums, calendulas and lilies have been reported to include induced tetraploids. These initiatives have improved and increased the variety of decorative plant types.

Table 2 : Induced mutation for change in flower morphology in flower crops.

S. no.	Crop	Mutagen	Character	References
1	*Begonia rex*	Gamma rays	Shape mutants	Buiatti *et al.* (1990)
2	Bougainvillea	Gamma rays	Variegated flower	Srivastava *et al.* (2002)
3	Carnation	Heavy ion beams	Change from serrate to rounded petals	Okamura *et al.* (2003)
4	*Catharanthus roseus*	Gamma rays	Development of four petals instead of five	El-Mokadem *et al.* (2014)
5	Chrysanthemum	Gamma rays	Tubular ray florets Tubular and Flat shaped floretsSpoon-shaped, tubular and irregular ray floretsReduced flower head size	Banerji and Datta (1992), Misra *et al.* (2003), Kumari *et al.* (2013)
6	Cyclamen	Ion-beams	Typical petal mutant	Sugiyama *et al.* (2008)
7	Dahlia	X-rays	Development of white tip	Broertjes and Ballego (1967)
8	Dendrobium Orchid	Gamma rays	Narrow, elongated or broad or curled petals, Veinous sepals. Short and broad lip.Small flower etc.	Ariffin and Basiran (2000)
9	Gerbera	Gamma rays	Change in flower morphology	Jain *et al.* (1998)
10	Hibiscus	Gamma rays	Single flower type	Banerji and Datta (1986)
11	Hydrangea	Ion-beams	Seeds	Kudo *et al.* (1998)
12	Limonium	Ion beam	Shoot cultures	Ogawa *et al.* (2014)
13	Petunia	MMS, MNNG	Dissected and *Dentate corolla*	Mahna and Garg (1989
14	Pelargonium	Ion-beams	Buds	Yu *et al.* (2016)
15	Prunus	Ion- beams	Scions	Hayashi *et al.* (2019)
16	Rose	Ion-beams X-rays	Change in no of petal, shape, and size Increase and decrease in petal number	Murugesan *et al.* (1993), Yamaguchi *et al.* (2003), Van and Broertjes (1989)
17	Spiraea	Ion beam	Seeds	Iizuka *et al.* (2001)
18	*Torenia hybrida*	Gamma rays	Erose petal margins, extra petals, extrastamens or missing petals Serrate petal margins	Suwanseree *et al.* (2011), Nishijima and Shima (2006)
19	Tuberose	Gamma rays	Large flower size	Patil *et al.* (1975)
20	Tulip	X-rays	Parrots, fringed and double	Van and Broertjes (1989)

Achievements of polyploidy in ornamentals

In a variety of ornamental crops, polyploidy has been a key factor in determining how species and cultivars have evolved. There are numerous well-known plants in this category, including tulips, dahlias, anthuriums, bougainvilleas, lilies, cactus, primulas, narcissus, roses, and more. Notably, induced tetraploids have been successfully produced in a number of ornamental species, including lilies, calendulas, chrysanthemums, petunias, snapdragons and marigolds. Those achievements have greatly increased the variety and quality of ornamental plant varieties.

Genetic Modification of Ornamental plants

The ability to create novel varieties through the use of gene transfer techniques makes genetic modification an intriguing opportunity for breeders of ornamental plants. In some cases, creating new ornamental varieties through traditional methods like hybridization or mutagenesis can be exceptionally challenging, time-consuming, or even unfeasible, especially when dealing with completely sterile varieties such as orchids. Genetic modification offers a promising alternative avenue for enhancing these varieties.

Breeders now have the ability to introduce features that are challenging or impossible to breed for using traditional techniques. These characteristics cover a wide variety of potential outcomes, such as alterations in floral color, smell, resistance to abiotic stressors, disease resistance, pest resistance, adaptations to plant and flower structure, modifications to flowering period, and lengthening post-harvest longevity, among others. Notable examples of ornamental plants that have benefited from genetic modification include Chrysanthemum, Torenia, Cyclamen, Petunia and more.

The act of introducing a specific DNA sequence, usually a gene, into an organism without fertilization or conventional crossbreeding is known as genetic transformation. Transgenic plants are referred to be plants that have undergone genetic modification. This technique increases the potential for genetic improvement by allowing the regulated integration of nucleic acids into the recipient genome.

Along with improvements in tissue culture techniques and genetic engineering, numerous genetic transformation approaches have been developed. The use of *Agrobacterium tumefaciens*, particle acceleration (biolistics), polyethylene glycol treatment, electroporation, silicon carbide fiber-mediated transformation (siliconization), silica carbonate microparticles, microlaser techniques, micro- and macro-injection techniques, and direct DNA application are some of the methods covered by these techniques. These methods each have particular benefits and uses in the area of genetic modification for ornamental plants.

The successful development of the first transgenic petunia during the 1990s was a crucial turning point for the field of genetic engineering of ornamental plants. Rose, chrysanthemum and carnation are three of the most widely grown cut flower crops that have undergone genetic transformation. For Rosa hybrida cv. 'Royalty,' the transformation procedure comprised co-cultivating the plant material with *Agrobacterium*. After that, friable embryogenic callus formed, and the plants were then converted by embryogenesis.

Agrobacterium has also been used to genetically alter *Chrysanthemum grandiflora* and indicum. The infection of either leaves or peduncles led to the regeneration of transformed plants through organogenesis or the formation of transformed callus capable of generating transformed plants. Transformed *Dianthus caryophyllus* (carnation) cultivars were produced by co-cultivating leaves, petals, or stems with Agrobacterium, followed by either direct or indirect organogenesis. Other flowering plants that have undergone Agrobacterium-mediated transformation include Narcissus, Gladiolus, *Lilium longiflorum, L. leichlnii* var. *maximowiczii* and Tulipa. Other flower crops, such as Gerbera, Dendrobium, Antirrhinum, Anthurium, Eustoma and Pelargonium, have also been investigated for genetic modification. For genetic alteration in ornamental plants, a variety of methods have been used, including:

a. RNAi or Gene Silencing

b. Chimeric Repressor Gene-Silencing Technology (CRES-T)

c. MicroRNA

These methods provide many ways to control gene expression and produce desirable features in ornamental plants, creating new opportunities for the development of ornamental variety.

RNAi or Gene silencing is a powerful method for silencing gene expression, offering a straightforward approach to regulate gene function. Both transcriptional gene silencing (TGS) and posttranscriptional gene silencing (PTGS), commonly known as RNA interference (RNAi) are separate methods by which this unique gene regulation mechanism might lower transcript levels. When mRNA is broken down into short RNAs, which in turn activate ribonucleases to target homologous mRNA of a particular gene, gene silencing occurs. The resulting phenotypes may resemble an allelic series of mutants or genetic null mutants.

These methods are frequently used for loss-of-function investigations because they enable the precise silencing of specific genes, inhibiting the manifestation of specified features. For instance, in a work by Noor *et al*. (2014), two C-class MADS-box genes, pMADS3 and FBP6 were targeted by virus-induced gene silencing (VIGS) to induce double flower development in four cultivars of Petunia hybrida. In flowers exposed to pMADS3/FBP6-VIGS, the results revealed a complete conversion of stamens into petaloid tissues combined with a considerable increase of upper limb-like tissues, giving the flowers a decorative aspect. Additionally, according to Heijmans *et al*. (2012a), flowers in fbp6/fbp6 pMADS3-RNAi plants showed a full conversion of these methods are frequently used for loss-of-function research because they enable the precise silencing of specific genes, prohibiting the expression of specific traits For instance, in a work by Noor *et al*. (2014), two C-class MADS-box genes, pMADS3 and FBP6, were targeted by virus-induced gene silencing (VIGS) to induce double flower development in four cultivars of *Petunia hybrida*. In flowers exposed to pMADS3/FBP6-VIGS, the results revealed a complete conversion of stamens into petaloid tissues combined with a considerable increase of upper limb-like tissues, giving the flowers a decorative aspect. In addition, flowers in fbp6/fbp6 pMADS3-RNAi plants showed a complete conversion of carpels into secondary flowers, giving them a voluminous appearance, according to Heijmans *et al*. (2012a). Numerous other plants, such as *Japanese gentian* (Nakatsuka *et al*., 2015), *Thalictrum thalictroides* (Galimba *et al*., 2012), *Phalaenopsis orchids* (Hsieh *et al*., 2013) and *Aquilegia* (Gould and Kramer, 2007) have been the

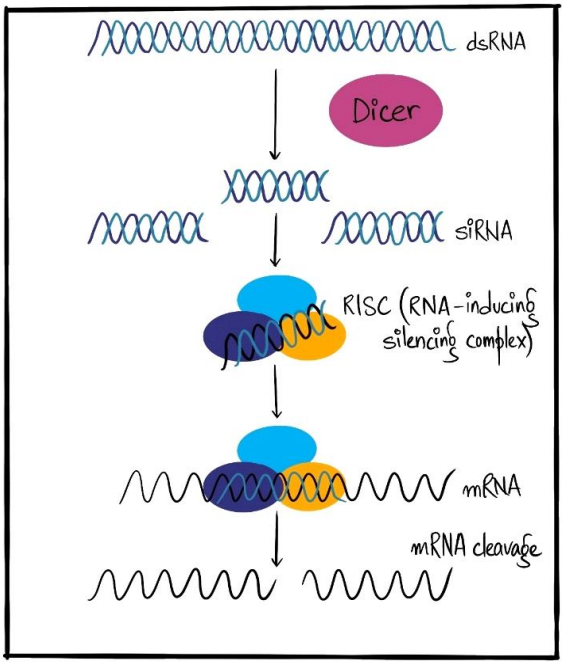

Fig. 5 : Mechanism of Gene silencing in RNA interference technology.

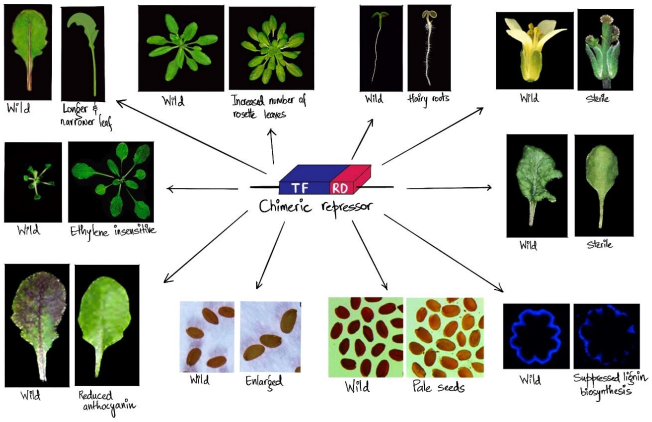

Fig. 6 : Chimeric Repressor Gene-Silencing Technology (CRES-T) induces phenotype.

subject of similar investigations for double bloom production. These results show how flexible and successful RNAi is for modifying gene expression and phenotypic features in ornamental plants.

Micro RNA is a class of small non-coding RNA molecules, typically about 22 nucleotides in length, that are present in eukaryotes. These molecules are essential for the post-transcriptional control of gene expression as well as RNA silencing. Nearly all biological and metabolic activities involve miRNAs. There have been many miRNAs found that are closely linked to plant architecture. For instance, miR156 has been linked to the control of plant architecture, according to research by Jiao *et al*. (2010). According to Carle *et al*. (2007)'s investigation of another miRNA, miR319, snapdragon plants' leaves and petals' morphology is influenced.

CRISPR/Cas9 Technology : The regularly spaced, clustered short palindromic repeats, the (CRISPR)/ CRISPR-associated protein (Cas) system has become a potent genome-editing tool for precisely altering DNA sequences in particular places. It offers great ways to genetically improving floricultural crops. The CRISPR/ Cas9 system plays an important role in agricultural crops, such as enhancing flowering characteristics including colour modification, extending the shelf life of flowers, promoting flower initiation and development and using genome editing tools to alter the colour of decorative foliage. Cas9/CRISPR gene editing could be helpful in creating new cultivars with improved essential oils and smell, among many other beneficial characteristics (Sirohi *et al.*, 2022). A CRISPR/Cas tool that can separate from the Cas9/sgRNA construct to prevent comparable changes by CRISPR/Cas generates stable gene mutations. CRISPR/Cas gene is a rapid and precise genetically engineered crop technology that produces crops resistant to abiotic and biotic stresses, viruses, fungus and bacteria in a fraction of the time compared to crops generated using conventional methods, which take ten to fifteen years to achieve resistance. Thus, CRISPR/ Cas is a helpful tool for producing agricultural products in a sustainable manner. This technology has been used to successfully modify plant characteristics. This has been used to successfully modify plant characteristics.

Gene knockouts have been the primary application of CRISPR/Cas9 technology in plants. Additionally *Petunia hybrid*, *Chrysanthemum morifolium*, *Dendrobium officinale*, Torenia, *Ipomoea nil*, *Lilium longiflorum*, *Lilium pumilum* and *Phalaenopsis equestris* have all benefited from its successful use in generating gene knockouts in ornamental plants to induce genetic alterations. According to these research, ornamental plants can effectively undergo mutagenesis produced by CRISPR/Cas9. Using the traditional Mendelian segregation, the modification generated is accurate and may be inherited by future generations (Sirohi *et al.*, 2022).

CRISPR/Cas systems are easy to use, dependable and capable of multiplex targeting, they provide many benefits for the establishment of resistance in agricultural crops. Because of their high precision and efficiency, these systems hold considerable promise for overcoming the limits of conventional breeding for the development of resistance. CRISPR/Cas9 still has a lot of restrictions despite its many benefits and wide range of applications. The direct targeting of S genes may result in some fitness

cost due to their connection with other desired genes, particularly the genes governing plant growth and development. There are only a few notable barriers that could prevent the CRISPR/Cas9 system from being effective in the development of disease resistance. Furthermore, any interruption of the S gene may interfere with the product's pathway and eventually, the pathways of many additional products. Without taking into account species boundaries, editing can produce desired S gene mutants in the majority of interesting plants for breeding. It is anticipated that more S genes will be identified, increasing the number of potential targets for genome editing. "Off target mutations" are a major constraint as well and are now a cornerstone of attempts to enhance the CRISPR system, especially in the transgene-free agricultural production process. Off-target genome editing is the term for DNA alterations at random and nonspecific locations that can happen via gRNA misguides or in a gRNA-independent way (Ahmad *et al*., 2020). Beyond the technological difficulties of bringing CRISPR/Cas9-developed crops from lab to the field, other barriers include ambiguous legal frameworks, disagreements over intellectual property rights and acceptability by customers. A number of useful Cas9-based applied approaches have emerged that allow scientists to quickly improve plants. Finally, it should be noted that the CRISPR/Cas9 system is an effective tool for genetically engineering crops.

Conclusion

The creation of novel flower forms in ornamental plants is a primary breeding objective, as it significantly enhances their commercial value due to their distinctiveness. It has been established that floral organ identity is determined by five classes of homeotic genes, denoted as A, B, C, D and E. To generate a new form, modifications of these genes are necessary. Various strategies, including hybridization, mutation, polyploidy induction, and genetic engineering, can be employed to achieve unique flower forms, including double flowers. In contemporary breeding efforts, innovative techniques such as RNA interference (RNAi), Chimeric Repressor gene-Silencing Technology (CRES-T), microRNA (miRNA) modulation, CRISPR/Cas9 technology and other gene-silencing approaches are available. These methods enable the targeted silencing of specific genes, thereby facilitating the development of plants with altered flower forms. Despite the array of techniques at our disposal, the commercial development of ornamental varieties through genetic transformation remains relatively limited.

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