Algopishi UB *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 3, Article number 14718
DOI:10.15835/nbha53314718
Review Article

The genetic applications of plant cell and tissue culture techniques: Essential tools for genetic manipulation and crop improvement

Uthman B. ALGOPISHI[1], Ahmed M. ABDELGHFFAR[2*], Nora M. AL ABOUD[3], Fatmah A. SAFHI[4], Eman FAYAD[5], Khadiga ALHARBI[4], Nada I. ALJWAIZEA[4], Abdallah A. HASSANIN[2*]

[1]*King Khalid University, College of Science, Department of Biology, Abha, 61413, Saudi Arabia; ualgopishi@kku.edu.sa*
[2]*Zagazig University, Faculty of Agriculture, Genetics Department, Zagazig, 44511, Egypt; ahmghaffar95@gmail.com (*corresponding author); asafan@zu.edu.eg (*corresponding author)*
[3]*Umm Al-Qura University, Faculty of Science, Department of Biology, Makkah, 24372, Saudi Arabia; nmaboud@uqu.edu.sa*
[4]*Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, Riyadh, 11671, Saudi Arabia; faalsafhi@pnu.edu.sa; kralharbi@pnu.edu.sa; nialjiwizaa@pnu.edu.sa*
[5]*Taif University, College of Sciences, Department of Biotechnology, Taif, 21944, Saudi Arabia; e.esmail@tu.edu.sa*

Abstract

Plant cell and tissue culture techniques are fundamental to contemporary plant science, providing essential tools for genetic manipulation and crop improvement. This review examines the primary applications of these techniques, emphasizing their role in advancing fundamental plant biology and developing novel agricultural strategies. Micropropagation is a technique that enables the rapid and efficient asexual propagation of superior genotypes, which is crucial for conserving and disseminating valuable plant material. Furthermore, meristem culture effectively eradicates viruses from infected plants, ensuring the production of disease-free planting stock. Tissue culture techniques are also instrumental in generating genetic variability through somaclonal variation, *in vitro* mutagenesis, and *in vitro* selection. These methods provide a substantial source of genetic diversity, facilitating the development of new plant varieties with advantageous characteristics. Beyond generating variation, tissue culture is indispensable for genetic engineering, allowing for the stable integration of exogenous DNA into plant cells to produce transgenic plants with novel traits. Embryo rescue is another significant application. It overcomes challenges in seed development and enabling successful hybridization between otherwise incompatible plant species. By salvaging immature embryos and culturing them to maturity, this technique allows for the creation of hybrid plants possessing desirable trait combinations that would be unattainable through conventional breeding. In conclusion, these techniques have profoundly transformed plant science, offering diverse applications for genetic manipulation, crop improvement, and basic research. By enabling precise control over plant development at the cellular and tissue levels, these techniques are critical for developing improved crops with enhanced yield, nutritional quality, and resilience to environmental stressors.

Keywords: embryo rescue; genetic engineering; genetic variation; micropropagation; plant cell culture; plant tissue culture; virus elimination

Received: 17 Aug 2025. Received in revised form: 04 Sep 2025. Accepted: 08 Sep 2025. Published online: 22 Sep 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Plant tissue culture (PTC) has become a cornerstone of plant science, offering significant economic advantages across various sectors. This technique enables rapid *in vitro* multiplication, producing thousands of clonal plants from a single small explant. This continuous process ensures a reliable supply of genetically uniform plant material (Amalahyacinth and Asaf, 2019; Chadipiralla *et al.*, 2020). Essentially, PTC allows for large-scale clonal propagation. Under controlled aseptic conditions, one explant can be efficiently propagated into millions of plants in a short timeframe, independent of seasonal variations (Chugh *et al.*, 2009). This significantly benefits the ornamental industry by enabling rapid, large-scale production of uniform plants. Furthermore, PTC stands out as a powerful tool for plant production and improvement, solidifying its importance in plant biotechnology.

Under field conditions, plants are susceptible to biotic stresses such as viruses, fungi, nematodes, and bacteria. These stresses significantly impact plant growth, development, and overall yield (Benke *et al.*, 2021). Viruses are particularly damaging due to their obligate intracellular nature and lack of effective control methods. Viral infections can cause devastating crop losses, sometimes reaching 100% (Prasanna *et al.*, 2015; Singh *et al.*, 2021). Meristem tip culture offers a practical solution for producing virus-free plants. This technique exploits the fact that shoot meristems, due to their rapid cell division and limited vascularization, are typically free of viral pathogens. The process involves the aseptic isolation and culture of the apical meristem, a tiny structure measuring only 100-200 micrometres (Rai *et al.*, 2022).

Plant tissue culture offers a diverse array of techniques for targeted manipulation and improvement of plant traits. Techniques like another culture, somaclonal variation, gametoclonal variation, microspore culture for haploid production, protoplast culture, somatic hybridization, and transgenic plant development all contribute to this goal (Singh *et al.*, 2021; Sarma *et al.*, 2023). Additionally, mutagens can be utilized to induce controlled genetic variability for crop improvement programs.

Somaclonal variation presents a particularly intriguing approach for rapidly achieving increased genetic diversity within a population. This variation arises spontaneously during the tissue culture process and can be exploited to introduce novel traits without requiring complex technology (Ferreira *et al.*, 2023).

The application of physical and chemical mutagens has been instrumental in generating genetic diversity and developing elite plant cultivars with desirable agronomic characteristics (Ahloowalia *et al.*, 2004; Pharmawati, 2024). Biotechnology techniques, particularly those involving *in vitro* plant cell and tissue cultures alongside molecular biology tools, offer promising solutions to address various challenges in plant breeding (Raina *et al.*, 2023). Unlike traditional crossbreeding methods, which involve the random mixing of entire genomes, mutagenesis allows for targeted modification of specific traits in an existing cultivar without significantly altering its overall genetic background. Furthermore, mutagenesis applied to *in vitro* plant cultures offers a robust and efficient approach to induce genetic variability for crop improvement (Penna and Bhagwat, 2023).

When combined with advanced molecular techniques, tissue culture methods have been successfully used to integrate specific, desirable traits into plant genomes through targeted gene transfer (Singh *et al.*, 2021). *Agrobacterium*-mediated gene transfer, utilizing *A. tumefaciens*, remains a premier technique for plant transformation. This method often necessitates tissue culture to regenerate complete plants from transformed explants under sterile conditions (Jagdish and Koundal, 2020). This review aimed to comprehensively evaluate the potential of plant tissue culture techniques for plant production, disease elimination, and trait improvement, highlighting their economic and scientific significance.

Micropropagation

High heterozygosity in many fruit trees and ornamental plants leads to significant phenotypic variation in their seed-derived offspring, causing them to deviate from parental phenotypes. In contrast, asexual reproduction generates progeny that are genetically identical to the parent, thereby preserving the unique genotypes and phenotypes of cultivars (Laurentin Táriba, 2023). Asexually derived populations with identical genotypes are termed clones, and the process of generating these populations is known as clonal propagation (Pandey, 2022; Abdelghaffar *et al.*, 2023a; Shalan *et al.*, 2023). This method ensures the genetic homogeneity of the donor plant across successive generations.

Micropropagation, a commercially significant application of *in vitro* culture techniques, enables the mass production of clonal plant material from diverse species (Duta-Cornescu *et al.*, 2023). Plant tissue culture (PTC) is a powerful technique for the rapid clonal propagation of plants, ensuring the consistent production of high-quality plant material for agricultural and horticultural applications. Micropropagation can be achieved through three primary methods (Figure 1): enhancing axillary bud breaking, producing adventitious buds directly or indirectly via callus, and somatic embryogenesis directly or indirectly on explants (George, 1993).

Enhancing axillary bud proliferation exhibits the lowest multiplication rate but offers the highest degree of genetic fidelity. Conversely, somatic embryogenesis possesses the potential for the highest number of plantlets but has limited applicability across plant taxa. Consequently, commercial production of many ornamental plants primarily relies on axillary bud proliferation due to its assured genetic uniformity (Richardson and Varkonyi-Gasic, 2023). Producing adventitious buds directly or indirectly via callus allows for rapid multiplication of large numbers, however, some species face challenges or failures in regeneration, and there is a high risk of genetic or cytogenetic alterations (D'Amato and Bayliss, 1985). Somatic embryogenesis directly or indirectly on explants possesses the potential for the highest number of plantlets, making asexual embryogenesis a promising technique for rapid plant clonal propagation (Muguerza *et al.*, 2022). However, its applicability is limited across plant taxa, and achieving synchronous embryo development and implementing effective protective measures are crucial for its successful application in plant production systems (George, 1993).

With increasing global trade, tissue culturists face both local and international competition. Micropropagation, commonly used for ornamental and medicinal plants (Bertsouklis *et al.*, 2024), is also applied to crops like potatoes, bananas, and some forest trees. Annually, hundreds of millions of plants, spanning tens of thousands of varieties, are micropropagated (Singh, 2015).

Global ornamental plant production has significantly increased, boosting the economies of over 50 countries. According to (Gabellini and Scaramuzzi, 2022), global cut flower and potted plants production counts for a value of about 35.5 billion USD with a cultivation area of 745,000 ha.

Micropropagation has become a dominant and dependable method for commercially producing tropical foliage plants (houseplants) (Irfan *et al.*, 2022). This is evident by the estimated annual global production of 254 million ornamental foliage plants in 2006, with a wholesale value of US $219 million. *Syngonium podophyllum* Schott serves as a prime example, being a key tropical foliage plant species, widely propagated *in vitro* for commercial production (Chen *et al.*, 2010). Roses (*Rosa* spp.) as one of the earliest domesticated ornamentals, have been a target for developing micropropagation protocols. These protocols aim to produce roses that are true-to-type and surpass traditionally propagated plants in terms of cost, quality, and growth characteristics (Pati *et al.*, 2006).

Plantation crops, staple crops like pineapple, banana, and sugarcane, play a vital role in global food and energy security. Maintaining their health, yield, and compliance with international export regulations necessitates a consistent supply of large quantities of clean, true-to-type planting material. This has driven producers to explore biotechnological solutions, with micropropagation emerging as a successful strategy for

growers worldwide to address challenges, enhance quality and yield, and improve production efficiency (Abdalla *et al.*, 2022; Abdelghaffar *et al.*, 2023b).

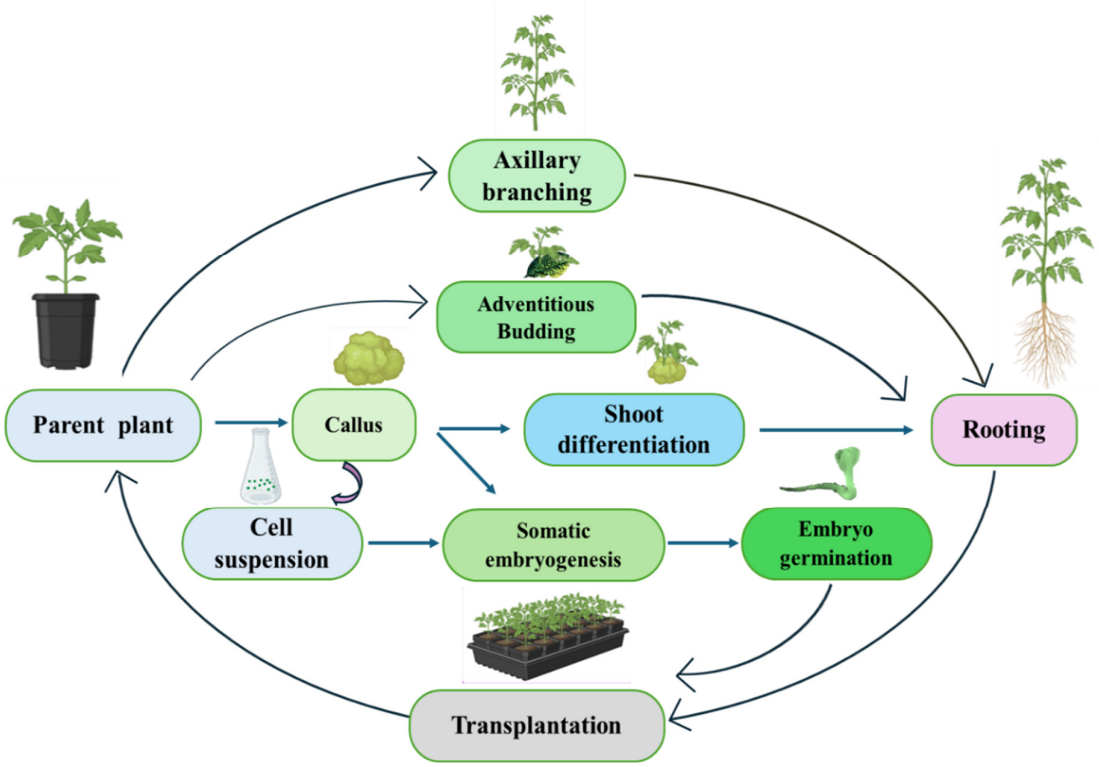

Figure 1. A detailed summary of the steps involved in micropropagation through tissue culture

Virus elimination in plants

Plant viruses pose a significant threat to global agricultural productivity, capable of causing substantial crop losses, in some cases reaching 100% (Prasanna *et al.*, 2015; Singh *et al.*, 2021). These pathogens are particularly problematic in vegetatively propagated crops, where systemic infections, often involving multiple viruses, are prevalent. *In vitro* meristem culture (Figure 2) is a highly effective strategy for generating virus-free plant material in such scenarios (Pramesh and Baranwal, 2015). The foundation of this technique dates back to the pioneering work of Morel and Martin (1952), who successfully applied apical meristem culture for virus elimination in dahlias. The effectiveness of meristem culture stems from the inherent properties of the shoot apical meristem (SAM). Studies consistently demonstrate that meristematic tissues are either completely virus-free or contain extremely low viral concentrations (Krishna *et al.*, 2022; Yao *et al.*, 2022). Another study conducted by Bettoni *et al.* (2022) improved procedure for producing virus-free planting material for the potato industry. It could also underpin the global exchange of virus-free germplasm for conservation and breeding programs. Kereša *et al.* (2021) stablished a protocol for the regeneration of virus-free garlic plants through somatic embryogenesis of two Croatian garlic ecotypes. This relative immunity can be attributed to several factors: The shoot tip and young leaf primordia are typically disconnected from the main vascular system, which serves as the primary transport route for viruses within the plant. The rapid cell division and growth processes in the meristem create an environment unfavorable for viral replication. Meristematic cells may possess intrinsic mechanisms for eliminating or suppressing viral activity compared to other plant tissues.

The high concentration of auxin hormones in the shoot apex might further restrict viral multiplication (Lai and Lai, 2019).

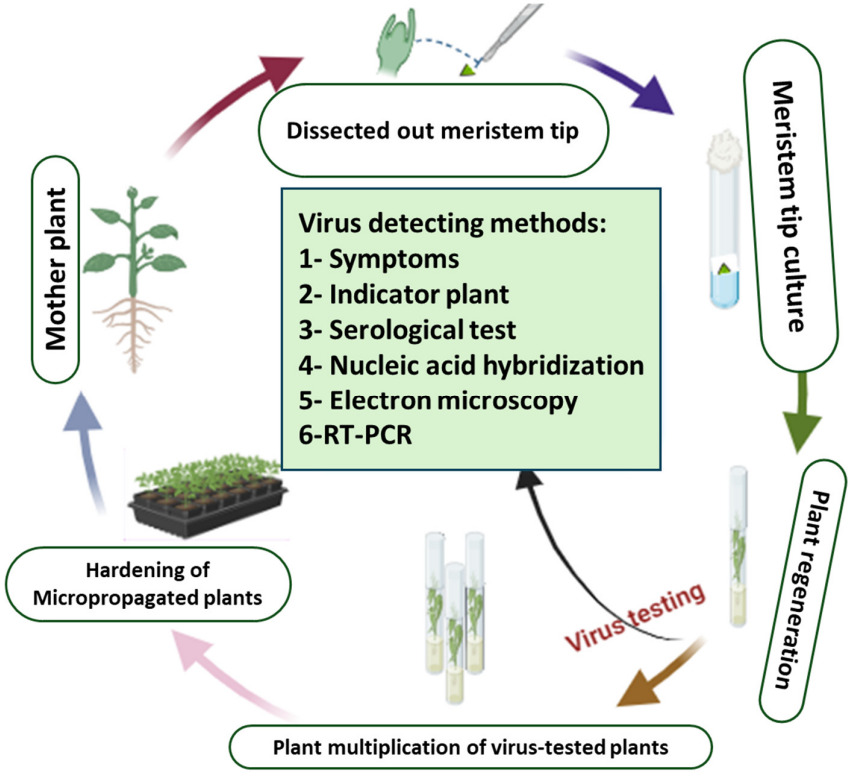

Figure 2. Production of virus-free plants through shoot-tip culture

The aseptic isolation of meristematic tissue is a critical step in successful meristem culture. Both the sterility of the process and the appropriate explant size are paramount for establishing healthy cultures, promoting subsequent plantlet development, and achieving complete virus elimination. The isolation procedure typically begins with the surface sterilization of the collected shoot tip. This involves sequential washes with tap water to remove gross debris, followed by sterile distilled water to eliminate any sterilization residues. Disinfection steps are then performed under laminar airflow to minimize microbial contamination. Commonly used disinfectants include 70% ethanol, various fungicides, sodium hypochlorite, or mercuric chloride, with the specific choice depending on the plant species and the anticipated pathogen profile (Benke *et al.*, 2021; Babu *et al.*, 2022). Following disinfection, the meristematic tissue is meticulously dissected under a stereo microscope. Sterile forceps are used to stabilize the bud, while fine needles are employed to precisely remove surrounding leaf primordia. The desired explant, the apical dome, characterized by its glistening appearance, is then carefully excised and transferred to a pre-prepared, sterile culture medium designed to support its growth and development (Sahraroo *et al.*, 2019).

Factors affecting virus eradication by meristem-tip culture

Culture medium

The selection of culture medium significantly influences the establishment rate of complete plant regeneration. Early studies on shoot-tip culture (Morel, 1948; Morel, 1952) employed media formulations

heavily based on the compositions established by White (1943) and Gautheret (1951) for meristem-tip cultures. Sucrose or glucose, typically ranging from 2% to 5% (w/v), are the most commonly used carbon sources (Srivastava *et al.*, 2017).

Supplementation with low concentrations of auxin or cytokinin (0.1-0.5 mg L^{-1}) can be beneficial for meristem cultures (Salem *et al.*, 2022). Exogenous auxin plays a crucial role in the successful culture of species like *Coleus blumei*, *Daucus carota*, *Nicotiana tabacum*, *N. glauca*, *Tropaeolum majus*, and *Lilium candidum* (Smith and Murashige, 1970). Conversely, both cytokinin and auxin are necessary for optimal regeneration of *Dianthus caryophyllus* meristems (Shabde and Murashige, 1977).

Explant size

The apical meristem of a shoot, situated distally to the youngest leaf primordium, possesses a diameter of up to 100 µm and a length of up to 250 µm (Krishnamurthy *et al.*, 2015). In conjunction with one to three leaf primordia (ranging from 100-500 µm), it forms the shoot apex. Explant size is critically important for meristem tip survival under optimal *in vitro* conditions. Explants must be sufficiently small to effectively eliminate potential viral contamination, yet large enough to retain the capability to develop into whole plants (Gupta *et al.*, 2022). The presence of two to three leaf primordia is deemed essential for meristem development into complete plants (Walkey, 1968).

Incubation conditions

Light incubation generally demonstrates greater efficacy compared to dark incubation for meristem-tip cultures (Bhat *et al.*, 2020). In the case of *Lolium multiflorum*, research has shown that 59% of meristem tips regenerated into plants under light exposure (6000 lux) compared to only 34% in darkness (Conger, 2018). The influence of temperature on plant regeneration is not extensively documented; however, cultures are routinely maintained at 25 ± 2 °C.

Physiological condition of the explant

Meristem tips should be excised from actively dividing tissues within growing buds. For carnations, terminal buds have been shown to yield superior outcomes compared to axillary buds (Hughes, 2018). However, such a distinction was not observed in strawberries (Boxus *et al.*, 1977). The timing of bud excision is a critical factor. For a majority of potato varieties, meristem tips collected during spring and early summer exhibit a greater propensity for successful rooting compared to those collected later in the season (Stace-Smith, 2018).

Virus indexing

Meristem culture, a technique for plant propagation, does not guarantee the elimination of viral pathogens. Consequently, claims of virus-free plants necessitate verification through analytical techniques. Enzyme-Linked Immunosorbent Assay (ELISA), a serological method for viral detection, is a commonly employed technique (Hayrapetyan *et al.*, 2023). Due to the delayed resurgence of many viruses in cultured plants, it is necessary to index plants multiple times within the first 18 months (Kumar *et al.*, 2022). Only plants that consistently test negative should be labeled as 'virus-tested' and released for commercial use.

Somaclonal variation

Somaclonal variation, the spontaneous genetic and epigenetic changes observed in plants regenerated from *in vitro* tissue culture, is a phenomenon of significant interest in plant science and breeding (Figure 3). Initially perceived as a drawback to maintaining genetic uniformity during micropropagation, it is now recognized as a valuable source of novel genetic traits for crop improvement. The induction of somaclonal variation is primarily attributed to the stressful conditions inherent in tissue culture environments. These stresses include, but are not limited to, exposure to various plant growth regulators, physical wounding during explant preparation, and nutrient imbalances or deficiencies within the culture medium (Wijerathna-Yapa and Hiti-Bandaralage, 2023). Such factors can induce a range of genetic and epigenetic alterations, including point mutations, which occur due to changes in a single nucleotide within a gene, potentially leading to altered protein function (Linacero and Ballesteros, 2024). Somaclonal variation can result from chromosomal aberrations: Duplications, deletions, or rearrangements of chromosomal segments, which can have significant phenotypic effects. Also, epigenetic modifications alter the gene expression patterns without altering the DNA, can trigger various genetic alterations.

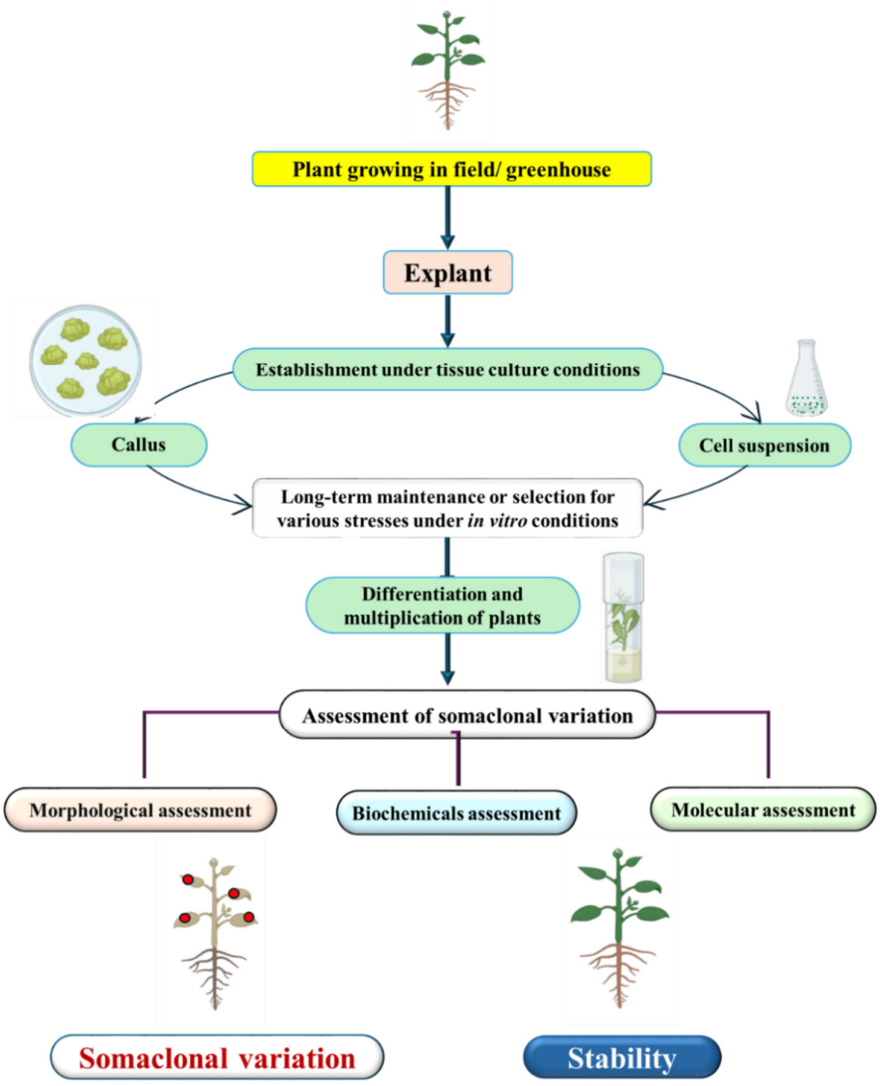

Figure 3. Generation and selection of somaclonal variation in plants

Somaclonal variation serves as a novel source of genetic diversity, exploitable in agricultural crop improvement programs. This approach has facilitated the generation of agronomically superior variants with desirable traits. Studies by Dey *et al.* (2015) demonstrated the successful application of somaclonal variation in *Cymbopogon winterianus*, leading to increased oil production. Biswas *et al.* (2009) reported the generation of strawberry variants exhibiting superior flowering and fruiting capacity through somaclonal variation. Rastogi *et al.* (2015) utilized somaclonal variation to create sugarcane varieties with significantly elevated sucrose yield, Baer *et al.* (2007) employed somaclonal variation to develop finger millet cultivars displaying enhanced seed and biomass production. Thieme and Griess (2005) successfully generated non-browning potato varieties using somaclonal variation. Furthermore, somaclonal variation has proven effective in generating disease-resistant cultivars across various crops, such as sugarcane resistant to red rot disease and eyespot (Rastogi *et al.*, 2015), Fusarium wilt-resistant banana (Muhammad and Othman, 2005), and wheat resistant to white blotch disease (Arun *et al.*, 2003). The applicability of somaclonal variation also extends to generating variants resistant to abiotic stresses such as drought and salt-tolerant sugarcane (Rastogi *et al.*, 2015) and drought-tolerant rice (Adkins *et al.*, 1995).

In vitro mutagenesis

Induced mutagenesis is a crucial technique in plant breeding, accelerating the development of superior cultivars with enhanced agronomic and quality traits (Kharkwal, 2023; Prasad *et al.*, 2024). *In vitro* plant cell and tissue culture mutagenesis provides an effective approach for generating novel genetic diversity (Larkin and Scowcroft, 1981).

Among the various mutagens utilized for plant mutation induction (Figure 4), ionizing radiation (e.g., X-rays, gamma rays, alpha and beta particles, protons, and neutrons) creates ion pairs upon interaction with biological matter. In contrast, ion beams induce a higher frequency and different types of mutations in plants. This technology has been instrumental in the commercial production of new rice and wheat mutant varieties in China. Furthermore, ion beam mutagenesis has led to the commercial development of ornamental plant cultivars with desirable traits in species such as *Verbena*, *Petunia*, *Dianthus caryophyllus* (carnation), and *Capsicum* (pepper) (Honda *et al.*, 2006).

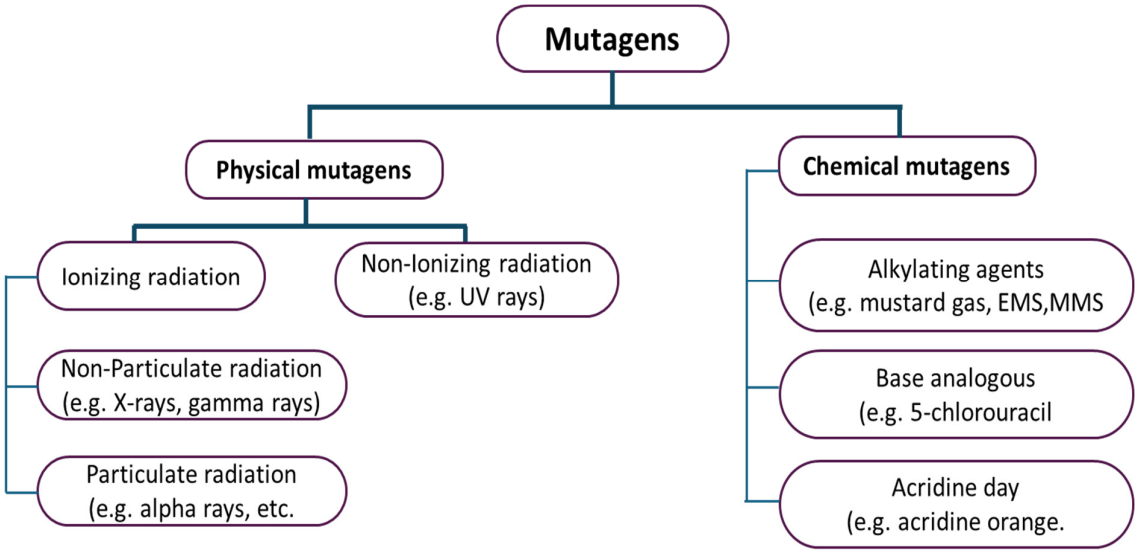

Figure 4. The most common mutagens used in plant mutation induction

In vitro chemical mutagenesis involves treating explants and calli with mutagens such as MNNG, EMS, NaN3, and N2H2 to generate mutations, which are heritable changes in the DNA sequence (Figure 5). For instance, studies have demonstrated that exposing soybean and carrot cells to EMS and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) can enhance their resistance to 5-fluorouracil and cycloheximide by tenfold (Penna *et al.*, 2012). Similarly, EMS treatment of carrot cells has shown a tenfold increase in resistance to 5-methyltryptophan (Widholm, 1977).

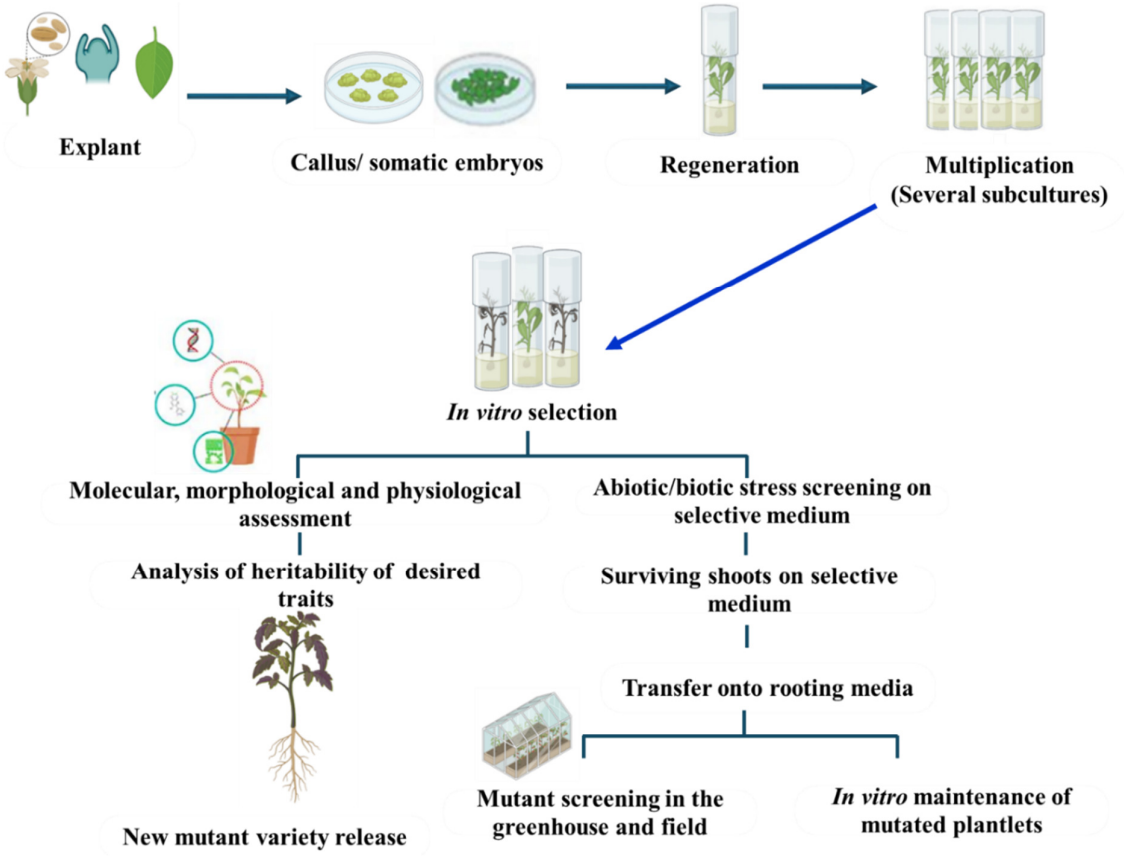

Figure 5. *In vitro* mutagenesis and selection in plant tissue

In vitro culture offers a powerful platform for mutagenesis due to its ability to handle large cell populations and facilitate selection before plant regeneration (Van Harten, 1998). Somatic embryogenesis, where embryos arise from single cells, is particularly advantageous. This method allows for chimera separation, ensuring mutations occur in single cells and are expressed in regenerated plants (Gajecka and Szarejko, 2023) and increased efficiency of mutagenized population selection. Haploid callus cultures derived from microspores or ovules represent ideal targets for mutagenesis due to their single-celled nature. Haploid cell and protoplast cultures are advantageous for mutant selection because recessive mutations can be directly identified in the first generation. Additionally, doubled haploidy (DH) allows for immediate fixation of these mutations(Szarejko and Forster, 2007). For example, EMS treatment of rice anthers followed by a 10-day culture period resulted in a high frequency (20%) of stable mutants with desirable traits like semi-dwarfism, grain shape, and glabrousness (Yeob Lee *et al.*, 2003). The key difference is in the type of DNA damage they cause. Chemical mutagens like EMS tend to produce specific, single-point mutations, making them ideal for targeted changes with less collateral damage to the plant. Radiation, on the other hand, causes more random and extensive

damage, including large chromosomal changes, which can lead to a wider variety of mutations but also increases the risk of harmful side effects like plant sterility.

In vitro selection

In vitro selection offers a powerful approach to circumvent the limitations of low population sizes encountered in *in vivo* mutagenesis. For effective *in vitro* selection, the targeted trait must be expressed in cultured cells, enabling efficient selection prior to plant regeneration. Furthermore, the induced mutation should be heritable and consistently expressed in the regenerated plants.

Traits with complex genetic control, such as yield, seed color, and plant height, which are typically polygenic, are generally not amenable to *in vitro* selection (Ahloowalia *et al.*, 2004). However, certain agronomic traits can be effectively selected by incorporating specific selective agents into the culture medium. *In vitro* selection protocols can be implemented using either single-step or multi-step methods. Typically, a selective agent, such as an inhibitor or antimetabolite, is introduced into the medium to suppress or eliminate the growth of non-mutated cells.

Selection for abiotic stress tolerance

In vitro selection has been successfully employed to enhance abiotic stress tolerance in diverse plant species, including cereals, vegetables, fruits, and other economically important crops (Rai *et al.*, 2011). This technique involves the exposure of callus, cell suspension, or protoplast cultures to growth-inhibitory levels of abiotic stress-inducing agents, such as sodium chloride, polyethylene glycol, sorbitol, and mannitol, in the culture medium. Mehmandar *et al.* (2023) have used polyethylene glycol (PEG) to simulate drought stress *in vitro* and select for drought-tolerant plant lines. This method was recently applied to develop drought-tolerant doubled haploid plants which were selected based on their growth and survival in the presence of PEG. Similarly, salt tolerance has been a major focus, with studies demonstrating the selection of salt-tolerant rice somaclones by exposing callus cultures to varying concentrations of NaCl (Sharmin *et al.*, 2025). This approach effectively screens for genotypes that can maintain growth and regeneration under high salt conditions. These illustrate how *in vitro* selection, by offering a controlled and rapid screening platform, continues to be a vital tool for developing climate-resilient crops and plants for environmental remediation.

Selection for biotic stress tolerance

In vitro selection is a powerful technique to enhance plant resistance to biotic stresses, such as fungal and bacterial infections. By exposing plant tissues, like callus or cell suspension cultures, to specific pathogens or their elicitors, researchers can select for individuals exhibiting enhanced defense mechanisms. These selected individuals can then be regenerated into whole plants with improved resistance. This approach has been successfully applied to develop crop varieties that are more resilient to diseases, leading to significant advancements in agriculture and food security. For instance, studies have successfully used fungal culture filtrates as a selective agent to screen for disease resistance. In one such case, researchers developed a rust-resistant safflower (*Carthamus tinctorius*) variety by exposing immature leaf calli to a fungal culture filtrate, leading to the regeneration of plants that showed enhanced resistance in pathogenicity assays and increased defense enzyme activity (Vijayakumar *et al.*, 2022). Another study conducted by Chakraborty *et al.* (2020) revealed that the *in vitro* selection of *Withania somnifera* against the fungal pathogen *Alternaria alternata*. By

applying a partially purified fungal toxin to callus cultures, scientists were able to select for and regenerate plants that exhibited a significant reduction in leaf spot disease incidence. Furthermore, Shaunak *et al.* (2023) developed resistance in tomato against soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici*, through in vitro cell line selection approach. These examples highlight how *in vitro* selection, by leveraging naturally occurring somaclonal variation and applying specific selective pressures, continues to be a crucial and rapid method for crop improvement.

Genetic engineering

Recent advancements in plant tissue culture techniques have facilitated the introduction of both endogenous and exogenous genes into plant genomes, resulting in the generation of transgenic plants (Su *et al.*, 2023). This synergistic relationship between tissue culture and genetic transformation has become a critical tool in the field of plant molecular biology research. It enables the elucidation of gene function and the targeted improvement of plant qualities to address future agricultural needs (Rajput *et al.*, 2023). The process of gene insertion into plant cells represents the initial step in a multifaceted genetic engineering workflow that encompasses cell selection, sustained tissue culture, whole plant regeneration, and successful acclimatization (Figure 6). The use of selectable markers like *nptII* has become increasingly controversial in plant biotechnology and is now discouraged by many scientific journals and regulatory bodies. This growing concern is primarily driven by the potential for horizontal gene transfer. In this case, there are fears that the *nptII* gene, which confers resistance to the antibiotic kanamycin, could be transferred from genetically modified plants to soil or gut bacteria. This event, while considered a low-probability risk, could potentially contribute to the global problem of antibiotic resistance, making these life-saving drugs less effective for human and animal health.

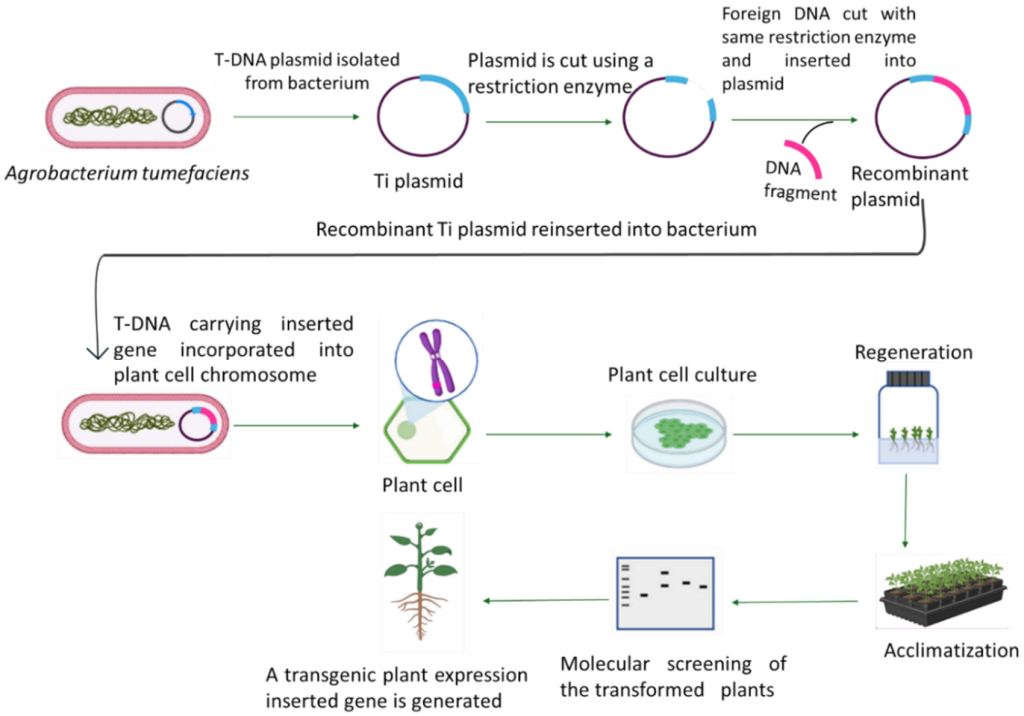

Figure 6. *Agrobacterium*- mediated transformation technique

Gene transfer methods in plants

Plant genetic transformation refers to the stable integration of a foreign DNA sequence into the host plant genome. This process allows for targeted manipulation of plant traits. Two main approaches are employed for gene transfer:

Agrobacterium-mediated Transformation

The development of *Agrobacterium*-mediated transformation marked a significant advancement in plant genetic engineering (Azizi-Dargahlou and Pouresmaeil, 2024). This technique involves co-cultivating plant explants (e.g., leaf discs, seedling segments) with *Agrobacterium* cultures. The bacteria preferentially infect wounded cells at the explant margins, transferring the T-DNA into the host genome. Subsequent tissue culture techniques allow for the regeneration of whole plants from transformed cells. Advancements in this method have expanded the range of suitable explants depending on the plant species' regeneration capabilities (Curtis and Grossniklaus, 2003). Following co-cultivation, selection media containing antibiotics eliminate untransformed cells, allowing for the identification and propagation of transformed lines.

Particle Bombardment

The biolistic method, invented by Sanford *et al.* (1987), was developed to overcome limitations associated with the host range of *Agrobacterium*. This method offers several advantages, including ease of use, broad applicability to various plant cells and tissues, and high transformation efficiency. The technique involves accelerating DNA-coated microcarriers towards target plant tissues using pressurized helium gas. The microcarriers penetrate the cell wall and membrane, delivering the DNA for potential integration into the host genome.

Following gene transfer via either *Agrobacterium*-mediated transformation or biolistics, only a minute fraction of cells within the target tissue typically undergoes successful integration of the foreign DNA (Hansen and Chilton, 1996). To enrich for these rare transformed cells, a selectable marker gene is typically co-introduced along with the gene of interest within the plant transformation vector. This marker gene confers a selective advantage to transformed cells when grown on media supplemented with a specific selection agent.

One of the most widely employed selectable marker genes in plant transformation is *nptII*, which encodes Neomycin phosphotransferase (Miki and McHugh, 2004). This enzyme confers resistance to the antibiotics kanamycin and geneticin (G418), allowing transformed cells to survive and grow on media containing these antibiotics, while untransformed cells are eliminated.

The ultimate goal of plant genetic transformation is often the recovery of whole plants expressing the introduced transgene. Therefore, the selection of explants with high regeneration potential and the development of efficient regeneration protocols are crucial steps in the workflow. Beyond just frequency, the type of regeneration is also critical for the successful recovery of transformed plants. Regeneration must occur within the region of the explant that has been exposed to the transforming agent (*Agrobacterium* or gene transfer machinery) to ensure the recovered plants harbor the transgene. In plant species where regeneration from isolated cells or callus is challenging, the use of intact explants (e.g., whole embryogenic calli or petioles) becomes a preferred strategy (Krikorian, 1982).

Embryo rescue

Embryo rescue, a vital tool in plant tissue culture, has revolutionized breeding programs by overcoming challenges in seed development and hybridization (Al-Ashkar *et al.*, 2023; Pathirana and Carimi, 2024). One

of the most significant applications of embryo rescue is facilitating the creation of interspecific and intergeneric hybrids (Amiteye, 2023). When crosses between different species or genera result in weak or immature embryos that wouldn't mature naturally, embryo rescue steps in. Isolating these immature embryos and providing them with a nurturing *in vitro* environment allows them to develop into healthy, viable plants. This paves the way for the introduction of valuable traits from diverse genetic backgrounds, expanding the potential for disease resistance, improved yields, and novel crop characteristics.

Embryo rescue isn't limited to interspecific crosses. It can also be used to recover embryos from plants with inherently weak seed development or those facing environmental challenges. This technique proves invaluable for preserving and propagating valuable genetic material from endangered or rare species, ensuring their continued existence and potential use in future breeding programs (Rogo *et al.*, 2023).

By overcoming seed inviability and incompatibility barriers, embryo rescue fosters the development of new plant varieties with increased genetic diversity. This broadened genetic pool allows for the introduction of desirable traits like improved adaptability to environmental stresses, enhanced nutritional value, and extended shelf life. This diversification strengthens agricultural resilience and paves the way for more sustainable food production systems.

Interspecific hybridization programs within the genus Helianthus have been extensively employed to transfer genes for resistance to biotic and abiotic stresses from wild Helianthus species into cultivated sunflower (*Helianthus annuus*) (Makarenko *et al.*, 2023). Ovule culture has been employed to successfully rescue hybrid embryos from the *N. stocktonii* × *N. tabacum* interspecific cross, circumventing their inherent lethality. (Muraida and Marubashi, 2015).

In chrysanthemum, the embryo rescue technique has been employed to develop interspecific hybrids resistant to biotic stress (Deng *et al.*, 2010) and tolerant to abiotic stresses (Cheng *et al.*, 2010). For instance, Zhu *et al.* (2013) obtained salinity tolerance, generated an intergeneric hybrid between *Chrysanthemum* × *morifolium* ($2n = 6x = 54$) and *Artemisia japonica* ($2n = 4x = 36$).

Conclusions

Plant cell and tissue culture techniques have become a cornerstone of modern plant science. This review has showcased the diverse applications of these techniques, from rapid plant multiplication and disease eradication to generating genetic variation and creating transgenic plants. By offering tools for micropropagation, virus elimination, somaclonal variation, genetic engineering, and embryo rescue, these techniques empower researchers and breeders to develop improved crops with enhanced qualities. As research continues to advance these methods, the future of plant science will likely enable further advances in areas like crop yield, stress tolerance, and nutritional value.

Authors' Contributions

Conceptualization: UBA, AMA., NMA, FAS, EF, KA, NIA, AAH; Data curation: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Formal analysis: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Funding acquisition: UBA, AMA, AAH; Investigation: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Methodology: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Project administration: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Resources: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Software: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Supervision: UBA, AMA, FAS, AAH; Validation: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; Visualization: XX; Roles/Writing - original draft: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH; and Writing - review & editing: UBA, AMA, NMA, FAS, EF, KA, NIA, AAH.

All authors read and approved the final manuscript.

Acknowledgements

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/279/46.

Funding

This work was supported by the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/279/46.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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