SUNFLOWER (*HELIANTHUS ANNUUS L.*) TISSUE CULTURE: TRENDS AND ADVANCES

CULTURI DE ȚESUTURI LA FLOAREA SORARELUI (HELIANTHUS ANNUUS L.): TENDINȚE ȘI PROGRESE

PINTILIA Sabina[1], PETCU Elena[1], ANTON Gabriel[1], BORDEI Marius[1]

*Corresponding author e-mail: saninapintilia17@gmail.com

Abstract.
Between 2020 and 2025, research on in vitro culture of sunflower (Helianthus annuus L.) has led to the refinement of explant use, including cotyledons, cotyledonary nodes, and immature embryos. Murashige and Skoog (MS)-based media, supplemented with specific combinations of auxins (IAA, NAA) and cytokinins (kinetin, 6-BA), have been optimized to enhance organogenesis—the main regeneration pathway. Somatic embryogenesis remains difficult and inconsistent. A major limitation is the genotype-dependent response and the generally low regeneration efficiency. These challenges are being addressed through genotype-specific protocols and by investigating the expression of key genetic regulators of totipotency, such as SERK and BBM. Improved in vitro systems now allow efficient genetic transformation, particularly through Agrobacterium tumefaciens-mediated methods, facilitating the development of transgenic or genome-edited lines. Moreover, these technologies support the conservation of genetic resources from wild Helianthus species and the rapid clonal propagation of elite agronomic genotypes. Overall, these advances underpin the development of cutting-edge biotechnological strategies in sunflower breeding, with applications in sustainable production and improved tolerance to biotic and abiotic stress factors.

Key words: Explant sources, cotyledon segments, immature embryos, regeneration techniques, genetic transformation

Rezumat.
Între anii 2020 și 2025 cercetările privind cultura in vitro a florii-soarelui (Helianthus annuus L.) au condus la perfecționarea utilizării explantatelor, precum: cotiledoane, noduri cotiledonare și embrioni imaturi. Mediile bazate pe formularea Murashige și Skoog (MS), suplimentate cu combinații specifice de auxine (IAA, NAA) și citochinine (kinetină, 6-BA), au fost optimizate pentru a stimula organogeneza — principala cale de regenerare. Embriogeneza somatică rămâne dificilă și inconsistentă. O limitare majoră este răspunsul dependent de genotip și eficiența general scăzută a regenerării. Aceste provocări sunt abordate

[1] National Agricultural Research and Development Institute Fundulea, Romania

prin protocoale specifice genotipului și prin investigarea expresiei regulatorilor genetici-cheie ai totipotenței, precum SERK și BBM. Sistemele in vitro îmbunătățite permit acum transformarea genetică eficientă, în special prin metode mediate de Agrobacterium tumefaciens, facilitând dezvoltarea liniilor transgenice sau editate genomic. În plus, aceste tehnologii sprijină conservarea resurselor genetice din speciile sălbatice de Helianthus și multiplicarea clonală rapidă a genotipurilor agronomice de elită. Aceste progrese susțin dezvoltarea unor strategii biotehnologice de ultimă generație în ameliorarea florii-soarelui, cu aplicații în producția durabilă și în îmbunătățirea toleranței la factori de stres biotici și abiotici.

Cuvinte cheie: Surse de explant, segmente cotiledonare, embrioni imaturi, tehnici de regenerare, transformare genetică

INTRODUCTION

Sunflower is the fourth most important oilseed crop worldwide, cultivated across diverse agro-ecological zones [Vijay *et al.*, 2020]. Its oil is valued for high unsaturated fatty acid content, particularly oleic and linoleic acids, which contribute to cardiovascular health and industrial uses [Islam *et al.*, 2021]. Traditional breeding has generated hybrid cultivars with improved yields, yet remains constrained by the narrow genetic base and extended breeding cycles [Purwantoro *et al.*, 2018].

Plant tissue culture offers solutions through clonal propagation, in vitro conservation, genetic transformation, and haploid production [Blinkov *et al.*, 2022]. However, sunflower is recalcitrant to regeneration, with outcomes strongly dependent on genotype and influenced by tissue browning and hyperhydricity [Mendoza and González, 2016]. Over the last decade, advances in media optimization, the use of silver nitrate and antioxidants, and novel genome editing approaches have significantly improved in vitro outcomes [Chen *et al.*, 2024; Lebedeva *et al.*, 2025].

MATERIAL AND METHOD

A systematic literature review was performed covering studies published between 2015 and 2025, complemented with selected classical works. Databases included Scopus, PubMed, Web of Science, and Google Scholar. Keywords were: “sunflower tissue culture,” “organogenesis Helianthus annuus,” “somatic embryogenesis sunflower,” “CRISPR sunflower,” “haploid sunflower,” and “synthetic seed Helianthus.” Both experimental and review papers were included.

RESULTS AND DISCUSSIONS

Explant Sources and Regeneration Pathways

The choice of explant is among the most critical determinants of in vitro regeneration in sunflower, as morphogenic competence varies widely between tissue types and genotypes. Cotyledonary nodes are widely recognized as the most responsive explant, with regeneration efficiencies exceeding 90% in optimized

conditions combining kinetin with low levels of auxins [Chen *et al.*, 2024]. Hypocotyls and leaf segments have been used extensively, although regeneration frequencies are more variable and strongly genotype dependent [Mendoza and González, 2016; Badouin *et al.*, 2017]. Immature embryos exhibit high plasticity and remain the most effective explants for somatic embryogenesis due to their undifferentiated meristematic tissues [Moradi *et al.*, 2018]. In wild relatives such as *H. verticillatus*, leaf explants have proven successful under cytokinin-auxin combinations, described in (Table 1), offering a pathway for conservation of rare species [Edwards *et al.*, 2020]. Interestingly, direct organogenesis from shoot tips and meristems provides a rapid and stable route with reduced somaclonal variation compared to callus-mediated regeneration [Shormee *et al.*, 2016]. Explant orientation and physiological stage at excision also influence outcomes, with juvenile tissues being more responsive than mature ones [Radova *et al.*, 2021]. In (Table 1) are presented all the explant sources that are commonly used, some of the main advantages and disadvantages along with the success rate. Collectively, understanding explant physiology and genotype-specific responses is essential to designing efficient, reproducible regeneration systems for sunflower and related Helianthus species.

Table 1

Comparative efficiency of regeneration pathways

Explant source	Regeneration pathway	Success rate (%)	Advantages	Disadvantages	References
Cotyledonary node	Direct organogenesis	70-95%	High regeneration frequency; stable; low somaclonal variation	Genotype-dependent; requires juvenile tissue	[Witrzens *et al.* 2004; Mendoza *et al.* 2016]
Hypocotyl	Indirect organogenesis	30-60%	Accessible; useful for transformation studies	Variable response; prone to callus-induced variation	[Singareddy *et al.* 2018; Garcia-Perez *et al.* 2021]
Leaf segment	Organogenesis / callus	10-35%	Non-destructive sampling; suitable for wild species	Low regeneration efficiency; high browning and necrosis	[Pavlović *et al.* 2020; Nowakowska *et al.* 2024]
Immature embryo	Somatic embryogenesis	60-85% (under optimized PGRs)	High morphogenic potential; ideal for synthetic seed production	Time-sensitive; labor-intensive; highly sensitive to culture conditions	[Ortiz *et al.* 2025; Martinez *et al.* 2021]

Explant source	Regeneration pathway	Success rate (%)	Advantages	Disadvantages	References
Meristem / shoot tip	Direct shoot regeneration	50-70%	Virus elimination; clonal fidelity	Low multiplication rate; technically demanding	[Ivanov *et al.* 2014]
Anther / pollen	Androgenesis (haploids)	<1-5%	Potential for DH line development; accelerates breeding	Very low efficiency; strong genotype dependency	[Singh *et al.* 2019; Blinkov *et al.* 2022]
Ovule / ovary	Gynogenesis (haploids)	<1-3%	Alternative to anther culture; possible DH production	Extremely low regeneration frequency; requires specialized protocols	[Meena *et al.* 2022]
Root segment	Callus induction only	<5%	Easy to obtain; good for metabolic studies	Very poor shoot regeneration; unsuitable for clonal propagation	[Radonic *et al.* 2015]

Organogenesis and Somatic Embryogenesis

Sunflower regeneration occurs primarily via two major morphogenic pathways: organogenesis and somatic embryogenesis. Direct organogenesis, typically from cotyledonary nodes, as shown in (Figure 1) is favored for its stability, shorter timelines, and reduced genetic variability [Mendoza *et al.*, 2016]. Cytokinins such as BA or kinetin, in combination with auxins like IAA or NAA, are the most effective in stimulating shoot organogenesis, as it is revealed in (Figure 2) [Purwantoro *et al.*, 2019]. Indirect organogenesis involves callus formation prior to shoot development and is useful for transformation but carries a higher risk of somaclonal variation [Edwards *et al.*, 2020]. Somatic embryogenesis, though less routine in sunflower compared to other crops, offers the potential for mass clonal propagation and synthetic seed production [Azadi *et al.*, 2021]. High sucrose concentrations and auxin analogs such as picloram or 2,4-D stimulate somatic embryo induction [Moradi *et al.*, 2018]. Stress factors, including osmotic stress and cold pretreatment, have also been shown to increase embryogenic competence [Ceriani *et al.*, 1992]. Although somatic embryos can germinate into viable plants, their conversion frequency remains a bottleneck, requiring optimization of maturation media and desiccation treatments. Future protocols may combine organogenesis for stable regeneration with embryogenesis for scaling-up, maximizing the potential of sunflower micropropagation systems.

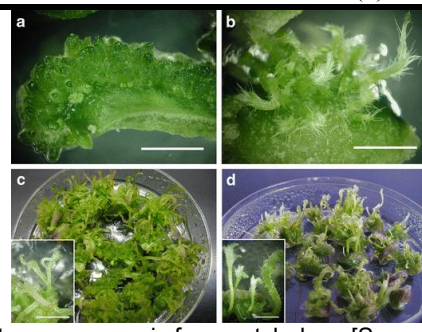

Fig. 1. Shoot organogenesis from cotyledons [Source: Zhang *et al.*, 2015]

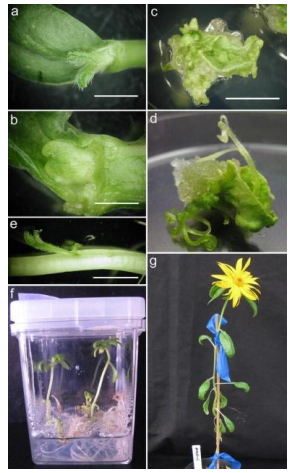

Fig. 2. Organogenesis from primary leaves of young seedlings preconditioned by cytokinin [Source: Zhang *et al.*, 2015]

Factors Influencing In Vitro Culture

Several intrinsic and extrinsic factors strongly influence the efficiency of sunflower tissue culture.

- **Genotype dependency** is the most widely reported constraint, with some cultivars showing prolific regeneration while others fail under identical protocols [Nowakowska *et al.*, 2024].
- **Plant growth regulators** (PGRs) are pivotal: cytokinins such as BAP or kinetin promote shoot induction, while low concentrations of auxins (IAA, NAA) encourage rooting [Purwantoro *et al.*, 2019]. Thidiazuron (TDZ) at micromolar concentrations has also proven highly effective in inducing adventitious shoots [Shormee *et al.*, 2016].
- **Additives such** as silver nitrate and silver thiosulfate inhibit ethylene accumulation, reducing tissue necrosis and increasing regeneration frequency [Khalil *et al.*, 2015]. Main additive that are used are presented in Table 2. Antioxidants including ascorbic acid, glutathione, and citric acid

are used to counteract phenolic exudation and browning [Radova *et al.*, 2021].

Table 2

Key additives and their role

Additive	Role	Effect on culture response	References
$AgNO_3$ (Silver nitrate)	Ethylene inhibitor	Prevents explant necrosis, delays senescence, increases shoot induction	[Pai *et al.*, 2018; Khalil *et al.*, 2015]
Ascorbic acid	Antioxidant	Reduces browning and oxidative stress, improves callus survival	[Radova *et al.*, 2021; Garcia-Perez *et al.*, 2021]
Putrescine	Polyamine	Enhances embryo maturation, promotes somatic embryogenesis	[Inoka *et al.*, 2015;] [Martinez *et al.*, 2021]
Activated charcoal	Adsorbent of phenolics	Prevents medium browning, supports root induction	[Moradi *et al.*, 2018]
Glutathione (GSH)	Antioxidant	Protects tissues from oxidative stress, increases regeneration efficiency	[Sharma *et al.*, 2020]
Polyvinylpyrrolidone (PVP)	Adsorbent	Binds phenolic compounds, reduces tissue necrosis	[Pavlović *et al.*, 2020]
Myo-inositol	Vitamin / osmoprotectant	Enhances cell division, promotes shoot elongation	[Purwantoro *et al.*, 2019]
Coconut water	Natural growth supplement	Provides vitamins, sugars, hormones; enhances callus proliferation	[Mendoza *et al.*, 2016]
Casein hydrolysate	Amino acid source	Improves embryogenic callus induction, enhances shoot quality	[Blinkov *et al.*, 2022]
Silver thiosulfate (STS)	Ethylene blocker	Stronger ethylene inhibition than $AgNO_3$, prolongs morphogenic competence	[Li *et al.*, 2023]
TDZ (Thidiazuron)	Cytokinin-like growth regulator	Highly efficient in shoot induction, induces direct organogenesis; may reduce rooting if concentration too high	[Sharma *et al.*, 2020;] [Nowakowska *et al.*, 2024]

- **Environmental conditions** further modulate responses: light quality, photoperiod, osmotic stress, and temperature pretreatments all contribute to enhancing morphogenesis [Chraibi *et al.*, 1992]. Even medium composition, particularly the source and concentration of carbon (sucrose, maltose), influences both callus proliferation and embryo development. Understanding

the interplay between genotype, PGR balance, and environmental factors is critical for optimizing protocols and ensuring reproducibility across different sunflower genotypes.

Genetic Transformation and Genome Editing

Genetic transformation in sunflower has historically been challenging due to genotype dependence, low regeneration frequency, and tissue necrosis during selection. Agrobacterium-mediated transformation (Figure 3) remains the most widely used approach, relying on the infection of cotyledonary nodes, hypocotyls, or leaf discs with *Agrobacterium tumefaciens*. Protocols combining vacuum infiltration, sonication-assisted infection, and antioxidant supplementation have significantly increased transient expression efficiencies, reaching >90% in optimized systems [Darqui *et al.*, 2021]. Stable transformation rates, however, remain limited to 5-10% depending on the cultivar [Tishchenko *et al.*, 2014]. Recent studies in cultivar ZADT reported reproducible stable transformants using EHA105 strain and kanamycin selection [Chen *et al.*, 2025]. Beyond Agrobacterium, particle bombardment and electroporation have been attempted, though with lower reproducibility and higher costs [Sosnowski *et al.*, 2023]. The advent of CRISPR/Cas9 technology has opened new frontiers for sunflower improvement. Knockout of the FAD2-1 gene via CRISPR led to altered fatty acid composition with elevated oleic acid content [Uslu *et al.*, 2022], while editing HaNSP1a disrupted strigolactone biosynthesis, conferring resistance to *Orobanche cumana* [Lebedeva *et al.*, 2025]. Multiplex CRISPR strategies could allow simultaneous editing of multiple traits, such as drought tolerance and disease resistance. Overall, genome editing is emerging as a transformative tool for functional genomics and targeted breeding in sunflower, complementing tissue culture-based regeneration systems.

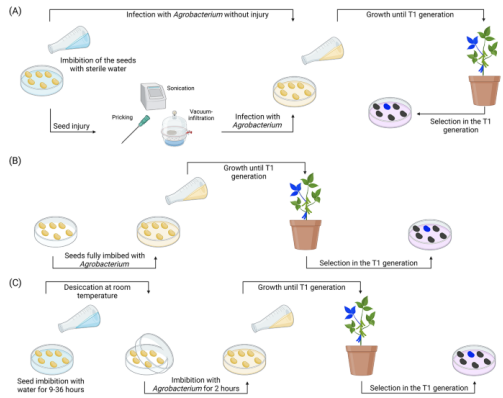

Fig. 3. Agrobacterium-mediated transformation [Source: Bélanger et al 2024]

Haploid and Double Haploid Systems

Haploid and double haploid (DH) technologies provide an accelerated route to homozygous lines, drastically reducing the breeding cycle from several generations to a single step. In sunflower, haploid production has been attempted through anther culture (Figure 4), isolated microspores, and irradiated pollen techniques, although success rates remain lower compared with cereals [Blinkov *et al.*, 2022]. Anther culture has occasionally yielded callus and plantlets, but the frequency of haploid plant recovery is typically <1% [Sosnowski *et al.*, 2023]. In vitro gynogenesis, involving unpollinated ovules cultured under stress conditions, represents a promising but underexplored pathway. Recent efforts have also focused on wide hybridization and in vitro rescue of haploid embryos from interspecific crosses, followed by chromosome doubling with colchicine [Zhang *et al.*, 2015]. Advances in flow cytometry and molecular markers now enable precise identification of haploid regenerants, facilitating their use in breeding. DH sunflower lines are particularly valuable for developing mapping populations and for producing stable homozygous parents in hybrid programs. Nevertheless, the strong genotype dependence and low reproducibility remain significant challenges, and integrating stress pretreatments with optimized PGR regimes may enhance efficiency. The development of reliable haploid induction systems in sunflower could be a game changer for genomics-assisted breeding.

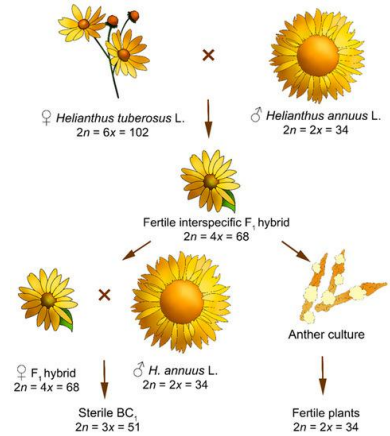

Fig. 4. Manipulation of ploidy level in the anther culture of an interspecific hybrid between cultivated sunflower and H. tuberosus L. [Source: Blinkov *et al.*, 2022]

Somaclonal Variation and Genetic Stability

Regeneration through callus culture, while enabling plant recovery, is often associated with somaclonal variation due to chromosomal rearrangements, point mutations, and epigenetic changes [Edwards *et al.*, 2020]. Although variation is undesirable in clonal propagation, it can generate novel traits for breeding, such as tolerance to abiotic stresses or altered metabolic profiles [Mendoza *et al.*, 2016]. The use of molecular markers such as SSRs, AFLPs, and SNP arrays has been

instrumental in assessing the genetic fidelity of regenerants [Radova *et al.*, 2021]. Studies on *Helianthus verticillatus* micropropagated lines revealed moderate variation among regenerants, yet most retained morphological and physiological stability [Nowakowska *et al.*, 2024]. To minimize somaclonal variation, direct organogenesis from meristematic tissues is preferred over indirect pathways involving friable callus. Antioxidants such as ascorbic acid and glutathione have also been reported to reduce oxidative stress and DNA damage during culture [Finer *et al.*, 1987]. Cryopreservation of shoot tips may further safeguard genetic integrity in long-term conservation programs [Fu *et al.*, 2017]. Thus, balancing regeneration efficiency with genetic stability is crucial when designing tissue culture-based breeding pipelines in sunflower.

Biotechnological Innovations

Innovations in tissue culture are broadening the scope of sunflower biotechnology. Somatic embryos encapsulated in sodium alginate and calcium chloride solutions form synthetic seeds, which can be stored, transported, and germinated under controlled conditions [Azadi *et al.*, 2021]. Synthetic seed technology offers opportunities for germplasm exchange and large-scale propagation of elite lines, particularly in recalcitrant genotypes. Advances in encapsulation matrices enriched with nutrients, PGRs, and antifungal agents have improved synthetic seed germination and conversion rates to plantlets [Onișan *et al.*, 2025]. Parallel to this, bioreactor systems such as temporary immersion bioreactors (TIBs) and air-lift systems are being tested for large-scale sunflower regeneration [Witrzens *et al.*, 2004]. Bioreactors reduce labor costs, ensure uniform microenvironment conditions, and support automated scaling of plantlet production. Although sunflower-specific applications remain limited, successful implementation in other oilseed crops suggests strong potential. Moreover, coupling bioreactors with somatic embryogenesis protocols may facilitate mass production of synthetic seeds or uniform regenerants. Together, synthetic seed and bioreactor technologies represent promising frontiers to overcome current bottlenecks in sunflower micropropagation and conservation.

Conservation Applications

Beyond breeding, tissue culture plays a vital role in conserving sunflower germplasm and rare Helianthus species. In vitro propagation protocols for the endangered *H. verticillatus* have enabled successful shoot regeneration and acclimatization [Edwards *et al.*, 2020; Nowakowska *et al.*, 2024]. Conservation efforts also extend to cryopreservation, where shoot tips, embryogenic callus, and zygotic embryos are stored in liquid nitrogen for long-term germplasm banking [Voronova *et al.*, 2016]. Slow-growth storage under osmotic stress and reduced temperature further complements cryopreservation by allowing medium-term maintenance. Molecular fingerprinting of in vitro conserved lines ensures genetic

integrity and aids in monitoring possible variation during long-term storage [Trigiano *et al.*, 2021]. Additionally, tissue culture facilitates the recovery of virus-free plants through meristem culture combined with thermotherapy or cryotherapy [Fernandez *et al.*, 2018]. These methods not only safeguard genetic diversity but also enable the clean exchange of germplasm between breeding programs. Given the increasing threats posed by climate change, habitat loss, and pathogen pressure, integrating tissue culture and cryobiology into sunflower conservation strategies is essential for ensuring the resilience and sustainability of both cultivated and wild germplasm.

Challenges in Sunflower Tissue Culture and Cropp

Despite the remarkable advances in sunflower tissue culture over the past decade, several challenges continue to limit its reproducibility, efficiency, and large-scale application.

- *Genotype dependency*- The single most significant barrier is the strong genotype effect on regeneration. While certain hybrids and inbred lines respond efficiently to organogenesis or embryogenesis, others fail under identical protocols [Kishor *et al.*, 2018]. This restricts the development of universal protocols applicable across breeding programs.
- *Low and variable transformation efficiency*- Agrobacterium-mediated transformation in sunflower remains inconsistent, with stable integration frequencies often below 10% [Marek *et al.*, 2019]. Factors such as explant age, bacterial strain, infection conditions, and plant genotype all contribute to poor reproducibility.
- *Somaclonal variation and genetic instability*- Regeneration via callus is frequently associated with karyotypic changes, transposon activation, and epigenetic alterations [Pavlović *et al.*, 2020]. While sometimes useful as a source of variation, it compromises clonal fidelity and trait stability.
- *Oxidative browning and phenolic exudation*- Sunflower explants often accumulate phenolic compounds upon wounding, leading to medium darkening, necrosis, and culture loss [Garcia-Perez *et al.*, 2021]. Although antioxidants and adsorbents (e.g., ascorbic acid, activated charcoal, silver nitrate) help, reproducibility remains a problem.
- *Limited efficiency of haploid technologies*- Despite potential for rapid breeding, haploid induction via androgenesis or gynogenesis is still inefficient (<5%) and highly genotype-dependent [Mushke *et al.*, 2019; Blinkov *et al.*, 2022].
- *Scalability constraints*- While synthetic seed technology and bioreactors are promising, their large-scale application in sunflower remains at the experimental stage, with cost, contamination risks and conversion efficiency as major obstacles [Li *et al.*, 2023].
- *Cryopreservation and germplasm stability*- Long-term conservation protocols are not yet standardized. While promising results exist for shoot tips and

embryos, genotype and cryoprotectant choice strongly influence survival and regrowth [Seiler *et al.*, 2017].

• *Integration with omics and genome editing*- Although transcriptomics and CRISPR have been applied to sunflower, integration into tissue culture pipelines is still in its infancy [Lebedeva *et al.*, 2025]. Establishing robust, genotype-independent protocols compatible with genome editing remains an unresolved challenge.

• *Herbicide resistance*- represents a central challenge but also an opportunity in sunflower production. The widespread use of herbicides for weed control has led to the selection of resistant weeds, while simultaneously creating demand for herbicide-tolerant sunflower hybrids. Conventional approaches to herbicide tolerance in sunflower have relied primarily on mutation breeding and natural variability within *Helianthus annuus* populations. The most prominent example is the development of imidazolinone-tolerant sunflower (Clearfield technology), which resulted from the identification of spontaneous mutations in the *AHAS* (acetohydroxyacid synthase) gene. These hybrids enabled effective weed control (Table 3) but raised concerns about the evolution of resistant weed populations [Bozic *et al.* 2015; Gaines *et al.*, 2020].

In vitro and biotechnological approaches have expanded the possibilities of generating herbicide resistance:

- *In vitro mutagenesis* combined with selection on herbicide-containing media has produced resistant calli and regenerants carrying point mutations in AHAS.
- *Somaclonal variation* has occasionally produced novel herbicide-tolerant phenotypes, though often at low frequency.
- *Transgenic and genome editing* approaches allow precise engineering of herbicide tolerance while avoiding undesired traits linked to random mutations [Lebedeva *et al.*, 2025].

Despite these advances, challenges remain. Clearfield hybrids face increasing weed resistance; public acceptance of GM crops is limited in some regions; and tissue culture bottlenecks restrict rapid deployment of engineered lines. Future strategies are likely to integrate CRISPR-based precise editing with conventional breeding to ensure durable herbicide tolerance.

Table 3

Conventional vs. in vitro approaches to herbicide resistance in sunflower

Approach	Mechanism	Advantages	Limitations	References
Conventional mutation breeding	Spontaneous/induced mutation in *AHAS*	Non-GMO; already commercialized (*Clearfield*)	Slow; resistance breakdown in weeds	[Bozic *et al.* 2015]

Approach	Mechanism	Advantages	Limitations	References
In vitro mutagenesis	Callus exposed to herbicide, resistant cells regenerated	Faster than field mutation; selectable markers	Low efficiency; somaclonal variation	[Brosnan *et al.* 2016]
Somaclonal variation	Spontaneous genetic/epigenetic changes	May produce novel herbicide tolerance	Rare, unpredictable; linked to instability	[Kaya, 2015]
Transgenic approaches	Transfer of resistant *AHAS* alleles	High precision, stable inheritance	Regulatory restrictions; low transformation rate	[Gaines *et al.*, 2020]
CRISPR/Cas9 genome editing	Targeted point mutation in *AHAS*	Precise, non-transgenic edits possible	Protocols not yet genotype-independent	[Lebedeva *et al.*, 2025]

- *Abiotic stresses* such as drought, heat, and salinity are increasingly critical under climate change, as sunflower is often cultivated in semi-arid regions with limited irrigation [FAO, 2020]. Furthermore, rising temperatures accelerate phenological development, reducing grain filling and oil accumulation.
- *Soil fertility constraints*- especially nitrogen and micronutrient deficiencies- reduce seed oil yield, and over-fertilization can lead to lodging and increased susceptibility to diseases [Khalil *et al.*, 2015].

Future Perspectives

The future of sunflower tissue culture lies in overcoming genotype dependency and achieving reproducible, genotype-independent regeneration systems. Advances in molecular biology and bioinformatics are paving the way for integrating omics-driven approaches (transcriptomics, proteomics, and metabolomics) to identify key regulators of morphogenesis [Bélanger *et al.*, 2024]. Such data-driven insights may allow the rational design of culture media and treatments tailored to specific genotypes or even universally applicable across diverse cultivars. CRISPR/Cas9-based genome editing is expected to expand beyond single-gene knockouts to multiplex editing, base editing, and prime editing, enabling fine-tuning of metabolic pathways such as oil biosynthesis and resistance against *Orobanche cumana* [Uslu *et al.*, 2022; Lebedeva *et al.*, 2025]. Moreover, coupling genome editing with haploid and doubled haploid systems will accelerate the development of homozygous edited lines, providing powerful resources for functional genomics and breeding [Blinkov *et al.*, 2022]. On the applied side, the integration of synthetic seed technology and temporary immersion bioreactors holds promise for scaling up propagation and reducing production costs [Witrzens

et al., 2004]. Conservation of wild Helianthus species through cryopreservation and in vitro slow-growth storage will further ensure genetic diversity in the face of climate change [West *et al.*, 2025]. Collectively, these innovations point toward a future where sunflower tissue culture becomes a routine and scalable platform for both basic research and applied crop improvement, bridging the gap between fundamental biotechnology and sustainable agriculture.

CONCLUSIONS

Over the past decade, sunflower tissue culture research has transitioned from being viewed as a niche and technically challenging field to becoming a central pillar of crop biotechnology. The refinement of regeneration systems using cotyledonary nodes and immature embryos, coupled with optimization of plant growth regulator regimes and additives such as silver nitrate and antioxidants, has substantially improved in vitro success rates [Khalil *et al.*, 2015; Trigiano *et al.*, 2021]. Parallel advances in Agrobacterium-mediated transformation and transient expression systems have laid the foundation for more efficient genetic manipulation, while CRISPR/Cas9 applications have demonstrated the feasibility of precise genome editing in sunflower [Uslu *et al.*, 2022; Lebedeva *et al.*, 2025]. The establishment of haploid and doubled haploid protocols, albeit limited in efficiency, offers promising avenues for rapid breeding. Somaclonal variation, once seen solely as a drawback, is now being reconsidered as a source of novel genetic diversity. Looking forward, integrating synthetic seed technology and bioreactor-based scaling with omics-driven media optimization will be crucial for industrial-scale applications. Moreover, in vitro conservation strategies, including cryopreservation, provide an essential safeguard for maintaining the genetic diversity of both cultivated and wild Helianthus species. Ultimately, overcoming genotype dependency and developing universally applicable regeneration and transformation protocols will be key to fully exploiting sunflower tissue culture in meeting the global demand for sustainable food and bioresources under changing climatic conditions.

ACKNOWLEGMENTS

This work was supported by a grant of the Ministry of Agriculture and Rural Development, within the ADER Program, project number 2.1.4 – *'Cercetări privind perfecționarea managementului integrat de combaterea buruienilor la principalele culturi de câmp, în contextul schimbărilor climatice și a restricțiilor Green Deal privind exploatarea durabilă a resurselor agricole de mediu'*.

REFERENCES

1. Azadi, P., Moieni, A. & Ahmadi, M.R. 2014- *Shoot organogenesis from cotyledons of sunflower*, Helia, 25, pp.19–26. doi:10.2298/HEL0237019A.

2. Badouin, H., Gouzy, J., Grassa, C.J., Murat, F., Staton, E., Cottret, L., Lelandais-Brière, C., Owens, G.L., Carrère, S., Mayjonade, B., et al. 2017- *The sunflower*

genome provides insights into oil metabolism, flowering and asterid evolution, Nature, 546, pp.148–152.

3. **Bélanger, R., Tavares, S., Roy, M. & Perret, M. 2024**- *A comprehensive review of in planta stable transformation strategies*, Plant Methods, 20(1), p.12. doi:10.1186/s13007-024-01200-8.
4. **Blinkov, Y., Gorbachenko, O., Iakovenko, O. & Davydov, V. 2022**- *The production of Helianthus haploids: Current status and future prospects*, Plants, 11(21), p.2919. doi:10.3390/plants11212919.
5. **Bozic, D., Pavlovic, D., Bregola, V**., **2015**- *Gene flow from herbicide-resistant sunflower hybrids to weedy sunflower*, Journal of Plant Diseases and Protection, 122(5–6), pp. 183–188. doi:10.1007/BF03356548.
6. **Brosnan, J.T., Vargas, J.J., Breeden, G.K., Grier, L., Aponte, R.A., Tresch, S. and Laforest, M. 2016**- *A new amino acid substitution (Ala-205-Phe) in acetolactate synthase (ALS) confers broad spectrum resistance to ALS-inhibiting herbicides*, Planta, 243(1), pp. 149–159. doi:10.1007/s00425-015-2399-9.
7. **Ceriani, M.F., Hopp, H.E., Hahne, G. & Alejandro, S. 1992**- *Cotyledons: an explant for routine regeneration of sunflower plants*, Plant Cell Physiology, 33(2), pp.157–164.
8. **Chen, G., Li, H., Zhang, Y. & Wang, Q. 2024**- *Tissue culture and Agrobacterium-mediated genetic transformation of the oil crop sunflower'* PLOS ONE, 19(2),
9. **Chen, G., Wang, J., Liu, H. & Zhang, Y**. **2025**- *Establishment of Agrobacterium-mediated transient transformation system in sunflower*, Plants, 14(15), p.2412.
10. **Chraibi, B.K.M., Castelle, J.C., Latche, A., Roustan, J.P. & Fallot, J. 1992-** *Enhancement of shoot regeneration potential by liquid medium culture from mature cotyledons of sunflower (Helianthus annuus L.), Plant cell reports, 10(12), 617–620.*
11. **Darqui, F., Radonic, L., Beracochea, V., Hopp, H., Lopez Bilbao, M. 2021-** *Peculiarities of the Transformation of Asteraceae Family Species: The Cases of Sunflower and Lettuce.* Frontiers in Plant Science. 12. 10.3389/fpls.2021.767459.
12. **Edwards, T.P., Trigiano, R.N., Ownley, B.H., Windham, A.S., Wyman, C.R., Wadl, P.A. & Hadziabdic, D**. **2020**- *Genetic diversity and conservation status of Helianthus verticillatus, an endangered sunflower of the Southern United States*, Frontiers in Genetics, 11, p.410.
13. **FAO 2020** -The *State of Food and Agriculture 2020: Overcoming water challenges in agriculture.* Rome: Food and Agriculture Organization of the United Nations.
14. **Fernandez, J., Morales, P. & Torres, M. 2018**- *Meristem culture and thermotherapy for virus elimination in sunflower germplasm,* Plant Cell, Tissue and Organ Culture, 134(1), pp.45–54.
15. **Finer, J.J. 1987**- *Direct somatic embryogenesis and plant regeneration from immature embryos of hybrid sunflower (Helianthus annuus L.) on a high sucrose-containing medium*, Plant Cell Reports, 6, pp.372–374. doi:10.1007/BF00269564.
16. **Fu, X., Qi, L., Hulke, B., Seiler, G. & Jan, C. 2017**- *Somatic embryogenesis from corolla tubes of interspecific amphiploids between cultivated sunflower (Helianthus annuus L.) and its wild species*, Helia, 40(66), pp.1–19. doi:10.1515/helia-2017-0006.
17. **Gaines, T. A., Duke, S. O., Morran, S., Rigon, C. A. G., Tranel, P. J., Küpper, A., & Dayan, F. E. 2020**- *Mechanisms of evolved herbicide resistance.* The Journal of biological chemistry, 295(30), 10307–10330.
18. **Inoka, K.P. & Dahanayake, N. 2015**- *Effect of plant growth regulators on micro-propagation of sunflower (Helianthus annuus L.)*, International Journal of Scientific and Research Publications, 5(1), pp.1–7.
19. **Islam, K., Ahmed, T., & Sharmin, T. 2021-** *In Vitro Growth of Sunflower (Helianthus annuus)* via Direct Organogenesis. *American Journal of Pure and Applied Biosciences*.

20. Ivanov, P., Encheva, J., Nenova, N. & Drumeva, M. 2014- *Application of some biotechnological achievements in sunflower breeding,* Helia, 37, pp.9–18. doi:10.2298/HEL0237009I.

21. Kaya, Y. 2015- *Herbicide resistance breeding in sunflower, current situation and future directions,* Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţele vieţii, 2(326), pp. 101–106.

22. Khalil, R., El-Sherif, H. & Gad, A. 2015- *Silver nitrate is essential for successful regeneration of Egyptian sunflower (Helianthus annuus L.) cv. Giza 102*, International Journal of Advanced Research, 3(2), pp.33–41.

23. Kishor, P., Rajam, M., Pullaiah, T. 2021- *Genetically Modified Crops Current Status, Prospects and Challenges*, Volume 1: Current Status, Prospects and Challenges Volume 1. 10.1007/978-981-15-5897-9.

24. Lebedeva, N., Ivanov, P., Pavlov, V. & Smirnova, O. 2025- *CRISPR/Cas9-mediated editing of HaNSP1a alters strigolactone biosynthesis in sunflower roots*, Agronomy, 15(1), p.129.

25. Marek, L.F. 2019- *Crop wild relatives of sunflower in North America*, in Greene, S.L., North American Crop Wild Relatives, Volume 2: Important Species. Cham: Springer, pp.453–483.

26. Meena, H.P. & Sujatha, M. 2022- *Sunflower breeding*, in Yadava, D.K., Dikshit, H.K., Mishra, G.P. & Tripathi, S. (eds) Fundamentals of Field Crop Breeding. Singapore: Springer. doi:10.1007/978-981-16-9257-4_19.

27. Mendoza, R. & González, F. 2016- *Explant type and regeneration potential in sunflower tissue culture*, Plant Cell Reports, 35(12), pp.2357–2364.

28. Moradi, R., Karimi, R. & Shaban, M. **2018**- *Direct somatic embryogenesis from immature embryos of sunflower hybrids*, International Journal of Field Crop Science, 13(1), pp.73–80.

29. Mushke, R., Rajesh, Y. & Kirti, P. **2019**- *Improved salinity tolerance and growth performance in transgenic sunflower plants via ectopic expression of a wheat antiporter gene (TaNHX2)*, Molecular Biology Reports, 46. doi:10.1007/s11033-019-05028-7.

30. Nowakowska, J., Kowalczyk, A. & Piotrowski, W. 2024- *In vitro regeneration from leaf explants of Helianthus verticillatus*, Plants, 13(2), p.285.

31. Onișan, E., Petolescu, C., Tenche-Constantinescu, A.-M. & Sarac, I. 2025 - *Application of biotechnology for sunflower inbred line development: Immature embryo rescue*, Applied Sciences, 15(3), p.1576. doi:10.3390/app15031576.

32. Pai, S. & Desai, N. 2018- *Effect of TDZ on various plant cultures'*, in. doi:10.1007/978-981-10-8004-3_25.

33. Purwantoro, A., Purwestri, Y.A., Lawrie, M.D. & Semiarti, E. 2022- *Genetic transformation via plant tissue culture techniques: Current and future approaches*, Advances in Plant Tissue Culture. Academic Press, pp.131–156. doi:10.1016/B978-0-323-90795-8.00001-1.

34. Radonic, L.M., Zaccolo, J., Zavallo, D., López, N. & Bilbao, M.L. 2015- *Sunflower (Helianthus annuus L.*), in Methods in Molecular Biology, 1224, pp.47–55. doi:10.1007/978-1-4939-1712-3_4.

35. Radova, R., Milan, K., Petra, R. & Jana, M. 2021- *Effect of antioxidants and silver nitrate on sunflower regeneration and phenolic oxidation*, Plant Biotechnology Reports, 15(3), pp.225–233.

36. Seiler, G.J., Qi, L.L. & Marek, L.F. 2017- *Utilization of sunflower crop wild relatives for cultivated sunflower improvement'*, Crop Science, 57, pp.1083–1101.

37. Shormee, S.Z. 2016- *Rhizogenesis of regenerated shoot in Bangladeshi Sunflower (Helianthus annuus L.) varieties*.

38. Singareddy, V., Sheri, V.R., Muddanuru, T., Tatineni, R., Jain, R.K., Sankaraneni, C.R., Kodeboyina, V.S. & Mulpuri, S. 2018- *Genetic engineering of sunflower (Helianthus annuus L.) for resistance to necrosis disease through deployment of the TSV coat protein gene*, Plant Cell, Tissue and Organ Culture, 135, pp.263–277.

39. Sosnowski, J., Truba, M. & Vasileva, V. 2023- *The impact of auxin and cytokinin on the growth and development of selected crops*, Agriculture, 13(3), p.724. doi:10.3390/agriculture13030724.

40. Tishchenko, O., Komisarenko, A., Mykhalska, S., Sergeeva, L., Adamenko, N., Morgun, B. & Kochetov, A. 2014- *Agrobacterium-mediated transformation of sunflower (Helianthus annuus L.) in vitro and in planta using LBA4404 strain harboring binary vector pBi2E*, Cytology and Genetics, 48, pp.218–226. doi:10.3103/S0095452714040094.

41. Trigiano, R.N., Boggess, S.L., Wyman, C.R., Hadziabdic, D. & Wilson, S. 2021- *Propagation methods for the conservation and preservation of the endangered whorled sunflower (Helianthus verticillatus)*, Plants, 10(8), p.1565. doi:10.3390/plants10081565.

42. Uslu, E., Kahriman, A. & Yalçın, M. 2022- *CRISPR/Cas9-mediated targeted mutagenesis of FAD2-1 gene for oleic acid composition in sunflower*, Genetics & Applications, 6(2), pp.63–69.

43. Vijay, S., Muddanuru, T. & Sujatha, M. 2020- *Genetic engineering of sunflower (Helianthus annuus L.) for important agronomic traits,* in. doi:10.1007/978-981-15-5897-9_9.

44. Voronova, O. 2016- *Callus formation and plant regeneration in sunflower (Helianthus L., Asteraceae) in vitro tissue culture'.*

45. West, Owen & Yordanova, Elena & Yordanov, Yordan. 2025- *Enhancing sunflower regeneration: genetic and molecular insights from Helianthus eggertii*. Plant Cell, Tissue and Organ Culture (PCTOC). 160. 10.1007/s11240-024-02932-0.

46. Witrzens, B., Scowcroft, W.R., Downes, R.W. & Larkin, P.J. 2004- *Tissue culture and plant regeneration from sunflower (Helianthus annuus) and interspecific hybrids (H. tuberosus × H. annuus)*, Plant Cell, Tissue and Organ Culture, 13, pp.61–76.

47. Zhang, Z. & Finer, J.J. **2015**- *Sunflower (Helianthus annuus L.) organogenesis from primary leaves of young seedlings preconditioned by cytokinin*, Plant Cell, Tissue and Organ Culture, 123, pp.645–655. doi:10.1007/s11240-015-0867-3.