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Application of Nanotechnology, Artificial Intelligence (AI) and use of Yeast extract in Plant Tissue Culture: An Updated Review

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Abstract

Plant tissue culture is a fastest *in vitro* cloning technique. Somatic embryogenesis (SE) is a critical process in plant tissue culture, enabling the regeneration of entire plants from somatic cells rather than through traditional sexual reproduction pathways. Transcription factors like WUSCHEL-RELATED HOMEOBOX (*WOX2*) are crucial for maintaining cellular totipotency and regulating the developmental pathways of somatic embryo. Additionally, plant cell and organ cultures are of interest for the production of secondary metabolites of industrial and pharmaceutical interest. CRISPR/Cas9 genome editing represents a revolutionary advance in plant genetic transformation, offering unparalleled precision in making targeted genetic modifications. Artificial Intelligence (AI) technology has enhanced cannabis crop production and improved real-time monitoring, harvesting, processing and marketing. Nanotechnology is one of the most exciting and rapidly advancing fields in modern science, offering innovative solutions by integrating various disciplines, including life sciences and materials engineering. $AgNO_3$ is also known for its role as an ethylene action inhibitor, influencing *in vitro* tissue responses by modulating growth and morphogenesis. The addition of $AgNO_3$ to plant tissue culture media has been shown to enhance shoot development by effectively inhibiting ethylene production. Nanoparticles have proven effective in enhancing regeneration, morphological development, morpho-physiology, and biochemical parameters of plantlets produced under *in vitro*. To increase the accumulation of bioactive compounds, yeast extract was used as elicitor. This elicitor induced a remarkable increase in total polyphenol content, with chlorogenic acid, procyanidin B2, and epicatechin being the most abundant. Yeast extract at 50 mg/L (YE50) was particularly effective, boosting biomass growth and the accumulation of key metabolites such as proteins, proline, phenolics, flavonoids, and condensed tannins.

Keywords: Artificial Intelligence (AI); Cannabis Sativa; CRISPR/Cas9 Genome Editing; Nanotechnology; Plant Tissue Culture; Yeast Extract

1. Introduction

Plant tissue culture techniques are the most frequently used biotechnology tools ranging from basic to applied investigation purposes in plant sciences [86-204, 223, 224]. Tissue culture technique offers several advantages over plant propagation under natural conditions [86-204, 217, 223, 224-228]. It is a rapid procedure as thousands of seedlings can be produced from small fragments (explants) of plants in a short period of time in contrast to conventionally propagated flora [223, 224-228]. Plant tissue culture methods, including plant regeneration through

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cell culture, somatic embryogenesis, and shoot organogenesis are fundamental to advancements in plant biotechnology and genetic engineering for plant improvement [86-204, 223, 224-228]. Plant tissue culture has many commercial applications and can be applied for the production of disease resistant plants either *via* organogenesis or somatic embryogenesis, protoplast isolation and fusion, cryopreservation, and artificial seeds production [86-204, 217, 223, 224]. The induction of somatic embryogenesis using adult shoot apical thin layers has been successful by Malabadi and Co-workers in few conifers such as *Pinus roxburghii, Pinus kesiya, Pinus wallichina, Pinus patula*, and *Pinus sylvestrus* (Scots pine) [110-218]. Malabadi and Coworkers induced and established somatic embryogenesis and plant regeneration in many commercially important plants in India such as grape, sugarcane, *Catharanthus roseus, Vigna aconitifolia, Clitoria ternatea*, papaya, and mango [110-218]. In addition to this, Malabadi and Coworkers also established an *in vitro* micropropagation and *in vitro* seed germination methods for many commercially important orchids in India such as *Pholidota pallida, Xenikophyton smeeanum, Liparis elliptica, Aerides maculosum, Eria dalzelli*, *Cymbidium bicolor, Dendrobium nobile, Vanda parviflora, Vanda coerulea*, and *Cymbidium elegans* [110-218]. Furthermore, Malabadi and Saxena (2012) [218] established a successful *in vitro* cloning and plant regeneration of sugar maple (*Acer saccharum*) [218] at the Department of Agriculture, University of Guelph, Ontario, Canada (Unpublished work) [218].

Another powerful tool in plant biotechnology is genetic transformation in order to transfer relevant genes from bacteria, fungi, animals or plants into plants of interest [110-218]. The fifth application of plant tissue culture is the anther culture [86-204]. Another culture technique is the most viable and efficient method of producing homozygous doubled haploid plants within a short period [110-218]. The sixth application of plant tissue culture is the mass *in vitro* propagation of medicinal plants for the isolation of secondary metabolites of pharmaceutical interest [110-218]. Applications of cell sorting techniques, embryogenic cell culture identification by Artificial Intelligence (AI) should be applied for the future studies of initiation of embryogenic cultures using thin cell layers of shoot apical domes of mature conifers [14, 15]. In the following section, the application of nanotechnology, artificial Intelligence (AI) and use of yeast extract in plant tissue culture has been discussed and updated.

2. Application of Artificial Intelligence (AI) in Plant Tissue Culture

Cannabis sativa has been used for thousands of years for recreational, medicinal, or religious purposes [1-51, 227]. *Cannabis sativa* has been commercially used as hempcrete in the building construction, biochar, biodiesel, bioethanol, and bioplastic preparation [1-51, 227]. It is used as a medicine in controlling viral diseases, wound healing, and cancer etc [1-51]. Now a days, *Cannabis sativa* has been domesticated, cultivated and distributed throughout the world [1-51]. *Cannabis sativa* is a flowering plant from the *Cannabaceae* family and genus cannabis [1-51]. *Cannabis sativa* has a long history in India, recorded in legends and religion [1-51, 227]. It was found in various habitats ranging from sea level to the temperate and alpine foothills of the Indian Himalaya Region from where it was probably spread over the last 10,000 years [1-52]. Many historians believed that Indian Himalayan Region was the centre of origin of *Cannabis sativa* L. and *Cannabis indica* L. [1-51]. *Cannabis sativa* is a plant known for narcotic substance of notorious psychoactive effect, but when used correctly, it provides a plethora of medicinal benefits [1-51, 227]. Industrial *Cannabis sativa* (Hemp or fibre type) can also be consumed as a cannabis tea in remote Himalayan villages of India [1-51, 227]. In remote area, the use of *Cannabis sativa* totally depends on traditional knowledge, which transmitted through family traditions basically through oral conversations [1-50]. *Cannabis sativa* is also a wild noxious weed with notorious psychoactive principle, Λ9-tetrahydrocannabinol (THC) found growing in India, China, Bhutan, Nepal, Pakistan, Afghanistan, Iran, and Morocco [1-51, 227]. Tribal people in the Indian Himalayan region used *Cannabis sativa* as a homemade herbal medicine for many diseases [1-50]. During, Covid-19, the infusion of *Cannabis sativa* flower with a morning cup of tea has saved the life of many people [1-50, 227]. **Artificial neural networks** (ANNs) are widely used in science and technology, and have been successfully applied in *Cannabis sativa* plant tissue cultures [1-51]. Furthermore, Artificial neural networks (ANNs) can also simulate the growth of plants under different *in vitro* conditions [14-15, 21]. *Cannabis sativa* micropropagation has largely been an underground effort with few peers reviewed studies [1-51, 227]. This lack of insight concerning *in vitro* cannabis techniques has limited the biotechnological utility of cannabis crop [1-50, 227]. This is mainly due to the fact that *Cannabis sativa* found to be recalcitrant under *in vitro* conditions, restrictions, long legacy of prohibition and stigmatization surrounding this Indian origin medicinal plant [1-51, 108]. Machine Learning (ML) and Deep Learning (DL) are two of the most exciting technological areas of Artificial Intelligence (AI) [14, 15, 21]. Data is a power today, and artificial intelligence (AI) can help cannabis businesses to gather and analyze data in a wide variety of ways [14, 15, 21]. Artificial Intelligence (AI) technology has enhanced cannabis crop production and improved real-time monitoring, harvesting, processing and marketing [14, 15, 21]. These technologies save the excess use of water, pesticides, herbicides, maintains the fertility of the soil, and also helps in the efficient use of man power and elevated the productivity and improved the quality of cannabis products [1-50]. However, very few and limited *in vitro* regeneration protocols have been developed in cannabis and existing protocols highlights only organogenesis [1-50]. Therefore, there is a golden opportunity for the development of new *in vitro* regeneration protocols particularly induction of somatic

embryogenesis, cryopreservation, protoplast isolation and culture, genetic transformation, production of synthetic seeds, and another culture for the production of haploids in *Cannabis sativa* [1-50]. Automation and artificial intelligence (AI) are transforming plant tissue culture, markedly improving efficiency, precision, and scalability [14-15, 21]. These technologies enable precise control over growth conditions and automate routine tasks such as micropropagation and explant handling, reducing labor requirements and minimizing human error [14-15, 21]. Moreover, AI algorithms analyze extensive datasets to predict optimal growth outcomes and dynamically adjust protocols, enhancing the adaptability and efficiency of tissue culture practices. For instance, robotic systems now perform delicate operations such as cutting and transplanting tissue cultures, ensuring consistent handling and reducing contamination risks [14-15, 21]. These innovations not only streamline production but also significantly reduce costs, making advanced tissue culture techniques more accessible and economically viable [14, 15, 21]. As AI and automation continue to evolve, they are setting new industry standards for producing high-quality, genetically uniform plantlets, and supporting scalable and sustainable agricultural operations [14-15, 21].

3. Application of Nanotechnology in Plant Tissue Culture

One of the application of plant tissue culture is the production of nanoparticles [53-90, 220- 228]. Nowadays, the addition of nanoparticles as elicitors has gained worldwide interest because of its success in microbial decontamination and enhancement of secondary metabolites [53-90, 220- 228]. Nanotechnology is one of the most exciting and rapidly advancing fields in modern science, offering innovative solutions by integrating various disciplines, including life sciences and materials engineering [53-90, 220- 228]. Nanoparticles are entities in the nanometric dimension range [53-90, 220-228]. Applications of nanotechnology are found across a wide range of sectors, such as pesticide degradation and dispersion, creation of nano sensors, use of micronutrients in agriculture, and the protection and nutrition of plants [53-90, 220- 228]. These applications suggest that nanoparticles (NPs) may play a pivotal role in driving innovation within agricultural systems [53-90, 220-228]. Despite the promise of nanotechnology is widely recognized, its use in agriculture to increase crop yields remains under debate [53-90, 220- 228]. They possess unique physicochemical properties. Among all the nanoparticles, silver-nanoparticles (AgNPs) are well-known for their antimicrobial and hormetic effects, which in appropriate doses, led to the improvement of plant biomass as well as secondary metabolite accumulation [53-90, 220-228]. Therefore, the evaluation of the integration of nanotechnology with plant tissue culture is a new advancement of plant biotechnology [53-90, 220- 228]. Among various nanomaterials, Ag-NPs have gained significant attention due to their antibacterial properties and have been utilized in various applications, including reducing microbial contamination, inducing somaclonal variation, increasing proliferation rates, and producing bioactive compounds *in vitro* [53-90, 220-228]. Parallel to this, $AgNO_3$ is also known for its role as an ethylene action inhibitor, influencing *in vitro* tissue responses by modulating growth and morphogenesis [53-90, 220- 228]. For instance, the addition of AgNO3 to culture media has been shown to enhance shoot development by effectively inhibiting ethylene production [53-90, 220-228]. Moreover, silver ions, particularly in nitrate form, are widely used in promoting somatic embryogenesis and organogenesis across different plant species due to their regulatory role in morphogenetic pathways [53-90, 220-228]. It is anticipated that advances in nanotechnology will be more effective when combined with compounds like silver nitrate ($AgNO_3$) and silver nanoparticles (Ag-NPs), as well as *in vitro* tissue culture techniques [[53-90, 220-228]. In this context, several studies have shown that NPs can positively influence regeneration efficiency, morphological traits, morphophysiology, and biochemical responses in plantlets generated in vitro [53-90, 220- 228]. However, while these substances offer substantial benefits, their effects are not universally positive [53-90, 220- 228].

The bioaccumulation of Ag- NPs within plant tissues has also been correlated with the system's redox potential, further complicating their use in plant systems [53-90, 220- 228]. Therefore, while both $AgNO_3$ and Ag-NPs exhibit promising effects on shoot and root development across various species, caution is warranted regarding concentration-dependent toxicity [53-90, 220- 228]. Interestingly, recent findings also point to the potential of NPs in facilitating genetic transformation, suggesting a new frontier in plant biotechnology [53-90, 220, 228]. The impact of Ag-NPs, especially at higher concentrations, varies depending on factors such as plant species, developmental stage, and nanoparticle size and dosage [53-90, 220- 228]. Some studies suggest that $AgNO_3$ acts as an inhibitor of ethylene action. Some studies have reported growth inhibition, reduced root length, and biomass accumulation under excessive Ag-NP exposure, highlighting potential phytotoxicity [53-90, 220- 228]. The highlight is especially conveyed on secondary metabolite enhancement, effects on plant growth and biomass accumulation as well as their possible mechanism of action [53-90, 220- 228]. In addition, the use of nanomaterials as potential therapeutic agents is gaining interest worldwide [53-90, 220- 228]. Elicitation of silver-nanoparticles, as well as nanomaterials, function as therapeutic agents for animal well-being is expected to play a major role in the process [53-90, 220- 228]. Additionally, interactions between nanomaterials and plants have been shown to affect seed germination, root initiation, and growth, with these effects varying based on the properties, concentration of NPs, and the plant species involved [53-90, 220-228].

Türkoğlu et al., (2025) [54] reported that nanoparticles have proven effective in enhancing regeneration, morphological development, morpho physiology, and biochemical parameters of plantlets produced in vitro [54]. This research by Türkoğlu et al., (2025) [54] aimed to examine the effects of silver nanoparticles (Ag-NPs) compared to silver nitrate ($AgNO_3$) on shoot organogenesis in the regeneration of quinoa (*Chenopodium quinoa* Willd.) [54]. To establish an efficient regeneration system the culture medium was then supplemented with $AgNO_3$ and Ag-NPs at a range of concentration, including 0, 2, 4, 6, and 8 mg L^{-1} of three types of explants (first node, middle node, and apical node) [54]. Ag-NPs yielded higher values than $AgNO_3$ across all measured parameters, including shoot length, callus count, leaf number, and shoot weight [54]. A concentration of 6 mg L^{-1} produced the optimal results for these parameters, while 8 mg L^{-1} led to reduced growth, indicating a potential toxicity threshold[54]. Apical node explants showed superior regeneration capacity across treatments [54]. Biochemical analyses revealed increased antioxidant enzyme activity and malondialdehyde content under Ag treatments, suggesting oxidative stress, while hydrogen peroxide levels decreased, indicating enhanced reactive oxygen species scavenging at higher concentrations (4–8 mg L−1) [54]. These experimental results by Türkoğlu et al., (2025) [54] showed the Ag treatments enhanced the uptake of phosphorus, potassium, and magnesium, while high concentrations resulted in decreased sodium and calcium levels, suggesting potential toxicity and impaired ion transport [54]. Principal component analysis was employed to explore relationships among factors associated with tissue culture, revealing a strong association between high AgNO3 concentrations, explant position, and shoot development [54]. This study by Türkoğlu et al., (2025) [54] found that Ag-NPs significantly influenced the growth parameters and other traits of the plantlets [54]. Furthermore, results of this study by Türkoğlu et al., (2025) [54] suggested that the efficiency of quinoa tissue culture can be enhanced by increasing the application of Ag in the form of nanoparticles [54]. In conclusion, this work underscores the potential of using Ag-NPs in quinoa tissue culture [54].

This study by Türkoğlu et al., (2025) [54] highlights the complex interactions between silver-based compounds, particularly Ag-NO3 and Ag-NPs, and plant physiological processes [54]. The findings demonstrated that both Ag-NO_3 and Ag-NPs influence essential plant growth parameters such as shoot length, callus forma-ion, leaf number, and shoot weight, with Ag-NPs generally showing more pronounced positive effects [54]. The observed enhancement in antioxidant enzyme activities and the rise in MDA levels indicate that silver ions trigger oxidative stress in plants, leading to the activation of defense mechanisms [54]. At medium to high concentrations (4–6 mg L^{-1}), plants exhibited the highest enzymatic activity, suggest- ing a dose-dependent response to mitigate ROS stress [54]. The increase in macro elements like P, K, and Mg implies that plants attempt to adapt to high Ag ion stress by regulating cellular energy metabolism and ion balance [54]. However, the decrease in Na and Ca levels at higher concentration indicates potential disruptions in ion transport and membrane stability, which could impair osmotic regulation and structural integrity [54]. While Ag-NPs offer promising applications in enhancing tissue culture processes and plant regeneration, the data also highlights the potential risks of toxicity at higher concentrations [54]. These findings underscore the need for further research to optimize the use of silver nanoparti- cles in agriculture while minimizing environmental and biological risks. Understanding the delicate balance between beneficial and harmful effects of Ag-based compounds is crucial for future applications in sustainable agriculture and environmental management [54].

Another study by Twaij et al., (2025) [221] investigated the optimization of callus induction using plant growth regulators (PGRs) in *Datura innoxia* and *Datura stramonium* and secondary metabolites (SMs) production from calli treated with aluminium oxide (Al_2O_3) and tungsten oxide (WO_3) nanoparticles [221]. For callus induction, leaf explants were inoculated on Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Thidiazuron (TDZ) [221]. Inducted calli were treated with varying concentrations of Al_2O_3 and WO_3 nanoparticles [221]. Data analysis indicated that both PGRs significantly contribute to callus induction in both species [221]. In the case of *Datura innoxia*, the maximum percentage (100%) of callus was achieved in 2,4-D 0.5, 1.0, and 1.5 mg/L supplemented media 1.5 mg/L TDZ supplemented media [221]. The combinations, 1.0 + 0.5 mg/L, 1.5 + 0.5 mg/L, 0.5 + 1.5 mg/L and 1.0 + 1.5 mg/L 2,4-D and TDZ respectively provided the same callusing percentage [221]. On the other hand, experiments showed maximum callus induction (100%) in the media supplemented with only 1.0 and 1.5 mg/L 2, 4-D [221]. Phytochemical analysis of the nanoparticles treated calli in both species demonstrated the enhanced production of key alkaloids, phenolic compounds, and flavonoids compared to the non-treated calli [221]. In terms of alkaloid production, both species showed height production (increment 263% and 283% respectively compared to the controls) with a supplantation of 60 mg/l tungsten oxide (WO_3)[221]. Around 80.8% increment of phenolic compounds was observed in *Datura stramonium* with 60 mg/g tungsten oxide (WO_3) supplementation [221]. Again, 81.81% higher flavonoids production was observed in D. innoxia in response to WO_3 [221]. These findings suggest that aluminium oxide (Al_2O_3) and tungsten oxide (WO_3) nanoparticles can effectively enhance SMs production in Datura spp., offering a novel approach for maximizing the yield of valuable bioactive compounds [221]. This research provides a foundation for the large-scale production of pharmaceutically important compounds from Datura through biotechnological interventions using nanoparticle technology [221].

The Vinh et al., (2024) [222] reported the effects of iron nanoparticles (FeNPs) on *in vitro* rooting, chlorophyll content, antioxidant and hydrolysis enzyme activities, acclimatization and subsequent growth of *Gerbera jamesonii* var. Revolution Yellow plantlets under greenhouse conditions [222]. The single shoots (2 cm in length) were cultured in different medium treatments, including: MS0 (MS medium - control), MS0 replacing 27.8 mg/L $FeSO_4.7H_2O$ with FeNPs (ranging from 0 to 11.2 mg/L) [222]. The results showed that 2-cm shoots cultured in medium supplemented with 100 mM FeNPs induced in vitro rooting 2 days earlier than shoots cultured in MS0 medium [222]. In addition, plantlet height (6.83 cm), number of main roots and lateral roots (13.00 roots and 4 roots), root length (5.27 cm), leaf length and width (2.07 cm and 1.83 cm), fresh and dry weights (1188.67 and 155 mg), dry matter ratio (13.04%), and chlorophyll a, b, a + b content (25.25, 18.35 and 43.6 mg/g, respectively) of the shoot culture in 5.6 mg/L FeNPs treatment were also recorded higher than other amounts of FeNPs and control (MS0) treatments after 4 weeks of culture [222]. Furthermore, the enzyme activities of SOD (41.32 U/g), CAT (308.70 U/g), and APX (0.55 U/g) were higher in the 100 µM FeNPs treatment than those in the MS0 and MS-Fe treatments, and hydrolysis enzyme activities (cellulase and pectinase) showed opposite results after 4 weeks of culture [222]. The plantlets derived from the 5.6 mg/L FeNPs treatment had the highest survival rate (93.00%) in comparison to those in MS0 medium (83.67%) and without FeNPs (73.33%) after 2 weeks under greenhouse conditions, and the early flower bud formation (10.17 weeks), increased flower diameter (7.87 cm), and peduncle length (22.17 cm) after 12 weeks [222].

Lankarani er al., (2024) [223] investigated the effects of different levels of zinc oxide nanoparticles (ZnONPs) (0, 10, 20, and 30 mg/L) and iron sulfate (13.9, 27.8, and 55.6 mg/L) on morphological and physiological responses of *Stevia rebaudiana* Bertoni plant under *in vitro* conditions [223]. Results indicated that the combined application of ZnONPs at 10 mg and iron at 27.8 mg led to the highest increase in shoot number, height, and biomass, showing a respective rise of 17.37%, 39.66%, and 45.02% compared to control cultures [223]. The highest pigment content and tissue antioxidant activity (83.48%) was observed with the combined presence of 10 mg/L ZnONPs and 27.8 mg/L iron [223].

As ZnONP concentration increased in the culture medium, the combined effect on lipid peroxidation rate became more pronounced [223]. The impact of ZnONPs on phenolic compound production varied depending on the specific substance. The iron content of shoots increased significantly by 41.11% under the influence of 27.8 mg/L iron and 10 mg/L ZnONP compared to control cultures [223]. Interaction effects of treatments at various levels resulted in increased zinc content in shoots, peaking at 27.8 mg/L iron when ZnONP reached 20 mg/L, representing a 56.28% increment over control levels before slightly decreasing [223]. The most increases in stevioside and rebaudioside were observed with the combination of 10 mg/L ZnONP and 27.8 mg/L iron, showing enhancements of 75.04% and 63.08%, respectively [223]. These findings suggest that ZnONPs could stimulate the growth and enhance the bioactive components of stevia plants, making them a viable option as elicitors in in vitro batch cultures [223].

One of the study by Dasauni and Nailwal, (2025) [227] explores the impact of green synthesized sulphur nanoparticles (gSNPs) on *in vitro* regeneration of *C. sativa*. [227]. Out of 50 axillary buds inoculated, approximately 40 buds showed shoot induction and proliferation on MS medium supplemented with 2.5 µM TDZ, 5.0 µM GA_3, and 50.0 mg L^{-1} gSNPs, significantly outperforming the control without gSNPs [227]. Rooting experiments with 50.0 mg L^{-1} gSNPs in ½ MS medium with up to 5.0 µM NAA and IBA revealed that 2.5 µM indole- 3-butyric acid (IBA) with 50.0 mg L^{-1} gSNPs yielded highest number of roots (17.7 ± 1.20) and root length (12.0 ± 1.52 cm) [227]. This standardized protocol presents a novel approach to *C. sativa* micropropagation using gSNPs with potential applications in both research and industrial applications of *Cannabis* [227].

However, ethylene build up during plant tissue culture is a major challenge to *in vitro* plant growth and development [228]. One of the study conducted by Oluwasegun et al., (2025) [228] examined the effects of silver nitrate ($AgNO_3$), a known ethylene action blocker on the *in vitro* growth and development of four accessions of *D. rotundata* in a 7*6*4 factorial outline in a completely randomized design [228]. Addition of 1.0 mg L^{-1} $AgNO_3$ to the culture medium significantly improved *in vitro* growth and development of the shoots, while 2.0 mg L^{-1} $AgNO_3$ significantly influenced root formation [228].This study demonstratrated the effectiveness of silver nitrate ($AgNO_3$) in enhancing the morphogenesis of *D. rotundata* [228].

4. Use of Yeast extract in Plant Tissue Culture

Today, plant cell cultures represent a valid alternative method to produce secondary metabolites [51-228]. To increase the accumulation of bioactive compounds, yeast extract was used as elicitor [52, 207-216]. This elicitor induced a remarkable increase in total polyphenol content, with chlorogenic acid, procyanidin B2, and epicatechin being the most abundant [52, 207-216]. The antioxidant potential of extracts from the callus cultures was investigated and results showed that the use of the elicitor improved the protective antioxidant effect of the extracts on UVA-stressed keratinocytes [52, 207-216]. Yeast extract is used in plant tissue culture as an elicitor to stimulate the production of

valuable secondary metabolites, such as phenolics and iso-flavones. It acts as a rich source of nutrients, amino acids, vitamins, and growth factors, which triggers plant defense responses and enhances the expression of biosynthetic pathway genes [52, 207-216]. While it can improve growth and metabolite content, excessive amounts may inhibit growth in some species, so the optimal concentration must be determined experimentally [52, 207-216]. Lescano et al., (2025) [52] reported that *Limonium algarvense* Erben, a medicinal halophyte, holds significant pharmacological promise due to its rich bioactive compound [52]. This study by Lescano et al., (2025) [52] aimed to established robust callus cultures as a sustainable, *in vitro* model for studying the plant's metabolic responses, particularly focusing on synthesising and accumulating primary and secondary metabolites under various elicitation treatments [52]. Callus cultures were initiated from leaf explants on Murashige and Skoog's medium supplemented with 1 mg/L picloram for 4 weeks. Afterwards, callus cultures were subjected to two elicitor treatments, including salicylic acid–SA and yeast extract–YE at 50 and 100 mg/L for four weeks [52]. Water extracts were assessed for their shifts in primary (total soluble sugars and proteins, and proline), and secondary metabolism (total phenolics, flavonoids, and condensed tannins). In addition, a detailed metabolic profiling was conducted using high-performance liquid chromatography with electrospray ionization mass spectrometry (HPLC–ESI–MS/MS) [52]. Elicitation induced signifcant shifts in the metabolite synthesis of elicited cultures compared to controls. While YE50 markedly increased the callus yield, the total levels of phenolics, flavonoids condensed tannins and total soluble proteins, the SA50 led to the highest increase in proline content [52]. Metabolomic analysis identifed 10 metabolites, including 4-hydroxyphenyllactic acid, hydroxybenzoic acid, ribofavin (Vitamin B2), and di-hydroferulic acid methyl ester 4-O-sulfate, that were increased in the YE50 elicitation treatment [52]. This suggests that elicitation can effectively enhance the biosynthesis of primary and secondary metabolites in *L. algarvense* callus cultures, offering great potential for nutritional and medicinal applications [52].

Yeast extract at 50 mg/L (YE50) was particularly effective, boosting biomass growth and the accumulation of key metabolites such as proteins, proline, phenolics, flavonoids, and condensed tannins [52]. Phytochemical profiling identifed a diverse range of bioactive compounds, including 4-hydroxyphenyllactic acid, hydroxybenzoic acid, ribofavin (Vitamin B2), and di-hydroferulic acid methyl ester 4-O-sulfate, known for their potent antioxidant and health-promoting properties [52]. These findings underscore the metabolic fexibility of *L. algarvense* callus cultures under elicitation, offering a sustainable source of high-value compounds with potential applications in health, cosmetics, and nutraceuticals [52]. This study provides a solid framework for leveraging plant cell culture systems for metabolite production, contributing to the conservation of *L. algarvense* and fostering innovative biotechnological advancements [52].

5. Conclusion

Tissue culture is a fastest *in vitro* cloning technique. Additionally, plant cell and organ cultures are of interest for the production of secondary metabolites of industrial and pharmaceutical interest. New technologies, such as the genome editing ones combined with tissue culture and *Agrobacterium tumefaciens* infection, are currently promising alternatives for the highly specific genetic manipulation of interesting agronomical or industrial traits in crop plants. The limitations of plant tissue culture methods include difficulties with continuous operation, product removal, and aseptic conditions. A few culture systems appear to have the potential to become commercially viable because of these limitations. Plant cell development under in vitro conditions and regeneration into full plants is an asexual process that involves just mitotic division of the cell and, ideally, should not result in variation. Clonal multiplication of genetically homogenous plants is the ideal scenario. Uncontrolled and unpredictable spontaneous variation throughout the cultural process is thus an unanticipated and largely undesirable phenomenon. Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in Cannabis sativa plant tissue cultures. Among all the nanoparticles, silver-nanoparticles (AGNPS) are well-known for their antimicrobial and hermetic effects, which in appropriate doses, led to the improvement of plant biomass as well as secondary metabolite accumulation. Therefore, the evaluation of the integration of nanotechnology with plant tissue culture is a new advancement of plant biotechnology. For instance, the addition of $AgNO_3$ to culture media has been shown to enhance shoot development by effectively inhibiting ethylene production. Moreover, silver ions, particularly in nitrate form, are widely used in promoting somatic embryogenesis and organogenesis across different plant species due to their regulatory role in morphogenetic pathways. Yeast extract at 50 mg/L (YE50) was particularly effective, boosting biomass growth and the accumulation of key metabolites such as proteins, proline, phenolics, flavonoids, and condensed tannins.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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