

In vitro culture and genomics tools to improve ornamental tuberose: A comprehensive review

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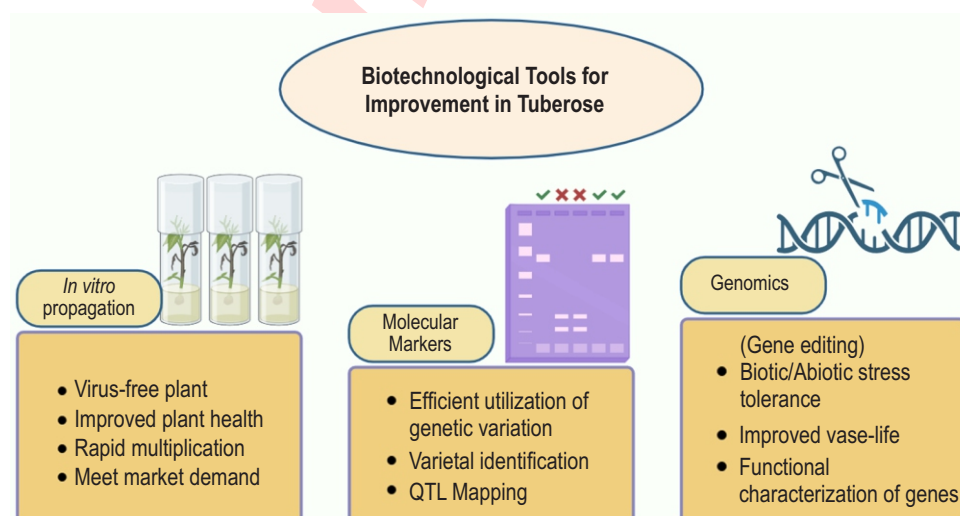
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Abstract

Tuberose *Agave amica* (Medik) is one of the most critical flowering plants in tropical and subtropical areas. Tuberose blooms' essential oils and aromas are commonly used in perfumes, making them well-known internationally. New flower varieties are needed to meet rising demand for unique, high-quality, and diverse blooms. Conventional breeding approaches—such as clonal selection, somaclonal variation, and hybridization—are often time-consuming and limited in their ability to introduce desirable traits efficiently.

In contrast, recent advances in modern biotechnological and genomic tools, including genetic engineering, CRISPR/Cas9-mediated genome editing, somatic hybridization, and tissue culture, provide more precise and accelerated routes for the development of improved cultivars. This review evaluates the efficiency of *in vitro* plantlet regeneration and the key factors influencing its success. It highlights the application of molecular markers in the genetic improvement of tuberose (*Polianthes tuberosa* L.). To support the ongoing tuberose improvement programs, this research review further proposes integrated strategies that combine genomics-assisted selection with *in vitro* methodologies to optimize trait enhancement and accelerate cultivar development.

Key words: Genome editing, *In vitro* propagation, Molecular markers, Plant growth regulators



Introduction

Tuberose (*Polianthes tuberosa* L.) is a perennial, fragrant flowering plant belonging to the family Asparagaceae, widely cultivated for its attractive, long-lasting, and highly aromatic flowers. In tropical and subtropical climates, it is frequently cultivated for commercial purposes as a cut flower and is also used to obtain essential oil (Maiti *et al.*, 2014). Cut flowers and garden displays are made with double-type tuberoses, which have more than two rows of corolla section; however, single-flowered genotypes, which have only one row of corolla, are frequently employed in floral arrangements, loose flower creation, essential oil extraction, etc. *Agave amica* (Medik), earlier known as *Polianthes tuberosa* Linn., or tuberose, is one of the most significant flowering bulbs in the Agavaceae family. It is native to Mexico and was cultivated long before the year 1522. The genus *Polianthes* contains fifteen species (Espejo, 1992). Datta (2017) also reported fifteen species, three varieties, and a few species under *Agave amica* (Medik). The color spectrum of these species is broad, ranging from striped to orange, red, and white.

Food, cosmetics, pharmaceuticals, and both essential and volatile oils are extensively used in industry, and their demand increases continuously each year. Alkaloids, flavonoids, and phenolic compounds representing a phytochemical composition of the ornamental flower extracts have pharmacological antibacterial, antioxidant, and anti-inflammatory activities with potential applications in food, medicine, and cosmetics. Additionally, medicament seeds in pharmaceuticals can be extracted from nature flowers. Flower extracts are widely applied in the cosmetic industry for skin-conditioning and anti-aging effects. Tuberose flower extracts are also used in perfume for their intense scent. These extracts can add nutritional value in the food industry, such as in the natural and functional food markets, improve visual appeal, and prolong shelf life. In general, flower extracts are an excellent source of bioactive compounds (Lekshmi *et al.*, 2023).

Various methods such as hybridization (Datta 2017) and mutation breeding (Kumar *et al.*, 2021a) have been previously adopted for improving tuberose. It is a common practice for the improvement of plants through tuberose hybridization, and various hybrids are being widely cultivated in different parts of India because of their better yield (Sarkar *et al.*, 2010), however, seed setting is the principal concern of genetic improvement in this plant (Kumar *et al.*, 2022). Alternatively, *in vitro* methods may be advantageous for mass production of plants and bulblets (Tripathi *et al.*, 2024). Commercial cultivars of tuberose need to adopt these methods at the business level to bridge the demand gap. Nowadays, biotechnological approaches have significantly contributed to the production of new desirable ornamental plants (Chandler and Sanchez, 2012; Kumar *et al.*, 2017; Sirohi *et al.*, 2022). Homeotic especially genes to control floral growth can be beneficial for developing flowering plants with attractive colors through molecular breeding (Chandler and Sanchez, 2012). However, there is no report available on tuberose improvement

through genetic engineering. To address consumers' demand for novelty, molecular techniques are currently employed to develop appealing plants with unusual colors, shapes, fragrances rich in essential oils, attractive plant architecture, and a longer vase life. In this context, DNA markers are used to analyze genetic diversity, genotype inter-relationships, marker traits in selection, gene mapping, identification of genes or alleles related to economically relevant traits, clonal fidelity analysis, and boosting trait selection efficiency, among other things. Several researchers have used these markers in tuberose for genetic studies (Sirohi *et al.*, 2017, 2017a; Kutty *et al.*, 2020). Molecular breeding can be a potential method for improving tuberose, where DNA markers enhance variety by selecting a good phenotype with the help of various markers. Faster crop improvement outcomes might be possible with the help of genomics tools, including high-throughput genotyping, phenotyping, genomics-assisted breeding, and gene editing (Kumar *et al.*, 2023a). In the present review, we have discussed the general factors affecting *in-vitro* propagation and the use of biotechnology tools to improve tuberose.

Factors affecting the *in-vitro* propagation of ornamental tuberose

***In-vitro* culture systems:** Biotechnology has completely transformed the floriculture sector, particularly through large-scale *in-vitro* cloning of plants. Plant tissue culture is essential to this process since it allows the plant breeders to access more desired germplasms, improve plant health, and create genetic variability. There have been several attempts to grow regenerative plants in tuberose by direct organogenesis (Jyothi *et al.*, 2008; Khanchana *et al.*, 2019; Copetta *et al.*, 2020), indirect organogenesis (Nazneen *et al.*, 2003; Naloussi *et al.*, 2019), and somatic embryogenesis (Ahmad *et al.*, 2006; Kahrizi *et al.*, 2008). The general outline of sequential steps involved in the complete *in-vitro* development of plantlets is illustrated in Fig. 1. Several parameters in *in-vitro* culture methods determine how effectively micro-plants are produced (Kumar *et al.*, 2022; Kumar *et al.*, 2023b).

Choice of genotype: Among the factors that affect how well *in vitro* tuberose development progresses, genotype plays an important role. Different plant genotypes exhibit varying abilities to respond to the conditions required for regeneration, and this response is often genotype-specific. Some genotypes readily regenerate, while others may be recalcitrant. Bindhani *et al.* (2004) found that the "single type of tuberose" had the highest *in-vitro* establishment efficiency, followed by the "double type," and the "Swarna Rekha" genotype. Tuberose cultivar responses vary in *in-vitro* regeneration: Taksande *et al.* (2018) found Nagpur Local-1 superior to Nagpur Local-2 and Amravati Local-1 for direct organogenesis. Khanchana *et al.* (2019) reported 'Arka Shringar' excelling in shoot number, rooting time, and bud emergence among 'Arka Nirantara', 'Shringar', 'Calcutta Double', and 'Vaibhav'. Naloussi *et al.* (2019) established a beneficial regeneration process using cv. 'Pearl Double'. Tripathi *et al.* (2024) observed more effective *in vitro* establishment in Mexican Single compared to Prajwal, Phule Rajni, and Shringar. These

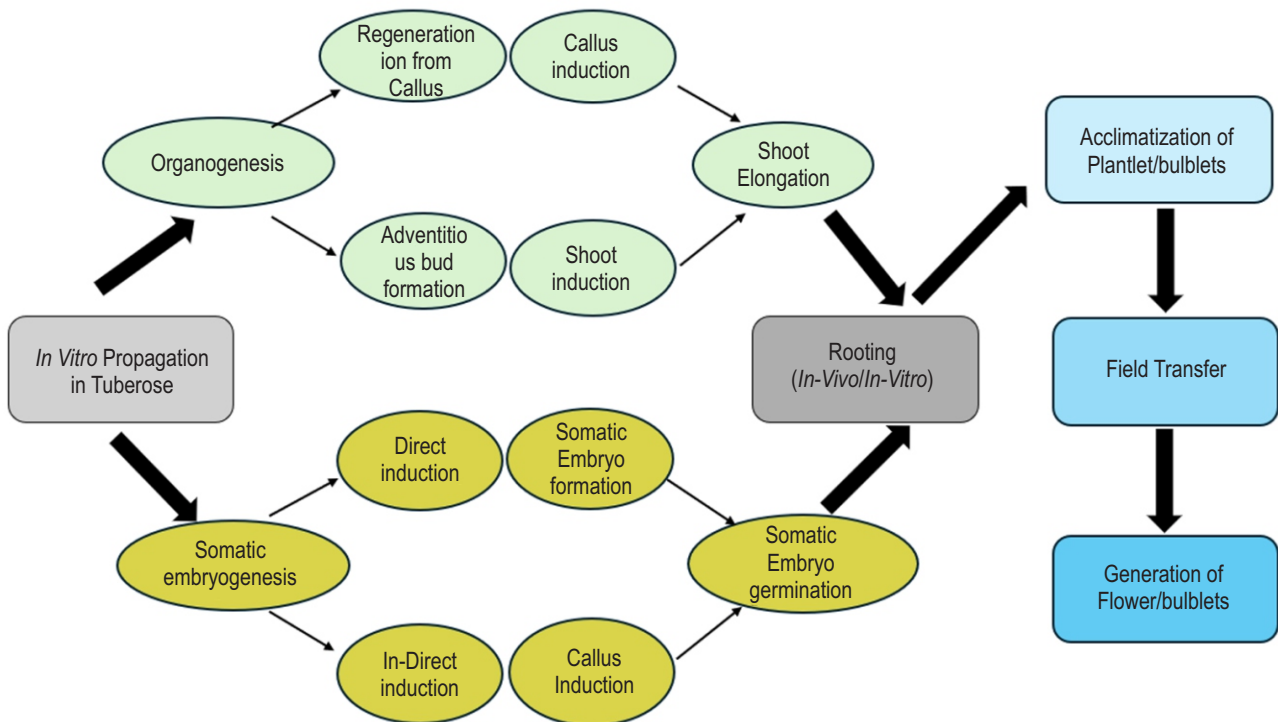


Fig. 1: A schematic chart involving different methods for developing *in vitro* plant lets and bulbets of ornamental tuberose.

findings support optimizing cultivar-specific protocols for tuberose genetic improvement.

Choice of explants: Differences in *in-vitro* morphogenesis have been observed by a variety of explants, including bulb scale (Nazneen *et al.*, 2003; Mishra *et al.*, 2005), rhizomes/bulbets (Samanta *et al.*, 2015), leaf disc (Bindhani *et al.*, 2004), stem disc (Raghuvanshi *et al.*, 2013; Taksande *et al.*, 2018; Tripathi *et al.*, 2024), pedicels (Pourkhaloe *et al.*, 2023), terminal stem scale (Patil *et al.*, 2025) etc. Compared with other types of explants, it was discovered that the inner leaf was appropriate for initiation and development of delicate calluses (Upadhyay *et al.*, 2001). Kahrizi *et al.* (2008) observed that, in comparison to leaf, the node and inter-node are the ideal explants for introducing embryogenic callus through the peduncle. Khanchana *et al.* (2019) utilized bulbets and axillary buds as explants.

The axillary bud showed a suitable outcome with the higher regeneration rates and better growth. Several researchers, including Surendranath *et al.* (2016), observed that explants from different sources can show varied responses. They found that axillary bud explants of the varieties 'Arka Prajwal' and 'Arka Suvasini' were most active, with high response rates of 95.00% and 92.50%, respectively. Nalousi *et al.* (2019) observed a compact callus in green color using leaves as an explant, as the petals, scales, and stems formed a delicate yet distinct callus. Moreover, Singh *et al.* (2020) discovered a large nodular callus

with a leaf explant, a very soft callus on the flower, a massive but compact callus on the roots, and a smooth, tiny nodular callus on the tuber.

Explant sterilization: Bulb or bulbet are typically used to propagate tuberose in the ground; these organs can also be used as explants. Organs generated from soil, such as bulbs and bulbets, are used as explants; therefore, they must undergo appropriate cleaning and sterilization. Different explants, such as aerial and soil-borne parts, are thoroughly cleaned in an air flow cabinet. They are first washed with 6% Teepol for 10 min. Thereafter, the explants are treated with 1% HgCl₂ for 15 min, then rinsed with clean water. This cleaning process is detailed by Singh *et al.* (2020), Samanta *et al.* (2015), Upadhyay *et al.* (2001) and Rajasekharan *et al.* (2000). Furthermore, bulbs were sterilized by Nalousi *et al.* (2019) employing 4% sodium hypochlorite (w/v) for 30 min and 70% ethanol (v/v) for 30 sec. Subsequently, the bulbs underwent three separate 10-min washes in sterile water. For sterilizing bulbs, Copetta *et al.* (2020) used 70% ethanol for 30 sec and 2.5% NaClO solution for 20 min. In contrast, the seeds were sterilized for 20 min using a 1% NaClO solution. After sterilization, both were cleaned twice, using autoclaved distilled water for 10 min each time. Other sterilization agents, including detergents, fungicides (Lakshmi *et al.*, 2021, Taksande *et al.*, 2018), were recommended (Kumar *et al.*, 2022). The choice of sterilization agent and duration of exposure varies depending on the type of explant employed; one procedure may

or may not work for another researcher; therefore, it is recommended to self-standardize the processes as needed.

Choice of culture media and plant growth regulators:

Different researchers have used various types of media to propagate tuberose *in-vitro*, including MS basal medium (Naloussi et al., 2019; Singh et al., 2020; Pourkhaloe et al., 2023), LS medium (Otsuji et al., 1994), and WH medium (Gajbhiye et al., 2011; Taksande et al., 2018). Gajbhiye et al. (2011) and Raghuvanshi et al. (2013) assessed various media, including WH (White, 1963), B5 (Gamborg et al., 1968), and MS (Murashige and Skoog, 1962), to discover the best response for tuberose *in vitro* plant development. Following early investigation, it was observed that the WH basal medium outperformed MS and B5 media. Similarly, Taksande et al. (2018) and Tripathi et al. (2024) observed that WH media produced longer shoots and roots *in-vitro*. The culture media contains a balanced nutrient composition, including macro and micronutrients and vitamins, creating an ideal environment to promote plant growth, organogenesis and regeneration.

In plant tissue culture, limited gas exchange in culture vessels reduce photosynthesis, so an external carbon source is needed. Sucrose, a stable and cost-effective disaccharide, is added to MS media to supply energy and carbon for cell growth and is preferred by tissue-culturists due to its efficiency and accessibility (Rajasekharan et al., 2000; Naz et al., 2012). Agar and phytigel are used as gelling agents in tuberose *in-vitro* propagation, providing solid support for growth. Phytigel forms a clear, strong gel, making it easier to detect microbial contamination. Plant growth regulators (PGRs), especially auxin and cytokinins, control *in-vitro* plant growth by influencing cell division, elongation, and organ formation. Among cytokinins, benzylaminopurine (BAP) or benzyladenine (BA) is more effective in enhancing tuberose *in-vitro* production compared to others (Mishra et al., 2005; Kumar et al., 2021b). Jyothi et al. (2008) regenerated micro-plantlets using 4 mg l⁻¹ BAP in two tuberose cultivars. Patil et al. (2025) used 17.74 µM BAP and 0.57 µM IAA to get the best shoot multiplication. Numerous research reports recommend higher IAA and lower BAP concentrations, including Copetta et al. (2020), who obtained direct micro-plantlets in media fortified by 1.5 mg l⁻¹ BA and 0.5 mg l⁻¹ IAA. Likewise, greater BAP concentrations combined with lower naphthalene acetic acid (NAA) concentrations are more efficient in tuberose, where excellent *in vitro* production of plants was observed with 2.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA (Khanchana et al., 2019). Moreover, Rajsekharan et al. (2000) reported successful *in-vitro* production of micro-plants in tuberose with kinetin alone and additional growth regulators.

Conditions of culture chambers: Plant tissue culture rooms require specific conditions for growth, such as controlled temperature, humidity, light, and air circulation. Studies have reported variable temperatures for successful *in-vitro* production of tuberose, including 22-25°C, a photoperiod of 16 hr light/ 8 hr dark with 2000-3000 lux illumination, and humidity ranging

between 40-60%, which were proven superior to organogenesis, both direct and indirect (Gajbhiye et al., 2011; Raghuvanshi et al., 2013; Singh et al. 2020). Installation of thermostat is recommended for proper temperature control. High humidity in culture rooms often create problems when *in vitro*-grown plants are transferred to field conditions. Adequate air circulation is also required for uniform temperature and humidity distribution throughout the culture room.

The cultural conditions of tissue culture laboratory are one of the most important factors for the successful development of micro-plantlets. Limited information is available on tuberose about variation in cultural conditions for *in-vitro* multiplication. Most researchers throughout the entire micropropagation process maintain same temperature, light intensity, and photoperiod. Previous studies have reported variable temperatures for successful *in-vitro* production of tuberose, including 25±2°C and the photoperiod for 16 hr, which have proven superior to both direct and indirect organogenesis (Gajbhiye et al., 2011; Raghuvanshi et al., 2013; Singh et al., 2020), while 25±1°C temperature and 16 and 8-hr light and dark periods have been optimized (Nazneen et al., 2003; Mishra et al., 2005). In one research report, Naloussi et al. (2019) adjusted the temperature to 24 ± 2°C in light at 80 nmol m⁻² s⁻¹. In a recent study, Pourkhaloe et al. (2023) maintained cultures at 27 ± 1°C with a daily 16 hr illumination regimen of 40 µmol m⁻² s⁻¹ photosynthetic photon flux density.

***In-vitro* rooting and acclimatization:** Few efforts have been made to increase the efficiency of root growth in various media and cultural contexts. MS media is frequently used with growth regulators for both *in vitro* tuberose rooting. Suitability of MS media in different forms for tuberose rooting *in vitro*, as reported by earlier Mishra et al. (2005) and Singh et al. (2020), employed half-strength MS media for *in-vitro* tuberose rooting, whereas Surendranath et al. (2016) employed one-third-strength MS media. A comparative study of *in-vitro* rooting of tuberose was carried out with different media like MS, B5 and WH (Gajbhiye et al., 2011; Raghuvanshi et al., 2013), and WH media was found to work well compared to MS and B5 media. Plant growth regulators (PGRs) are added in different forms and combinations to produce *in vitro* rooting efficiently. PGRs such as IBA (Rajasekharan et al., 2000; Mishra et al., 2005) and NAA (Nazneen et al., 2003) have been reported to induce *in-vitro* rooting in tuberose genotypes. Root length and quantity increased well at 2.0 mg l⁻¹ IBA. Media containing 1.0 mg l⁻¹ IBA caused the most significant number of roots to sprout (Jyothi et al., 2008). Taksande et al. (2018) observed that full-strength White's media supplemented with 2 mg l⁻¹ IAA resulted in the highest root proliferation (58.7%), maximum root length and early root formation. Recently, Patil et al. (2025) found that media containing MS with 4.90 µM IBA produced the maximum number of roots.

Hardening and acclimatization in plant tissue culture are critical for successfully transferring *in-vitro*-derived plantlets from sterile laboratory to natural environment (Naloussi et al., 2019;

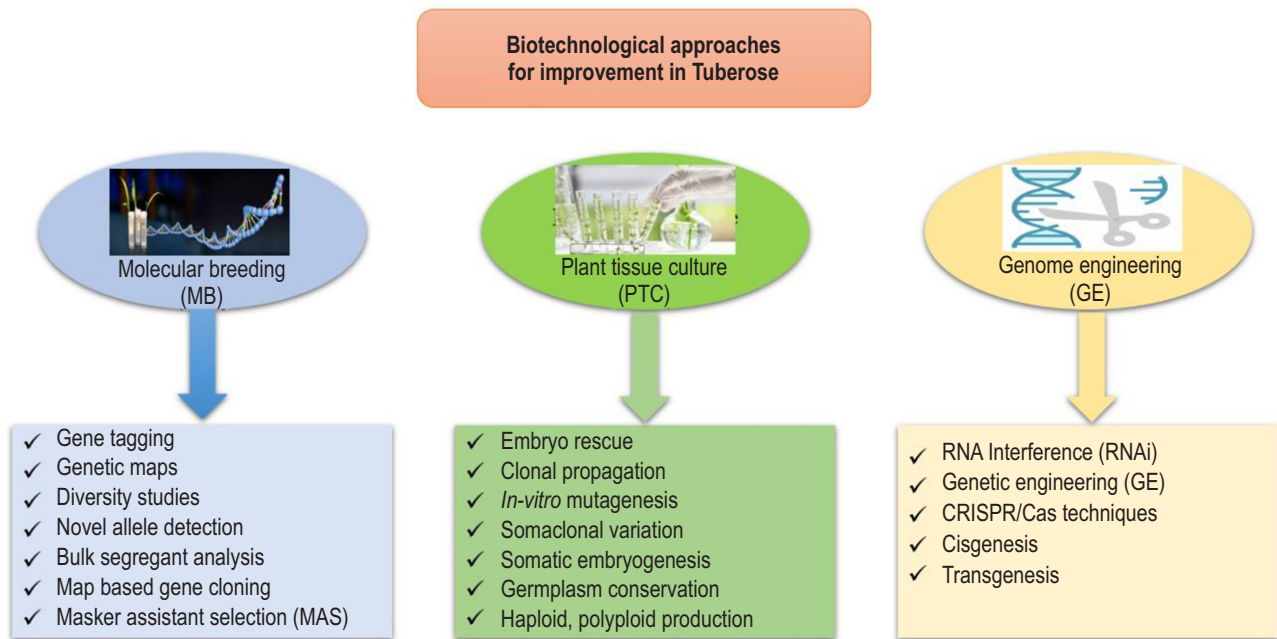


Fig. 2: Schematic representation of some biotechnological approaches toward the genetic improvement of ornamental tuberose.

Khanchana *et al.*, 2019; Taksande *et al.*, 2018). Hardening involves gradually exposing plantlets to less controlled conditions, like a greenhouse, while acclimatization is their adjustment to a new environment, such as field. Before transplanting, the roots are washed to remove culture media. Plantlets are then placed in pots with a sterile mix of cocopeat, vermicompost, sand, and perlite, covered with a clear plastic to maintain humidity. The plastic is slowly removed over several days before transferring the plants to greenhouse. Panigrahi and Saiyad (2013) kept plantlets in garden soil and leaf mold (3:1) for two months. Thereafter, they were kept in a greenhouse for two months before being moved into the field. Khanchana *et al.* (2019) used sand, cocopeat, and vermicompost in a 1:1:1 ratio and observed a survival rate of 97.17%.

New approach for enhancing ornamental tuberose: Researchers and rising flower farmers seek to overcome traditional breeding limitations and limit a continuous supply of superior planting materials for disease-free culture programs through *in vitro* culture and genomics (Fig. 2). These techniques speed up the production of good-quality flowers that are economically competitive. Although *in-vitro* flowering induction looks promising, there are still restrictions with respect to scaling, standardization, and molecular dissection of the system. Mekapogu *et al.* (2022) discussed the challenges posed by chrysanthemum's complex genome (polyploidy, heterozygosity). Genetically modified or derived varieties are selected for improving traits like flower color, form, blooming time, longevity in vase and resistance (biotic and abiotic). Different types of *in vitro* and genetic approaches for the enhancement of tuberose are presented here.

Biotechnological tools for virus detection: Viral infection is the major threats, which severely affects the quality and yield of tuberose. There is a growing concern in relation to the trade between different countries, particularly India's import and export of tuberose bulbs (growing or flowering) through different quarantine centers, leading to an increase in the risk of spreading bulb-borne viruses like Tuberose mild mosaic virus (TuMMV) and Tuberose mild mottle virus (TuMMoV), etc. These viruses can easily spread through vegetative propagation and insect vectors, threatening local horticulture. Therefore, strict quarantine measures and virus indexing are essential to ensure the safe exchange of tuberose germplasm globally (Vimal *et al.*, 2024).

Quick and accurate detection methods like ELISA and one-step RT-PCR are essential to prevent unintentional virus introduction and ensure virus-free tuberose production (Ganesh Selvaraj *et al.*, 2009). Lin *et al.* (2004) amplified 4607 nucleotides via RT-PCR with degraded primers, detecting various potyviruses and revealing TuMMV identities of 71.1 and 76.6%, proposing a new potyvirus called TuMMoVs. Kulshrestha *et al.* (2005) first detected potyvirus in tuberose in India using ELISA and RT-PCR, confirming infection by Tuberose mild mottle virus (TuMMoV). Raj *et al.* (2009) confirmed RT-PCR amplicons closely related to TuMMV, with 98-99% nucleotide and amino acid similarity. Dey *et al.* (2018) detected potyvirus in tuberose in Mexico using lateral flow immunoassays and ELISA. Complete genomic RNA sequences of TuMMoV and TuMMV revealed 72.4–77% nucleotide similarity (Prajapati *et al.*, 2022). Ghorbani *et al.* (2024) identified two novel potyviruses in Iran that form a unique, genetically diverse group outside known phylogenetic clusters.

Table 1: Recent studies on tuberose (*Agave amica* (Medik) transcriptomics)

Category	Plant organs/ developmental stage	Study objectives	Methodology	References
Abiotic and biotic stresses including nematodes	All development stage including abiotic and biotic stresses	Gene expression	RNA extraction and cDNA synthesis/ PCR and RT-qPCR	Madhvan et al. (2015)
Floral scent volatiles in the floral lifespan	All development stage	Physiological and cell biological aspects of floral fragrance production	Reverse transcription –PCR (RT–PCR)	Maiti and Mitra (2017)
Floral scent formation	Developmental stage	Gene expression patterns associated with floral smell creation	RNA-seq technology	Fan et al. (2018)
Transcriptome sequence of tuberose flower and de novo analysis for identification of flowering genes and transcription factors	Flowers	Identification of genes and metabolic pathways	RNA-seq technology	Madhavan et al. (2018)
Markers development	-	Identification of SSR	miRNA from transcriptome	Madhavan et al. (2019)
Change of floral scent volatiles synthesis, storage and emission	Flowers	Gene expression patterns involved in floral scent formation	Reverse transcription –PCR (RT–PCR)	Kutty et al. (2021)
Molecular mechanism in the host-pathogen interactions and transcriptome profiles of infected Root-knot nematodes (<i>Meloidogyne incognita</i>) tuberose plants, during early, mid, and late infection stage	Roots	Identification of coding sequences (CDS) and candidate gene expression gene associated with Root -knot nematodes	Reverse transcription –PCR (RT–PCR)	Singh et al. (2022)

These discoveries are important for tuberose cultivation, breeding, and viral disease management.

Molecular marker technology: Molecular marker technology uses specific DNA sequences to identify genetic differences among plants. It help the breeders to study genetic diversity, map genes and QTLs, and select plants with desirable traits more precisely and efficiently than traditional methods based on visible traits (Amiteye, 2021).

DNA markers are also useful for determining genetic variability, genotype relationships, and clonal fidelity in *in vitro* plants. Different DNA markers, including ISSR, SSR, RAPD, RFLP, AFLP, EST, STS, SCAR, and SNP, are applied in flower breeding programs (Kumar et al., 2019). RAPD markers have been successfully used to determine genetic diversity among accessions in tuberose, and they have shown marked polymorphism (Majd et al., 2013). Studies by Sarkar et al. (2010) reported high genetic relatedness among Guwahati Double and Swarn Rekha genotypes. Sirohi et al. (2017) also recorded significant RAPD marker-based genetic diversity. RAPD primers

were used to evaluate the change induced by gamma on polymorphism (Seyed Hajizadeh et al., 2023). ISSR markers revealed close genetic similarity between Calcutta Single and Arka Prajwal genotypes (Kameswari et al., 2014), while highlighting differences among Hyderabad Single, Kalyani Single, and Pearl double-petaled cultivar (Sirohi et al., 2017a). In comparison analysis, mean level of polymorphism observed for RAPD and ISSR ranged from 53 to 73% (Khandagale et al., 2014). Barba-Gonzalez et al. (2013) studied wild populations in Mexico with AFLP. A high similarity coefficient (0.95) was observed in *Polianthes tuberosa* 'Double,' indicating uniformity of asexually propagated cultivar. Yet, it has sufficient genetic diversity for breeding and is comparable to species like *P. platiphylla*, *P. longiflora* and *P. howardii*. Genetic variation within and between plant populations is crucial for effective plant utilization and breeding. Advances in molecular markers have greatly improved breeding strategies by enhancing genetic understanding (Kumar et al., 2019). Integration of next-generation sequencing (NGS) with precise phenotyping, association mapping, and omics approaches help to identify candidate genes and allelic variations linked to important traits. Functional markers, genotype-by-

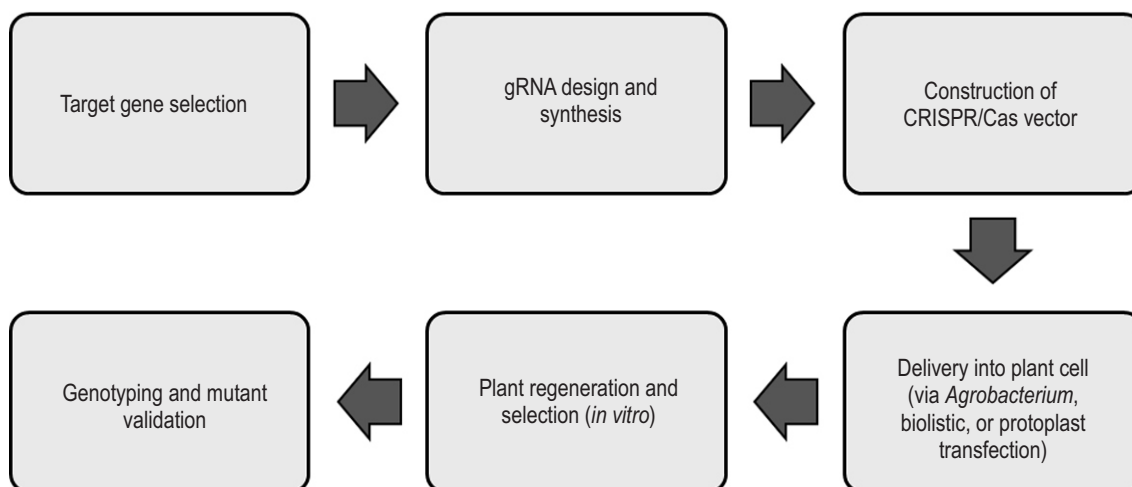


Fig. 3: Simplified steps of genome editing in plants.

sequencing, and association mapping enable discovery of new genetic resources, accelerating breeding for higher yield, better nutrition, and stress tolerance (Ramesh *et al.*, 2020).

Transcriptomics and genomics for tuberose improvement:

Transcriptomics uses high-throughput methods to analyze all RNAs in a specific tissue or cell (Wang *et al.*, 2009). In tuberose, limited transcriptomic and genomic studies exist (Table 1). Madhavan *et al.* (2019) identified 1,656 SSRs and 87 miRNAs from tuberose sequences. Tuberose transcriptome sequencing can be considered an effective tool for clarifying the genetic control of the biosynthesis of floral fragrance. In genomics studies of tuberose, Madhavan *et al.* (2015) evaluated stable reference genes in tuberose cultivars. Maiti and Mitra (2017) found developmental expression of Actin, DXR, and PAL genes. Transcriptome analysis detected 66 transcripts and 4,591 altered genes linked to stress responses, with APETALA2 and EREBPs being the most prevalent markers related to plant stress reactions (Madhavan *et al.*, 2018). Fan *et al.* (2018) identified 4,694 differentially expressed genes during flowering, with some related to terpene and benzenoid biosynthesis. Kutty *et al.* (2021) observed the highest expression of biosynthesis and sucrose metabolism genes in early bud and late shoot, which was associated with sugar metabolite concentration. Singh *et al.* (2022) described some important genes and TFs related to tuberose defense against infection with *Meloidogyne incognita* stress that could help us understand the molecular basis of nematode-induced stress. Singh *et al.* (2023) recently reported the production of first transgenic tuberose using an *Agrobacterium*-mediated transformation system, which employed a host-induced RNA silencing to demonstrate the potential of RNAi and artificial miRNAs as powerful tools with gene-silencing properties in the development of nematode-resistant crops.

Tuberose enhancement by genome editing: Genome editing, and in particular CRISPR (Nobel Prize 2020—invented by

Doudna and Charpentier), can be transformative in ornamental plant breeding. Modern genetic manipulations now mean cultivars can exist with abnormally colored, shaped, or scented flowers or even something such as additional stress resistance and larger blooms. Initially, limited to insect and herbicide resistance in crops, recent studies are mainly focusing on the quality traits for industrial and ornamental applications (Passeri *et al.*, 2016; Sarode *et al.*, 2024). The fundamental process is summarized in Fig. 3 as genome editing, which is also a rapid and efficient tool to improve tuberose with respect to characters such as disease resistance, vase life, quality, essential oil yield, and extracellular polysaccharide yield (Sadhukhan and Huo, 2020), by Ali *et al.* (2015) established the efficacy CRISPR/Cas9 system's in interfering with plant viruses, expanding the potential application of technology and creating plants immune to several viral infections. In tuberose, where sequence information is crucial, whole-genome sequence analysis may be the best strategy to improve the use of this practical technology. High-efficiency targeted mutagenesis utilizing CRISPR/Cas9 technology will help in identifying the function of a desired gene. The main difficulty researchers is the lack of sufficient sequence data in tuberose.

CRISPR/Cas9 gene editing technology, while potentially revolutionizing various floricultural crops like tuberose, faces challenges due to lack of suitable protocols. Currently, lilies (Yan *et al.*, 2019), petunias (Yu *et al.*, 2021), roses (Wang *et al.*, 2022), Torenia (Nishihara *et al.*, 2018), Japanese gentian (Watanabe *et al.*, 2017), and chrysanthemums (Kishi-Kaboshi *et al.*, 2017) have benefited from the successful application of gene editing technology. Target genes for flower color, fragrance, and longevity in tuberose can be selected from its genome or transcriptome homologs. Established CRISPR/Cas9 delivery methods from petunia and chrysanthemum, along with multiplexed guide RNA designs for polyploid genomes, can improve tuberose editing. DNA-free RNP-based editing reduces transgene risks and may speed commercial use (Zhang *et al.*,

2021). With targeted gene selection and protocol adaptation, these strategies are highly applicable to advancing tuberose genetic improvement for ornamental and commercial value.

Tuberose breeding face challenges like self-incompatibility, seed sterility, long cycles, and limited variation. Editing genes to overcome self-incompatibility and seed sterility can boost seed production and breeding efficiency. Accelerating the development of desired features without lengthy generation time or linkage drag, that come with traditional breeding might help overcome the limitations of tuberose breeding. Several genomic technologies beyond CRISPR/Cas have advanced tuberose improvement and hold future potential. These include RNA sequencing/transcriptomics, interspecific hybridization, mutant breeding, marker-assisted selection (MAS), and alternative gene-editing tools like TALENs and ZFNs. MAS uses DNA markers linked to traits such as stress tolerance, disease resistance, and floral qualities for early and effective selection in breeding programs (Vogel, 2009). Mutation breeding employing physical mutagens (gamma rays, X-rays) and chemical mutagens (EMS, MMS) has broadened the genetic base and created novel tuberose cultivars with unique flower colors, shapes and longer spikes (Kumar et al., 2021a). Hybridization between tuberose and related *Polianthes* species is used to introduce traits such as enhanced self-compatibility or new flower colors (Kumar et al., 2021a). Even in the absence of direct genome editing, transcriptome (RNA-Seq) research can aid in selecting breeding or modification strategies by identifying important flowering genes and regulatory networks (Madhavan et al., 2018).

Conclusion and future research directions: Tissue culture techniques provide a promising strategy to generate high-quality planting material in tuberose using low-cost micropropagation systems based on somatic embryogenesis and bioreactor-assisted culturing. Despite the commercial potential of tuberose, existing cultivars are limited in their low essential oil concentration and short floral lifespan. Notably, tuberose essential oil has strong antioxidant and DNA-protective effects, indicating its potential in cosmetic and medicinal applications. With the expected increase in demand for premium-grade essential oils and floral concretes, there is a rising need for research focused on improving terpene and benzene derivative biosynthesis pathways to optimize both productivity and quality in tuberose. The extended breeding cycle and seed setting issues limit traditional tuberose breeding approaches.

In recent years, genome-editing and gene-silencing technologies such as CRISPR/Cas9 and RNA interference (RNAi) have emerged as effective tools for crop development. However, there have been no published findings demonstrating the use of CRISPR/Cas9 or RNAi for genetic improvement in tuberose. Despite CRISPR/Cas9's extraordinary promise for precise genome editing, its larger applicability is hampered by issues such as off-target effects, poor transformation and delivery efficiency, and unresolved ethical concerns. As a result, further research is required to optimize these factors and enable the successful use of

genome-editing technologies in tuberose improvement.

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