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ADVANCES IN TISSUE CULTURE FOR LEGUME IMPROVEMENT

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ABSTRACT
Legumes are vital for global food security, nutrition, and sustainable agriculture, yet their improvement is often hindered by species-specific recalcitrance and conventional breeding limitations. Tissue culture technologies have emerged as transformative tools, enabling rapid regeneration, somatic embryogenesis, haploid production, and in vitro mutagenesis to generate elite genotypes and novel genetic diversity. Their integration with molecular breeding, genome editing, and in vitro conservation has facilitated the development of stress-tolerant, disease-resistant, and nutritionally enhanced cultivars while safeguarding germplasm resources. Recent advances highlight the role of artificial intelligence, particularly machine learning models, in optimizing plant growth regulator combinations to overcome regeneration bottlenecks in recalcitrant legumes, thereby enhancing shoot regeneration frequency and efficiency. Coupled with high-throughput phenotyping, mutational breeding, and genomics-based approaches, these computational innovations accelerate the exploitation of genetic diversity and introgression of novel traits beyond the natural legume gene pool. Collectively, tissue culture technologies, strengthened by AI-driven predictive modeling and precision phenotyping, stand as a cornerstone for sustainable legume improvement, addressing escalating global food and feed demands while meeting environmental challenges.

INTRODUCTION
Legumes are vital for global food security, offering crucial protein sources and contributing to soil fertility through nitrogen fixation. However, despite their significance, legume crops often face challenges such as low yields and susceptibility to various stresses, necessitating the development of improved varieties (Calles, 2016). Tissue culture techniques have emerged as powerful tools to address these limitations by enabling rapid propagation, genetic manipulation, and the generation of novel genetic variations within legume species (Dita et al., 2006). These biotechnological approaches facilitate the circumvention of traditional breeding barriers, offering pathways for enhanced crop productivity and resilience (Sidik et al., 2024). The integration of sophisticated synthetic biology and precise genome editing techniques, such as CRISPR/Cas, has further refined the accuracy of genetic modifications, allowing for targeted improvements in legume characteristics (Koti & Bill, 2025; Sangari et al., 2024). This review aims to explore recent advancements in tissue culture methodologies and their application in legume crop improvement, focusing on key areas such as in vitro regeneration, protoplast culture, and genetic transformation. Despite these advancements, inherent recalcitrance to in vitro regeneration through conventional tissue culture methods remains a significant challenge for legumes, necessitating refined protocols that optimize explant selection, hormonal balance, and environmental conditions (Begum et al., 2025). A meticulous fine-tuning process is therefore imperative to address the genotypic diversity and enhance the reproducibility of superior genotypes (Pasternak & Steinmacher, 2024). This recalcitrance often extends to genetic transformation, hindering the efficient application of advanced genome editing tools in many legume species (Nivya & Shah, 2023). Overcoming this recalcitrance is crucial for successful genetic engineering and the widespread adoption of molecular breeding strategies for legume enhancement (Bennur et al., 2024). Indeed, while genome editing tools have broadened the scope for crop improvement, their successful implementation in legumes is frequently bottlenecked by the efficiency of the underlying tissue culture systems, particularly in achieving stable transformation and regeneration (Bekalu et al., 2023). Specifically, limitations in regeneration efficiency and tissue-specific transformability present considerable hurdles for deploying gene editing strategies to enhance traits like drought tolerance or salinity resistance in legumes (Nivya & Shah, 2023; Sangari et al., 2024). This necessitates exploring advanced approaches such as protoplast isolation and transfection, despite the challenges associated with maintaining cell viability and regeneration capacity in these systems (Singh et al., 2025). The high water content in explants such as seeds, roots, shoots, ovaries, ovules, anthers, and axillary buds significantly impairs nutrient uptake and cell division, further inhibiting regeneration under controlled environments, often resulting in regeneration failure under cold or freezing conditions (Pandey et al., 2025). Such physiological constraints highlight the critical need for optimizing tissue culture protocols to enhance the regeneration potential of recalcitrant legume species, thereby improving their susceptibility to genetic modification (Christiaens et al., 2021; Ludvíková & Griga, 2022). Further research into understanding the molecular mechanisms underpinning regeneration recalcitrance could provide pathways for developing universally applicable tissue

culture protocols across diverse legume genotypes, moving beyond the current genotype-specific limitations (Bohra et al., 2014). Specifically, the lack of efficient tissue culture methods for regeneration and transformation remains a major drawback for the widespread applicability of gene-editing technologies in many recalcitrant legume varieties (Kaur et al., 2025). However, ongoing advancements in understanding plant developmental pathways and refining plant growth regulator concentrations offer promising avenues to overcome these regeneration barriers and facilitate more efficient genetic manipulation (Baloğlu et al., 2022). This includes the precise modulation of phytohormone ratios, media components, and environmental cues, which are critical for inducing totipotency and subsequent organogenesis or embryogenesis in a wider range of legume genotypes. This would enable the effective integration of advanced gene-editing techniques for targeted trait improvement (Nivya & Shah, 2023; Tek et al., 2026). Beyond refining media components, novel transformation strategies are also being developed to bypass the recalcitrance associated with traditional methods, paving the way for more efficient genetic modification (Fernandez-Gutierrez et al., 2026). One promising alternative involves nodal culture, which has demonstrated efficacy in recalcitrant horticultural crops and could be adapted for legumes to improve regeneration efficiency, particularly for CRISPR/Cas9-mediated genetic modifications (Pandey et al., 2025). Another emerging strategy involves leveraging morphogenic regulators to enhance in vitro regeneration, thereby circumventing the genotype-dependent recalcitrance observed in many crop species (Kowalik et al., 2026). The development of robust and reproducible tissue culture protocols is therefore paramount for the efficient deployment of genome editing tools like CRISPR/Cas9, which enable precise modifications for enhancing traits such as stress resistance and nutritional value in legumes (Baloğlu et al., 2022). This approach, while still in its nascent stages for many legume species, offers a direct means to stimulate regeneration in difficult-to-transform genotypes, potentially accelerating the development of improved cultivars. Furthermore, the integration of artificial intelligence offers predictive insights into gene functions and interactions, which can streamline the research process and facilitate CRISPR/Cas-mediated genome modifications in important species, accelerating cultivar development (Bennur et al., 2024). The application of advanced genome-editing technologies, such as the CRISPR/Cas system, holds significant promise for further enhancing genetic improvements in legumes by enabling precise manipulation of single or multiple genetic loci (Baloğlu et al., 2022; Huang, 2024). This technology allows for targeted alterations, such as enhancing disease resistance, improving nutritional content, and increasing yield, thereby addressing critical agricultural challenges (Maia et al., 2024). The CRISPR/Cas9 system, particularly noted for its widespread application in grain legumes, allows for specific gene targeting through guide RNA sequences, overcoming some transformation hurdles traditionally associated with these crops (Asiamah et al., 2025; Lippolis et al., 2023; Sangari et al., 2024). This precision in gene editing is crucial for developing climate-resilient crops with enhanced resistance to abiotic stresses like drought, salinity, and extreme temperatures (Albalawi et al., 2025). Moreover, CRISPR/Cas9 technology has already been successfully deployed in soybeans to develop mutants with delayed flowering times and increased pod yields, underscoring its potential for practical agricultural applications (Ambika et al., 2022). The ongoing integration of artificial intelligence with plant tissue culture and genome editing, particularly with CRISPR/Cas9 platforms, represents a transformative advancement, enabling the optimization of protocols and predictive modeling of complex nonlinear data (Narra et al., 2025). This synergy facilitates an expedited and more precise approach to cultivar development, moving beyond conventional breeding limitations by identifying crucial genes and predicting their functional outcomes (Nadeem et al., 2023; Wu et al., 2025).

Legume Crops: Importance and Challenges

This interdisciplinary approach, leveraging AI and CRISPR/Cas9, promises to significantly enhance the efficiency and precision of genetic modifications in legumes, thereby accelerating the development of superior crop varieties. Such advancements are critical given the recalcitrant nature of many legumes to in vitro gene transfer and regeneration, which has historically hindered the widespread application of gene-editing technologies (Popoola et al., 2022). Moreover, the advent of sequence-specific nucleases, including CRISPR/Cas9, has revolutionized plant genome editing by enabling targeted mutagenesis, which is pivotal for creating mutants in otherwise inaccessible genes and for speeding up plant breeding without introducing transgenes (Punia et al., 2022). This not only streamlines the development of novel traits but also circumvents the stringent regulatory frameworks often associated with genetically modified organisms (Raina et al., 2023).

This capability is particularly beneficial for improving neglected and underutilized legume crops, which often lack comprehensive genomic resources and have not yet been subjected to extensive biotechnological interventions, including sophisticated tissue culture micropropagation and genetic engineering (Popoola et al., 2019). Furthermore, the application of CRISPR/Cas9 for gene knockout and targeted base editing in legumes has demonstrated its potential to enhance critical agronomic traits, including improved drought tolerance in chickpea through gene editing (Ali et al., 2022) and enhanced yield and quality in soybean (Guan et al., 2022). The integration of artificial intelligence in agricultural practices further amplifies these advancements, offering powerful means to discern intricate relationships within large datasets and predict outcomes based on historical and real-time data (Na & Na, 2024). This allows for the identification of optimal breeding strategies and the acceleration of crop improvement programs by leveraging predictive analytics (Borgohain et al., 2026). This integrated approach, combining advanced biotechnologies with computational intelligence, aims to overcome the traditional limitations of crop improvement, paving the way for the development of more resilient and productive legume varieties (Li et al., 2025). By leveraging the capabilities of AI to analyze extensive genomic data and guide CRISPR/Cas9 applications, researchers can precisely target genes responsible for desired traits, thereby enhancing resistance to pests, diseases, and environmental stressors, while simultaneously improving nutritional profiles (Wu et al., 2025).

The judicious application of these advanced tools, therefore, is pivotal in addressing global food security challenges by developing high-yielding, nutrient-dense legume varieties capable of thriving in diverse and changing environmental

conditions (Borgohain et al., 2026). This confluence of technologies also facilitates the development of transgene-free, climate-resilient legumes, circumventing regulatory hurdles and accelerating their adoption in agricultural systems (Nivya & Shah, 2023). The use of AI-powered robotic harvesting systems represents another significant stride in legume agriculture, promising to revolutionize traditional harvesting methods by improving efficiency and sustainability (Ah & Lee, 2025). These systems, integrated with real-time environmental data, optimize harvest timing and minimize crop loss, thus contributing to enhanced productivity and reduced resource expenditure (Kim & AlZubi, 2024).

Nutritional Value of Legumes

Legumes are renowned for their substantial protein content, ranging from 18% to 40%, and their capacity for nitrogen fixation, which can reach up to 200 kg/ha, significantly enriching soil fertility and reducing the need for synthetic fertilizers (Acheampong et al., 2025). This inherent ability makes legumes indispensable for sustainable agricultural practices, particularly in promoting soil health and mitigating environmental impact. Beyond their direct nutritional benefits, legumes play a vital role in complementing cereal-based diets by providing essential amino acids, thereby addressing protein deficiencies prevalent in many regions globally (Varshney et al., 2018). Their nutritional profile, encompassing high fiber content, complex carbohydrates, and various micronutrients, further underscores their importance in human diets for promoting overall health and preventing non-communicable diseases (Jiménez-López et al., 2022). As a result, the United Nations declared 2016 as the International Year of Pulses, emphasizing their role as "nutritious seeds for a sustainable future" and advocating for a "second green revolution" to ensure global food and nutritional security amidst climate change challenges (Foyer et al., 2016). Given their critical role in global food security and sustainable agriculture, enhancing the productivity and resilience of legume crops through advanced tissue culture techniques and biotechnological interventions is paramount (Jiménez-López et al., 2023). This urgency is amplified by the projected global population of nearly 10 billion by 2100, which necessitates substantial increases in food production that current systems are ill-equipped to provide (Udawat, 2023). Moreover, legumes are recognized for their favorable greenhouse gas and water footprints, presenting a sustainable protein source compared to animal-derived products (Semba et al., 2021). Their capacity for biological nitrogen fixation further reduces the environmental impact of agriculture by minimizing the reliance on synthetic nitrogen fertilizers, which are significant contributors to greenhouse gas emissions and water pollution (Tiwari et al., 2022). Furthermore, legumes contribute significantly to food security, poverty alleviation, and sustainable agriculture by providing 27% of global primary crop production and fulfilling 33% of protein requirements, second only to cereals (Afkar, 2023). Despite these substantial benefits, enhancing legume production faces challenges due to their susceptibility to various biotic and abiotic stresses, necessitating innovative approaches for crop improvement (Papa et al., 2024). Therefore, advancements in tissue culture protocols, particularly those enhancing regeneration efficiency and genetic transformation, are crucial for developing elite legume varieties capable of thriving in diverse environmental conditions (Hasanuzzaman et al., 2023). Such innovations are increasingly important as the global population grows and climate change intensifies, creating an urgent demand for enhanced crop yields and nutritional content (Jha et al., 2022; Polowick & Yan, 2023). These advancements are particularly critical given the increasing demand for protein in human diets, where legumes offer a sustainable and nutritious alternative to animal-based proteins (Vilakazi et al., 2025).

Further, the ability of legumes to grow in marginal lands with minimal input, coupled with their capacity for nitrogen fixation, positions them as key components in strategies aimed at achieving food security and sustainable agriculture, particularly in regions vulnerable to climate change (Mayes et al., 2019; Pierre, 2024). The ongoing intensification of agricultural systems globally necessitates the optimization of nitrogen utilization, making legumes, with their inherent nitrogen-fixing capabilities, central to sustainable agricultural transformations and the mitigation of environmental impacts associated with synthetic fertilizer use (Irisarri et al., 2021). The environmental advantages of legumes are further underscored by their significantly lower greenhouse gas emissions compared to other crops and dairy or beef agriculture (Abobatta et al., 2021).

Agricultural Benefits of Legumes

Legumes contribute to sustainable agriculture by enhancing soil fertility through biological nitrogen fixation, thereby reducing the reliance on synthetic nitrogen fertilizers (Özköse, 2023; Wang et al., 2024). This process, mediated by symbiotic rhizobia, enriches the soil's nitrogen content, diminishing the environmental burden of industrial nitrogen production and application (Kebede, 2021). Additionally, their cultivation can lead to improved soil structure, increased water infiltration, and enhanced biodiversity within agricultural ecosystems (Abobatta et al., 2021). These benefits collectively promote sustainable land management practices and contribute to climate change mitigation by reducing greenhouse gas emissions associated with conventional farming (“Maximizing Nitrogen Fixation in Legumes as a Tool for Sustainable Agriculture Intensification,” 2022).

Furthermore, the integration of legumes into crop rotation systems diversifies agricultural production, aids in soil recovery, and mitigates issues related to soil pests and weeds, leading to productivity gains (Schwember, 2020). Moreover, legumes serve as an affordable plant-based protein source that is crucial for global food and feed security, especially considering the escalating global population and increasing demand for high-protein food and feedstuff (Jiménez-López et al., 2022; Rubiales et al., 2021). This makes them a pivotal component in strategies aimed at reducing the environmental footprint of food production, as their cultivation generates significantly fewer greenhouse gas emissions compared to animal-based protein sources (Keller et al., 2024). This intrinsic capability for biological nitrogen fixation renders legume cultivation particularly important for agricultural sustainability, offering a stark contrast to cereal crops which typically necessitate substantial external inputs of nitrogen fertilizers (Goyal et al., 2021).

The nitrogen-fixing capacity of legumes not only reduces the need for synthetic fertilizers but also offers substantial environmental benefits by minimizing the emission of nitrous oxide, a potent greenhouse gas (Koushal et al., 2025). Indeed, integrating legumes into cropping systems can lead to an 18–33% reduction in nitrous oxide emissions and a 24–38% decrease in nitrogen fertilizer usage in both arable and forage systems, respectively, when compared to systems lacking legumes (Lisciani et al., 2024). This reduction in fertilizer dependence translates to decreased fossil energy consumption and greenhouse gas emissions linked to fertilizer manufacturing (Rubiales, 2023). Moreover, the cultivation of legumes significantly enhances soil health by improving soil organic matter balance and overall agrotechnical conditions, as evidenced by their substantial contribution of dry matter to the soil (Jerzak, 2021).

Challenges in Legume Production

Despite these compelling agricultural and environmental advantages, legume production globally faces considerable constraints, primarily stemming from biotic and abiotic stresses that significantly limit yield potential (Stagnari et al., 2017). These stresses encompass a wide array of challenges, including susceptibility to various pathogens and pests, drought, salinity, and extreme temperatures, all of which contribute to unstable and often low yields (Chen et al., 2022). Furthermore, the shift towards industrialized animal farming systems exacerbates the demand for grain and other plant proteins as animal feed, thereby creating production challenges concerning waste, pollution, deforestation, and greenhouse gas emissions (Payne et al., 2019). Addressing these challenges is critical for leveraging legumes as a cornerstone of sustainable agri-food systems and mitigating the environmental impact of global food production (Ferreira et al., 2021). However, conventional breeding methods often prove time-consuming and inefficient in developing stress-tolerant legume varieties, necessitating the integration of advanced biotechnological tools. This includes the application of tissue culture techniques, which offer a powerful platform for rapid propagation, genetic manipulation, and the development of novel genotypes with enhanced resilience to adverse environmental conditions and improved nutritional profiles.

Tissue culture, therefore, offers a promising avenue for accelerating the development of improved legume varieties that can better withstand biotic and abiotic stresses, thereby enhancing productivity and sustainability in agriculture. This review aims to explore the recent advancements in tissue culture techniques and their application in legume crop improvement, focusing on their potential to overcome current production constraints and contribute to global food security. This exploration will delve into how these biotechnological strategies can enhance legume adaptability to challenging environmental conditions, such as drought and salinity, which are increasingly prevalent due to climate change (Nadeem, Li, Yahya, Sher, et al., 2019; Nadeem, Li, Yahya, Wang, et al., 2019). Specifically, tissue culture techniques provide a controlled environment for the rapid generation of plantlets and offer a strategic approach for screening and selecting varieties with enhanced stress tolerance (Abdelaleem et al., 2019; Irum et al., 2020). This approach is particularly valuable for developing stress-tolerant crops, as it enables the development of new varieties and the study of plant responses to various environmental pressures at the cellular level (Wijerathna-Yapa & Hiti-Bandaralage, 2023).

Basics of Tissue Culture

Plant tissue culture involves the aseptic in vitro cultivation of plant cells, tissues, or organs under precisely controlled environmental conditions, allowing for the propagation of genetically uniform and disease-free plants (Sidik et al., 2024). This biotechnological approach capitalizes on the principle of cellular totipotency, where a single plant cell can regenerate into a whole plant (Sokra et al., 2025). This technique, pioneered by Gottlieb Haberlandt in 1902, leverages cellular totipotency, dedifferentiation, and redifferentiation to enable plant regeneration (Mahato et al., 2023). The controlled environment and sterile conditions inherent in in vitro culture offer distinct advantages for studying plant responses to various stressors and for large-scale plant propagation (Wijerathna-Yapa & Hiti-Bandaralage, 2023).

This includes the ability to apply selective pressures, such as specific concentrations of abiotic stress-inducing agents, to cultured cells, enabling the identification and regeneration of stress-tolerant plant lines (Algopishi et al., 2025; Manoj et al., 2010). The utility of in vitro selection in developing stress-tolerant crops has been demonstrated across various species, even though its application in legumes has not been fully explored (Dita et al., 2006). However, recent advancements in tissue culture techniques have made significant strides in improving legume crops, particularly in developing resistance to both biotic and abiotic stresses through methods like in vitro selection (Ma & Gómez-Cadenas, 2012).

What is Plant Tissue Culture?

This process involves exposing plant tissues, such as callus or cell suspension cultures, to specific selective agents, allowing researchers to isolate individuals exhibiting enhanced tolerance to particular stressors, which can then be regenerated into whole plants with improved resilience (Algopishi et al., 2025; Sahu et al., 2023). This method significantly enhances the efficiency of identifying and propagating desired traits compared to conventional breeding techniques, especially for complex, multigenic traits associated with abiotic stress tolerance (Leva & Rinaldi, 2012). This methodology enables the handling of extensive populations and the rapid cloning of chosen variants, thereby increasing the effectiveness of mutagenic treatments (Ma & Gómez-Cadenas, 2012).

Furthermore, in vitro selection facilitates the screening of a large number of cells or tissues in a confined space, offering a more efficient and rapid selection process than field-based methods (Leva & Rinaldi, 2012). From a fundamental perspective, in vitro selection can also be used to unravel the mechanisms underlying the acquisition of stress tolerance (Elmaghrabi et al., 2018). However, the successful application of in vitro selection necessitates a profound understanding of plant growth regulation and regeneration pathways, which are often empirically determined (Pasternak & Steinmacher, 2024).

Despite these empirical approaches, ongoing research aims to elucidate the precise molecular and genetic mechanisms governing in vitro plant regeneration to optimize these protocols (Pasternak & Steinmacher, 2024). Indeed, the development of robust regeneration protocols remains a critical bottleneck for many legume species, directly impacting the broader application of advanced biotechnological approaches like in-vitro selection (Dita et al., 2006). Challenges associated with regenerating legumes via tissue culture have prompted the refinement of protocols, particularly in optimizing explant selection, hormonal balance, and environmental conditions to overcome recalcitrance and improve regeneration success rates (Begum et al., 2025). One promising approach to overcome these limitations involves refining media composition for all components and micropropagation stages, alongside effective initial explant selection (Sota et al., 2025).

Further optimization of regeneration protocols frequently involves a precise manipulation of phytohormone concentrations, particularly the removal of auxins during somatic embryogenesis to facilitate robust plantlet conversion (Elmaghrabi et al., 2018). However, challenges persist, particularly for large-seeded legumes, which frequently exhibit rooting difficulties in vitro, hindering the establishment of stable regeneration protocols crucial for commercial production (Sangari et al., 2024).

Key Components of Tissue Culture

The successful application of tissue culture techniques hinges on several key components, including the careful selection of explants, precise control over the physiochemical environment, and optimized growth media composition (Sidik et al., 2024). The culture medium, a critical factor, typically comprises macronutrients, micronutrients, vitamins, organic compounds, plant growth regulators, a carbon source, and gelling agents for solid media, with Murashige and Skoog medium being a commonly utilized formulation (Malabadi et al., 2023). Moreover, the specific combination and concentration of these constituents within the medium profoundly influence explant viability and regeneration efficiency, often requiring empirical optimization to alleviate tissue culture recalcitrance in various species (Bennur et al., 2024). Beyond medium composition, other environmental factors such as light intensity, photoperiod, temperature, and humidity are also critically important for successful tissue culture and regeneration (Pandey et al., 2025).

These parameters are meticulously controlled to mimic an optimal growth environment, thereby maximizing the morphogenetic potential of the cultured tissues. Moreover, the age and physiological state of the explant significantly impact its regenerative capacity, with younger, metabolically active tissues often exhibiting a higher propensity for successful differentiation and subsequent plant regeneration (Long et al., 2022). Beyond these intrinsic factors, the genetic makeup of the source plant (genotype) plays a substantial role in determining regeneration efficiency, necessitating genotype-specific protocol development for optimal outcomes (Mehbub et al., 2022). Furthermore, the precise balance of plant growth regulators, especially auxins and cytokinins, is paramount, as their interplay orchestrates the developmental pathways of cells from totipotency to organized tissue regeneration (Hasnain et al., 2022; Long et al., 2022).

This intricate hormonal regulation, coupled with the proper selection of explant material and basal medium, determines the success of inducing morphogenic responses such as callus formation or shoot regeneration (García-Gonzáles et al., 2010; Liu et al., 2025). For instance, a higher ratio of auxin to cytokinin generally promotes callus and adventitious root formation, whereas a lower ratio Favors bud development (Liu et al., 2025). The effectiveness of these growth regulators is further modulated by their concentration, with even minute variations significantly altering the morphogenetic outcome (Hundessa et al., 2019; Mahato et al., 2023). The pH of the culture medium, typically maintained between 5.4 and 5.8, also critically influences plant growth and the activity of these plant growth regulators (Hussain et al., 2012). Moreover, the type and concentration of exogenously supplied phytohormones in vitro, particularly the ratio of cytokinin to auxin, are critical determinants of the regenerative ability of explants and subsequent plant development (Bennur et al., 2024; Wu et al., 2024). The differential responses observed across studies underscore the impact of endogenous phytohormone levels, genetic variation, explant age, and distinct culture environments on callus induction and subsequent regeneration (Wu et al., 2023).

For instance, the optimal balance between auxins and cytokinins is crucial for de-novo shoot organogenesis, where a sequence of media with varying hormone levels is often employed to induce callus and subsequently promote shoot development (Raspor et al., 2021). Studies have shown that the type of callus itself is a significant factor in indirect de-novo shoot regeneration, with epigenetic regulation and endogenous biochemicals influencing its varying in-vitro responses (Jafari & Daneshvar, 2023). The precise ratio of exogenous auxins and cytokinins significantly influences the differentiation direction of regenerating seedlings, with specific optimal ratios yielding maximum regeneration efficiency depending on the plant species (Xuetong et al., 2024).

Advantages for Crop Improvement

Tissue culture techniques offers numerous advantages for crop improvement, primarily by enabling rapid propagation of desirable genotypes, facilitating the generation of disease-free plant material, and supporting the creation of novel genetic variations through somaclonal variation or genetic engineering. The inherent totipotency of plant cells allows for the regeneration of whole plants from various explant sources, providing a powerful platform for biotechnological applications aimed at enhancing crop traits (Raspor et al., 2021). This includes improving yields, nutritional content, and resistance to biotic and abiotic stresses, thereby directly addressing global food security challenges.

For example, micropropagation, a tissue culture technique using explants such as seeds, embryos, calli, anthers, protoplasts, or meristematic tissues, enables the large-scale production of elite plant lines (Malabadi et al., 2023). This approach is particularly valuable for species that are difficult to propagate conventionally or for maintaining genetic

uniformity in high-value crops. This method is also instrumental in the rapid multiplication of new cultivars and the conservation of endangered plant species.

In legume crops, tissue culture techniques have been instrumental in developing improved varieties with enhanced characteristics such as increased nitrogen fixation, disease resistance, and stress tolerance. The application of tissue culture to legumes has led to breakthroughs in regeneration protocols, often leveraging growth factors and developmental regulators to overcome recalcitrance in many species (Atia et al., 2024). This capability is further bolstered by the ability to manipulate plant regeneration pathways, including both direct and indirect organogenesis and somatic embryogenesis, which are pivotal for developing robust and resilient legume varieties (Ibáñez et al., 2020).

Micropropagation, a key tissue culture technique, significantly contributes to this by enabling rapid, large-scale reproduction of selected legume genotypes from minimal starting material, thereby preserving gene pools and genetic diversity (Long et al., 2022). This rapid cloning method is crucial for secondary metabolite production and for conserving species that are difficult to regenerate or are at risk of extinction (Malabadi et al., 2025). Tissue culture's capacity to multiply valuable genotypes and preserve elite lines further underscores its importance in plant breeding (Duta-Cornescu et al., 2023). Moreover, this approach facilitates the efficient production of disease-resistant and genetically uniform plant materials, which are crucial for sustainable agriculture and horticulture (Thakur et al., 2024). Furthermore, the strategic application of tailored culture media and specific plant growth regulators has been pivotal in optimizing outcomes such as callus induction, shoot and root regeneration, and large-scale micropropagation, directly contributing to enhanced crop productivity and quality (Sidik et al., 2024).

Traditional Tissue Culture Techniques for Legumes

The traditional tissue culture methods for most of the crops including legume crops involve the establishment of aseptic cultures from various explants, followed by their manipulation under controlled in vitro conditions to induce desired developmental pathways (Malabadi et al., 2025). This method generally involves surface sterilization of explants, inoculation onto a nutrient medium containing specific growth regulators, and subsequent transfer to different media for regeneration and acclimatization (Huyghe, 1990). A critical aspect of these traditional methods is the precise formulation of the culture medium, which typically includes Murashige and Skoog medium supplemented with various plant growth regulators like auxins and cytokinins, whose ratios are finely tuned to promote specific developmental responses such as callus induction, shoot proliferation, or root formation (Jangid et al., 2023).

The selection of appropriate explant type, such as nodal segments, meristems, or cotyledonary nodes, is also paramount, as their inherent regenerative capacity and endogenous hormone levels significantly influence the success of the culture (Monnet et al., 2016). This meticulous control over the physiochemical environment allows for the consistent production of disease-free plantlets, a significant advantage over conventional propagation methods (Bhardwaj et al., 2025). Furthermore, the ability to produce disease-free plant material is particularly beneficial for commercial horticulture, ensuring healthier and stronger plants (DEMİREL et al., 2024).

These advancements in plant tissue culture also provide a platform for the storage of plantlets at temperatures between 0 and 15 °C, which is crucial for genetic resource conservation and maintaining virus-free plant collections (Gautam et al., 2024). While traditional methods have been foundational, recent advancements have focused on refining these techniques, such as optimizing media components and growth regulator concentrations, to improve regeneration efficiency and broaden the range of amenable legume species (Cenkci et al., 2009). For instance, certain legumes are considered recalcitrant to in vitro culture, necessitating specialized approaches such as nodal culture for effective regeneration and germplasm conservation (D.S. et al., 2016). These refinements often involve tailoring phytohormone combinations and concentrations to specific genotypes, moving beyond generalized protocols to address species-specific physiological requirements for enhanced regeneration and successful plantlet development (Yaroshko et al., 2023). Specifically, the judicious application of cytokinins such as 6-benzylaminopurine or Kinetin, often in conjunction with auxins, has been shown to induce robust shoot proliferation from cotyledonary node explants in various legumes (Yarra et al., 2016). This fine-tuning of plant growth regulator concentrations, alongside factors like light intensity and temperature, is crucial for maximizing regeneration success and minimizing somaclonal variation, particularly in recalcitrant species (Pandey et al., 2025).

So, the conventional methods for crop improvement using tissue culture may involve optimizing explant selection and carefully adjusting the pH of the culture medium to ensure optimal plant growth and phytohormone activity (Bidabadi & Jain, 2020; Leva & Rinaldi, 2012). However, challenges such as genetic stability and transformation efficacy remain, along with the need to address regulatory barriers and public skepticism regarding genetically modified crops (Koti & Bill, 2025). Despite these challenges, tissue culture remains a cornerstone for advanced legume breeding programs, particularly through techniques like micropropagation, which offers efficient clonal propagation and the production of disease-free material in a short timeframe (Majumder et al., 2025; Salgotra et al., 2019).

Callus Culture and Regeneration

Callus culture, involving the induction of an undifferentiated mass of cells from explants, serves as a versatile platform for regenerating whole plants and for generating genetic diversity through somaclonal variation or induced mutagenesis (Dita et al., 2006). This technique is particularly valuable for species recalcitrant to direct organogenesis, providing an intermediate stage from which shoots and roots can subsequently be regenerated (Luo et al., 2026). The successful regeneration of plants from callus often necessitates a precise balance of auxins and cytokinins in the culture medium to steer differentiation towards organogenesis or embryogenesis (Yarra et al., 2016).

This process is highly genotype-dependent, and the efficacy of regeneration can be significantly influenced by the specific explant source and the physiological state of the mother plant (Yaroshko et al., 2023). For instance, high concentrations of auxins coupled with low concentrations of cytokinins are typically employed for callus initiation, whereas the reverse ratio often promotes shoot-bud differentiation (Venkatachalam & Jayabalan, 1997). However, even with optimized hormonal balances, challenges persist in achieving efficient and reproducible plant regeneration from callus in many legume species, often attributed to factors such as genotype recalcitrance and the propensity for chromosomal aberrations during early cellular divisions (Huyghe, 1990).

Such challenges necessitate careful monitoring and optimization of culture conditions, as well as the exploration of alternative regeneration pathways like somatic embryogenesis, which can offer a more reliable route to producing true-to-type clones compared to direct regeneration from calli or explants (Nivya & Shah, 2023). However, despite its potential for high-frequency plantlet production, somatic embryogenesis in legumes is often limited by issues such as asynchronous embryo development and species-specific applicability, making it difficult to achieve consistent and large-scale propagation (Algopishi et al., 2025). This necessitates continued research into identifying optimal protocols and genetic factors that enhance both the frequency and fidelity of somatic embryogenesis in recalcitrant legume species.

Embryo Culture

The technique of embryo culture involves the aseptic isolation and in vitro growth of immature or mature embryos to bypass barriers to seed development, such as hybrid inviability or dormancy. This method is particularly valuable for accelerating breeding cycles and rescuing embryos from wide crosses that would otherwise fail to develop, thereby expanding the genetic diversity available for crop improvement. It also serves as a critical tool for overcoming seed dormancy and for the rapid generation of new plantlets from early-stage embryos, which can be particularly challenging in some legume species. in legume crops this techniques facilitates the propagation of genotypes that are difficult to germinate via conventional methods (AL-Oqab et al., 2023).

Moreover, embryo culture has proven effective in rescuing embryos from interspecific and intergeneric crosses, which often exhibit developmental arrest due to endosperm failure, thereby enabling the introduction of novel genetic traits from wild relatives into cultivated legumes (Ludvíková & Griga, 2022). This approach is especially critical for orphan crops where traditional breeding methods are hampered by genetic bottlenecks and poor seed viability (Croser et al., 2006). Furthermore, embryo rescue techniques can significantly reduce the breeding cycle duration by allowing the culture of immature embryos, thus enabling multiple generations per year (Journal, 2019).

This capability is particularly beneficial for genetic improvement programs aiming to incorporate resistance to biotic and abiotic stresses from wild germplasm into cultivated varieties. Embryo culture also plays a crucial role in breaking seed dormancy, which can shorten breeding cycles by months or even years, and can be used to salvage important seed lots with compromised viability (Smith & DREW, 1990). Another related technique, embryo rescue, is particularly instrumental in addressing issues such as hybrid inviability, which frequently arises from incongruities in endosperm development in wide crosses (Duc et al., 2014).

This technique has been widely applied in overcoming pre-zygotic and post-zygotic reproductive barriers, enabling the successful hybridization of species that would otherwise be reproductively isolated (Onișan et al., 2025). So for the successful application of embryo rescue, the maturity stage of the embryo, the specific composition of the culture medium, and the genotype of the plant are critical determinants (Kumari et al., 2018). For instance, the precise stage of embryo development at which rescue is attempted significantly impacts viability, with immature embryos often requiring more complex media formulations than mature ones (Kumari et al., 2018). hence in legume crops this techniques can ameliorate sexual incompatibility barriers and facilitate the production of doubled haploids for accelerated breeding programs (Mohammed & Baldwin, 2024).

Micropropagation for Legume Production

The easiest method under tissue culture is micropropagation, which involves the rapid clonal propagation of plants under aseptic conditions from small explants, offering a powerful avenue for the mass production of elite legume genotypes and disease-free planting material. This technique is particularly valuable for accelerating the dissemination of improved varieties, especially those developed through genetic engineering or conventional breeding, that possess desirable traits such as enhanced yield, nutritional quality, or resistance to various stresses (Chongtham et al., 2022).

It also allows for the efficient production of large numbers of genetically identical plants in a short period, which is crucial for commercial agriculture and conservation efforts. Micropropagation protocols have been successfully established for several legume species, enabling the rapid multiplication of superior genotypes (Encina & Regalado, 2022). This approach bypasses challenges associated with sexual reproduction, such as heterozygosity and prolonged breeding cycles, by directly producing clones from selected parent plants (Gana & Bañón, 2011). Furthermore, micropropagation facilitates the recovery of whole plants from protoplasts and genetically modified cells, making it an indispensable tool for biotechnology applications in legume improvement. Beyond simple clonal multiplication, advancements in micropropagation techniques, such as somatic embryogenesis, further enable large-scale production of elite lines, particularly in crops like alfalfa where efficient regeneration systems are well-established. the recent advances in micropropagation have focused on optimizing media composition and environmental conditions to improve regeneration rates and reduce somaclonal variation, crucial for maintaining genetic fidelity in propagated legumes. For instance, the clonal propagation of tetraploid L. leucocephala has been facilitated through optimized tissue culture systems, demonstrating the potential for broader adaptation across diverse legume species (Journal, 2019). This technology is particularly significant for species like Astragalus membranaceus mongholicus, where conventional propagation is

hampered by self-incompatibility and complex genetic backgrounds, making micropropagation an essential tool for rapid resource utilization and breeding (Li et al., 2024). Micropropagation also offers a solution for the preservation of elite allelic compositions, especially in allogamous and auto-incompatible species, by allowing the clonal multiplication of superior genotypes (Amokrane et al., 2016).

This technique allows for the rapid production of a large number of genetically uniform plants from a chosen genotype throughout the year, in a short period of time, and in a reduced space (Castro-Camba et al., 2023). So in legume crops , micropropagation provides a reliable method for the mass production of genetically identical, disease-free plant material, which is critical for establishing plantations and germplasm banks (Devanand et al., 2024). This ensures the continuous availability of desirable traits for future breeding programs and ecological restoration efforts (Kulus & Tymoszuk, 2024).

Advanced Tissue Culture Methods

The advances in tissue culture methods have led to the development of sophisticated techniques such as somatic embryogenesis, which provides an efficient pathway for large-scale clonal propagation and germplasm conservation in legumes (Pugalendhi & Taji, 2000). This method allows for the regeneration of whole plants from somatic cells through a process that mimics zygotic embryogenesis, making it an invaluable tool for crops where sexual propagation is challenging or undesirable (McKersie & Brown, 1996).

Additionally, bioreactor systems and temporary immersion systems have emerged as advanced tools for automating and scaling up micropropagation, significantly enhancing multiplication rates and reducing production costs compared to traditional semi-solid culture methods (Bello et al., 2025; Murthy et al., 2023). These advanced approaches offer improved control over environmental parameters, such as nutrient supply and gas exchange, leading to more uniform and vigorous plantlets (Sota et al., 2025).

These innovative systems also minimize manual labor and the risk of contamination, thereby improving the economic viability and efficiency of tissue culture propagation in legume crops (Kulus & Tymoszuk, 2024). Furthermore, the utilization of liquid media in conjunction with bioreactors has been shown to escalate the production of healthy, disease-free plants, enabling rapid multiplication of rare species and facilitating genetic transformation processes (Murthy et al., 2023). Further, the integration of somatic embryogenesis with synthetic seed technology offers a promising approach for the efficient delivery and storage of elite legume genotypes, particularly for species with recalcitrant seeds or those requiring specialized germination conditions. This is particularly important for enhancing breeding programs by providing a consistent supply of uniform plant material for selection and genetic analysis.

The application of somatic embryogenesis also extends to cryopreservation, offering a robust strategy for long-term genetic resource conservation by enabling the storage of valuable germplasm in a stable, dormant state (Välimäki et al., 2021). Somatic embryogenesis offers several advantages over other regeneration methods, such as organogenesis, including the production of a functional meristem with a vascular system and a root/shoot axis in a single step (Bakhshaie et al., 2010).

This bipolar nature allows for direct development into a plantlet, bypassing the rooting stage often required in organogenic regeneration (Rastgoo, 2021). The capability to induce dormancy in somatic embryos further enables their long-term storage or even the creation of artificial seeds, which is crucial for germplasm preservation and efficient distribution (Landey, 2013). So the advancement for legume crop improvement through somatic embryogenesis is centered on developing high-yielding, stress-tolerant, and disease-resistant varieties, as demonstrated in studies with soybean genotypes (AD et al., 2024). Also the tissue culture technique helps in mitigating numerous problems facing by the farmers in legume crops.

Anther and Ovule Culture for Haploid Production

These techniques are instrumental in generating homozygous lines more rapidly than conventional breeding methods, thereby accelerating breeding cycles and facilitating the identification of desirable traits. This acceleration is particularly beneficial for genetic studies and cultivar development, as it allows for the quick expression and analysis of recessive genes. Such approaches shorten the time required to develop new varieties with improved characteristics, ultimately enhancing agricultural productivity and sustainability. In addition to haploid production, these methods contribute to gene mapping and the study of complex genetic traits by simplifying genetic backgrounds, which can be particularly advantageous in understanding the genetic architecture of legume crops.

For example, efficient plant regeneration through somatic embryogenesis is a prerequisite for successful genetic transformation and the application of modern crop improvement techniques like CRISPR-based genome editing in legumes (Raza et al., 2019). Indeed, somatic embryogenesis, particularly from immature cotyledons, has proven to be a highly effective method for plant regeneration in legumes such as soybean, providing suitable explants for initiating cultures (Van et al., 2008).

Synthetic Seed Technology

This technology involves encapsulating somatic embryos or other asexual propagules in a protective coating, which allows for their direct sowing and offers an efficient system for germplasm preservation and dissemination, particularly for species that are difficult to propagate conventionally. Furthermore, synthetic seeds provide a scalable solution for the mass propagation of elite genotypes, ensuring genetic stability and disease-free plant material for agricultural applications (Carra et al., 2024).

This innovative approach combines the benefits of tissue culture with direct planting, reducing the need for costly and labor-intensive greenhouse acclimatization stages. Additionally, synthetic seed technology facilitates the global exchange

of genetic resources, minimizing biosecurity risks associated with traditional plant material exchange while ensuring viability and genetic integrity. This method also offers a promising avenue for the conservation of endangered legume species by providing a means for ex-situ propagation and reintroduction. Moreover, the integration of synthetic seed technology with mutation breeding techniques, such as in vitro mutagenesis, could enable the rapid development and deployment of novel mutants with enhanced traits, thereby diversifying genetic resources for legume crop improvement (Suprasanna et al., 2012).

The utility of synthetic seeds extends to facilitating rapid propagation of superior genotypes on a commercial scale, bypassing many limitations of conventional seed propagation (Alghamdi et al., 2020). This approach not only reduces production costs and lead times but also ensures the clonal fidelity of elite varieties, which is critical for maintaining desirable agronomic characteristics. The precise encapsulation of somatic embryos within a protective matrix ensures uniform development and simplifies handling, allowing for direct field planting and reducing the dependence on controlled nursery environments. The successful implementation of synthetic seed technology hinges on optimizing various factors, including the choice of explant, the composition of the culture medium, and the encapsulation technique, to ensure high conversion rates into viable plants and maintain genetic stability (Özbay et al., 2024).

This technology combines the advantages of micropropagation with the convenience of zygotic seeds, making plant material easier to handle, store, and transport while preserving genetic uniformity (Regni et al., 2024). Additionally, artificial seed production offers a cost-effective and efficient method for large-scale propagation, allowing for the widespread dissemination of improved legume varieties (AL-Jaf et al., 2023). This can significantly shorten the breeding cycle, as synthetic seeds can be produced at any time of the year, independent of seasonal reproductive cycles (Magray et al., 2017). The ability to produce synthetic seeds also facilitates the long-term conservation of rare, endangered, and commercially important forestry species by maintaining valuable germplasm (Abbas et al., 2022).

This method is particularly valuable for species that exhibit recalcitrant seed behavior or have extended breeding cycles, offering a robust alternative for their propagation and conservation (Özbay et al., 2024; Wu et al., 2026). Specifically, the encapsulation of somatic embryos or other vegetative propagules, such as shoot buds or auxiliary buds, into synthetic seeds allows for their direct sowing and conversion into plantlets under both in vitro and ex vitro conditions (Rihan et al., 2017).

Integrating Tissue Culture with Genetic Engineering

The synergy between tissue culture and genetic engineering techniques has revolutionized legume crop improvement by enabling the precise introduction of desired traits, such as herbicide resistance or enhanced nutritional content, directly into elite germplasm. This integration permits the development of transgenic legumes with improved abiotic stress tolerance, pest resistance, and yield characteristics, which are crucial for sustainable agriculture (Begum et al., 2025). This combination facilitates the accelerated creation of novel legume varieties that exhibit enhanced resilience and productivity, directly addressing global food security challenges.

Additionally, advancements in gene editing tools, such as CRISPR-Cas9, when coupled with efficient tissue culture regeneration protocols, enable targeted modifications to the legume genome with unprecedented precision and efficiency, further accelerating crop improvement (Punia et al., 2022). This approach has been instrumental in engineering drought-tolerant legumes through genetic modifications (Fernandez-Gutierrez et al., 2026). However, the success of these genome editing approaches is frequently hampered by the inherent recalcitrance of many legume species to efficient transformation and regeneration protocols (Nivya & Shah, 2023). Nonetheless, continued research into optimizing these protocols is crucial for realizing the full potential of genetic engineering in legume breeding (Sidik et al., 2024). Addressing these challenges necessitates concerted efforts to develop stable regeneration protocols and enhance transformation efficiencies, particularly for large-seeded legumes that often exhibit poor rooting in vitro (Sangari et al., 2024). Such improvements in regeneration and transformation are critical for leveraging advanced biotechnological tools to enhance the genetic potential of neglected and underutilized legume crops (Popoola et al., 2019).

These efforts also include the development of more efficient plant regeneration systems that incorporate customizable regulatory circuits to fine-tune regeneration pathways in a species-specific manner (Pandey et al., 2025). Beyond these foundational advancements, the emergence of direct transformation methods, which circumvent the dependency on tissue culture-based plant regeneration, represents a significant leap forward in accelerating crop trait development (Anjanappa & Gruissem, 2021). For instance, methods like Agrobacterium-mediated transformation, followed by techniques such as microprojectile bombardment, direct gene transfer to protoplasts, and electroporation, have emerged as prominent strategies for introducing foreign genes into legume genomes (Ali et al., 2022). Such advancements are pivotal for overcoming the challenges associated with the limited host range of Agrobacterium and the genotype-dependent regeneration in legumes, thereby broadening the applicability of genetic modification strategies (Huang, 2024; Sangari et al., 2024). These direct methods often reduce the time and effort required for plant regeneration, making them particularly attractive for challenging legume species. Despite these advancements, successful Agrobacterium-mediated genetic transformation, particularly for recalcitrant legume species like green gram, remains a significant challenge (Patra et al., 2021). The low transformation efficiency in many legumes often necessitates the exploration of alternative or complementary strategies (Li et al., 2017). One such strategy involves optimizing the Agrobacterium tumefaciens strain itself, or even exploring alternative microbial delivery systems like Ensifer or Rhizobium, to enhance DNA transfer into recalcitrant plant cells (Altpeter et al., 2016). Moreover, advancements in Agrobacterium-mediated transformation protocols have significantly improved efficiency and reproducibility across various legume species, often through careful optimization of co-cultivation conditions, bacterial density, and plant growth regulator concentrations (Bélanger et al., 2024; Peña & Pueyo, 2011; Prasad et al., 2024). so in a nutshell , Agrobacterium-mediated genetic transformation is

widely accepted as the standard method for introducing foreign DNA into plant cells in pulse crops (Bohra et al., 2014). This method typically involves inoculating explants with Agrobacterium strains carrying desired genes, followed by selection on a suitable medium and subsequent regeneration of whole plants (Singh et al., 2022).

Gene Transfer Techniques

This is the most favoured strategy for stable gene integration due to its simplicity, cost-effectiveness, and tendency for low transgene copy number (Han et al., 2025). However, achieving high transformation efficiency, especially in recalcitrant legumes, continues to be challenging (Yıldız et al., 2016). Therefore, researchers frequently explore alternative methods, such as biolistic delivery (gene gun) and electroporation, to overcome these limitations and expand the range of transformable legume genotypes (Shivashakarappa et al., 2025). Agrobacterium-mediated transformation, despite being the preferred method due to its advantages in accurate DNA sequence integration and consistent gene expression, often faces challenges with low transformation frequencies, especially with certain recalcitrant strains or large plasmids (Collado et al., 2016; Liu et al., 2023).

This has led to the exploration of approaches such as manipulating histone genes to increase transformation susceptibility, which has shown promise in expanding the range of recalcitrant crops amenable to Agrobacterium-mediated transformation (Bennur et al., 2024). However, direct gene transfer methods such as particle bombardment (biolistic method) were developed to circumvent the host range limitations of Agrobacterium and are particularly useful for species that are not amenable to Agrobacterium infection (Algopishi et al., 2025). Nonetheless, Agrobacterium tumefaciens remains the primary platform for generating engineered crop varieties globally (Zuniga-Soto et al., 2019). Its widespread adoption is largely attributed to its ability to transfer single-copy T-DNA into the plant genome, minimizing the issues of DNA rearrangement often associated with other direct DNA transfer methods (Temizgül & Akbulut, 2011). While particle bombardment offers an alternative, it presents its own disadvantages, including higher costs, potential DNA damage, lower transformation efficiency, and an increased risk of gene silencing due to multiple copy insertions into the plant genome (Geetha et al., 2022). Despite these limitations, biolistics remain a viable option for species recalcitrant to Agrobacterium-mediated transformation, and continuous optimization efforts aim to mitigate their inherent drawbacks (Chen et al., 2013). Conversely, the biolistic method offers benefits in its capacity to transform organelles and deliver various biomolecules, including RNA, proteins, nanoparticles, and dyes, directly into cells, circumventing some of the limitations of Agrobacterium-mediated approaches (Xu et al., 2022). However, biolistic methods are often associated with the integration of multiple gene copies into the host genome, which can lead to gene silencing and instability across generations, thus increasing the financial cost of regulatory approval for commercial use (Basso et al., 2020; Tirnaz et al., 2022). Moreover, the high copy number of transgenes introduced via biolistics can lead to complexities in transgene expression, including gene silencing, which is less prevalent with Agrobacterium-mediated transformation due to its typically lower transgene copy number (Chu & Agapito-Tenfen, 2022; Eck, 2018).

Developing Disease Resistant Legumes

Beyond transformation efficiency, a critical area of focus in legume crop improvement is the development of disease-resistant varieties. This involves leveraging genetic engineering to introduce genes conferring resistance to prevalent pathogens, thereby enhancing crop resilience and reducing yield losses. This approach often involves the identification and cloning of resistance genes from wild relatives or other plant species, followed by their stable integration into elite legume cultivars using the aforementioned gene transfer techniques (Parrott et al., 2024).

For instance, targeted gene editing technologies, such as CRISPR-Cas systems, are increasingly being employed to precisely modify native legume genes to enhance disease resistance or introduce novel resistance mechanisms. However, conventional genetic engineering techniques, including Agrobacterium-mediated transformation and biolistic delivery, often face challenges such as low efficiency, tissue damage, and the integration of multiple gene copies, which can lead to unpredictable gene expression and hinder the regeneration of transformed plants (Rani et al., 2025; Thorpe et al., 2025). To circumvent these issues, novel gene delivery systems, such as nanoparticle-mediated approaches, are being explored to improve the efficiency and precision of genetic modifications by overcoming cellular barriers and enabling targeted delivery to various cellular compartments (Kandhol et al., 2025). These advanced delivery systems exploit the plant's innate immune response to bypass cellular defenses and introduce DNA vectors, offering a promising alternative, particularly for crops resistant to traditional Agrobacterium-mediated transformation (Shivashakarappa et al., 2025).

Moreover, achieving high transformation efficiency remains a significant bottleneck, particularly for many legume species, where low transformation rates impede the widespread application of genome-editing technologies (Nivya & Shah, 2023). This necessitates the development of more robust and species-independent gene delivery systems, such as those utilizing nanomaterials, to efficiently introduce genome editing tools into legume cells (Zhi et al., 2022). This focus on enhancing gene delivery is particularly relevant given the emergence of genome editing as a transformative tool for accelerating crop improvement through precise genomic alterations (Manzoor et al., 2024).

Indeed, the application of genome-editing technologies like CRISPR has significantly advanced legume breeding by enabling precise manipulation of genes for traits such as disease resistance, thereby accelerating the development of improved crop varieties (Baloğlu et al., 2022). However, the broad application of CRISPR/Cas technology in legumes is constrained by the lack of routine and efficient genome editing protocols, largely due to difficulties in plantlet regeneration and genetic transformation across various legume species (Baloğlu et al., 2022). The utilization of nanotechnology in plant genetic engineering presents a promising avenue for overcoming these obstacles by facilitating efficient biomolecule delivery into plant cells, thereby enhancing the applicability of CRISPR/Cas systems in legumes (Rani et al., 2025). Specifically, nanoparticle-mediated gene delivery methods offer superior transformation efficiencies and can mitigate the

limitations associated with conventional gene delivery, such as tissue damage and off-target integrations (Ahmar et al., 2021; Shivashakarappa et al., 2025).

This innovative approach leverages the unique properties of nanoparticles to encapsulate and deliver genetic material directly into plant cells, bypassing rigid cell walls and minimizing cellular toxicity (Zhao et al., 2024). For instance, magnetic nanoparticles and carbon nanotubes have demonstrated considerable promise in delivering genetic material independent of genotype, leading to efficient transgene expression in various plant systems (Arshad et al., 2025). These advanced nanocarriers can overcome the plant cell wall, a significant barrier to genetic transformation, enabling more effective gene delivery than traditional methods (Jat et al., 2020).

Improving Nutritional Content

Beyond enhancing disease resistance, another crucial aspect of legume crop improvement focuses on augmenting their nutritional profiles, particularly by increasing micronutrient content to combat widespread deficiencies (Tiwari et al., 2022). This involves leveraging tissue culture techniques to develop legume varieties with enhanced levels of essential vitamins, minerals, and proteins, thereby addressing malnutrition (Asiamah et al., 2025). Tissue culture methods, in particular, enable the rapid screening and propagation of variants exhibiting superior nutritional traits, contributing to the development of biofortified legume crops.

Additionally, advancements in nanoparticle technology are being explored to enhance nutrient uptake and improve nitrogen fixation efficiency in legumes, contributing to more sustainable agricultural practices and enriching the nutritional value of the crops (Shahroz et al., 2024). The integration of nanoparticles with CRISPR technology, for example, offers a powerful strategy for precise gene editing to enhance both yield and nutritional quality in legumes by directly delivering CRISPR tools to target sites within plant cells (Afzal et al., 2023). This synergistic approach not only promises to overcome current delivery limitations but also enables the targeted modification of complex metabolic pathways involved in nutrient synthesis and accumulation (Afzal et al., 2023). For example, nanoparticles are being used to deliver essential elements directly to plant cells, enhancing the bioavailability of nutrients and improving crop productivity (Irum et al., 2020). This direct delivery mechanism can bypass uptake limitations from soil, ensuring higher concentrations of critical micronutrients within the plant tissues, thereby contributing to biofortification efforts (Mosqueda-Frómeta et al., 2025; Tiwari et al., 2022). These nanotechnological interventions also extend to genetic engineering by facilitating the targeted delivery of CRISPR/Cas9 components, allowing for precise modifications that can upregulate specific metabolic pathways responsible for synthesizing and accumulating desired nutrients (Afzal et al., 2023).

This precision in genetic modification allows for the development of legume varieties with improved nutritional value, addressing issues such as "hidden hunger" by increasing the density of essential micronutrients like iron and zinc (Jha et al., 2022; Raina et al., 2023). Biofortification, whether achieved through traditional breeding, genetic engineering, or nanotechnological approaches, represents a sustainable strategy for addressing micronutrient deficiencies in human populations (Altaf et al., 2024; Manideepak et al., 2025). Specifically, genetic biofortification strategies, including both conventional breeding and transgenic methods, have significantly advanced the development of metal-efficient crop varieties with enhanced nutritional profiles and increased stress resilience (Garg et al., 2025). This approach integrates diverse strategies such as genetic modification and advanced breeding techniques to introduce or enhance traits crucial for better nutrient absorption and stress tolerance in legumes (Badiyal et al., 2024).

Genetic biofortification further encompasses innovative methods like speed breeding, transgenic approaches, and advanced genome editing techniques, alongside integrated omics approaches, to enhance the nutritional profile of crops (Zulfiqar et al., 2024). The development of transgenic crops, while initially resource-intensive, offers a sustainable long-term solution for addressing hidden hunger, particularly in developing nations, by enabling a one-time investment to enhance micronutrient concentrations and simultaneously reduce anti-nutrients (Lakshmanan et al., 2025). Hence tissue culture may help in propagating these enhanced varieties efficiently and rapidly, ensuring widespread availability of nutritionally superior legume crops (Azeem et al., 2025).

Case Studies of Legume Improvement

Notable examples of legume improvement through tissue culture methods include the development of disease-resistant cultivars and the creation of plants with enhanced nutritional content, demonstrating the broad applicability of these techniques (Banerjee et al., 2023). These advancements highlight the potential of tissue culture to accelerate the breeding process, facilitating the introduction of desired traits into commercially viable legume varieties.

Additionally, efficient tissue culture protocols are vital for enabling sophisticated molecular genetics applications like gene overexpression, suppression, and promoter analysis, which are crucial for developing new legume varieties with improved traits (Dita et al., 2006). This integration of tissue culture with molecular techniques offers a powerful platform for engineering legumes with improved yield, stress tolerance, and nutritional quality. One area where tissue culture has been particularly impactful is in the development of varieties resistant to biotic and abiotic stresses, thereby safeguarding crop yields and nutritional quality under challenging environmental conditions (Dita et al., 2006). For instance, the application of tissue culture in conjunction with genetic engineering has led to the successful integration of genes conferring herbicide tolerance and insect resistance in several legume species, improving overall crop management and reducing yield losses.

Beyond direct genetic modification, tissue culture is also indispensable for micropropagation of elite germplasm, enabling the rapid multiplication of superior accessions that possess desirable agronomic traits or enhanced nutritional value. This ensures a consistent supply of high-performing plants for agricultural production and further research.

Additionally, in vitro regeneration techniques through tissue culture are considered a foundational step for any subsequent genetic improvement research aimed at reducing anti-nutritional compounds and increasing the bioavailability of nutrients in legumes (Senapati et al., 2023). These methods are also crucial for screening stress tolerance and studying physiological and biochemical changes in response to various environmental challenges, such as drought and salinity, offering a sustainable approach to crop improvement (Wijerathna-Yapa & Hiti-Bandaralage, 2023).

Soybean Improvement through Tissue Culture

Soybean regeneration from tissue culture, while historically challenging, has become a routine procedure, enabling the widespread application of advanced breeding techniques (RADHAKRISHNAN & Kumari, 2008). This has facilitated the development of new varieties with improved vegetative and yield characteristics, particularly under biotic and abiotic stress conditions (Mangena, 2021). The ability to regenerate whole plants from various explant types, such as immature cotyledons, hypocotyls, and primary leaf tissues, is critical for the genetic manipulation and improvement of soybean (Begum et al., 2019). Such advancements in regeneration protocols have paved the way for successful genetic transformation experiments, allowing for the stable integration of beneficial genes into the soybean genome.

This genetic transformation, particularly for soybeans, has yielded high-performing transgenic plants that exhibit enhanced resistance to various biotic and abiotic stresses, thereby improving crop productivity and resilience (Mangena, 2019). For example, the development of glyphosate-resistant soybean varieties was achieved through the transfer of a gene from Agrobacterium sp. strain CP4, encoding a glyphosate-tolerant EPSP synthase, which significantly streamlined weed control and reduced production costs (Atak et al., 2022). Furthermore, the use of Agrobacterium-mediated transformation, alongside alternative methods like electroporation and PEG-mediated protoplast transformation, has broadened the scope for genetic modification in legumes, including soybean (Huyghe, 1990). These developments are critical as efficient and reproducible plant regeneration is essential for introducing genes of interest into crops like soybean, which can otherwise be difficult to manipulate in vitro due to genotype-dependent responses and protocol variability (Soto et al., 2013).

The continued refinement of tissue culture techniques, specifically in soybean, has directly enabled progress in genetic engineering, leading to cultivars with improved secondary metabolites and disease-free plants, especially when regenerated under aseptic conditions (Mangena, 2019). This methodology has been instrumental in producing transgenic soybean plants through Agrobacterium-mediated gene transfer, leading to improved traits such as herbicide resistance (Hinchee et al., 1988). This transformation process often involves the transfer of specific transgenes, delivered via the tumor-inducing plasmid of Agrobacterium tumefaciens, into soybean cells, followed by regeneration from cotyledonary node explants (Arias et al., 2021). Despite the advances, achieving high transformation efficiency in soybean remains a critical challenge, with established protocols often yielding less than 10% (Mangena, 2019). However, ongoing research aims to optimize various factors, including Agrobacterium inoculum conditions, regeneration media components, and Agrobacterium strains, to enhance transformation success rates in soybean (Bennur et al., 2024; Ludvíková & Griga, 2022).

Common Bean Enhancement

Similarly, advancements in tissue culture techniques have significantly contributed to the genetic enhancement of common bean, allowing for the development of improved varieties with desirable agricultural traits. This is particularly important given the inherent challenges in common bean breeding, such as narrow genetic variability and susceptibility to various diseases and environmental stresses (Atak et al., 2022). For instance, the application of Agrobacterium rhizogenes has enabled the induction of transgenic hairy roots from common bean petioles, providing a robust system for functional genomic studies and the development of disease-resistant lines (Y et al., 2024). This method, leveraging the natural genetic transfer capabilities of Agrobacterium, facilitates the efficient incorporation of foreign genes into the common bean genome, thereby circumventing limitations associated with traditional breeding approaches (Malabadi et al., 2023). Moreover, the capacity for reliable and efficient in vitro culture, facilitating shoot development and whole plant regeneration, is crucial for genetic transformation and mutagenesis in common bean, enabling the introduction of traits like enhanced stress tolerance or altered seed quality (Gatica-Arias et al., 2010). These approaches, coupled with genetic modification and genome editing, address challenges posed by the crop's narrow genetic base and susceptibility to pathogens and abiotic stressors (Koti & Bill, 2025; Zhong et al., 2024). Furthermore, the integration of advanced molecular techniques with tissue culture methods has enabled the precise incorporation of desired traits into common bean genomes through targeted gene transfer, offering a powerful avenue for cultivar improvement (Algopishi et al., 2025). While genetic engineering offers substantial benefits by introducing genes inaccessible through sexual hybridization, reliable transformation systems for common beans, leading to stable transgenic plants, continue to be a primary limitation despite considerable research efforts (Song et al., 2020). Nevertheless, Agrobacterium-mediated transformation, particularly utilizing strains like A. rhizogenes 15834, has shown promise in common bean by inducing normal primary roots and enabling the transfer of genes conferring resistance to agricultural chemicals, cold, diseases, and pests (Saglam et al., 2005). This approach has also proven useful as a rapid prototyping tool for in planta screening, particularly for assessing sgRNA editing efficiency, prior to the resource-intensive process of generating stable transgenic lines (Goralogia et al., 2025; Suprasanna et al., 2023). Beyond stable transgenic lines, the utility of Agrobacterium rhizogenes extends to generating composite plants, which consist of transgenic roots and wild-type shoots, offering an ideal system for investigating root biology and gene function in interactions with pathogens and rhizosphere organisms (Aggarwal et al., 2018). This versatility underscores the remarkable advantages of A. rhizogenes-mediated transformation, particularly its wide species applicability, procedural simplicity, and inherent efficiency (Sun et al., 2025). A. rhizogenes-

mediated hairy root induction also facilitates the rapid assessment of sgRNA efficiency in plants, offering a streamlined approach to evaluate gene editing constructs prior to the generation of stable transgenic lines (Koning et al., 2023). This rapid assessment capability is especially valuable for identifying optimal CRISPR/Cas9 constructs for gene knockout studies (Pacheco et al., 2024). This technique is particularly useful in woody ornamental crops, where accelerating the breeding process and reducing regulatory burdens associated with transgenic crops are critical (Duan et al., 2025).

Pea and Lentil Advances

Significant strides have also been made in the genetic improvement of pea and lentil through sophisticated tissue culture protocols, enabling enhanced resistance to biotic and abiotic stresses and improved nutritional profiles. These advances often leverage gene editing technologies, such as CRISPR-Cas systems, to precisely modify target genes for traits like enhanced stress tolerance or improved seed quality, as exemplified by modifications to lipoxygenase genes in peas to reduce off-flavors (Cheng et al., 2024). Similarly, CRISPR/Cas9 genome editing offers a promising avenue for rapid and precise genetic modifications, overcoming limitations of traditional and transgenic breeding by introducing small deletions in native genes that are often regulatory body-accepted (Sharma et al., 2023). Such systems can also be employed to target organelle genomes or RNA, expanding the scope of genomic modification, and modified gene-editing systems can generate transgene-free plants more readily (Ercolano & Wang, 2023). However, successful integration of CRISPR components and subsequent plant regeneration remain crucial prerequisites for fully realizing the potential of gene editing (Polowick & Yan, 2023). Despite these advancements, persistent challenges exist in developing efficient regeneration protocols for many legume species, making them recalcitrant to somatic embryogenesis and stable plant transformation via Agrobacterium tumefaciens (Güngör et al., 2023). This recalcitrance often necessitates alternative strategies, such as Agrobacterium rhizogenes-mediated hairy root transformation, which bypasses the need for complete plant regeneration for studying root-related traits like symbiotic nodule development (Güngör et al., 2023). The optimization of these protocols, including the specific combination of mineral media and hormones, is crucial for maximizing shoot regeneration and, consequently, the efficiency and success of CRISPR/Cas9-based gene editing in legumes (Ali et al., 2022). However, challenges such as the low regeneration capacities of genetically transformed cells and tissues in many legumes, coupled with the inherent difficulties in their transformation, continue to limit the widespread application of genome editing (Lippolis et al., 2023). To address these limitations, researchers are exploring novel tissue culture methodologies, including nodal culture, to facilitate CRISPR/Cas9-mediated genetic modifications, particularly in recalcitrant legume crops (Pandey et al., 2025). Soybean and Medicago truncatula have emerged as key model legumes for CRISPR/Cas9 applications due to their amenability to genetic manipulation, allowing for advancements in traits such as abiotic stress tolerance and altered plant architecture (Bhowmik et al., 2023; Ludvíková & Griga, 2022). Beyond model legumes, the development of robust and efficient CRISPR-Cas systems in diverse legume species holds considerable promise for accelerating crop improvement programs, particularly for those with complex genomes or limited genetic resources (Lokya et al., 2025). Addressing the recalcitrance of various legumes to transformation and regeneration is a critical area of ongoing research, with efforts focused on optimizing explant selection, culture media, and transformation protocols to enhance genome-editing rates (Nivya & Shah, 2023; Sangari et al., 2024).

Challenges and Limitations

Despite these advancements, the widespread applicability of gene editing technologies in legume crop improvement faces significant hurdles, notably the occurrence of off-target edits which can lead to unintended genomic alterations (Kaur et al., 2025). Another significant challenge pertains to the legal and regulatory frameworks governing genetically modified organisms, which often impede the commercialization and public acceptance of CRISPR-edited crops (Costa et al., 2020). Moreover, the development of efficient and reproducible transformation and regeneration protocols remains a substantial barrier for many legume species, directly impacting the efficacy of CRISPR/Cas9 applications (Maia et al., 2024). The low amenability to transformation and regeneration efficiencies in legumes pose a significant hurdle to the broad application of CRISPR technology, despite notable successes in species like cowpea, soybean, Medicago truncatula, and Lotus japonicus (Popoola et al., 2022). This recalcitrance often makes the use of Agrobacterium-mediated transformation challenging, requiring continuous innovation in CRISPR/Cas9 technology and strategic collaborations to unlock its full potential in plant genetic improvement (Sangari et al., 2024). The continued refinement of gene editing tools, including optimizing guide RNA design and delivery methods, is crucial for overcoming these obstacles and enhancing the precision and efficiency of genetic modifications across diverse legume germplasm (Huang, 2024). Further research endeavours are focused on developing transgene-free CRISPR/Cas-edited plants to navigate regulatory complexities and enhance public acceptance, alongside exploring advanced screening methods for off-target mutations. Furthermore, despite advancements, several challenges persist that restrict the broad applicability of CRISPR/Cas9-based genome editing in plants, particularly concerning the efficiency of editing and the complexities associated with constructing guide RNAs (Son & Park, 2022; Venezia & Krainer, 2021). These include optimizing CRISPR cassette design, improving transformation frequency, increasing the editing efficiency ratio of targeted plant cells, and overcoming tissue-specific transformability and regeneration issues, especially for economically important legumes (Freitas-Alves et al., 2024; Sangari et al., 2024). So its mandatory to develop the protocols for tissue culture in more legume crops and additionally, achieving high transformation and regeneration efficiency, particularly for protoplast systems, continues to be a major impediment to the widespread implementation of genome editing techniques in several legume crops, including pea (Singh et al., 2025).

Regeneration Difficulties in Legumes

The inherent recalcitrance of many legume species to efficient regeneration remains a primary bottleneck for successful gene editing and genetic transformation, often exhibiting genotype-dependent responses and prolonged culture durations (Prasad et al., 2024). For instance, recalcitrant crops like pigeonpea and groundnut exhibit significant challenges in gene editing due to the intricate nature of their genomes and their resistance to regeneration, often resulting in issues such as chimera generation and genotype-specific limitations (Prasad et al., 2024). This recalcitrance often stems from the absence of robust and universal tissue culture protocols that can consistently induce high-frequency organogenesis or somatic embryogenesis across a wide range of genotypes. These difficulties are further compounded by the necessity for labor-intensive, time-consuming processes to develop transgene-free lines, alongside a limited number of genotypes amenable to in-vitro regeneration, particularly in the context of advanced genome-editing applications like CRISPR/Cas9 (Ahmar et al., 2023). Overcoming these regeneration challenges is critical for translating CRISPR technology from model systems to a broader spectrum of agriculturally important legume crops, thereby maximizing its potential for yield enhancement and stress tolerance improvements (Baloğlu et al., 2022). The genotype-dependent nature of regeneration protocols and the challenges in obtaining stable, heritable epigenetic changes through CRISPR-mediated methods further underscore the need for sophisticated tissue culture approaches (Prasad & Shetty, 2026). Moreover, the low efficiency of plant regeneration, even from transformed cells, directly impedes the application of genome editing tools for crop improvement (Bekalu et al., 2023).

Genetic Instability

Moreover, challenges persist in generating stable, transgene-free edited plants, often necessitating rigorous screening to ensure the absence of foreign DNA and mitigate regulatory concerns associated with genetically modified organisms (Singh et al., 2025). This difficulty is exacerbated by the often laborious and time-consuming processes required for plant regeneration from transformed cells, presenting a significant bottleneck for the broader application of gene-editing technologies (Tek et al., 2026). Further complications arise from the limited success in applying Agrobacterium-mediated CRISPR/Cas9 gene editing in several recalcitrant legume crops, like pigeonpea and groundnut, which often exhibit low transformation efficiency and reproducibility (Prasad et al., 2024). This limited success is often attributable to the absence of efficient tissue culture systems or effective transformation methods for these species, which are critical for the successful deployment of CRISPR/Cas9 (Das et al., 2024). Consequently, developing streamlined strategies that integrate optimized regeneration protocols with enhanced transformation techniques is paramount for unlocking the full potential of CRISPR/Cas9 in improving recalcitrant legume crops (Pandey et al., 2025). Furthermore, the current transient transformation systems, while offering transgene-free genome editing, exhibit low efficiency, which significantly limits their widespread application, especially for crops like pigeonpea and groundnut (Pak & Li, 2022; Prasad et al., 2024).

Therefore, research efforts are increasingly focused on developing highly efficient plant regeneration systems that overcome the intrinsic recalcitrance of these species, often involving the optimization of growth regulators and environmental conditions (Begum et al., 2025). This includes exploring novel delivery systems for genome editing reagents, such as direct delivery to apical meristems or pollen grains, which could bypass the need for tissue culture altogether, thereby accelerating the development of edited plants (Adhikari & Poudel, 2020). However, a significant challenge arises from the genomic instability frequently observed in plants regenerated from protoplasts and Agrobacterium-transformed explants, often manifesting as large-scale chromosomal deletions, duplications, and trisomies, which can adversely affect the phenotypic stability of CRISPR/Cas-edited plants (Chincinska et al., 2022). Such instability can lead to unintended phenotypic variations, thus complicating the identification of desired traits and potentially compromising the agronomic performance of improved varieties (Sharma et al., 2022). These issues underscore the critical need for advanced tissue culture methodologies that prioritize genomic integrity throughout the regeneration process, particularly when employing methods like Agrobacterium-mediated transformation, which, despite its prevalence, often exhibits limitations in efficiency and genotype specificity (Geng et al., 2025; Mushtaq et al., 2021). A promising avenue to enhance transformation efficiency and mitigate genomic instability in recalcitrant legumes involves exploring alternative delivery systems beyond traditional Agrobacterium methods, such as utilizing novel bacterial strains or optimizing biolistic approaches (Bhavanee et al., 2024; Nivya & Shah, 2023). Despite these advancements, delivering exogenous DNA into plant cells for genome editing, whether through biolistic transformation or Agrobacterium-mediated approaches, still faces limitations, particularly concerning low regeneration efficiency and few transformable genotypes in many modern wheat varieties (Laforest & Nadakuduti, 2022; Li et al., 2021).

Cost and Scale-Up Issues

The main issue that hinders broader adoption is the high cost associated with developing and optimizing these sophisticated tissue culture protocols, especially for a diverse array of legume species (Shipman et al., 2021). This economic burden is further compounded by the extensive labor and specialized infrastructure required for large-scale production, which collectively limit the widespread commercial application of these advanced techniques (Salgotra et al., 2024). Additionally, the potential for somaclonal variation during in-vitro propagation poses a risk to the genetic stability of regenerated plants, necessitating stringent monitoring and screening processes to maintain desired traits and minimize economic repercussions (Hasnain et al., 2022).

These financial and practical constraints often restrict the deployment of advanced tissue culture to a few well-funded institutions and economically important crops, neglecting many orphan legume species with significant potential for food security. Furthermore, the substantial financial investment and regulatory compliance associated with biosafety education for genetically modified crops often deter their broader utilization, confining their adoption to a limited number of crops

and countries (Singh et al., 2023). This situation highlights the urgent need for cost-effective and scalable tissue culture solutions that can democratize access to advanced biotechnological tools for a wider range of legume crops, particularly those vital for food security in developing regions. Recognizing these challenges, there is an increasing imperative to explore innovative, non-tissue culture-based transformation methods that bypass labor-intensive culture practices and circumvent issues like somaclonal variations and genotype-specific recalcitrance (Bohra et al., 2014). These tissue culture-independent approaches offer distinct advantages, such as avoiding somaclonal variation, streamlining methodological processes, and increasing accessibility for non-experts (Quiroz et al., 2024). Such advancements are crucial given that traditional crop improvement methods are time-consuming and often constrained by the limited genetic diversity within related species (Phogat et al., 2023).

Future Directions and Opportunities

Moving forward, the integration of advanced biotechnologies, including molecular markers and genetic engineering, with traditional breeding methods holds significant promise for accelerating legume crop improvement (Abobatta et al., 2021). This integrated approach can overcome limitations such as regeneration recalcitrance and somaclonal variation, which are common challenges in conventional tissue culture techniques (Trauger et al., 2021). The ongoing development of transient and stable plant transformation, coupled with the discovery of new genes influencing critical developmental stages, along with the integration of novel synthetic biology strategies, collectively foreshadows a promising future for tissue culture-independent transformation and gene editing (Quiroz et al., 2024).

New genome editing technologies, such as CRISPR, offer promising avenues for targeted genetic modifications in legumes, potentially circumventing some of the regeneration bottlenecks associated with traditional transformation methods (Popoola et al., 2019). Moreover, the application of DNA-free editing techniques using pre-made ribonucleoprotein complexes and nanoparticles for targeted genome modification presents a notable advancement, sidestepping concerns related to unintended genomic integrations (Patel et al., 2023).

These techniques, coupled with ongoing advancements in plant regeneration methods, can significantly accelerate the development of improved legume varieties with enhanced agronomic traits. To further overcome the limitations of traditional tissue culture and enhance transformation efficiency, direct transformation methods that bypass tissue culture bottlenecks are being explored (Anjanappa & Gruissem, 2021). These in planta transformation methods directly target meristematic tissues, floral structures, or embryos, thus minimizing or eliminating the need for complex tissue culture steps (Kumar & Singh, 2025). Specifically, new approaches to improve transformation and regeneration efficiency are critical for realizing the substantial improvement promised by new genome-editing techniques in legumes (Baloğlu et al., 2022).

For instance, the application of CRISPR/Cas9 has been instrumental in enhancing specific agronomic traits in grain legumes, thereby contributing to food security (Asiamah et al., 2025). This includes gene editing of drought-tolerance mechanisms to enhance climate resilience (Fernandez-Gutierrez et al., 2026) and the use of artificial intelligence for optimizing tissue culture protocols in recalcitrant crops like common bean (Raina et al., 2023). These AI-driven strategies can refine media formulations, optimize growth conditions, and predict favorable genotypes for successful regeneration, thereby expanding the applicability of tissue culture to a broader range of legume species (Narra et al., 2025).

New Technologies in Tissue Culture

The integration of artificial intelligence and automation is emerging as a transformative approach to streamline complex tissue culture processes, offering solutions to the labor-intensive nature of current methodologies and accelerating the generation of transgenic events (Bennur et al., 2024; Parrott et al., 2024). These advanced computational approaches facilitate high-throughput screening and optimization of culture conditions, significantly reducing the empirical effort traditionally required (Wu et al., 2024).

Furthermore, machine learning algorithms can predict optimal media compositions and growth regulator concentrations, enhancing regeneration frequencies and reducing genotype dependency across diverse legume varieties. (Parrott et al., 2024) Beyond optimizing existing protocols, AI-driven models are increasingly being deployed to guide CRISPR/Cas9 genome editing, enhancing precision and mitigating off-target effects by predicting optimal guide RNA sequences and evaluating potential insertion sites (Narra et al., 2025). These technological advancements represent a significant paradigm shift towards more efficient and genotype-independent plant transformation systems, ultimately broadening the applicability of genetic engineering to a wider array of legume crops (Sebiani-Calvo et al., 2024).

CRISPR and Gene Editing Applications

Genome editing technologies, particularly CRISPR-Cas systems, have revolutionized crop improvement by enabling precise modifications to plant genomes, which is crucial for developing crops with enhanced disease resistance and resilience to environmental challenges (Manzoor et al., 2024). This precision allows for the targeted alteration of transcriptional activity or epigenetic status, offering avenues beyond simple gene knockouts (Losa et al., 2021).

This precision is paramount for engineering elite alleles and designing novel functional proteins with enhanced traits, thereby leveraging natural genetic diversity to overcome environmental constraints and improve crop resilience (Li et al., 2025). Despite the transformative potential of CRISPR-Cas systems, their practical application in legumes often remains constrained by the reliance on efficient tissue culture and plant regeneration protocols, highlighting a key bottleneck in translating genomic insights into improved crop varieties (Han et al., 2025). However, the challenges associated with developing universally reproducible and reliable regeneration and transformation protocols for various legume species often impede the full exploitation of CRISPR/Cas technology (Baloğlu et al., 2022).

This necessitates continued research into optimizing in vitro culture conditions and developing robust regeneration systems that are broadly applicable across diverse legume genotypes, thereby unlocking the full potential of genome editing for legume crop improvement (Nivya & Shah, 2023). For instance, established gene-editing protocols for crops like soybean, cowpea, and chickpea demonstrate the feasibility of precise genetic manipulation in certain legumes (Ambika et al., 2022).

However, the efficacy of CRISPR-Cas9 in various legume species can be limited by the availability of sufficient genomic sequence information for identifying appropriate target sites and assessing off-target risks (Sharma et al., 2025). Therefore, a comprehensive understanding of legume genomic sequences is essential for the effective design of guide RNAs and accurate prediction of off-target effects, which is pivotal for the successful deployment of CRISPR-Cas technology in these crops (Sangari et al., 2024).

Sustainable Legume Production

Enhancing the sustainability of legume production through advanced tissue culture techniques directly contributes to global food security by improving yield, nutritional value, and resilience against biotic and abiotic stressors. This includes leveraging tissue culture to integrate beneficial traits such as enhanced symbiotic nitrogen fixation, which reduces reliance on synthetic fertilizers and fosters environmentally friendly farming practices (Mahto et al., 2025). Moreover, tissue culture facilitates the rapid propagation of elite legume genotypes with improved stress tolerance and nutrient uptake efficiency, ensuring consistent crop performance under varying environmental conditions (Mahto et al., 2025).

These advancements also enable the rapid deployment of pathogen-free planting materials, minimizing disease spread and supporting sustainable agricultural systems. Further, these methods support the conservation of genetic diversity by enabling the efficient regeneration and storage of rare or endangered legume germplasm, securing future breeding efforts and maintaining ecosystem stability.

Still, the development of synthetic seeds and cryopreservation techniques through tissue culture offers promising avenues for long-term conservation and efficient distribution of valuable legume genetic resources. The development of genomic selection techniques, which integrate both minor and major genetic factors, also plays a crucial role in accelerating effective selection gains in legume crops (Nadeem et al., 2023). This approach, coupled with high-throughput genotyping platforms, allows for cost-effective and large-scale screening, thereby increasing the utility of marker-assisted selection in legume breeding programs (Atak et al., 2022). This integrated approach not only streamlines the breeding process but also ensures the development of legume varieties that are better adapted to diverse agro-ecological zones, thereby underpinning sustainable agricultural practices (Pierre, 2024).

CONCLUSION

This review highlights the transformative potential of tissue culture technologies, from optimizing regeneration protocols with AI to enabling precise genome editing and fostering sustainable legume production, all of which are critical for addressing global food demands and environmental challenges. Future research endeavours should prioritize the development of more efficient and broadly applicable regeneration systems across recalcitrant legume species to fully harness the power of advanced biotechnological tools, including high-throughput phenotyping and genomics-based breeding. The integration of artificial intelligence tools, particularly machine learning models for plant growth regulator optimization, offers a promising pathway to overcome regeneration recalcitrance in legumes by predicting optimal in vitro culture conditions. Such predictive modeling can significantly increase shoot regeneration frequency and overall regeneration efficiency in recalcitrant crops. These advanced computational approaches, when combined with high-throughput precision phenotyping and mutational breeding, can further accelerate the exploitation of genetic diversity and the introgression of novel traits not naturally present in the legume gene pool. So the tissue culture technologies for the improvement of the legume crop improvement have been instrumental in addressing the escalating global demand for food and feed.

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