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Review

In vitro techniques for propagation and breeding of Edible Flowers - recent achievements

Técnicas *in vitro* para propagação e melhoramento de flores comestíveis - avanços recentes

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Abstract
In vitro propagation is widely used in different crops for mass multiplication and the creation of new cultivars. While edible flowers are a part of the ornamental industry, the use of *in vitro* techniques for these plants has only recently begun. Considering the importance of these species, this review aimed to examine recent advances in the use of in vitro techniques for the propagation and improvement of edible flowers, highlighting their applications, challenges, and recent findings. The number of publications in this field is still limited, with most focusing on shoot proliferation. Callogenesis studies are particularly relevant as they enable the detection, identification, and production of secondary metabolites. Biotechnological approaches, such as genetic transformation and CRISPR, were also identified, though only in a few studies. In conclusion, despite ongoing research, *in vitro* propagation of edible flowers remains a largely unexplored area, with significant potential as new alternative food and medicinal crop.
Keywords: antioxidants, metabolites, micropropagation, ornamental plants, biotechnology, tissue culture, nutraceutical plants.

Resumo
A propagação *in vitro* é amplamente utilizada em diferentes culturas para a multiplicação em massa e a criação de novas cultivares. Embora as flores comestíveis façam parte da indústria de ornamentais, o uso de técnicas *in vitro* para essas plantas é ainda está iniciando. Considerando a importância destas espécies, objetivou-se revisar os avanços recentes no uso de técnicas *in vitro* para a propagação e melhoramento de flores comestíveis, destacando suas aplicações, desafios e os últimos resultados. O número de publicações nessa área ainda é limitado, com a maioria concentrando-se na proliferação de brotações. Estudos de calogênese são particularmente relevantes, pois permitem a detecção, identificação e produção de metabólitos secundários. Abordagens biotecnológicas, como a transformação genética e o CRISPR, também foram identificadas, embora em poucos estudos. Em conclusão, apesar das pesquisas em andamento, a propagação *in vitro* de flores comestíveis continua sendo uma área amplamente inexplorada, com grande potencial como uma nova alternativa de espécie para alimentação e medicinal.
Palavras-chave: antioxidantes, biotecnologia, cultura de tecidos, plantas nutraceuticas, plantas ornamentais, metabólitos, micropropagação,

Introduction

The floriculture industry is driven by innovation, and new products or new uses of already known crops are always offered to the market. Over the years, the consumers' requirements have changed with sustainability and care about the benefits that plants can give to humans being the key points. In this light, ornamental plants are appreciated not only for their aesthetic side, but also for other important characteristics related to human uses for cooking or for therapeutic applications.

The term "edible flower" refers to ornamental plants whose flowers are suitable for culinary use. Edible flowers have been used since ancient times, with records showing that Greeks and Romans incorporated them not only for decorative or religious purposes but also in culinary preparations and medicine. In medieval Europe, flowers such as roses, violets, and calendula were used to create dishes, drinks, and even for medicinal practices (Scariot et al., 2022). Some more recent examples include the use of chrysanthemum, lotus, and peony flowers in China and Japan, for tea preparation, for incorporation in various dishes, for both flavour and health benefits. In Indonesia, the boiled flowers of *Etlingera elatior* are traditionally served hot with peanut sauce. Moreover, the popularity and relevance of edible flowers have significantly increased in recent years across several regions of the world (Kumar et al., 2025).

Edible flowers are notable for their nutraceutical properties, including antioxidant levels that can be up to ten times higher than those found in common fruits and vegetables (Kwon et al., 2019). These flowers are usually rich in phytochemicals, especially phenolic compounds, and exhibit strong antioxidant activity, contributing to potential health benefits (Kumar et al., 2025). In addition to antioxidants, edible flowers contain a wide range of bioactive compounds, including minerals and vitamins. Their use in gastronomy not only adds aesthetic and sensory appeal but can also enhance the nutritional quality of meals (Fernandez et al., 2017). Moreover, several species are recognized for their medicinal properties, further supporting their relevance in both culinary and health-related contexts (Fernandez et al., 2017; Zhang et al., 2023; Sood et al., 2024). However, adverse impacts could be observed too, with hypersensitivity reactions. Therefore, it is of fundamental importance to conduct allergen and toxicity tests before recommending the use of an edible flower (Santos and Reis, 2021).

As previously mentioned, edible flowers have a wide range of applications, including in the food, nutraceutical industries, and culinary sectors as garnishes and nutritional additives. This versatility is driving a positive market outlook. In 2022, the global edible flowers market was valued at USD 317.90 million, with projections indicating growth to over USD 503.22 million by 2032 (Fact.MR report - Edible Flower Market, available online: https://www.factmr.com/report/2979/edible-flowers-market). The rising demand for natural and organic nutraceutical supplements, the increased use of plant-based products in the pharmaceutical industry, and the growing interest in skin care and anti-aging supplements are considered key drivers for this trend.

The biodiversity of edible flowers is extensive, including ornamental, wild, and cultivated species. As a result, establishing a definitive count of all edible flowers remains a challenge. In addition to traditionally used ornamental and wild species, several widely consumed floral vegetables such as broccoli, cauliflower, squash blossoms, and globe artichoke also hold significant commercial value in global markets (Kumar et al., 2025). Lim (2014) edited a list of edible medicinal and non-medicinal plants, including edible flowers, resulting in 1,729 species, belonging

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to 631 genera and 133 botanical families. Botanists and culinary experts estimate there are over 250-400 edible flower species consumed across cultures (https://www.bloomybliss.com/2025), and according to Sarode et al. (2024), about 180 flower species are suitable for human consumption.

Despite the increasing market interest in edible flowers and their recognized nutraceutical potential, technologies related to propagation, cultivation, postharvest preservation, and commercialization remain underdeveloped (Fernandes et al., 2020). As a result, research and development are essential to support producers and strengthen supply chains. In this context, the development of propagation technologies plays a key role, as high-quality and sufficient quantities of planting material are critical to ensure successful production (Paiva et al., 2024).

Tissue culture techniques are increasingly used in edible flower production, since they offer many benefits (Domingues et al., 2024). Micropropagation allows fast, efficient, and year-round production of plants, and this is important to quickly reach the market or to obtain the production of rare species (Rout et al. 2006; Mehbub et al. 2022); the axillary branching method is preferable to get a true-to-type production (Coelho et al., 2020). Moreover, the development of efficient methods to rapidly supply growers with clean and genetically uniform planting material is a key priority in agriculture (Govinden-Soulange et al. 2009). *In vitro* culture can preserve the germplasm of edible flowers, especially those with limited seed viability or those that have been selected for specific features. *In vitro* culture can also be used to produce secondary metabolites that derive from edible flowers. Advanced *in vitro* techniques such as protoplast fusion, anther, microspore, and ovule cultures; embryo rescue, and suspension culture can enhance the development of novel cultivars (Sarode et al. 2024).

Biotechnological tools, like genetic transformation and genome editing, can support the genetic improvements of edible flowers with regard to the flower colour, size, disease resistance, and nutritional content. All these techniques could have a great impact on edible flowers by facilitating the creation of new varieties with improved characteristics for culinary and aesthetic purposes or disease-resistant varieties, by enhancing the levels of beneficial compounds, and by performing production regardless of seasonal limitations (Sarode et al. 2024) and adapted to support climate change mitigation and resilience (Paiva, 2023).

In this paper, we aim to highlight selected examples of *in vitro* applications in edible flowers. To achieve this, a comprehensive literature search was conducted across major scientific databases, including Web of Science, Scopus, PubMed, Google Scholar, and Google Search. Thus, among the published articles, those addressing the central theme: *in vitro* propagation and cultivation of edible flowers, being selected with the aim of analyzing recent findings and research focuses, mainly considering publications from the last 10 years. Predefined keywords were employed to select studies based on their relevance and scientific quality as edible flower, *in vitro* propagation, edible plants, micropropagation, biotechnology. As a result, the most significant and recent studies on edible flowers, excluding those focused on species cultivated mainly for commercial decorative purposes are summarized in Table 1, with the ensuing discussion organized thematically throughout this review.

Table 1. Summary of recent research studies and the techniques used for edible flowers.

Family	Species	Explant	Research Topic	Reference
ASTERACEAE	*Calendula maritima Guss*	Nodal segments	*In vitro* establishment and rooting	Catalano et al. (2022)
	Calendula officinalis	Seeds	*In vitro* propagation	Victorio et al. (2012)
	Calendula officinalis	Leaf segment	Callogenesis	Fatima et al. (2024)
	Calendula officinalis	Leaf segment Cotyledonary node	*In vitro* propagation Callogenesis	Aboshama (2005)
	Carthamus tinctorius	Anthers	Shoot and root regeneration	Kassa et al. (2024)
	Chrysanthemum spp.	Shoot tips	Cryopreservation	Ozudogru et al. (2010)
	Dahlia spp.	Nodal segment	*In vitro* propagation	Jiménez-Mariña (2020)
	Tagetes erecta	Anthers	Androgenesis	Kumar et al. (2019)
BEGONIACEAE	*Begonia malabarica*	Leaf segment Shoot tips	*In vitro* propagation	Aswathy and Murugan (2019)
	Begonia rex	Petioles	*In vitro* propagation	Davoudipahnekolayi et al. (2024)
	Begonia semperflores-cultorum	Leaf segment	Regeneration after genetic transformation	Hirutani et al. (2020)
	Begonia spp.	Male flower buds	*In vitro* propagation	Nabieva and Fershalova (2023)
CARYOPHYLLACEAE	*Dianthus* spp.	Leaf segment	Somatic hybridization	Nakano et al. (2001)
		Shoot tips	Cryopreservation	Ozudogru et al. (2010)
GENTIANACEAE	*Gentiana* spp.	Soot tips	Cryopreservation	Ozudogru et al. (2010)
GESNERIACEAE	*Saintpaulia ionantha*	Leaf segment	*In vitro* propagation and rooting	Deltalab et al. (2024)

Table 1. cont.

LAMIACEAE	*Lavandula angustifolia*	Foliated nodal segments	*In vitro* propagation and rooting	Darouez and Werbrouck (2024)
	Lavandula angustifolia	Single-node shoots	*In vitro* propagation Biocompound analysis	Andrys et al. (2018)
	Lavandula angustifolia	Shoots	*In vitro* propagation and essential oil production	Andrys and Kulpa (2017)
	Lavandula angustifolia	Nodal segments	*In vitro* propagation	Yahya et al. (2024)
	Lavandula angustifolia (var. Krajová and Beta *Lavandula x intermedia* (var. Budrovka and Grosso).	Apical and nodal segments	*In vitro* propagation	Gubišová and Čičová (2023)
	Lavandula dentata	Seeds	*In vitro* propagation Acclimatization	Koefender et al. (2021)
	Lavandula stoechas	Shoots tips	*In vitro* propagation and Metabolite production	Benyammi et al. (2023)
	Lavandula stoechas	Single-node explants	*In vitro* propagation Slow-growth Conservation	Domingues et al. (2024)
LINDERNIACEAE	*Torenia fournieri*	Not defined	*Agrobacterium-mediated* transformation	Nishihara et al. (2024)
MALVACEAE	*Hibiscus sabdariffa*	Seed	Sterilization protocol *In vitro* propagation	Bello et al. (2025)
	Hibiscus sabdariffa	Nodal segment	*In vitro propagation*	Kumar et al. (2016)
	Hibiscus sabdariffa	Seeds	*In vitro* propagation	Govinden-Soulanget al. (2009)
ORCHIDACEE	*Dactylorhiza fuchsii*	Seeds	Cryopreservation	Ozudogru et al. (2010)
	Oncidium spp.	Protocorm-like body	Cryopreservation	Ozudogru et al (2010)
PAEONIACEAE	*Paeonia lactiflora*	Shoot tips	Cryopreservation	Ozudogru et al. (2010)
PLANTAGINACEAE	*Antirrhinum majus L.*	Seeds	*In vitro* propagation Callogenesis	Kiymaz and Acemï (2024)
PLUMBAGINACEAE	*Limonium spp*	Shoot tips	Cryopreservation	Ozudogru et al. (2010)
ROSACEAE	*Rosa spp.*	Petals	*In vitro* enzyme inhibition	Zhang et al. (2024)
		Shoot cultures	Slow growth storage	Ozudogru et al. (2010)
		Shoot tips	Cryopreservation	Ozudogru et al. (2010)
SOLANACEAE	*Petunia* hybrida	Leaf segment	Genetic transformation	Schwinn et al. (2014)
	Petunia hybrida	Nodal segments	CRISPR	Li et al. (2023)
THEACEAE	*Camellia japonica* *Camellia reticulata*	Shoot cultures	Slow growth storage	Ozudogru et al. (2010)

Examples of *in vitro* propagation applications for edible flowers

The genus *Calendula* (Asteraceae), commonly known as marigold, comprises several species recognized for their medicinal and cosmetic applications. *Calendula officinalis* L. is widely regarded as an edible flower valued for both culinary and medicinal uses, owing to the chemical composition and strong antioxidant capacity of its flowers (Escher, 2019). Tissue culture techniques are applied for improving *Calendula* crops by ensuring a consistent supply of plant material for food, medicinal, and cosmetic applications, while supporting research and genetic enhancement efforts.

Direct organogenesis through leaf tissues of *C. officinalis* L. was achieved when explants were cultured on MS (Murashige and Skoog, 1962) basal medium supplemented with 200 mg L^{-1} glutamine, 500 mg L^{-1} casein hydrolysate, and 1.0 mg L^{-1} 2,4-D (Aboshama, 2005). No information is provided regarding the true-to-type nature of the regenerated plantlets, and it is well known that using the growth regulator 2,4-D can increase the risk of non-conformity in *in vitro* cultured plantlets (Coelho et al., 2020). In another study, a micropropagation protocol for *C. officinalis* was achieved by culturing nodal segments of *in vitro* seedlings on modified MS supplemented with growth regulators (Victorio et al., 2012). Shoot proliferation was dependent on cytokinins, with the best results obtained using TDZ at concentrations between 0.2 - 0.8 mg L^{-1}. However, TDZ also suppressed shoot elongation. On the contrary, the use of 0.5 or 0.8 mg L^{-1} BAP increased shoot production without reducing elongation. The highest rooting rate (80%) was achieved with 0.1 mg L^{-1} IAA (Victorio et al., 2012).

Calendula maritima Guss. is an endemic species from western Sicily that has been identified as a promising "farm plant species". This species

is rare and critically endangered due to the difficulties in conservation under its natural habitat, the competition with other species, and possible hybridization with the species *C. suffruticosa* subsp. Fulgida (Raf.) (Catalano et al., 2022). In this case, the *in vitro* techniques could be a useful biotechnological tool for the conservation and production of this species. A micropropagation protocol for *C. maritima* was established using nodal segments from selected plants cultured on MS medium. Although phloroglucinol (PG) did not enhance shoot multiplication, the greatest shoot length (3.4 cm) was achieved when the explants were cultured in the presence of 3 µM PG. Zeolite provided the best rooting performance, increasing the percentage of plants suitable for ex vitro establishment while also reducing the overall costs of the *in vitro* propagation process (Catalano et al., 2022).

Another species belonging to the Asteraceae family, *Carthamus tinctorius* L. (the so-called safflower, because also used as a saffron substitute), is used in cooking and as a dish garnish, although traditionally employed as a dye and in traditional medicine. Another culture originated the highest degree of callus formation (46%) when 0.5 mg L^{-1} TDZ was added to MS medium, but genotype differences could be observed in the efficiency of both shoot and root regeneration. However, despite successful *in vitro* development, the acclimatized plantlets failed to survive under glasshouse conditions (Kassa et al., 2024).

Also in the case *Antirrhinum majus* L. (snapdragon), a widely known edible flower, cytokinin type and balance are required across different *in vitro* stages. Thidiazuron (TDZ) and N^6-(2-chlorophenyl) adenine (CPA) effectively promote shoot multiplication and callogenesis, supporting the commercial propagation of *A. majus* (Kiymaz and Acemi, 2024).

Hibiscus sabdariffa (Malvaceae family), commonly known as roselle, is an edible flower widely valued in both culinary and traditional medicine practices for its rich phytochemical composition. However, the short longevity of the flowers underscores the need for technological strategies to efficiently extract and preserve their bioactive compounds (Vargas et al., 2024). In this case, micropropagation can enhance the standardization and reliability of the extracts of these plants (Juraev et al., 2025). Kumar et al. (2016) faced the micropropagation of *H. sabdariffa* to enrich the production of ascorbic acid in tissues. For this purpose, they developed a micropropagation protocol through axillary bud cultures from *in vitro* seedlings. Nodal explants were cultured on MS with 2.0 mg L^{-1} 6-benzylaminopurine, 0.5 mg L^{-1} indole-3-acetic acid, and 10 µM silver nitrate or 20 µg L^{-1} triacontanol (TRIA). A satisfactory multiplication was obtained: 7-8 shoots/explant and 4-5 shoots/explant when silver nitrate and TRIA were added, respectively. The observations carried out for the *ex-vitro* plantlets showed that the micropropagated plants had 2.8-fold more ascorbic acid than the in vivo tissues of the same age. Recently, Bello et al. (2025) performed the micropropagation of *H. sabdariffa* using seedlings. Shoot proliferation was improved by supplementing the culture medium with 15% coconut water, used as a natural plant growth regulator. Root formation was observed in all the explants. Another interesting study was carried out by Govinden-Soulange et al. (2010) aimed at detecting somaclonal variation in micropropagated *H.sabdariffa*. Although the most reliable micropropagation method was used (single-node explants), the authors observed plantlets that normally grew and other plantlets showing symptoms of chlorosis, likely due to a mineral imbalance affecting chlorophyll formation.

Begonia semperflorens-cultorum is among the most popular species, with a wide range of commercial cultivars available (Hirutani et al., 2020). The flowers of certain begonias (*Begonia* x *semperflorens-cultorum* and *B.* x *tuberhybrida*) are edible with a slightly sour citrus flavor and a light, crisp texture. Begonias propagated through conventional methods, such as stem and leaf cuttings, can be affected by pathogens, which compromise their ornamental value. *In vitro* propagation is particularly useful for begonias because it can overcome issues associated with traditional propagation methods, such as disease susceptibility and the difficulty of propagating some species. Notwithstanding this, there is still no well-established strategy for achieving direct regeneration of plantlets, which is the preferred approach for *in vitro* propagation of begonias (Aswathy and Murugan, 2019). Nabieva and Fershalova (2023) achieved the regeneration from somatic flower tissues of three *Begonia* species and one hybrid cultured on N6 medium (Chu et al., 1975) supplemented with 2-isopentenyl adenine (2iP) at 0.3 mg L^{-1} and naphthaleneacetic acid (NAA) at 0.1 mg L^{-1}.

The Lamiaceae family includes over 240 genera and around 7,200 species, featuring well-known culinary herbs such as basil, mint, rosemary, thyme, oregano, and lavender. These plants are valued for their aromatic leaves and edible flowers. Lavender, in particular, stands out for its versatile uses, economic importance, and positive environmental impact through the potential for sustainable agronomic practices. Micropropagation is valuable for lavender as it enables the large-scale production of disease-free and true-to-type plants. This ensures a consistent supply of high-quality plant material for industry, helping to prevent loss of valuable natural germplasm and facilitating the introduction of new selections into the market. Additionally, micropropagation is a promising strategy to produce large quantities of genetically uniform plantlets for extracting high-value bioactive compounds (Yahy et al., 2024).

Several lavender varieties are commonly micropropagated, and *Lavandula angustifolia* Miller and *L. x intermedia* are mostly studied. *L. angustifolia*, the so-called English lavender, is the most popular type of lavender, often used in aromatherapy, perfumes, and skincare products. It's also a popular choice for gardens and landscaping. This lavender is known for its rich content of organic compounds, including volatile oils and acids, triterpenic acids, coumarins, flavones, resins, and polyphenols, found in both fresh and dried forms. These compounds contribute significantly to the economies of various countries (Chrysargyris et al., 2016). *L. x intermedia* is known as lavandin, generally with less refined fragrance compared to English lavender, but with vigorous growth and high yield of essential oil.

L. angustifolia (var. 'Krajová' and 'Beta') and *L.* × *intermedia* (var. 'Budrovka' and 'Grosso') have been successfully micropropagated through apical or nodal segments on MS medium supplemented with 1 mg L^{-1} BAP (Gubišová and Čičová, 2023). Rooting was achieved in a growth regulator-free medium containing half-strength MS salts. After acclimatization, plantlet survival rates ranged from 78.9% to 87.7%, except for *L. angustifolia* var. 'Beta', which exhibited a lower survival rate of 50%. Generally, in *L. angustifolia*, *in vitro* rooting is often hindered by the species' sensitivity to natural auxins, with rooting frequently inhibited by callus formation. Light quality and plant hormone combinations play a crucial role in influencing various physiological and morphological traits, contributing to more effective and sustainable micropropagation protocols for lavender and other species (Darouez and Werbrouck, 2024).

L. stoechas subsp. *luisieri* and *Pterospartum tridentatum* are valuable aromatic and medicinal plants that have been successfully propagated on MS medium supplemented with BAP and IBA (Domingues et al., 2024). For rooting, it is recommended that explants be treated with IBA or a commercial IBA-based product such as Clonex® (Domingues et al., 2024). Optimal *in vitro* shoot proliferation and biomass accumulation in *L. stoechas* were obtained with a combination of 0.5 mg L^{-1} 6-benzyladenine (BA) and 0.5 mg L^{-1} gibberellic acid (GA_3).

In vitro germination of *L. dentata*, a lavender not intensively fragrant as the other lavender but of some interest for aromatic compounds and ornamental gardening, has been carried out by Koefender et al. (2021). The authors treated seeds with gibberellic acid (GA_3) at 1.5 mg L^{-1} for 48 hours before inoculation on a simple medium composed of agar and water, and an 80% germination rate was obtained. For the *L. angustifolia* multiplication, supplementation of the MS medium with BAP at 1.0 mg L^{-1} provides a 9.05 shoots/explant, a good multiplication rate compared to previous findings reported by Machado et al. (2013), of 1.9 - 3.3 shoots $explant^{-1}$ on a similar multiplication medium. Elongation and rooting stages did not require the addition of growth regulators, and plantlets were successfully transferred to *in vivo* conditions (Koefender et al., 2021).

Examples of *in vitro* plant transformation and breeding applications in edible flowers

In vitro plant transformation and breeding techniques can significantly enhance edible flowers by improving their quality and aesthetic traits. These methods enable target modifications in crops such as increased disease resistance, stress tolerance, and nutritional content; thereby boosting the appeal of edible flowers for culinary applications and broader agricultural uses. Several techniques can be used, ranging from *in vitro* plant breeding such as somatic hybridization and embryo rescue, somaclonal variation, and *in vitro* pollination and fertilization, to genetic modification methods like *Agrobacterium*-mediated transformation and biolistics-gene gun. For example, these genetic engineering techniques

have been applied to petunia to increase levels of specific anthocyanins known for their antioxidant properties (Schwinn et al., 2014). Techniques like CRISPR-Cas9 enable targeted improvements in the plant genome with greater precision and efficiency than traditional breeding methods (Li et al., 2023). A recent review by Sarode et al. (2024) summarizes biotechnological and genome editing strategies applied to important edible flowers. Biotechnological approaches have been used to enhance drought tolerance and productivity of *Tagetes erecta* (African marigold), a widely appreciated edible flower. Successful optimization of plant tissue culture protocols for both *T. erecta* and *T. patula* (French marigold) has facilitated the production of doubled haploids, providing a solid foundation for modern breeding programs (Kumar et al., 2019). Somatic hybridization is a complex technique, with limited success in ornamentals, mainly due to low regeneration efficiency (Nakano et al. 2001). However, protocols have been developed to regenerate plants from leaf- and hypocotyl-derived protoplasts in some *Dianthus* species and cultivars (Nakano et al. 2001), which are also recognized as edible flowers. Conversely, embryo-rescue techniques have been applied to several edible flower species, including *Dianthus*, roses, orchids, lilies, and chrysanthemums.

Tissue culture can generate somaclonal variation, either spontaneously or through inductions in regenerated plantlets. When these variations are stable, they can serve as the basis for developing new cultivars with desirable features. Examples of edible flowers in which somaclonal variations have been reported include a pansy variety exhibiting a unique color, a rose with a more intense fragrance, and a hibiscus with improved disease resistance (Góvinden-Soulange et al., 2009; Suprasanna and Jain, 2022).

Genetic transformation was essential for the further modification of *Begonia* species, as it enables the introduction of desirable traits such as novel flower colors (Hirutani et al., 2020). Transgenic *B. semperflorens-cultorum* plants were successfully regenerated from leaf segments cultured on MS medium supplemented with growth regulators. Gene transfer was carried out using *Agrobacterium tumefaciens* strain EHA101 harboring the plasmid pIG121-Hm (Hirutani et al., 2020). Using *Agrobacterium*-mediated transformation, purple-reddish flowers were produced in the 'Crown Violet' cultivar of *Torenia fournieri*. This achievement marks the first successful expression of betalain pigments in the Linderniaceae family, demonstrating the accumulation of foreign betacyanins along with native pigments in *Torenia* (Nishihara et al., 2024). Genetic manipulation enables the development of transgenic plants capable of producing valuable biotechnological compounds, offering a more sustainable and cost-effective alternative to conventional production methods (Narayanan and Glick, 2023).

Examples of applications of *in vitro* metabolite production in edible flowers

Natural phenolic compounds occur naturally in plants and represent safer, more sustainable alternatives to synthetic chemicals used in human health treatments. Edible flowers are gaining recognition for their potential to produce high-value metabolites for applications in food, pharmaceuticals, and cosmetic industries. Advances in genetic engineering of higher plants suggest that, within the next 10 to 20 years, plants may largely replace microbial and animal cell cultures as a basis for the production of health-promoting compounds. Once the safety and efficacy of purified plant-derived compounds are validated, *in vitro* production methods will provide a sustainable and efficient alternative to conventional extraction methods from whole plants. Nonetheless, achieving large-scale production will require innovative cultivation strategies such as the use of bioreactors and elicitors (e.g., fungal extracts and salicylic acid), to stimulate cell or tissue cultures that can significantly enhance the production of specific secondary metabolites, including phenolic compounds, terpenoids, and flavonoids (Bapat et al., 2023). This field of industrial application still needs to face some important challenges related to the extraction systems, the scale-up of bioreactor cultivation, or the necessity to exclude or replace in the culture media the carcinogenic plant growth regulator 2,4-D, which is often employed (Vrancheva et al., 2018). In addition, it is necessary to implement studies on omics approaches (genomics, proteomics, metabolomics) to understand the complex metabolic pathways related to metabolite production in edible flowers (Sarode et al. 2024).

Edible chrysanthemums are a potential source of bioactive compounds (Doan et al., 2024), and roses are rich sources of polyphenols, polysaccharides, proteins, organic acids, minerals, and vitamins (Zhang et al., 2024). Ultrasonic treatment has been shown to enhance the accumulation of rose polyphenols, which in turn increased the inhibitory effect of rose extracts (REs) on α-glucosidase (α-Glu) activity, providing insight into the interaction mechanism between REs and α-Glu (Zhang et al., 2024). *Calendula* species exhibit diverse medicinal properties attributed to their phytochemical composition that includes terpenoids, flavonoids, coumarins, volatile oils, and quinones. *In vitro* culture offers a reliable alternative to field cultivation for the consistent production of these bioactive metabolites (Fatima et al., 2024). Callus formation and indirect organogenesis in *C. officinalis* on biochemical content were studied by Fatima et al. (2024). The results showed that the callus developed from *in vitro* leaf tissue is a reliable and powerful source of several bioactive compounds with a wide range of medicinal uses. The successful stimulation of callus development, indirect organogenesis, biochemical analysis, and GC-MS confirmation of the presence of significant phytocompounds are all described in this study. This work provides an alternative approach for ongoing and sustainable synthesis of chemicals.

In vitro cultivation conditions may affect the biochemical profile of lavender essential oils, increasing the concentration of certain compounds. This modification can enhance the antimicrobial and antioxidant activities of the oils. Indeed, essential oils extracted from *in vitro* propagated lavender plants demonstrate higher antioxidant and antimicrobial activities compared to those obtained from field-grown plants (Andrys and Kulpa, 2017).

A protocol was also developed for the *in vitro* propagation of *L. angustifolia* Miller, a notable source of volatile organic compounds (VOCs). Nodal explants formed callus on MS medium supplemented with 2 mg L^{-1} BAP. Shoot proliferation was induced from callus cultures using MS medium with 5 mg L 2-iP, followed by transfer to media containing 1 or 2 mg L^{-1} BAP to support callus growth. The application of different plant growth regulators led to variations in the phenolic and flavonoid profiles. Kinetin (KIN) resulted in the highest accumulation of phenolic acids, whereas the lowest concentrations were found in media supplemented with BAP + NAA. Conversely, the highest flavonoid content was observed in BAP + NAA media, while the lowest was found in callus cultures grown on MS medium with BAP alone. Jasmonic acid (JA) showed promising results in enhancing the antimicrobial properties of narrow-leaved lavender against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans*. The addition of JA to the culture medium also promoted an increase in essential oil content, contributing to greater antioxidant activity. However, higher concentrations of JA led to a reduction in the number of secretory trichomes, while lower concentrations proved ineffective (Andrys et al., 2018). *In vitro* cultures of *L. stoechas* have also been identified as a promising source of bioactive compounds with strong antioxidant properties (Benyammi et al., 2023).

Hibiscus sabdariffa Linn. features economically valuable parts such as seeds, leaves, and calyces with diverse applications (Chew et al., 2024). The seeds are rich in phenolics, fiber, oil, and protein. Meanwhile, the leaves and calyces contain phenols, flavonoids, and anthocyanins, making them useful in nanoparticle synthesis, food products, and natural colorants (Omole and Oranusi, 2019; Emetere, 2020; Omole et al., 2022; Chew et al., 2024).

Nasturtium (*Tropaeolum majus* L.), an annual species native to the Andes region (Bolivia, Peru, and Colombia) and belonging to the Tropaeolaceae family, is an edible plant with strong potential for biofortification (Lara et al., 2025). This sustainable and low-cost approach aims to enhance the mineral and vitamin content of edible plant parts, improving their nutritional value and health benefits. Although there are currently no studies on *in vitro* biofortification of edible flowers, such research exists for other species, primarily medicinal plants. For example, selenium supplementation during *in vitro* cultivation of *Digitalis lanata* and *Digitalis mariana* increased physiological parameters and cardenolide production (Moura et al., 2023).

Examples of applications of *in vitro* conservation of edible flowers

Techniques like micropropagation and cryopreservation are used to preserve edible flowers, minimizing the risk of contamination and maximizing their long-term viability.

Short-term conservation involves the maintenance of actively growing *in vitro* cultures through regular subculturing. For long-term storage, slow-growth techniques are employed, allowing species preservation for periods ranging from six months up to five years by slowing growth rates and reducing the frequency of subculturing, consequently saving time and costs. However, this approach has limitations, including the requirement for adequate storage space and the potential for somaclonal variation (Domingues et al., 2024).

Slow-growth conservation is effective at 4 – 5 °C, preferably in the dark, although some species (e.g. *Camellia japonica*, *Camellia reticulata*, *Humulus* spp.) require low light (3 - 8 µmol/m²/s) for optimal storage (Ozudogru et al., 2010). To ensure successful regrowth of *L. stoechas* and *Pterospartum tridentatum* shoots after storage, cultures should be conserved at 4°C for no more than 12 months at reduced sucrose concentrations (Domingues et al., 2024). However, the temperatures applied for short-term conservation, light conditions and photoperiod, as well as the length of the storage period, vary greatly from species to species, with differing regrowth percentages. For example, *C. japonica* and *C. reticulata* have shown 100% regrowth when shoot cultures were stored for one year at 2 – 4 °C, low light intensity, and a 16-h photoperiod. *Rosa* cultivars and *Gerbera* cv. Marleen exhibited strong regrowth rates, exceeding 80% and 96%, respectively, following storage at 4 °C in darkness for six months and three months, respectively (Ozudogru et al., 2010).

The use of synthetic seeds provides an alternative method for the *in vitro* conservation of ornamental plants. However, it is crucial to study the cold storage conditions, as the alginate beads surrounding the bud or shoot can become dehydrated (Ozden-Tokatli et al., 2008).

Cryopreservation involves freezing plant tissues at extremely low temperatures (-196°C) to preserve them for extended periods (Ozudogru et al., 2010). This technique was reported to be useful for virus-eradication (Wang et al., 2018) and should be important for long-term conservation of rare or endangered edible flowers. Several cryo-techniques (slow cooling, encapsulation-dehydration, droplet method and droplet-vitrification) have been proposed with different results. A survival rate of 76% was recorded after the cryopreservation of shoot tips for *Limonium*, approximately 70% for *Rosa* spp., 70% for *Gentiana* spp, and 74% for *Paeonia lactiflora*. For shoot tips of various cultivars of *Chrysanthemum*, *Dianthus*, and *Gypsophila*, survival rates after storage varied widely, ranging from 9% to 100%. Seeds and protocorm-like bodies of orchids such as *Dactylorhiza fuchsii* and *Oncidium* spp were cryopreserved with survival rates of 85% and 30%, respectively (Ozudogru et al., 2010).

Conclusions

The production and market for edible flowers have grown significantly in recent years, driven by rising consumer interest in healthy and visually appealing food options. This global trend is accompanied by a general lack of comprehensive data on the production, import, and export of edible flowers. This gap is partly due to their use often being associated to local traditions and consumption patterns. Additionally, the edible flowers market is highly segmented across food and beverage, foodservice, and retail sectors, reflecting their diverse applications.

Biotechnological tools can support edible flower producers and enable the development of new products. However, the application of these *in vitro* techniques should be integrated into the entire production process. Additionally, an important application lies in the production of bioactive compounds, which requires well-developed protocols for industrial use. This shows that, despite several studies already conducted, there is a need for further research, including the use of advanced techniques. For example, the development of protocols for most species is still lacking, particularly those integrating tissue culture techniques with the analysis and production of bioactive compounds. Moreover, there is significant potential for studies applying omics technologies, which could optimize metabolite production. From a sustainability perspective, the development of *in vitro* techniques for conservation and cryopreservation is crucial, supporting the establishment of germplasm banks to preserve and serve as sources of genetic resources.

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Author contribution

PDOP: Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing. **ACS:** Conceptualization, Investigation, Writing – Review & Editing. **RP:** Writing – Original Draft, Writing – Review & Editing. **MIB:** Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All the research data is contained in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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