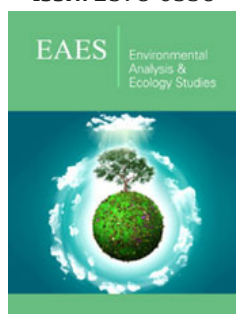


# Cutting-Edge Biotechnological Progress in The World of Edible Flowers

ISSN: 2578-0336



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**Submission:**  October 19, 2023

**Published:**  March 18, 2024

Volume 12 - Issue 1

**How to cite this article:** Prashant GK\*, Pritam RJ, Prasad KV, Devarumath RM, Dipak KS, Nalini Shinde, Madhuri CP and Prashant RS. Cutting-Edge Biotechnological Progress in The World of Edible Flowers. Environ Anal Eco Stud. 000779. 12(1). 2024. DOI: [10.31031/EAES.2024.12.000779](https://doi.org/10.31031/EAES.2024.12.000779)

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## Introduction

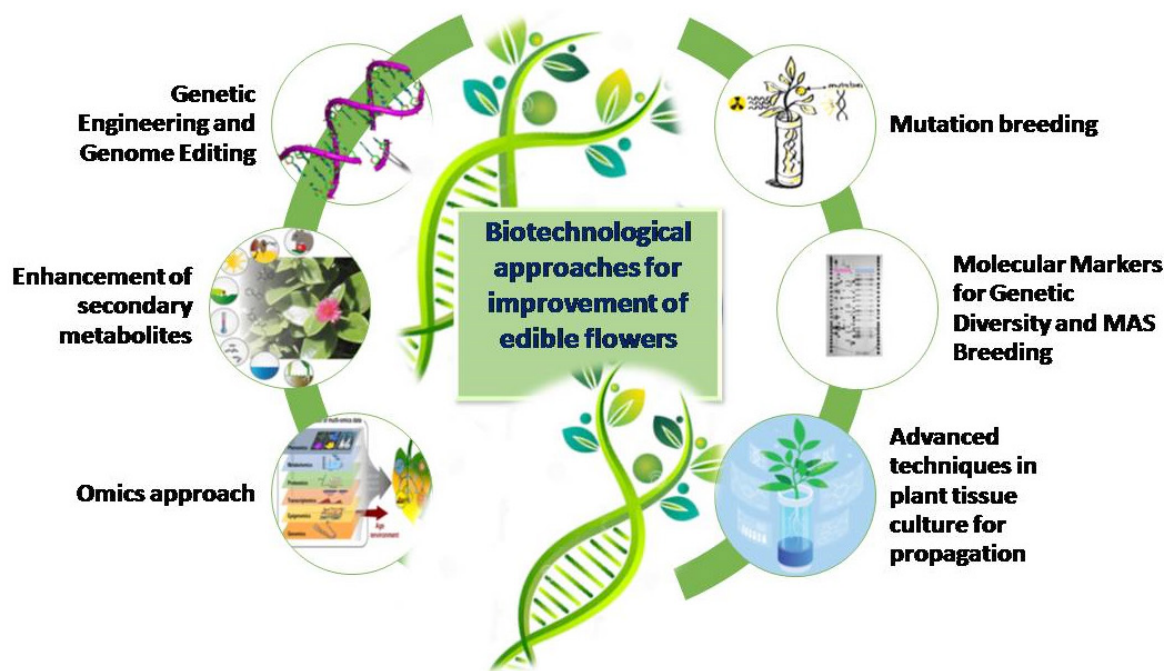
Edible flowers possess a rich history of consumption and documentation worldwide, spanning ancient civilizations like Greece, Rome, medieval Europe and Asian countries such as China and Japan [1,2]. Over time, globalization and heightened consumer awareness have rekindled interest in edible flowers due to their potential to enhance human well-being and health. Research has spotlighted their bioactive compounds, including natural pigments, essential oils and antioxidants, elucidating their health-promoting effects and folk medicinal uses. The common phytochemicals in edible flowers are depicted in (Table 1). In response to consumer preferences for natural, functional and healthy food products, edible flowers have gained considerable attraction in the market which led to the evaluation of several species such as chrysanthemum, hibiscus, lavender, marigold and rose for their potential benefits [13,14]. Approximately 180 flower species have been identified as suitable for human consumption, edible flowers offer more than just aesthetic enhancement; they present a safe and nutritious option. These blossoms play a crucial role as functional ingredients in food along with their aroma and have potential health advantages when included in range of dishes and beverages like teas, wines, fruit juices etc. [15,2]. Beyond culinary appeal, their extensive historical use in traditional medicine underscores their medicinal properties.

**Table 1:** Common phytochemicals in edible flowers.

Edible Flowers	Phytochemicals	Reference
Marigold, Rose, <i>Calendula</i> , <i>Chrysanthemum</i>	Carotenoids (Lutein, $\beta$ -carotene, Flavoxanthin, lycopene, zeaxanthin, etc)	Varzakas and Kiokias [3]; Wan et al. [4]; Pavelkova et al. [5]
Rose, <i>Hibiscus</i> , <i>Calendula</i> , <i>Chrysanthemum</i>	Anthocyanins (Cyanidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, peonidin 3-O-glucoside, etc)	Grajeda-Iglesias et al. [6]; Wan et al. [7]; Kumari et al. [8]
Rose, <i>Calendula</i> , Marigold, Lavender, <i>Hibiscus</i> , Daylily, <b>Chrysanthemum</b>	Flavonoids (Quercetin, myricetin 3,5-di-O-glucoside, kaempferol 3,7-di-O-rhamnoside, etc)	Cendrowski et al. [9]; Cao et al. [10]; Wan et al. [4]
Rose, Lavender, <i>Chrysanthemum</i> , <i>Calendula</i> , Daylily, Marigold	Phenolic acid (Gallic, Caffeic, Caftaric, Chlorogenic, Chicoric, Coumaric, Sinapic and Ferulic, etc)	Ryu et al. [11]; Krzyżmińska et al. [12]

As a niche market, edible flowers cater to a diverse range of consumers seeking natural and bioactive components in their diet, further highlighting their significance in contemporary food culture. In the present scenario, there is a global demand for novel traits in ornamental plant products, including improved anatomical attributes, floral colors, pigments, stress tolerance, disease

resistance and enhanced secondary metabolites with health benefits and medicinal values [16,17]. Research and advancements in biotechnology Figure 1 have paved the way to enhance edible flowers, ensuring their safety and augmenting their valuable traits for diverse purposes, including industrial applications, promising a future for these blossoms.



**Figure 1:** Biotechnological approaches for improvement of edible flowers.

## Biotechnological Approaches Employed for Improving Specific Traits in Edible Flower Crops

### Mutation breeding

Mutation breeding has been a fundamental approach in developing new plant varieties, especially in ornamental plants. Traditional breeding methods, although reliable, are laborious and time-consuming compared to mutation breeding. This technique involves the use of UV radiation, ionizing radiation and chemical mutagens like EMS and Sodium Azide to induce mutations. It has significantly enhanced breeding efficiency. Mutation breeding revolutionized ornamental plant breeding, accelerating the development of novel traits, particularly flower colors. This method is pivotal in satisfying the floriculture industry's demands for unique attributes. Various techniques, including gamma radiation optimization and chemical mutagen selection, have yielded promising results in plants like carnations, *dianthus*, *chrysanthemums*, roses and more. These methods have shown potential in creating new cultivars and enhancing characteristics like flower color and plant architecture. Research institutions have actively registered number of new mutant lines of *dianthus*, *chrysanthemums*, roses, marigold, Jasmine, tuberose, aster, gladiolus and many more, ensuring the continuous innovation of ornamental plant varieties [18-29] DFR Annual Report, 2022-23). Mutation breeding methods are pivotal for the growth and innovation of the

ornamental plant industry, enabling the creation of desirable traits to meet local and export market demands.

### Molecular markers for genetic diversity and MAS breeding

Molecular markers have emerged as valuable tools for genetic improvement in ornamental plants, offering insights into genetic diversity and aiding in various applications such as Marker-Assisted Selection (MAS). Various molecular techniques like SSR, ISSR and SNP have been effectively utilized to assess genetic diversity, determine parentage and identify specific traits. These markers have been employed in genetic studies of different ornamental species, revealing unique alleles and providing essential information for plant breeding program [30]. For example, SSR markers have been used to distinguish different rose varieties and evaluate genetic diversity in *chrysanthemums* [31,32] Additionally, GWAS studies have linked specific genes to traits like flower type and shape in *chrysanthemums* [33]. Agarwal et al. [34] identified the polymorphic nature of SCoT markers and established genetic diversity among different *Rosa* germplasms. *Rosa hybrida*'s genetic fidelity was affirmed with RAPD and ISSR markers [35] and SSR markers differentiated between cultivated and wild rose species [36,37] constructed the first individual maps of rose populations, linking them with 824 SNPs and 13 SSR bridge markers. In carnations, molecular markers unveiled the genetic basis of

resistance to *Fusarium oxysporum*, with a sequence-specific PCR-based SCAR marker developed [38]. SSR markers differentiated carnation varieties [39], while RAPD markers assessed genetic fidelity of *in vitro* regenerated plants [40]. Marigold research employed molecular markers for investigating male sterility [41] and assessing genetic diversity in genotypes. In *Lilium* species, ISSR and AFLP markers confirmed genetic stability and variations in regenerants and progeny [42,43]. ICAR-DFR is working on unique fingerprints for marigold, rose and *chrysanthemum*, employing SRAP, ISSR, and SCoT markers, with a specific focus on variety-specific markers in *chrysanthemums* (Unpublished data).

### Advanced techniques in plant tissue culture for propagation

The floriculture industry, driven by the demand for high-quality ornamental plants and flowers, consistently seeks new varieties with improved traits, including plant architecture, vase life, keeping quality, color, and disease resistance [16,17]. Ornamental horticulture encompasses a wide range of plants used in various applications, such as home gardening, landscaping and cut flower production, contributing significantly to the industry [44,45]. Tissue culture technology has brought about a revolution in the floriculture sector, particularly through large-scale propagation using *in vitro* cloning. Tissue culture has proven invaluable in generating genetic

variability, enhancing plant health and expanding germplasms available to breeders. This technology has successfully incorporated specific traits through gene transfer, resulting in new genetic variations within breeding lines [36]. Various *in vitro* techniques, such as protoplast culture, organ culture and meristem culture, have been employed to produce haploid lines and somaclonal variants, offering advantages over traditional methods in terms of time and mass multiplication [17]. In addition to mass production, tissue culture has enabled pathogen elimination from planting materials and the production of uniform, disease-free plantlets for large-scale cultivation. These techniques have also facilitated the rapid multiplication of chimeric-mutant plants obtained through mutagenesis [16]. *In vitro* techniques have significantly reduced the time required for developing new varieties and have facilitated the rapid dissemination of planting materials over large areas [46]. (Table 2) has summarized the tissue culture techniques employed for edible flower species. ICAR-DFR has developed efficient regeneration protocols for indigenous *chrysanthemum* varieties, which are particularly valuable for multiplying chimeric flowers induced by physical mutagens. The institute has also established regeneration and direct organogenesis protocols for marigold varieties, allowing for the development of transgenic and genome-edited plants (Unpublished data).

**Table 2:** Summary of tissue culture techniques employed for edible flower species.

Species/Cultivars	Explant	Response	References
Organogenesis			
<i>Chrysanthemum spp.</i>	Shoot tips, Nodal explants and leaf	Multiple shoot, root and plant formation	Jahan et al. 2021
<i>Gerbera jamesonii</i>	Shoot tips, floral buds, leaf, petioles and petals	Multiple shoot root and plant formation	Winarto B & Prama Yufdy M 2017; Akter et al. 2012
<i>Hemerocallis fulva</i>	Stem tissue	Callus and shoot formation	Matand et al. 2020
<i>Hibiscus rosa sinensis</i>	Nodal explants with axillary bud	Multiple shoot root and plant formation	Metwally et al. 2016
<i>Jasminum sambac</i>	Young leaves, stems and petioles	Callus and shoot formation	Farzinebrahimi et al. 2014
<i>Dianthus caryophyllus</i>	Axillary buds	Multiple shoot root and plant formation	Ahmadian et al. 2017
<i>Nelumbo nucifera</i>	Immature cotyledon and embryo and meristem and embryos	Callus induction and Multiple shoo, root and plant formation	Deng et al. 2020 and Yu et al. 2015
<i>Rhododendron arboretum</i>	Nodal explants	Multiple shoot root and plant formation	Mao et al. 2018
<i>Rosa spp.</i>	Axillary buds	Multiple shoot root and plant formation	Attia et al. 2012 and Baig et al. 2011
<i>Tagetes erecta</i> and <i>Tagetes patula</i>	Nodal segments, shoot tip	Multiple shoot root and plant formation	Kumar et al. 2018, Majumder et al. 2014
Soma clonal variation			
<i>Chrysanthemum spp.</i>	Adventitious shoots from two explant types, leaves and internodes	Eeckhau et al. 2020 and Zalewska et al. 2011	
<i>Gerbera</i>	Capitulum	Bhatia et al. (2009)	
Carnation	Leaf base explants	Esmaiel et al. (2012)	
Somatic embryogenesis			
<i>Rosa spp.</i>	Nodal stem segment	Li et al. 2002 and Kim et al. 2003	
Haploid development			
<i>Chrysanthemum spp.</i>	Anther, Ovule	Khandakar et al. 2014 and Wang et al. 2014 and Gao et al. 2010	

Carnation	Anther	Nontaswatsri et al. 2007
Lily	Anther	Arzate-Fernandez et al. 1997
Marigold	Anther	Kurimella et al. 2021
<i>Gerbera</i>	Ovule	Cappadocia et al. 1988

### Genetic engineering and genome editing for edible flower crops

Genetic manipulation techniques have emerged as powerful tools to overcome limitations of traditional breeding methods in the ornamental plant industry. Genetic engineering allows the introduction of desired genes into ornamental plants, providing the ability to modify traits such as color, fragrance, disease resistance and flower architecture. *Agrobacterium*-mediated plant genetic

transformation is a widely used method for gene transfer due to its simplicity and adaptability (Table 3). Through genetic engineering, it is possible to enhance the quality and traits of ornamental plants for both aesthetic and practical purposes. This approach offers a promising way to develop new cultivars with novel flower colors, improved post-harvest longevity and other desired characteristics, contributing to the advancement of the ornamental plant industry described below.

**Table 3:** *Agrobacterium*-mediated stable gene transformation in edible flower plants.

Species	Exogenous	Explant	Methods	Gene <i>A. tumefaciens</i> strain	Transformation Efficiency	Phenotype of Transgenic Plant	Ref
<i>Rosa hybrida</i>	GFP	Leaf	Somatic embryogenesis	GV3101	5~6%	Green fluorescence observed	Liu et al. 2021
<i>Rosa chinensis</i>	GUS	Somatic embryos	Somatic embryogenesis+Shoot regeneration	EHA105	ND	GUS positive	Vergne et al. 2010
<i>Tagetes erecta</i>	GFP	Flower	Floral dipping	EHA105	ND	Green fluorescence observed	Cheng et al., 2019
<i>Tagetes erecta</i>	GUS	Leaf	Shoot regeneration	LBA4404	ND	GUS positive in leaves of transgenic plants	Narushima et al., 2017
<i>Chrysanthemum</i>	Artemisinin biosynthesis genes	Leaf	Shoot regeneration	CBE21	0.17~0.33%	Artemisinin production	Firsov et al., 2020
<i>Chrysanthemum</i>	RsMYB1	Leaf	Shoot regeneration	GV3101	1%	Improved resistance to herbicides	Naing et al., 2016
<i>Chrysanthemum</i>	cry1Ab	Leaf	Shoot regeneration	LBA4404	ND	Improved insect resistance	Shinoyama et al., 2002
<i>Lilium</i> 0-	GUS	Meristematic nodular calli	Shoot regeneration	EHA101	11.10%	Stable expression of GUS gene	Abbasi et al., 2020
<i>Lilium</i>	GUS	Filament-derived calli	Shoot regeneration	EHA101	ND	GUS positive	Hoshi et al., 2004
<i>Gerbera hybrida</i>	GMYP10	Leaf	Shoot regeneration	C58C1	ND	Activation of Anthocyanin Biosynthesis	Elomaa et al., 2003

**Flower colour:** Plant flower colors depend on anthocyanins, water-soluble pigments that bring vibrant hues to fruits, flowers and leaves. These pigments, derived from six anthocyanidins, are pivotal in genetic and molecular research [47]. Anthocyanin creation involves a series of enzymatic reactions within the flavonoid biosynthetic pathway, encompassing phenylalanine, phenylpropanoid, flavonoid and anthocyanin metabolism. In the realm of genetic manipulation for flower color, *Petunia hybrida* with its well-studied genetics and transformability, stands out as a valuable model for engineering flower color [48]. *Chrysanthemum*,

known for its diverse flower colors, has been genetically engineered to intensify cyanidin content for vibrant red petals.

However, achieving blue flower color through the introduction of a pansy F3'5'H gene remains a persistent challenge [49]. Moon carnations and roses have been successfully engineered to display violet and blue flower colors, respectively. Introduction of *petunia* genes F3'5'H and DFR into white carnation mutants resulted in violet hues through co-pigmentation. Similarly, roses were modified to produce delphinidin-based anthocyanins, leading to blue and magenta flower colors via specific gene introduction [50]. Transient



transformation experiments in *Lilium* plants have demonstrated the alteration of flower color through the overexpression of key genes for instance, overexpressing the *Phalaenopsis* F3'5'H (Ph F3'5'H) gene shifted the color from pink to pale purple, with further deepening of the purple color achieved by co-expressing PhF3'5'H and Hyacinth DFR (HyDFR) genes [51]. Genome editing has emerged as a potent tool for modifying flower color across various plant species. Understanding anthocyanin biosynthesis and related genes offers exciting possibilities for creating unique flower colors, greatly impacting the ornamental plants industry with visually captivating varieties.

**Floral scent:** Floral scent plays a vital role in attracting pollinators, protecting plants from herbivores and pathogens and facilitating reproductive processes. The scent is a complex mixture of compounds, including fatty acid derivatives, benzenoids, phenylpropanoids and terpenoids. Understanding the intricate role of floral scent in plant biology offers opportunities to improve crop yields and enhance the visual and olfactory appeal of flowers [52,53]. Genetic engineering offers a solution to impart fragrance to modern cut-flowers, addressing the decline of natural scent due to selective breeding for other traits. A breakthrough involved isolating the S-Linalool Synthase (LIS) gene from *Clarkia breweri*, a sweet-scented California plant. By introducing the LIS gene into carnations, linalool production was successfully enhanced, showcasing the potential of genetic manipulation to enhance floral scents [54]. Transcription Factors (TFs) are key players in fragrance biosynthesis, regulating various pathways that lead to the production of volatile compounds. They control processes like the shikimate pathway, regulated by TF ODORNT1 (ODO1), specifically expressed in *petunia* petals. While some pathways are well understood, the regulation of terpenoid pathways remains unclear. The successful introduction of the PAP1 transcription factor from *Arabidopsis* into rose flowers resulted in heightened production of specific scent compounds, emphasizing the potential of genetic engineering to enhance floral fragrances and revolutionize the floral industry [55].

**Biotic and abiotic stress resistance:** Plants are susceptible to a wide range of environmental stresses viz., abiotic (e.g., radiation, salinity, drought) and biotic (e.g., pathogens, herbivores) that impair growth and productivity [17]. These stresses trigger diverse plant responses, altering gene expression, metabolic processes and physiological attributes. Research on stress tolerance in edible flower crops has made progress in understanding and improving their responses, exemplified by transgenic carnations with enhanced resistance to *Fusarium* wilt disease and caffeine-producing chrysanthemum plants exhibiting resistance to fungal attacks [56,57]. The abundance, organization and expression patterns of LWRKY genes were studied in *Lilium*, indicating their potential role in both abiotic and biotic stress tolerance [58]. Genetic transformation methods, like *Agrobacterium*-mediated transfer of specific genes, have been instrumental in conferring resistance against viruses and pests, enhancing crop resilience. Additionally, the role of critical transcription factors such as ZIP, WRKY and NAC in stress response pathways has been demonstrated, paving the way

for targeted approaches to enhance stress tolerance in ornamental plants [59,60]. Overall, employing biotechnological strategies holds promise in mitigating environmental stress effects and improving the productivity and stress resilience of ornamental plants.

**Keeping quality and post-harvest quality management:** Ornamental plants are integral to the horticulture industry and preserving their quality and freshness is commercially important. Ethylene and bacterial infections accelerate flower wilting, necessitating resistance. Genetic engineering offers a cost-effective solution by reducing ethylene-induced senescence [61]. Targeting ethylene-related genes enhances vase life and quality in flowers. Successful genetic modifications, as seen in carnations, illuminate this potential [62]. Additionally, optimizing tissue-specific ethylene insensitivity, demonstrated in transgenic petunias, is crucial for commercial viability. In roses, various genes and transcription factors have been implicated in the ethylene response, providing opportunities to manipulate floral senescence and extend flower life. In conclusion, genetic engineering focused on ethylene pathways holds promise for enhancing the shelf life of ornamental plants, ensuring better quality and commercial value. Understanding regulatory mechanisms and gene expression patterns in ethylene response pathways is pivotal for developing enduring ornamental plants.

**Enhancement of industrially important pigments, essential oils and secondary metabolites:** Highlighting the nutraceutical potential of edible flowers, underscoring their richness in nutrients, phytochemicals and the subsequent health benefits, these flowers demonstrate a broad range of medicinal attributes, including anti-diabetic, anti-cancer, anti-anxiety, anti-inflammatory, antimicrobial, diuretic and immunomodulatory effects [63,64]. Also, flower essential oils are highly valued for their aromatic appeal and pharmacological properties enriched with active constituents. These oils possess antimicrobial, antioxidant and anti-pest properties, crucial for food preservation, cosmetics and medicine [65,66]. Biotechnological advancements show potential in optimizing essential oil yield and composition, exemplified by studies focusing on lavender as a key example [67-69].

**Omics approaches:** Crop improvement endeavors aim to develop climate-smart crops with heightened stress tolerance, improved nutritional value and superior agronomic traits. The integration of "omics" technologies-genomics, transcriptomics, proteomics and metabolomics-has been pivotal in identifying pivotal genes, proteins and metabolic pathways governing desired traits, facilitating marker-assisted breeding in major crops. Additionally, harnessing natural variation in crop wild relatives and underutilized species is crucial.

## Conclusion

Edible flowers are highly valued for their functional properties, adding appeal and nutritional benefits to food products. With growing consumer demand for natural and healthy food options, there's a significant opportunity to use biotechnological tools to enhance the quality and yield of edible flowers to meet global demands. Progress has been notable in enhancing key traits through

mutation breeding and tissue culture techniques. Despite genome sequencing progress, the application of genome editing in edible ornamentals is still evolving. There's a need to extend transgenic, genome editing and omics technologies to enhance the nutritional and bioactive properties of valuable edible flowers.

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