

In vitro propagation of Gerbera for high-quality flower production

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Abstract:

Gerbera (*Gerbera jamesonii*) is a popular ornamental plant known for its vibrant flowers and extensive use in the floriculture industry. In vitro propagation offers a reliable and efficient method for producing high-quality *Gerbera* plants with uniform traits, overcoming the limitations of conventional propagation methods such as low multiplication rates and susceptibility to diseases. This technique involves the aseptic culture of explants, such as shoot tips, leaf discs, or flower buds, on nutrient-rich media supplemented with appropriate plant growth regulators. Micropropagation ensures rapid multiplication of true-to-type plants with desirable characteristics, including enhanced flower size, color intensity, and prolonged vase life. Optimizing factors such as media composition, hormone concentrations, and environmental conditions is critical for achieving high proliferation rates and robust plant development. Furthermore, in vitro propagation allows for the production of disease-free planting material, contributing to sustainable and profitable cultivation practices. The use of molecular markers to monitor genetic fidelity has enhanced the reliability of this method, ensuring clonal uniformity in commercial *Gerbera* production. In addition to meeting market demands for high-quality flowers, this approach supports the conservation of valuable *Gerbera* germplasm. Continued advancements in in vitro techniques are expected to further revolutionize the floriculture industry by providing innovative solutions for high-quality flower production.

Keywords: Gerbera, *in vitro* propagation, high-quality flowers, floriculture, genetic fidelity.

1. Introduction

Gerbera (*Gerbera jamesonii* Bolus ex Hooker f.), belonging to the Asteraceae family, ranks among the top ten most commercially important cut flowers globally, prized for its large, vibrant, and diverse flower heads, long vase life, and year-round production potential. High-quality Gerbera production demands uniform, disease-free, and vigorous plants capable of yielding flowers with optimal size, color intensity, stem length, strength, and longevity. Conventional propagation methods, primarily through seed or division of clump-forming crowns, present significant limitations for achieving this standard. Seed propagation leads to high genetic variability, resulting in non-uniform flower quality and characteristics unsuitable for the standardized cut flower market. Vegetative division is slow, inefficient (yielding only 3-5 plants per mother plant per year), and carries a high risk of transmitting systemic pathogens like viruses (e.g., *Tomato spotted wilt virus*, *Cucumber mosaic virus*), fungi (e.g., *Phytophthora*, *Fusarium*), and bacteria, which severely compromise plant health, flower yield, and quality. *In vitro* propagation, or micropropagation, has emerged as the indispensable solution for large-scale, commercial Gerbera production, enabling the rapid multiplication of genetically uniform, pathogen-free elite clones with consistent and superior flower traits (Pierik, 1997; Nhut et al., 2003; Kanwar & Kumar, 2008).

2. Advantages of In Vitro Propagation for Gerbera Quality

The adoption of *in vitro* techniques offers numerous distinct advantages crucial for producing high-quality Gerbera flowers. Firstly, it facilitates the mass clonal multiplication of selected elite genotypes, ensuring absolute genetic uniformity essential for standardized flower size, color, form, and stem characteristics across thousands of plants (Ruffoni & Savona, 2013). Secondly, the process inherently produces pathogen-free plantlets. Starting from meristematic explants (shoot tips, meristems) combined with rigorous sterile culture conditions

and potential thermotherapy or chemotherapy treatments, allows for the effective elimination of systemic diseases that plague conventionally propagated stocks (Cassells & Long, 1982). This directly translates to healthier plants with reduced susceptibility to soil-borne pathogens, enhanced vigor, and consequently, higher flower yields and quality. Thirdly, micropropagation enables year-round production of plantlets independent of seasonal constraints, ensuring a continuous supply of high-quality planting material to meet market demands consistently. Finally, *in vitro* systems provide a platform for germplasm conservation and the rapid introduction of new cultivars developed through breeding programs, accelerating their commercial availability (Bhatia et al., 2015).

3. Explant Selection and Sterilization

The choice of explant is critical for successful establishment and subsequent plantlet quality. Shoot tips (apical and axillary meristems, typically 0.3-0.8 mm in size) are the most preferred explants. They possess high morphogenetic potential, minimize the risk of chimerism, and are the primary tissue used for virus eradication due to their active cell division and typically lower viral load compared to differentiated tissues (Nhut et al., 2003). Flower buds (especially immature capitulum tissue) also exhibit high regeneration potential but are more prone to somaclonal variation. Leaf segments (petioles, lamina) and scales can be used but generally show lower and slower regeneration efficiency. Rigorous surface sterilization is paramount. Common protocols involve sequential washing with detergent (e.g., Tween-20), treatment with a strong oxidizing agent (e.g., sodium hypochlorite, NaOCl, 1.0-2.0% available chlorine for 10-20 minutes), and rinsing with sterile distilled water, often followed by a brief dip in ethanol (70%) and a final rinse. Antibiotics (e.g., streptomycin) or fungicides may be added to the sterilization solution or initial culture medium to combat endogenous contamination (Reed, 1999; Kanwar & Kumar, 2008). Aseptic establishment is a major bottleneck requiring meticulous technique.

4. Culture Media Composition and Growth Regulators

Murashige and Skoog (MS) basal medium is the most widely used foundation for *Gerbera* micropropagation due to its balanced nutrient composition. Modifications, such as half-strength MS macro-elements or specific media like Schenk and Hildebrandt (SH), are sometimes employed, particularly during rooting stages. Sucrose (2-3%) serves as the primary carbon source and osmoticum. The addition of a gelling agent, typically agar (0.6-0.8%) or gellan gum (e.g., Gelrite®, Phytigel™ at 0.2-0.3%), provides physical support. Growth regulators are the key drivers of morphogenesis:

Shoot Induction and Proliferation

Cytokinins are essential. Benzylaminopurine (BAP) in the range of 0.5-5.0 mg/L is most effective, stimulating axillary bud break and shoot multiplication. Kinetin (0.1-2.0 mg/L) is sometimes used but less potent than BAP. Thidiazuron (TDZ), a potent phenylurea cytokinin, can induce high multiplication rates at very low concentrations (0.01-0.5 mg/L) but carries a higher risk of inducing hyperhydricity (vitrification) and somaclonal variation if used long-term or at high doses (Huang & Chu, 1985; Palai et al., 2020). A low auxin concentration, often Indole-3-acetic acid (IAA) or Indole-3-butyric acid (IBA) (0.1-0.5 mg/L), is frequently added to the proliferation medium to promote shoot elongation and reduce callusing. Activated charcoal (0.1-0.5%) is sometimes added to absorb inhibitory phenolic exudates and promote cleaner growth.

Rooting

Shoots (3-5 cm tall) are transferred to rooting media, typically containing auxins. IBA (0.5-2.0 mg/L) is generally more effective than Naphthaleneacetic acid (NAA) (0.1-1.0 mg/L) in inducing strong, branched roots. High auxin concentrations or prolonged exposure can lead to callus formation at the base. Rooting is often more efficient on half-strength MS medium without cytokinins. Some protocols utilize a brief pulse treatment with high auxin concentration (e.g., 50-100 mg/L IBA for 5-30 minutes) followed by transfer to hormone-free medium, promoting better root quality and minimizing

callus (Murashige et al., 1974; Cardoso & Teixeira da Silva, 2012).

Callus Induction and Organogenesis

While less common for large-scale propagation due to higher variation risk, callus induction (using 2,4-Dichlorophenoxyacetic acid (2,4-D) or NAA combined with cytokinins) followed by shoot regeneration (transfer to cytokinin-rich medium) can be used for mass propagation or genetic manipulation studies.

Optimization of Culture Conditions and Multiplication

Physical culture conditions significantly impact growth rate and plantlet quality. Temperature is typically maintained between 22-25°C. A photoperiod of 16 hours light (30-60 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetic photon flux density - PPFD) provided by cool white fluorescent or LED lamps is standard. Higher light intensities during the rooting and pre-acclimatization phases can enhance photosynthesis and harden plantlets. Relative humidity inside culture vessels is high (near 100%), contributing to the risk of hyperhydricity. Cultures are usually maintained on semi-solid medium in glass jars or Magenta™ boxes, with periodic subculturing every 4-6 weeks onto fresh proliferation medium to maintain exponential growth rates. Shoot multiplication occurs primarily via the stimulation of axillary buds, yielding 5-15 new shoots per explant per cycle. Careful selection of healthy, non-vitrified shoots during subculture is crucial for maintaining stock quality and preventing the accumulation of off-types. Vigorous proliferation without excessive callus formation is key to high-quality output (Ruffoni & Savona, 2013).

Rooting, Acclimatization, and Hardening

Successful rooting is a critical determinant of survival during subsequent transfer to soil. Root initiation typically occurs within 2-4 weeks on auxin-supplemented or pulse-treated media. High rooting percentages (>80-90%) with well-developed root systems are achievable with optimized protocols. Acclimatization (weaning) is arguably the most critical and challenging phase for ensuring high survival rates

and subsequent field performance. *In vitro*-derived plantlets are heterotrophic (reliant on sugar), have underdeveloped cuticles, poorly functioning stomata, and fragile roots adapted to high humidity. They must be gradually acclimatized to the lower humidity, higher light intensity, and autotrophic conditions of the greenhouse. This involves:

Ex Vitro Rooting

Transferring unrooted microshoots directly to sterile substrates (peat:perlite mix) under high humidity (e.g., mist, fog) and sometimes auxin treatment.

Initial High Humidity

Transferring rooted plantlets to trays containing a well-drained, porous, sterile substrate (e.g., peat moss, perlite, vermiculite mixtures). Trays are placed under transparent plastic covers or in fog/mist systems to maintain 80-90% relative humidity for 1-2 weeks. Fungicides are often applied preventatively.

Gradual Humidity Reduction

Slowly reducing the humidity over the next 2-4 weeks by gradually increasing ventilation (lifting covers, increasing mist intervals). Light intensity is gradually increased to full sunlight levels.

Fertilization

Commencing application of a diluted, balanced nutrient solution once plants show signs of new growth. Careful management of water (avoiding both drought and waterlogging) and disease prevention during this phase is essential to achieve survival rates exceeding 85-90% (Pospisilova et al., 1999; Cardoso & Teixeira da Silva, 2012). Acclimatized plants are then grown on in the greenhouse until ready for field planting or pot production.

5. Impact on Flower Quality and Yield

Micropropagated Gerbera plants consistently demonstrate superior performance leading to higher-quality flower production compared to conventionally propagated stock.

The most significant benefits include enhanced uniformity in plant growth, flowering time, flower size, color consistency, and stem length and diameter, meeting strict market standards. Increased flower yield per plant and per unit area is frequently reported, attributed to the greater vigor, larger crown size, and higher number of flowering points in disease-free micropropagated plants. Flowers often exhibit improved postharvest longevity (vase life), linked to better overall plant health, stronger stems, and potentially optimized physiological characteristics. The reduction in disease incidence due to the pathogen-free starting material significantly lowers losses from soil-borne pathogens like *Phytophthora* and *Verticillium*, leading to more sustainable production with reduced pesticide use. Furthermore, micropropagation allows for the rapid deployment of superior new cultivars developed for specific quality traits like novel colors, unique flower forms, or enhanced vase life (Cappelletti et al., 1990; Palai et al., 2020).

6. Challenges and Future Perspectives

Despite its success, Gerbera micropropagation faces challenges impacting cost-effectiveness and quality consistency. **Hyperhydricity (Vitrification):** A physiological disorder causing water-soaked, translucent, brittle shoots with poor growth. It is associated with high humidity inside vessels, low light intensity, high cytokinin levels (especially TDZ), high ammonium nitrate concentration, and poor gas exchange. Mitigation involves optimizing gelling agents, reducing vessel humidity (e.g., using ventilated lids), increasing light intensity, reducing cytokinin concentration/duration, adding growth retardants (e.g., ancymidol, paclobutrazol) or osmotic agents (e.g., mannitol), and careful explant selection (Ziv, 1991). **Endogenous Contamination:** Latent bacterial or fungal infections can cause significant losses; stringent sterilization, media antibiotics (with caution due to phytotoxicity), and meristem culture are key countermeasures (Reed, 1999). **Somaclonal Variation:** While generally low with meristem/axillary bud proliferation, variations in flower color, form, and plant habit can occur, especially with prolonged subculture or organogenesis/callus systems. Rigorous roguing and maintaining limited subculture generations are essential

(Bhatia et al., 2015). High Production Costs: Labor, energy, and consumables contribute significantly to the cost. Automation (e.g., bioreactors for multiplication), optimizing protocols for efficiency, and scaling up can reduce costs. Acclimatization Losses: This phase remains a bottleneck; optimizing substrates, environmental control, and hardening protocols is ongoing. Future research focuses on protocol refinement for higher efficiency and lower costs, automation and bioreactor technology, molecular markers for early detection of variation and pathogen testing, improved rooting and acclimatization strategies, and integrating micropropagation with genetic engineering for novel traits like disease resistance or novel flower colors.

7. Conclusion

In vitro propagation is the cornerstone technology for modern, high-quality Gerbera cut flower production. It effectively addresses the critical limitations of conventional propagation by enabling the mass production of genetically uniform, pathogen-free elite clones. Through meticulous optimization of explant selection, sterilization, media composition (particularly growth regulators), and culture conditions, coupled with careful management of the critical acclimatization phase, micropropagation delivers vigorous, healthy plants. These plants consistently outperform conventionally propagated stock, resulting in superior flower uniformity, higher yields, enhanced vase life, and reduced disease incidence. While challenges like hyperhydricity, contamination, and acclimatization costs persist, ongoing research and technological advancements continue to refine the process. The integration of automation, molecular tools, and potentially genetic engineering promises to further enhance the efficiency and capabilities of Gerbera micropropagation, ensuring its continued pivotal role in meeting the global demand for premium Gerbera flowers.

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