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A review on crop improvement strategies and breeding methods in ornamental annuals

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Abstract: The availability of a broad variety of cultivars in many ornamental species has increased recently, in particular for attractive annuals, which are valued commercially for their use as cut flowers, potted plants, loose flowers, and in landscape gardening. The breeding of ornamental plants in the current scenario is a challenging endeavour with constantly evolving new obstacles. Modern genomic technologies provide prospects for improved precision breeding and selection for characteristics that are more difficult to determine. Traditionally, ornamental breeding has been focused on increasing resistance to biotic or abiotic stress, novelty, yield, and quality. However, accomplishing these objectives necessitates tedious cross-breeding, and exact breeding methods have been noticed to be not applied constantly. Though the purposes of ornamental crop breeding may vary, the process generally does not differ from the breeding of other crops. Furthermore, vegetatively propagated ornamentals constitute most of the crops. The expanding interest in ornamental crops that are produced by modern crop breeding methods such as genome editing, chromosome manipulation, molecular marker-assisted breeding, mutation breeding, and exploiting somaclonal variations, particularly in relation to altering desirable plant features and producing new ornamental traits of the crops which is the main objective of crop improvement practices. Hence, it has become obligatory to evaluate the current state of any technology created following an in-depth study carried out by several research organisations.

Keywords: breeding strategies; crop improvement; cross breeding; genome editing; mutation; precision breeding

Breeding for ornamentals has entirely different traits that are prioritised from breeding for edible crops. Ornamental crops have been bred using a variety of techniques to create new cultivars owing to their great diversity, from herbaceous seasonals to woody perennials. Due to their genetic diversity, ornamental crops have less economic turnover per individual species than agricultural or vegetable crops (Datta 2022). As a result, expenses for research and development are also consequently

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reduced. Nevertheless, public research initiatives are engaged in genetic research and the development of ornamental cultivars. In ornamental plant breeding, the main focus is on improving the variety of characteristics, such as new colours, shapes, sizes, flowering number, flower vase lifespan, repeated blooming, disease tolerance, nutrient absorption, and growth habit (De 2017). The features are suited for different categories of flowers, such as cut flowers, loose flowers, and potted plants. The important general features of ornamental breeding are improving the yield and quality of the plants.

Ashok and Velmurugan (2020) discussed a true breakthrough in flower seed production that happened in the 1950s, and since then, the field has made enormous strides, producing seeds using a variety of inventive methods like mutation breeding, etc. At present, in India, the area under flower seed production is around 600–800 ha. Punjab generates 60% of India's total seed output, whereas Karnataka produces 25% of the total seed output. In these regions, a high number of annual plants generate the majority of the open-pollinated seeds. Prakash et al. (2023) described that India offers ample opportunities for producing various hybrid flower seeds, including those for snapdragon, pansy, geranium, balsam, petunia and marigold. Worldwide, seed firms have created numerous promising hybrid series for various annual flowers. To further accommodate the needs of novel bedding plants, the F1 hybrid technology has been modified (Goldsmith 1968). The main areas of flower seed production in India are Punjab (Sangrur, Patiala and Ludhiana); Haryana (Panipat, Sirsa); Karnataka (Bengaluru, Rani Banur); Himachal Pradesh (Kullu Valley); Jammu and Kashmir (Sri Nagar Valley) and West Bengal (Kalimpong). By standardising processes for large-scale production and maintaining the germination of seeds, along with fairly inexpensive labour, scientific expertise, and other materials, the prohibitive cost of these seeds could be overcome. This would allow for the organisation of a robust flower seed production programme that is solely for export (Salunkhe et al. 1987). The major states of India that have increased their output value from the floriculture industry are represented in Figure 1. It is a constant challenge for breeders to predict the evolutionary tendencies and translate them into breeding objectives.

In this review paper, a brief discussion is carried out on both conventional and modern breeding techniques that outline the classical methods and recent advancements in breeding methods, as well as genetic and molecular tools that can provide ornamental breeders with some valuable information. This is not an exhaustive examination of the topic. Instead, the goal is to highlight the approaches discussed using some of the breeding methods from

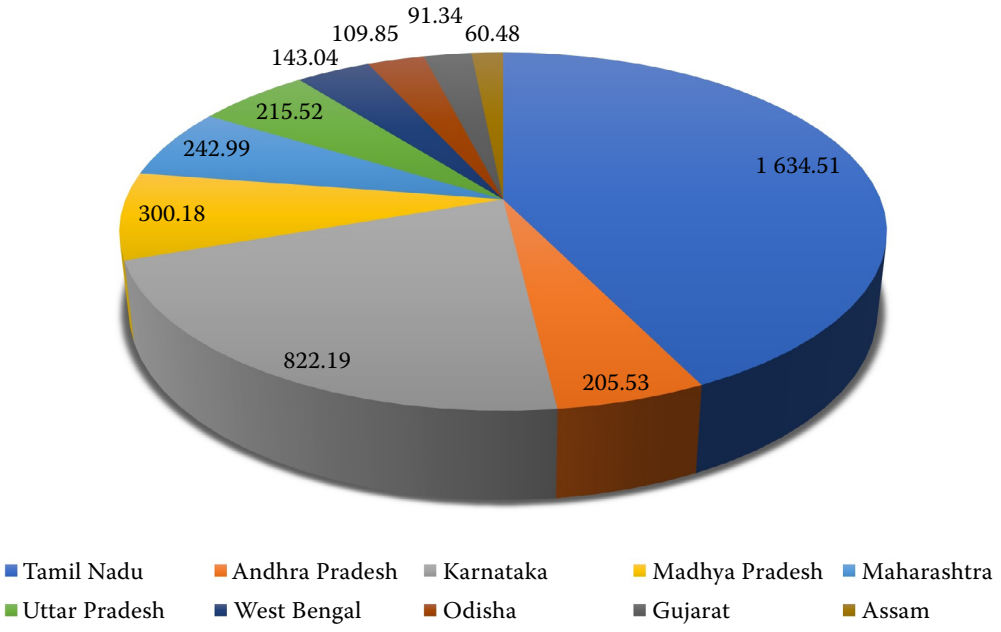

Figure 1. Value of output (million EUR) from major states of ornamental crop production in India (2021)
Data source: Ministry of Statistics and Programme Implementation, Government of India (2021)

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conventional and modern breeding techniques as examples. The major crops of ornamental annuals discussed in this review include celosia, marigold, zinnia, chrysanthemum, balsam and china aster.

Crop improvement strategies

Plant breeding entered a new era with Mendel's discovery of how traits are transmitted from one generation to the next. Ornamental plants have also progressively benefited from plant breeding based on cross-breeding and the subsequent selection of seedlings exhibiting the desired traits of both parents. In the 17th and 18th centuries, botanists and plant hunters introduced new plants from Asia and the New World to Europe, establishing the history of many decorative plants. The cultivated plants that exist at present have their progenitors as wild relatives grown in nature and have also evolved through natural variations and through human interventions through breeding techniques (Van Huylenbroeck 2019). There are several breeding strategies, including both traditional and modern methods for improving the traits of ornamental crops. The list of crop improvement research attempted in ornamentals is presented in Table 1, and the list of crop varieties developed through various breeding methods is presented in Table 2.

Selection. The exploration and collection of germplasms of the relevant genus or species are the first steps in plant breeding. Selection is a fundamental method of the breeding process as the best genotypes will be selected with desirable characteristics, which are subjected to other breeding methods. The selection of parents is the foremost criterion before the initiation of some advanced breeding methods. Agronomical and morphological descriptors are required to characterise the genetic variance of the collection. In the interim, research into their reproductive traits is required in order to determine the most effective methods to cope with the breeding process in the future. The general criteria for the selection methods are that every cultivar needs to be identified from its forebearers, which should have a commercial purpose and be resist-

Table 1. Crop improvement research attempted in ornamental annuals

Crop	Crop improvement methods	Nature of work	References
Celosia	selection	identifying the pre-breeding lines of the species for future breeding works	Ahmed et al. (2022) Bugallo and Facciuto (2023)
	hybridisation	cytological study of F_2 hybrids	Grant and William (1954)
	somaclonal variation	plant regeneration and cellular behaviour studies	Taha and Wafa (2012) Yaacob et al. (2014)
	genetic transformation	*Agrobacterium*-mediated genetic transformation	Meng et al. (2009a) Gholizadeh (2011)
	mutation breeding	mutation done by ethyl methane sulphonate (EMS) and gamma irradiation	Aisyah et al. (2021)
		sodium azide and fast neutron irradiation	Abubakar et al. (2022)
	marker-assisted breeding	molecular variability using amplified fragment length polymorphism (AFLP) marker and sequence-related amplified polymorphism (SRAP)	Feng et al. (2009) John et al. (2016)
Zinnia	hybridisation	embryo culture of inter-specific hybrids of zinnia	Shahin et al. (1971)
	mutation breeding	gamma ray irradiation	Venkatachalam and Jayabalan (1997) Pallavi et al. (2017) Kole and Meher (2005)
	marker-assisted breeding	comparative analysis between inbred lines through random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers	Tugbaeva et al. (2023)
	selection	identifying the pre-breeding lines of the species for future breeding works	Gulia et al. (2017)

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Table 1 to be continued

Crop	Crop improvement methods	Nature of work	References
Marigold	hybridisation	interspecific hybridisation between male sterile line and self-lines	Zhang et al. (2022)
	polyploidy induction	colchicine induced polyploidy	Bolz (1961) Mathew and Abraham (1980) Kapelev and Robotyagov (1981)
	mutation breeding	genetic variability studies by gamma irradiation and ethidium bromide	Karuppaiah et al. (2004) Aravind and Dhanavel (2021) Majumder et al. (2018) Keadtidumrongkul et al. (2018)
	marker-assisted breeding	transcriptome analysis, variability and correlation studies	Majumder et al. (2018) Cheng et al. (2023)
	selection	identifying the pre-breeding lines of the species for future breeding works	Pal et al. (2018)
Balsam	somaclonal variation	altering polyploidy levels by plant growth regulators Gibberrelic Acid (GA_3)	Mohamed et al. (2019)
	mutation breeding	mutagenesis through physical and chemical mutagen	Luo et al. (2021) Pal et al. (2023)
	selection	identifying the pre-breeding lines of the species for future breeding works	several research institutions
Chrysanthemum	hybridisation	cross-breeding of commercial cultivars	Anderson et al. (2014)
	somaclonal variation	somatic embryogenesis	Votruba and Kodytek (1987) Miller and Zalewska (2014) Ghosh et al. (2018)
	genetic transformation	gene transformation studies, microprojectile bombardment	van Wordragen et al. (1991) Pavingerová et al. (1994) Urban et al. (1994) Yepes et al. (1995) Shulga et al. (2011) Teixeira da Silva et al. (2013)
	mutation breeding	changes in morphology and flower characters through mutation	Wasscher (1956) Broertjes et al. (1976) Zalewska et al. (2010) Patil et al. (2017) Su et al. (2019)
	marker-assisted breeding	rapid detection of genetic variability	Wolff and Peters-van Rijn (1993) Huang et al. (2000)
	gene editing	improvement of phenotypic characters	Kishi-Kaboshi et al. (2017) Mekapogu et al. (2022)
	polyploidy induction	colchicine induced polyploidy, colchicine induced autoploidy	Kushwah et al. (2018) Yue et al. (2020)
Geranium	somaclonal variation	variation through *N*-nitroso-*N*-methyl urea (NMU)	Ravindra et al. (2004)

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Table 2. Crop varieties in ornamental annuals developed through various breeding methods

Crop	Variety	Institution/company	Method of breeding	Important features
Geranium	Sel-8 (Reunion type)	IIHR, Bangalore	selection	slender stems and dark pink flowers, giving it an elegant appearance
	Hemanthi	CIMAP, Lucknow	selection	rich in citronellol
	Bipuli	CIMAP, Lucknow	selection	rich in both geraniols and citronellol
Marigold	Calcutta marigold	IIHR, Bangalore	double-line hybrid breeding	vibrant colour and longer flowering period
	Arka Agni	IIHR, Bangalore	male sterility	early flowering, orange colour, suitable for loose flower cultivation
	Arka Alankara	IIHR, Bangalore	male sterility	photo insensitive, double-coloured and dwarf nature
	MDU 1	TNAU, Coimbatore	selection	early flowering, flowers are large with attraction sulphur yellow colour
	Pusa Basanthi Gainda	IARI, Delhi	pedigree method	produces medium-sized, lemon-yellow flowers
	Pusa Narangi Gainda	IARI, Delhi	pedigree method	produces deep orange flowers with ruffled florets, rich in carotenoids
	Pusa Arpita	IARI, Delhi	selection	produces medium-sized, light orange flowers
	Pusa Shankar 1, Nugget, Snow, Boat, Seven Star	IARI, Delhi	interspecific hybridisation	–
Celosia	Red velvet, Jewel box, Fire feather, Flamingo purple	private institutions	selection	suitable for specific geographic conditions, market requirements and landscape utilisation
	Century, Sparkler, Toreador		hybridisation	
Zinnia	Zinnia Orange, Zinnia Purple etc.	private institutions	selection	market preferences and improved landscaping characteristics
	Double Yellow		hybridisation	

IIHR – Indian Institute of Horticultural Research; CIMAP – Central Institute of Medicinal and Aromatic Plants; TNAU – Tamil Nadu Agricultural University; IARI – Indian Agricultural Research Institute

ant to diseases and pests. These selection criteria are, therefore, universal to plants across all crop types. The novelty, health, distinctive characteristics of each market, and the minimal environmental impact of its manufacturing are taken into account in every instance (Bugallo, Facciuto 2023).

The evaluation of cockscomb (*Celosia argentea* var. *cristata* 'Chief Mix') and wheat celosia (*Celosia spicata* 'Pink Candle') was undertaken by Green et al. (2010) for the flower yield, where the quality characteristics suggested that these crops have excellent performance as speciality flowers for semi-arid conditions. Ahmed et al. (2022) studied the performance of *Celosia cristata* L. genotypes in response to different potting media, where they segregated and selected the best-performing genotypes in terms of flowering traits. The genotype BD 2 of *Impatiens balsamina* was selected as the better-performing genotype based on the flower yield, quality, seed attributes and its growth and flowering characteristics among eighteen double-whorled genotypes collected from different locations (Pal et al. 2018). The varietal performance of the marigold (*Tagetes* sp.) has been evaluated for the se-

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lection of suitable varieties for specific geographical situations. Pursuing variation in marigolds while preserving their purity is a challenging task. Still, the selection has led to the evolution of three cultivars, 'Pusa Arpita' from the Indian Agricultural Research Institute (IARI), New Delhi, and the cultivars 'Hisar Beauty' and 'Hisar Jaffri-2' of the French marigold from Haryana Agricultural University, Hisar (Gulia et al. 2017). The selection of the variety or cultivar is mainly based on one important criterion, the yield, which can be increased through many physiological interventions, one of which is pinching. One such study was conducted in zinnia by Ullah et al. (2019), where the methods of single and double pinching were conducted in many cultivars of zinnia; among them, the cultivar 'Sun gold' produced more flowers with the double pinching method. Various institutes developed many enhanced chrysanthemum varieties such as 'Rakhee', 'Appu', 'Apsara', 'Apurva Singar', 'Arun Kiran', 'Birbal Sahni', 'Diana', 'Apurva', 'Kundan', etc., were evolved by selection. Several annual crops have been researched and are still under research to select the best characteristics, with growth and flowering being selected as the main characteristics of the parents for the breeding programme in future work.

Hybridisation. Hybridisation is the method of crossing two genetically dissimilar parents while hybridisation among entities from diverse species of the same genus, i.e. intrageneric hybridisation and two diverse genera of the same family, i.e. combined intergeneric hybridisation, known as distant hybridisation and such crosses, are known as distant crosses or wide crosses (Goswami, Kuchay 2023). The primary source of diversity in ornamental breeding is interspecific hybridisation. Interspecific hybrids possess the capacity to embody the vitality of a hybrid and blend characteristics that are exclusive to a single species (Volker, Orme 1988). The first person who succeeded in distant hybridisation was Thomas Fairchild, who crossed the 'Sweet William' (*Dianthus barbatus*) with the carnation (*Dianthus caryophyllus*) to develop a hybrid. Grant and William (1954) attempted to study *Celosia argentea* L. var. 'Cristata' and their hybrids, where eight F2 plants from hybrid seeds were studied cytologically. Reports of hybridisation programmes in ornamental *Celosia* species are found less due to their self-pollinating nature both naturally and through breeding studies. In *Celosia,* some of the F1 hybrids reported were 'Century', 'Sparkler' and 'Toreador'. The interspecific hybridisation between two cosmos species was reported by Kato and Mii (2012), and the process of hybrid development in cosmos is given in Figure 2.

Hybridisation programmes in other annuals have been reported in some crops like zinnia, where interspecific hybrids of *Zinnia peruviana* Jacq. and *Zinnia elegans* Jacq. through embryo culture were studied by Shahin et al. (1971), where F1 hybrids resembled *Zinnia elegans* Jacq. more than *Zinnia peruviana* Jacq. and were more vigorous than either parent and were sterile. Alturaifi et al. (2023) studied the effect of low and moderate salinity tolerance in *Zinnia marylandica,* which is an artificial hybrid between *Zinnia violaceae* and *Zinnia angustifolia* and observed that at greater salinity levels, it is unable to maintain the K^+/Na^+ ratio at an appropriate level. In marigold and zinnia, efficient and cost-effective hybrid seed production is facilitated by hereditary male sterility. Tetraploid zinnias are created by treating diploid plants with colchicine; they grow quickly and have larger flowers. 'Fairyland Gold', 'Fairyland Scarlet', 'Sunrise', 'Dreamland Coral', 'Sunrise Red', 'Yellow Zenith', 'Firecracker', 'Silver Sun', and so on are some of the F1 hybrids of zinnia where plants can be multiplied vegetatively, and hybrid vigour can be maintained. Carefree geranium plants

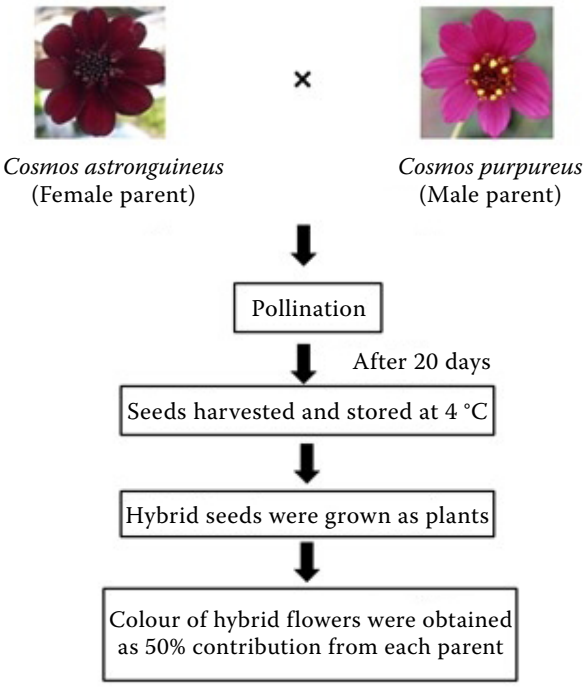

Figure 2. Development of interspecific hybrids in cosmos species
Source: Kato and Mii (2012)

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grown from cuttings in F1 hybrids are stronger and more uniform than F2 plants grown from seeds (Singh 2015). Zhang et al. (2022) identified two hybrid combinations for the commercialisation of marigold varieties as a result of interspecific hybridisation between two male sterile African marigold lines and six self-lines of French marigolds. Due to their sterility and lack of fertilisation, triploid hybrid flowers such as 'Nugget' in marigold are produced from the cross of *Tagetes erecta* (diploid) × *Tagetes patula* (tetraploid), allowing the seed sets to stay fresh on the plant for a longer period of time. The importance of hybridisation is to improve the quality of the cultivated species and resistance to pests and diseases. More than 25% of known plant species confront natural hybridisation on a regular basis (Mallet 2005).

In chrysanthemum, cross-breeding has produced a large number of commercial cultivars with favourable features, such as the garden chrysanthemum 'Lavender Daisy' from the Mammoth series (Anderson et al. 2014). Some hybrids of chrysanthemum released by the Indian Institute of Horticultural Research (IIHR) were 'Indira', 'Red Gold', 'Chandrika', 'Kirti', 'Nilima', 'Ravikiran', etc. (Singh 2015). Hybridisation studies in *Impatiens* were conducted by Merlin and Grant (1986), who studied the biosystematic relationships of *Impatiens walleriana* Hook. f. and other selected species through hybridisation experiments and cytological techniques. Many horticulturally useful phenotypes with distinct leaf types, flower shapes, colours and smells can be produced in natural hybrids due to the traits of distinct genetic combinations from parental species. The most feasible breeding strategy for raising productivity and production was discovered to take advantage of heterosis. A relative heterosis estimation for six lines of *Callistephus chinensis* was performed by Bhargav et al. (2018) where among the six lines of china aster taken for the study with thirty crosses, they discussed that in terms of the plant height, number of branches per plant, and flowering period, the cross L5 × T1 showed the highest positive significant relative heterosis. The cross L5 × T4 recorded the largest positive relative heterosis for the flower head diameter and 100 flower weight, while the cross L5 × T3 showed the maximum negative relative heterosis for the days to first flowering. Kumari et al. (2018) reported a crossability study between fifteen F1 hybrids and eight parents of china aster by documenting the seed-setting-related metrics such as the number of seeds/cross, the weight of each seed/cross and the number of days it takes for hybrid seeds to mature. Yet, due to the significant focus gap that exists between plant taxonomists and horticulturists around the world, natural hybrids have enormous potential to serve as a source for breeding new cultivars which has been frequently overlooked (Tian, Ma 2022).

Somaclonal variation. The genetic variation occurring in plants derived through tissue culture is referred to as somaclonal variation (Larkin, Scowcroft 1981). The simplest method of identifying somaclonal variation is to transplant the plants into the soil while maintaining the phenotypic variations. The factors that influence the somaclonal variation in *in vitro* culture are the regeneration system, type of tissue, explant source, media components and culture cycle duration (Sarmah et al. 2017). Somaclonal variation arises from two sources: genetic variation, which is produced by changes in the genes themselves, and epigenetic variation, which is caused by changes in the expression of the genes created during tissue culture. Taha and Wafa (2012) studied plant regeneration and cellular behaviour in *Celosia cristata* L.*,* which was grown in both *in vivo* and *in vitro* conditions, and the observation was the mean chromosome number, mitotic index, mean nuclear to cell area ratio of the *in vitro* root meristem cells were comparatively higher than the *in vivo* plants. Another report on *Celosia cristata* L. on the *in vitro* regeneration and acclimatisation was elucidated by Yaacob et al. (2014).

Studies on somaclonal variation in ornamental annuals are abundant, and experiments have been conducted on many annuals, such as in *Impatiens balsamina* L. by Mohamed et al. (2019) who reported that the *in vitro* blooming plants of *Impatiens balsamina* L. had the highest percentage of polyploid cells (30.7%) and discovered that the polyploidy level of the meristematic root cells was elevated by plant growth regulators, particularly Gibberrelic Acid (GA3). In the rose-scented geranium, *N*-nitroso-*N*-methyl urea (NMU) was used to create somaclonal variation in the Indian cultivar 'Bourbon' and its clone 'Narmada' both with and without *in vitro* mutagenesis and the first report on a chemovariant of the rose-scented geranium with a moderately high content of isomenthone was studied by Ravindra et al. (2004), somatic embryogenesis in chrysanthemum cv. 'Marigold' was experimented on by Gosal and Wani (2018). Stieve and Stimart (1996) examined the somaclonal varia-

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tion in zinnia since traditional methods of breeding remain ineffective for zinnia. Miller and Zalewska (2014) obtained three new, eye-catching varietals with altered the inflorescence colours from leaf explants of two cultivars ('Albugo' and 'Alchimist Tubular') through somaclonal variation in the chrysanthemum. Votruba and Kodytek (1987) observed the increasing variability for the plant height, flowering date, plant width, number of flowers and flower diameter of chrysanthemum through somaclonal variation. The somaclonal variation observed in ornamental annuals is presented in Table 3.

Genetic transformation. Genetic transformation can improve the traits derived from one or several kinds of genes. In a manner akin to mutation breeding, the strategy could be used to provide a one-point enhancement of the characteristics in original cultivars bred by cross-hybridisation (Shibata 2008). One of the target qualities that has been most successfully enhanced through gene transfer is the flower colour (Meyer et al. 1987). With the use of this technology, it may be possible to create incredibly precise programmes meant to enhance certain characteristics while preserving their current qualities. Petunia flowers bloomed red when the *dihydroflavonol 4-reductase* gene, obtained from *Zea mays*, was introduced. This was the first documented example of a flower colour changing through genetic transformation.

Meng et al. (2009b) observed the alternations in the dorsoventral side of leaves in transgenic *Celosia* caused by the *ASYMMETRIC LEAVES2-LIKE38/LBD41* gene of *Arabidopsis*. The identification of horizontal transfer of genetic information between co-existing proteobacteria and *Celosia* leaves was found by Gholizadeh (2011) in *Celosia cristata* L. Another study on the regeneration and genetic transformation in *Celosia cristata* L. and *Celosia plumosa* L. experimented with the transfer of the *PttKN1* gene (Meng et al. 2009a). A plant regeneration and genetic transformation study in *Tagetes erecta* L. was experimented on by Gupta and ur Rahman (2015), where an investigation was made on the effect of different types of explants (hy-

Table 3. The somaclonal variation observed in ornamental annuals

Serial number	Crop species	Explants	Observations	References
1.	*Celosia cristata* L.	shoot (1 month old aseptic seedlings)	no somaclonal variation detected lower mean cell areas of *in vitro* plants than nuclear areas of *in vivo* plants	Taha and Wafa (2012)
		shoot tip	pigment contents of amaranthin, betanin, betaxanthin increased in different coloured callus used betalamic acid were equal in three different coloured callus lines	Warhade and Badere (2015)
		leaf stem shoot tip	75% survival rate production of red and orange-coloured callus with only medium Benzyl Amino Purine (BAP) multiple shoot production with medium containing BAP and Naphthalene Acetic Acid (NAA)	Yaacob et al. (2014)
2.	*Zinnia marylandica* L.	seeds	variations in plant height, fertility, flower colour, morphology	Stieve and Stimart (1996)
3.	*Impatiens balsamina* L.	stem shoot	greater number of shoots *in vitro* flowering	Mohamed et al. (2019)
4.	*Tagetes erecta* L.	leaf segments	economically desired plant height, flower size, viable seeds, flower, days to full bloom etc.	Misra and Datta (2001)
	Tagetes minuta L.	cotyledons	significant percentage of tolerance to drought, low water potential, greater accumulation of biomass, higher relative growth rate	Mohamed et al. (2000)

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pocotyls, cotyledonary leaves, rachis and leaf sections) and different Benzyl Amino Purine (BAP) and Gibberrelic Acid (GA3) combinations on the regeneration frequency of *Tagetes erecta* L. which revealed that hypocotyl explants grown under conditions with 1.5 mg/L BAP and 5 mg/L GA3 had the best regeneration frequency (66%) with an average of 5.08 ± 0.09 shoot buds/explant. Many studies have been reported on the *Tagetes* species for genetic transformation. The creation of a straightforward and effective genetic transformation system mediated by *Agrobacterium* was attempted in *Impatiens balsamina* by Dan et al. (2010) and the process of the transformation is depicted in Figure 3.

Among the several vector-mediated transformations in chrysanthemum species, the regeneration of transgenic plants and the susceptibility to infection are highly cultivar-specific when it comes to *Agrobacterium*-mediated transformations (Deroles et al. 1997). Many cultivars have been studied through gene transformation in chrysanthemums by several researchers (van Wordragen et al. 1991; Pavingerová et al. 1994; Urban et al. 1994; Teixeira da Silva et al. 2013). The chrysanthemum was effectively transformed by micro projectile bombardment (Yepes et al. 1995). Genetic modification in ornamental plants has several important roles in different traits such as biotic stress resistance, abiotic stress resistance, flower colour modification, perfume modification, morphology modification, and the longevity of the flowers, etc. Early flowering in a transgenic chrysanthemum was induced by the overexpression of the *AP1* gene, a member of the MADS-box gene family (Shulga et al. 2011). There are many other ornamental plants examined with genetic modification methods and the obtained results have promising traits.

Polyploidy induction. Eeckhaut et al. (2020) discussed that, in ornamental breeding, one of the techniques used to overcome barriers to generate homogenous lines or induce unique variety is polyploidy induction. In addition, polyploid plants typically flower later or for a longer duration than similar diploid plants, which is a desirable trait for ornamental breeding, and the plants also grow more slowly (Sattler et al. 2016). The most widely used anti-mitotic treatment was colchicine, although it needed to be administered at rather large concentrations due to how weakly it binds to plant tubulins. Because they have a higher affinity for plant tubulin dimers and a polyploidisation capability, 25% of all herbicides can be employed at lower dosages. Mostafa and Alhamd (2016) treated *Celosia argentea* L. seeds at 0, 0.01, 0.02, 0.05, 0.1 and 0.2% colchicine for 48 h to induce polyploidy. In comparison to diploid plants, the putative tetraploid plants showed a considerable increase in all the examined parameters, including vegetative growth, flowering growth and phytochemical composition. When tetraploid plants were compared to diploid plants, there was a drop in pollen viability and seed germination percentage.

Bolz (1961) successfully produced fertile allotetraploids in the *Tagetes* species by using colchicine. Mathew and Abraham (1980) used colchicine to induce polyploidy in *Tagetes erecta* L. They saw a decrease in the plant height and an increase in the

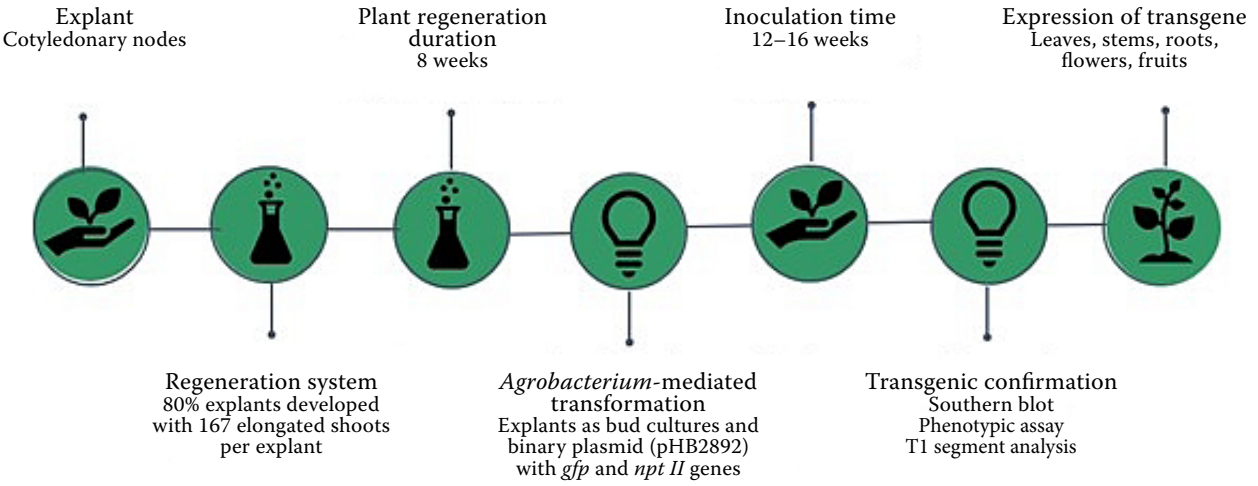

Figure 3. Steps involved in the genetic transformation method of *Impatiens balsamina*
Source: Dan et al. (2010)

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capitulum size. After being exposed to a 0.10% colchicine solution for 12 h, marigold seeds produced plants that outperformed the original variety in terms of flowering, flower output and essential oil content (Kapelev, Rabotyagov 1981). 'Jaguar', 'Zenith' and 'Nugget Supreme' are among the most well-known polyploid marigold cultivars. Kushwah et al. (2018) studied polyploidy induction in *Chrysanthemum carinatum* using colchicine and observed that the colchitetraploid plant's phenotypic traits included somewhat slower growth, a stronger stem, thicker and larger leaves, larger flowers, and seeds. Yue et al. (2020) treated 120 nodal segments of chrysanthemum 'Gongju' in colchicine to induce autoploidy and discussed that the morphological features of octoploid and tetraploid plants revealed no difference in the plant height, but they differed significantly in their leaves and flowers. It is possible to achieve chromosome doubling in numerous decorative crops, including lilies, sage, phlox, gladiolus, petunia, and marigold, by applying colchicine in various ways. Hanzelka and Kobza (2004) studied the characteristics of genome-induced mutation in *Callistephus chinensis* like the thousand seed weight (TSW, g), achene size (mm) and fertility in polyploid plants of C_0 (1999) and C_1 (2000) generations. They reported that, in comparison to diploid plants, polyploid plants often had far lower fertility, more than ten times lower in fact. There was only one tetraploid plant [genotype A (TM) 1] that had huge achenes, a high TSW, and abnormally high fertility. The TSW ranged from 2.0–2.3 g in diploid plants and 2.6–4.13 g in polyploid plants. In most cases, the achene size was between 3.7 and 4.8 mm for diploid plants and 4.0–4.8 mm for polyploid plants.

Mutation breeding. A fundamental contributor to the variety and evolution of species is mutation, which is a heritable alteration in the genetic makeup of living things. The ability to adapt more effectively to their surroundings, whether natural or artificial, gives mutants a genetic advantage. Mutations can occur spontaneously or through inducement. Wild species have been brought into domestication primarily through mutation (Bado et al. 2015). Direct DNA modifications or the use of chemical and physical mutagens can both induce mutations. These mutagenic agents alter the genetic components of the target materials by causing the chromosomes to double or the DNA to be deleted. Annual hybrid seeds, like seeds from the marigold, zinnia, cosmos, and celosia, can be exposed to radiation to cause mutations that often manifest as a chimaera. Thus, clonal propagation and seed care ought to go hand in hand. It is possible to sustain an enhanced cultivar by vegetatively reproducing selected mutations. Country-wise data on released mutant varieties is represented in Figure 4.

Many studies have been published in annual reports, including research by Aisyah et al. (2023), which investigated mutation studies in *Celosia cristata* L. using gamma irradiation and ethyl methane sulphonate to increase morphological diversity and

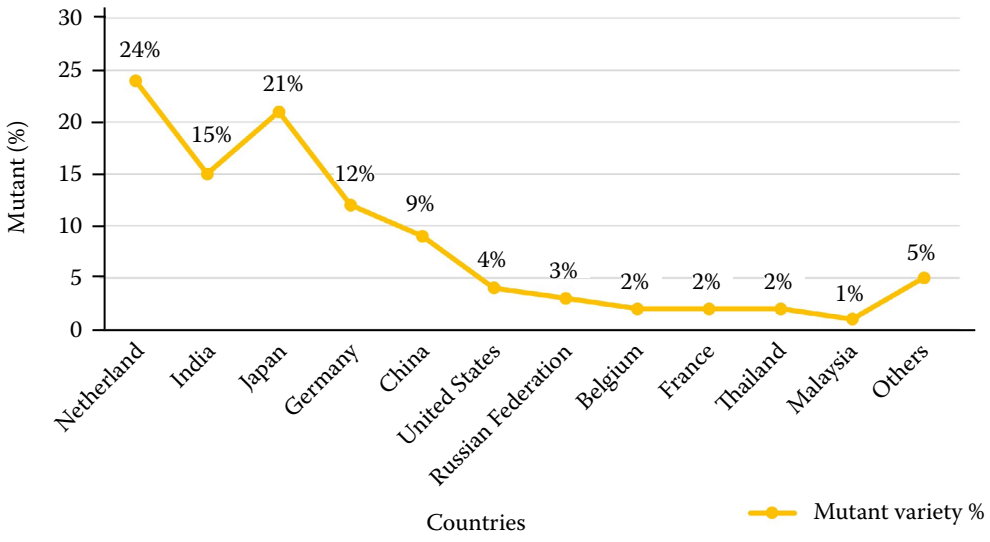

Figure 4. Country-wise data on released ornamental mutant cultivars
Adapted with modifications from Suprasanna and Jain (2021)

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improve biochemical properties. The study found that chronic alterations resulted in changes to the plant's morphology, such as the level of anthocyanin pigmentation at the base, leaf form, leaf colour, and flower shape. Mostafa et al. (2014) reported on the induced mutation in *Celosia argentea* L. using dimethyl sulfate as a mutagen and revealed at doses 2 000, 3 000, 4 000 ppm, dwarf plants were obtained, and changes in the leaf and inflorescence in M2 generation were noticed along with polymorphism of 41.8% with the inter simple sequence repeat (ISSR) markers. A gamma irradiation study in *Celosia argentea* var. 'Plumosa' was reported on by Aisyah et al. (2021) for morphological variation and discussed that the pink and rose genotypes exposed to 600 Gray (Gy) radiation had the greatest colour differences in the inflorescence, whereas the yellow genotype was exposed to 800 Gy.

Molecular diversity studies in mutants of *Celosia cristata* L. were experimented on by Abubakar et al. (2022), who discussed that the mutagenic agent used did not influence the clustering of the mutants and their parents into five different genetic groups by an unweighted pair group method with arithmetic mean (UPGMA) dendrogram or four groups by the main component analysis, indicating the high degree of produced diversity among the treatments. The mutagenic effect of sodium azide and fast neutron irradiation on the cytological parameters in *C. argentea* var. 'Cristata' was reported on by Abubakar et al. (2017), where a variety of abnormalities, including invagination of the leaf apex, dented edges, wrinkle forms, and leaf chlorosis, were revealed by the phenotypic expression of the mutant leaves. The edible leafy vegetable *Celosia argentea* L. showed considerable improvement in all the irradiated plants' morphological leafy criteria, indicating that fast neutron irradiation (FNI) is a useful strategy for improving Lagos spinach and that the ideal irradiation dosage is 60 min of exposure.

In zinnia, a mutation study was reported on by Venkatachalam and Jayabalan (1997), where they obtained four colour mutations (majenta, yellow, red, and red with white spots). Pallavi et al. (2017) experimented with a mutation study in the *Zinnia elegans* var. 'Dreamland' through gamma irradiation and obtained variations in the morphological characteristics and flower colour variations as a result. Kole and Meher (2005) experimented with a mutation study on dry seeds of two zinnia cultivars ('Suttons Gaint Double Orange' and 'Yellow'), which were irradiated with 5, 10 and 15 kR doses of gamma-rays using a Cobalt (^{60}Co) gamma cell at 1.6 kR/min for some qualitative and quantitative characteristics.

In the marigold, a mutation study was performed by Aravind and Dhanavel (2021) to find the seed germination, seedling survival and determination of lethal dose (LD 50) concentration of the mutagen doses and found that the dosage is inversely proportional to the seed germination and seedling survival. Majumder et al. (2018) studied the correlation and genetic variability in distinct putative mutants of the marigold var. 'Pusa Narangi Gainda' generated through gamma irradiation (*in vivo* and *in vitro*) conditions, while a study on the genetic variability and characteristic association in *Tagetes patula* L. was reported on by Karuppaiah et al. (2004). Keadtidumrongkul et al. (2018) experimented with induced mutagenesis in marigolds using ethidium bromide and determined the LD 50 value, and several other studies in marigolds on mutagenesis have been reported.

The National Botanical Research Institute (NBRI) has carried out an enormous amount of successful chrysanthemum mutation work in Lucknow, where 30% of the varieties were originally bud sports (Wasscher 1956). 'Kasturba Gandhi' (white), 'Sonar Bangla' (yellow), 'White Cloud' (white), 'Sharda' (yellow), 'Queen of Tamluk' (yellow), 'R. Venkatraman' (yellow), 'William Turner' (white), 'NBRI Pushpangadan', etc. are the more prominent bud sports in chrysanthemums. In chrysanthemums, many researchers have worked on unique changes in the morphology and flower characteristics of the crop (Broertjes et al. 1976; Zalewska et al. 2010; Patil et al. 2017; Su et al. 2019). Portulaca mutation research has been conducted by various researchers (Raghuvanshi, Singh 1979; Amirul Alam et al. 2014; Srivastava 2018; Nurcholis et al. 2023) for improving their phenotypic characteristics in flowering and growth traits. The primary goals of ornamental breeding are economic attributes, including the flower colour, extended shelf life and, to a limited extent, fragrance tampering. The plant and flower architecture refers to the size, shape, and form of the plant as well as to the flowers and inflorescences.

Anwar et al. (2020) analysed induced mutation in *Callistephus chinensis* by gamma rays on the performance of MV3 in lowland conditions. They discussed the gamma-ray-induced mutation of M1V3 *Callistephus chinensis* at lowland increased height,

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number of leaves, number of flowers, and length of the flower stem. The majority of the flowers were purple, but those with the Y1-U4-9 genotype (Y1-U4-9-5) were pink and concluded that due to the gamma-ray-induced mutation, *Callistephus chinensis* might adapt to lowland environments.

Marker-assisted breeding. Molecular marker-assisted breeding (MAB) is a technique which involves DNA markers that are, in conjunction with linkage maps and genomics, used for modifying and improving plant traits based on genotypic tests (Jiang 2013). The development of molecular (DNA) markers has facilitated the finest indirect selection for target genes at the DNA level, providing a powerful and valuable tool for gene selection in plant breeding. Nevertheless, it is important to note that this approach does not constitute true gene selection. In *Celosia argentea* L. molecular variability was detected using amplified fragment length polymorphism (AFLP) markers by John et al. (2016), where the study confirmed the variability, and this may encourage *Celosia argentea* L. germplasm conservation and enhancement to increase the genetic diversity of breeding programmes. Genetic diversity and population structure studies were experimented on by Feng et al. (2009) in *Celosia argentea* L. and *Celosia cristata* L. using sequence-related amplified polymorphism (SRAP) markers and confirmed to the genetic diversity. Qian et al. (2019) identified and sequenced the complete chloroplast genome of *Celosia argentea* L. where the genome size was found to be 153 474 base pairs (bp), the GC content (guanine and cytosine bases) was 36.7%. John et al. (2016) analysed the molecular variability using the AFLP marker and recorded the highest concentrations of genomic DNA for the total genomic DNA for the NG/TO/MAY/09/015 and NG/MA/MAY/09/015 genotypes.

Studies on MAB in other annual crops were experimented on by several researchers, where Ye et al. (2008) performed a comparative analysis between inbred lines of *Zinnia elegans* L. using morphological traits and through random amplified polymorphic DNA (RAPD) and ISSR markers. Tugbaeva et al. (2023) experimented with assisted breeding in zinnias. In marigold, Majumder et al. (2018) conducted variability and correlation studies in *Tagetes erecta* L. (Kumar et al. 2023). Cheng et al. (2023) conducted a transcriptome analysis on *Tagetes erecta* L. leaves in response to *Alternaria tagetica*. The cultivars and their ancestors were identified using a variety of molecular markers.

In a study on the quick detection of genetic variability, Wolff and Peters-van Rijn (1993) discovered that RAPD fragments were helpful for cultivar identification due to their high level of polymorphism and clonal stability and that genetic diversity among the related chrysanthemum species was excessively high. In their investigation into the intricacy of chrysanthemums, Huang et al. (2000) discovered that RAPD is an effective method for identifying several molecular markers in hybrid populations of chrysanthemum cultivars. A phylogenetic study in *Impatiens balsamina* L. was carried out by Yu et al. (2016) by integrating morphological and molecular evidence.

Bhargav et al. (2021) studied *Callistephus chinensis* using 26 polymorphic markers to estimate the genetic diversity of 42 genotypes. China aster genotypes were separated into five primary clusters using the Weighted Neighbour Joining method. These clusters were not correlated with their geographical locations, but they did correspond for the morphological features, primarily flower colour and form. The findings showed that the population would be helpful for mapping the genome-wide associations between the markers and traits. With the use of this collection of cross-species transferable simple sequence repeats (SSR) markers, China could use the SSR technology for future crop development.

Other breeding techniques

Embryo rescue method. Embryo rescue is a technique that is frequently employed to protect immature or mature deadly embryos as well as hybrid embryos produced from interspecific and intergeneric crosses that are incapable of surviving *in vivo* during conventional plant procedures. In an attempt to rescue immature embryos, the developmental differences between dicots and monocots must be taken into consideration (Rogo et al. 2023). In the 18th century, embryo rescue was first recorded by Charles Bonnet (1720–1793). The most common method for creating hybrids from several incompatible crosses is the embryo rescue technique. At the moment, embryo rescue shows a lot of potential for producing haploid plants, wide crossings, and plants from embryos that are naturally weak, as well as reducing the length of the breeding cycle (Sharma 1999). Hussein (2013) experimented with techniques of clearing and enzymic maceration to provide methods for separating and/or clearing the embryo sacs of *Impatiens glandulifera* Royle. to enable a microscopic analysis in preparation for further research on *in vitro* flow-

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ering plant fertilisation. Interspecific triploid hybrids (2*n* = 36) have been produced in marigold through the crossing of tetraploid (2*n* = 48) *Tagetes patula* and diploid (2*n* = 24) *Tagetes erecta* (Datta 2022).

CRISPR-cas technology. The development of more accurate and effective methods to cause mutations in plant genes, altering their expression or silencing them, is made possible by genome editing techniques, especially those that rely on clustered regularly interspaced short palindromic repeats (CRISPR)-derived technologies (Hahn, Nekrasov 2019). In *Chrysanthemum morifolium* Ramat., an experiment was conducted and is the first report on gene editing using the CRISPR/Cas9 system by Kishi-Kaboshi et al. (2017). Another work reported in chrysanthemum for the improvement of the phenotypic characteristics by using this technique was conducted by Mekapogu et al. (2022). In petunia, many works have been reported on, such as targeted genome mutagenesis by CRISPR/Cas9 (Zhang et al. 2016), flower longevity enhancement in petunia by editing in an ethylene biosynthesis gene (Xu et al. 2021), flower colour modification in petunia via CRISPR/Cas9 ribonucleoproteins (Lin, Jones 2022), and changing the leaf shape of petunia (Moazzam 2020).

Root-inducing (Ri) technology. Christensen et al. (2008) discussed, in their study about Ri technology in ornamentals, that *rhizobium* rhizogenes wild-type strains are used in an intriguing alternate transformation technique. Numerous dicotyledonous plant species are affected by the so-called crazy root disease, which is caused by a group of pathogenic bacteria that carry a root-inducing Ri plasmid. Natural transformation is the outcome of co-cultivating the bacteria and plants in a lab setting. Since they are not genetically modified, regenerated Ri phenotypic plants are free from regulatory restrictions and can be sold. The Ri technique is being utilised presently. The morphological alterations in the leaves, flowers, flowering time, root growth and other important trait growth habits are expressed by Ri phenotypes. In addition to the most intriguing characteristic, these Ri phenotypes can exhibit morphological changes in the leaves, flowers, flowering time, and root growth. Since Ri transformation typically results in small growing phenotypes, this technology presents an intriguing alternative method for producing more compact growing plants. Compact growth is a major economic consideration in many ornamental crops.

SUMMARY

Owing to the improved scientific methodologies and a steady supply of improved varieties, floriculture has grown to be a significant sector in many nations. Significant challenges in the floriculture trade include the development of novel varieties and their quick marketing. There is a constant need for novel varieties in today's industrialised and sophisticated flower industry. Genetic modification, either by modifying the gene architecture or by gene transfer, is required for the generation of novel phenotypes. Biotechnological tools for the manipulation of genes in biological systems, as well as several traditional and contemporary techniques, are currently available for crop improvement. Induced mutagenesis, chromosomal modification, and hybridisation are examples of traditional biotechnology techniques. Under the current biotechnology methods, genetic diversity can be manipulated through molecular approaches. Every method has benefits, drawbacks, and restrictions. Despite the fact that a large number of ornamental plants can be purchased globally, there is currently no collaboration in the development and execution of new regulations governing gene-edited plants, and there is no international harmonisation of the control of genetic modification. Utilising both conventional and contemporary methods in plant breeding results in the creation of new varieties that stimulate global trade. Plant breeders considering the adoption of these newer technologies must weigh technical concerns against the cost of development and the difficulty of securing regulatory approval. They also need to balance the estimate of any possible commercial benefits.

FUTURE PERSPECTIVE

Understanding traditional and modern breeding approaches will enhance the selection of the breeding methods suitable for the selected crop to improve its characteristics. Hence, wider knowledge is required in the area in order to undergo experimental work, which will involve higher costs and more labour. Particularly with the successful application of genome editing technologies, recent advancements in genomics have considerably boosted the basic research in horticultural plants, signifying a new channel for ornamental research and breed-

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ing. Since ornamentals have a complicated genetic background and breeding history, further research is necessary. Molecular biology and genome engineering will open up new avenues of ornamental plant cultivation. More efficient, systematic and focused breeding approaches will transform ornamental plant cultivation and increase the diversity of the ornamental characteristics.

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