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Cannabis sativa: Applications of Artificial Intelligence (AI) and Plant Tissue Culture for Micropropagation

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Abstract: - This review paper highlights about the important applications of Artificial Intelligence (AI) and in vitro micropropagation of Cannabis. Cannabis micropropagation has largely been an underground effort with few peer reviewed studies. This lack of insight concerning in vitro cannabis techniques has limited the biotechnological utility of Cannabis crop. This is mainly due to the fact that Cannabis found to be recalcitrant under in vitro conditions, restrictions, long legacy of prohibition and stigmatization surrounding this **Indian origin** medicinal plant. Machine Learning (ML) and Deep Learning (DL) are two of the most exciting technological areas of Artificial Intelligence (AI). Data is a power today, and artificial intelligence (AI) can help Cannabis businesses to gather and analyze data in a wide variety of ways. Artificial Intelligence (AI) technology has enhanced Cannabis crop production and improved real-time monitoring, harvesting, processing and marketing. These technologies saves 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. Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in Cannabis plant tissue cultures. Furthermore, Artificial neural networks (ANNs) can also simulate the growth of plants under different in vitro conditions. However, very few and limited in vitro regeneration protocols have been developed in Cannabis and existing protocols highlights only organogenesis. 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 anther culture for the production of haploids in Cannabis.

Key Words: Artificial Intelligence (AI), Artificial Neural Networks (ANNs), Cannabis, Plant Tissue Culture, Δ9-tetrahydrocannabinol (Δ-9-THC), Machine Learning, Psychoactive molecule.

I. Introduction

Cannabis sativa L. is a wind-pollinated, dioecious medicinal plant (i.e., the male and female reproductive structures are on separate plants), although monoecious plants (male and female flowers on same plant) can occur in some population (1-15). Male plants die shortly after flowering. The female plants live 3 to 5 weeks until seed is fully riped (1-17). Therefore, the plants are obligatory out-crossers. In commercial production, medical Cannabis (drug or marijuana type) plants are all genetically female and male plants are destroyed as seed formation reduces flower quality (1-20). Additionally, the species *Cannabis sativa* L. and *Cannabis indica* are a potential source of fibre, food, oil, and protein. However, cannabis research work remains years behind than other crops because of the long legacy of prohibition and stigmatization. Cannabis is the most commonly used illicit drug worldwide and the active constituents of the product were described several decades ago (1-20). The legal status of *Cannabis* is changing, fuelling an increasing diversity of *Cannabis* derived products (1-15). New laws leading to decriminalization and legalization have given rise to a global, multibillion dollar industry that is projected to continue to grow (1-20). The changing legal landscape and rising interest in its potential therapeutic utilities have opened new opportunities for

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therapy. In addition, Cannabis industrial companies face increased competition and lower revenue margins, further impacting development in the field.

Artificial neural network (ANN) technology is an effective alternative used for a reliable and objective assessment of biological processes (22-56). Although artificial neural network (ANNs) have shown significant progress in controlling bioprocesses, their use in the complex systems of **plant tissue cultures** is relatively infrequent (37-56). Artificial intelligence (AI) has already played a major role in agriculture and also applied in Cannabis industries in order to maintain the quality control, cost issues, and labour problems (22-56). This technology has protected the Cannabis crop yield from various factors like the climate changes, population growth, employment issues and the food security problems (22-56).

Initial uses of Cannabis date back to almost 5000 years in **India** which was well documented in A***yurveda*** and now cultivated for both medicinal and recreational applications (1-20). Cannabis is also known as the **Pot gold** of Indian Himalayan Region (1-20). Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes (1-45). *Cannabis sativa* and *Cannabis indica* are the native of Indian origin found as wild noxious weed in the Indian Himalayan Region and other parts of India, China, Nepal, Bhutan, Sri Lanka, Pakistan, Afghanistan, Persian, Iran, and Morocco (1-22).

Cannabis sativa has a mixed mating system with either dioecious or monoecious (with individuals that produce male and female flowers) lineages (1-22). Dioecious lineages have sex chromosomes where females are homogametic (XX), and males are heterogametic (XY) (1-15). Monoecious lineages appear to have two X chromosomes as well. Most of the Cannabis varieties in the market today are hybrids (**700** hybrid strains) with both *Cannabis sativa* and *Cannabis indica* genetics. *Cannabis ruderalis* flowers matures as a result of age, not light conditions, which is called Autoflowering (1-20). It is principally used in hybrids to enable the hybrid to have the Autoflowering property (1-20). Medical Cannabis (Drug or Marijuana-type) lineages used for human consumption has been focused on female domestication, since most of the Cannabinoid production is found in the trichomes of female flowers (1-17). There has been strong human selection against males and monoecious individuals. On the other hand, Industrial Cannabis sativa (hemp) cultivated for their stalks or seeds for fiber or grain production produces lower Cannabinoid or terpenes (1-18).

Cannabis sativa has been classified into 2 types depending on the Δ-9-THC (**Δ**9-tetrahydrocannabinol) level content. One is called as the Industrial Cannabis sativa (Hemp) containing very low levels (0.3%) of THC (1-20). Second type is known as Medical Cannabis sativa (Drug or Marijuana-type) containing very high levels (25-35%) of THC content. The Δ-9-THC (**Δ**9-tetrahydrocannabinol), the psychoactive molecule is largely concentrated around the flowering parts of the female Cannabis plant (1-15). The leaves and male plants have less Δ-9-THC (**Δ**9-tetrahydrocannabinol), while the stalks and seeds contain almost none. Industrial *C. sativa* (Hemp) produces fiber that can be used in paper, rope, or clothing, biofuel, and grain that can be used to extract oil for cooking, personal hygiene and beauty products, while Medical Cannabis sativa (drug or marijuana type) produces compounds used for medicinal and recreational purposes (1-15).

According to the recent literature, over 200 phytocannabinoids have been identified in the Cannabis plant (1-15). Cannabis sativa L. plants that contain a large variety of secondary metabolites, including phytocannabinoids, terpenoids, and flavonoids, which have profound anti-microbial activities, anti-inflammatory, anti-oxidative, and neuromodulatory properties (1-20). They are classified into different subclasses according to their chemical structure, Cannabidiol (CBD), narcotic psychoactive compound, Δ9-tetrahydrocannabinol (Δ9-THC), Cannabigerol (CBG), Δ8-tetrahydrocannabinol (Δ8 -THC), and Cannabinol (CBN) are the most studied. Cannabis contains a psychoactive compound called Δ9- tetrahydrocannabinol (Δ9-THC), that creates a psychogenic effect (1-22). Cannabinoids are a class of compounds that interact with the Endocannabinoid system (ECS) and many have medicinal or psychoactive properties (1-45). Two of the most widely known Cannabinoids are Δ-9-tetrahydrocannabinolic acid (THCA), and Cannabidiolic acid (CBDA), which are converted to the neutral forms of Δ-9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) respectively once heated (1-22).

In the following section, the application of artificial Intelligence (AI) and plant tissue culture for the micropropagation has been discussed.

II. Cannabis sativa: Application of Artificial Intelligence (AI)

Artificial intelligence (AI) is defined as a branch of computer science that focuses on creating machines that can think and act like humans. Artificial intelligence (AI) systems are designed to learn from their environment, analyze data, and make decisions without human intervention (22-37). Artificial intelligence (AI) can be used for a wide range of tasks, from playing games to driving cars and in agriculture including Cannabis industries (22-37). Artificial intelligence (AI) systems are powered by algorithms, which are sets of instructions that advise the computer how to process data and make decisions. These algorithms are based on mathematical models that use input data to generate output results (22-30). For example, an Artificial intelligence (AI) system might use an algorithm to analyze images and recognize objects in them. Artificial intelligence (AI) is being used in

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many different industries, from healthcare to finance, traffic recognition system using Machine learning (ML), virtual painter, Cannabis and plant tissue culture industries (22-56, 213, 214).

Machine Learning and Deep Learning are a growing diverse fields of Artificial Intelligence (AI) which studies algorithms that are capable of automatically learning from data and making predictions based on data (22-37, 212). Machine Learning and Deep Learning are two of the most exciting technological areas of Artificial Intelligence (AI). In healthcare, Artificial intelligence (AI) can be used for medical diagnosis and treatment planning (22-37, 212). In finance, Artificial intelligence (AI) can be used for automated trading and portfolio management. Additionally, Artificial intelligence (AI) can be used for natural language processing (NLP), which allows computers to understand human language and respond accordingly (22-37). Artificial intelligence is a rapidly growing field of technology with many potential applications in various industries including agriculture sector. Artificial intelligence (AI) is transforming healthcare in a number of ways (22-37). Artificial intelligence-powered medical imaging systems are helping doctors to diagnose diseases more accurately and quickly (22-37). Artificial intelligence-based **Chatbots** are providing patients with personalized health advice and support. AI-powered robots are assisting surgeons with complex procedures (30-37). All of these advances are making healthcare more efficient and accessible, improving patient outcomes and saving lives (22-37-56).

Artificial Intelligence (AI) is based on the vast domains like Biology, Linguistics, Computer Science, Mathematics, Psychology, engineering, Cannabis and plant tissue culture industries (22-57). The basic concept of AI is to develop a technology which functions like a human brain (22-37). This technology is perpetrated by studying how human brain thinks, how humans learn, make decisions, and work while solving a problem, and on this ground intelligent software and systems are developed (22-37). These softwares are fed with training data and further these intelligent devices provide us with desired output for every valid input, just like the human brain (23-37). Vast domains including Machine Learning and Deep learning are core part of Artificial Intelligence (AI) (22-37). Artificial Intelligence (AI) is the science of making intelligent machines and programs. Machine learning (ML) is the ability to learn something without being explicitly programmed and Deep Learning (DL) is the learning of deep neural networks. ANN is a processing algorithm or a hardware whose functioning is inspired by the design and functioning of a human brain (22-37). Neural networks have a remarkable ability of self organization, and adaptive learning. It has replaced many traditional methods in numerous fields like Computer Science, Mathematics, Physics, Engineering image/signal processing, Economic/ Finance, Philosophy, Linguistics, Neurology. Artificial neural network (ANN) undergoes the process of learning. Learning is the process of adapting the change in itself as and when there is a change in environment (22-57).

Artificial Intelligence (AI) is an emerging technology in the field of agriculture. Artificial Intelligence (AI)-based equipment and machines, has taken today's agriculture system to a different level (22-37). Agricultural robots are built in order to deliver high valued application of Artificial Intelligence (AI) in the agricultural sector like the crop yield, irrigation, soil content sensing, crop- monitoring, weeding, and crop establishment (22-37). Artificial Intelligence (AI) has the potential to deliver much-needed solution (22-57). AI-based technological solutions has enabled the farmers to produce more output with less input and even improved the quality of output, also ensuring faster go-to- market for the yielded crops (22-37). Artificial Intelligence (AI) technology has enhanced crop production and improved real-time monitoring, harvesting, processing and marketing. The latest technologies of automated systems using agricultural robots and **drones** have made a tremendous contribution in the agro-based sector (22-37). Various hi-tech computer based systems are designed to determine various important parameters like weed detection, yield detection and crop quality and many other techniques (22-37). The automated irrigation, weeding and spraying to enhance the productivity and reduce the work load on the farmers (22-37). Various automated soil sensing techniques are made available. The robots used in sensing were localized by GPS modules and the location of these robots was tracked using the Google maps. The data from the robots was fetched through Zigbee wireless protocol (22-37).

III. Artificial intelligence (AI): Cannabis Industries

First-generation artificial intelligence (AI) systems failed to explain the decision making algorithms clearly in medical cannabis (22-37). However, real-world utilization of First-generation artificial intelligence (AI) is limited because most algorithms need not necessarily results in better patient outcomes (29-30). Digital medical Cannabis is a Cannabis product controlled by a second-generation artificial intelligence (AI) system that improved patient responses by increasing adherence and dealing with tolerance to drugs. Second-generation artificial intelligence (AI) systems focused on a single patient's outcome and deal with the inter- and intra-subject variability in responses (29-37). The use of digital medical Cannabis is expected to improve product standardization, maximize therapeutic benefits, reduce health care costs, and increase the revenue of Cannabis industrial companies (29-30). Digital medical Cannabis offers several market differentiators for Cannabis companies. Ongoing trials and real-world data on the use of these systems further supported the use of digital medical Cannabis for improved global health (29-30, 32-57).

The automation in Industrial Cannabis sativa (Hemp) and medical Cannabis agriculture is the main concern and the emerging subject across the world (22-37). The traditional methods which were used by the farmers, were not sufficient enough to

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fulfill these requirements (22-57). Thus, new automated methods were introduced. Artificial Intelligence (AI) in outdoor and indoor Industrial hemp cultivation has brought an agriculture revolution. This technology has protected the crop yield from various factors like the climate changes, population growth, employment issues and the food security problems (22-37). There are various applications of Artificial intelligence (AI) in agriculture particularly hemp and medical Cannabis cultivation such as for irrigation, weeding, spraying with the help of sensors and other means embedded in robots and drones (22-37). These technologies saves the excess use of water, pesticides, herbicides, maintains the fertility of the soil, and also helps in the efficient use of man power and elevate the productivity and improved the quality (22-37).

Some of the important applications of artificial intelligence (AI) in the Cannabis industries are for example; Discovering the new Cannabis strains based on chemical families and compound properties using artificial intelligence (22-37). Track the growth rate of Cannabis plants in their vegetative stage using machine vision and artificial intelligence (Yield prediction) (22-37). Furthermore, offer artificial intelligence powered e-commerce personalization and delivery solutions, including personalized search, product recommendations, and advanced data analytics to online retailers of marijuana (Ecommerce Personalization) (22-37). Use of wheeled delivery robots to perform marijuana deliveries based on algorithms powered by artificial intelligence (Robotic Delivery of Marijuana Products). Use of artificial intelligence to predict Cannabis stock market changes and identify patterns that can be used to make more accurate forecasts and investment decisions (Reduced Business Risk in Marijuana) (22-37). BudGenius uses Cannabis test results from its own testing facility to gather data about each marijuana strain, and using artificial intelligence technology, it merges that data with scientific trial data and real patient user experiences (22-37). Machine learning allows BudGenius to improve each time a new scientific trial is released, and each time a patient submits a product review. Dispensaries can also use BudGenius to help their patients to choose the best products (Enhanced Customer Service Using Chatbots) (22-37). There are many Cannabis companies that are already using artificial intelligence (AI), machine learning and deep learning in their products and services. Use of artificial intelligence (AI), predictive, and machine learning algorithms to assess business risk for marijuana business stakeholders (22-37).

The artificial intelligence uses compliance data to predict which categories a marijuana license holder is the most at the risk to fail in (Adherence Compliance) (22-37). Predict energy consumed and labour cost by Cannabis operation using environmental conditions, nutrient feed, pH, CO_2, light spectrum (Energy Consumption & Labour Cost)(37-57). Identify chronic sickness by creating a human-level diagnosis tool for Cannabis plants (Identify Unhealthy Marijuana Leaves) (22-37). Artificial intelligence, machine learning, and deep learning are becoming essential tools of leading marijuana companies. Any company, in Cannabis or other industries, that leverages artificial intelligence (AI) will gain significant competitive advantage in the marketplace (22-37). Artificial intelligence (AI) has gone mainstream with the explosive growth of ChatGPT which is a great news for business operations in all industries, including Cannabis, because artificial intelligence enables companies to streamline operations, reduce costs, and seize opportunities (22-56). Artificial intelligence (AI) is already being used in a variety of ways across the Cannabis value chain. Both business to business (B2B) and business to consumer (B2C) brands use artificial intelligence to improve Cannabis sales (22-37). Cultivators use artificial intelligence (AI) to boost watering and lighting efficiencies, and manufacturers use it for quality control to detect problems and boost productivity (22-57). In simplest terms, this feature uses artificial intelligence (AI) and machine learning to analyze every record in the Cannabiz Media License Database and determine the best time to send email messages to each email address (22-37). The best time is based on each recipient's previous engagement with messages that have been sent to them in the past. Artificial intelligence (AI) tools can also help to improve the operations for businesses across the Cannabis supply chain (22-37). There are many Cannabis technology platforms and products that leverage artificial intelligence to help businesses improve operations. In the near future, undoubtedly many new possibilities and evolving technology to optimize the benefits of artificial intelligence (AI) for businesses and consumers (22-37).

Cannabis growers already uses artificial intelligence (AI) to automatically optimize lighting, humidity, watering, pest management, and quality control (22-57). This enables them to grow healthier plants and higher Cannabis crop yields (22-37). As a result, they can lower costs, reduce waste, and possibly sell more of their high-quality Cannabis crops for higher prices (Cultivation) (22-37). Artificial intelligence not only helps with quality control by detecting manufacturing problems earlier and automatically taking steps to reduce costs and delays associated with those problems, but it can also be used to improve the production overall by tracking inventory more effectively (22-37). Supply and demand can change quickly in the Cannabis industry, and artificial intelligence (AI) can detect and predict fluctuations to ensure the right products are being manufactured at the right times (Manufacturing) (22-37). Shipping and logistics are more efficient when artificial intelligence is used to manage the process. Artificial intelligence (AI) can predict problems and automatically modify logistics in real time to optimize labour, transportation, and other problems (22-37). As a result, distribution and delivery are timely and less expensive (Distribution and Delivery). Artificial intelligence (AI) is already being used by businesses to provide customer service via online chat bots. In addition, businesses are training customer service representatives to use artificial intelligence (AI) to answer customer questions and solve customer problems (22-37).

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A study from the National Bureau of Economic Research that was conducted by MIT and Stanford University found that artificial intelligence (AI) improves customer service in three specific ways: (Customer Service). Customer service agents respond faster, resolve more problems, and answer more customers problems with artificial intelligence (AI) than without AI (22-37). Complaining customers treat customer service agents better when the agents use artificial intelligence (AI). Customers are less likely to demand to speak with a supervisor when agents use artificial intelligence (AI). Based on the research data, artificial intelligence (AI) improved customer service metrics like response time, problem resolution, and so on, which leads to lower costs and better results for businesses (22-37).

Artificial intelligence can also help marketers make better use of customer data to develop marketing campaigns that are more personalized and effective (22-57). Marketers can use artificial intelligence (AI) to track engagement with their brands' websites, social media, advertising, and more in order to develop campaigns that deliver better results in the future (Marketing) (22-37). The sales team can identify the best prospects more efficiently and accurately with the help of artificial intelligence (AI). In fact, artificial intelligence (AI) can also be used to power automated events across the sales pipeline (22-37). As a result, conversions will increase, sales will go up, and fewer opportunities will be lost (Sales). Data is power today, and artificial intelligence (AI) can help Cannabis businesses to gather and analyze data in a wide variety of ways. Competitive analysis, compliance and regulatory changes, operational performance, business opportunities, and more can be tracked to improve the decision-making and profitability (Business Intelligence) (22-37).

IV. Artificial intelligence (AI) in Plant Tissue Culture

In vitro germination of Industrial Cannabis sativa (hemp) is challenging due to low germination and high contamination rates (37-57). Successful establishment of *in vitro* sterilization is the prerequisite of plant tissue culture studies (37). Recent advancements in the field of artificial neural network (ANN) and machine learning (ML) algorithms open new horizons for sustainable and precision agriculture (23-37-57). Artificial neural network (ANN) and machine learning (ML) algorithms are powerful tools to evaluate the results and make more precise and high accuracy predictions in the field of plant tissue culture, especially for industrial purposes (37). Keeping in view, the study was designed and investigated the possible response of variable concentrations of hydrogen peroxide (H_2O_2) on germination and morphological traits of *in vitro*-grown hemp seedlings by using ML algorithms (37). Five different machine learning (ML) algorithms were used in this study and evaluated the prediction of the output variables : Support Vector Classifier (SVC), Gaussian Process (GP), Extreme Gradient Boosting (XGBoost), Random Forest (RF) models, and Multilayer Perceptron (MLP) neural network utilizing accuracy, F1 score, precision, and recall values (22-37). Among the tested models, the RF model exhibited better prediction of output variables with a high F1 score in the range of 0.98–1.00 (37). The F1 scores of the other models ranged between 0.69 and 0.86 (37). Response surface methodology (RSM) was used to compute the optimum concentration of H_2O_2 revealed the statistically significant effect of H_2O_2 on *in vitro* germination and seedling growth (37-57). The optimum value of H_2O_2 for the maximum germination and seedling was optimized to about ~2.2% by using RSM (37-57). This work is a case study about the application of different ML and ANN models in plant tissue culture and reveals the possibility of application in many other economic crops (22-57).

Predictions of in vitro conditions to refine growth responses were subsequently tested in a validation experiment and data showed no significant differences between predicted optimized values and observed data. Another parallel study demonstrated the potential of machine learning and optimization algorithms to predict the most favourable light combinations and sucrose levels to elicit specific developmental responses in the in vitro micropropation of Cannabis (55-57). Based on these, recommendations of light and carbohydrate levels to promote specific developmental outcomes for in vitro Cannabis are suggested (55-57). Ultimately, this work confirmed the importance of light quality and carbohydrate supply in directing plant development as well as the power of machine learning approaches to investigate complex interactions in plant tissue culture (55-57). This machine learning—assisted, multivariable micropropagation study has demonstrated that distinct growth responses in Cannabis can be shaped by changing the influences of sugar and light dynamics in the absence of PGRs (55-57). The development of alternative protocols to guide plant growth toward specific responses shows endless value for numerous in vitro applications (55). Finally, the results obtained from this experiment allowed to recommend GRNN-SOS to be a more efficacious algorithm to study dynamic plant responses to multivariable stimuli under in vitro conditions for the development of new methods, and optimization of current protocols (55-57).

The in vitro method used for growing plants is one of the most popular methods employed in plant biotechnology (37-57). Without in vitro techniques, plant micropropagation, androgenesis, gynogenesis, somatic embryogenesis, or the production of secondary metabolites would not be possible (37-57). Such processes can be studied because they take place in controlled conditions like strict sterility, defined temperature, light and humidity as well as on solid or liquid media of a scheduled composition (37-54). Although there are many biological processes that can easily be observed in plant tissue cultures, none of them are linear, and, moreover, they are influenced by many other factors as well (37-57). Thus, appropriate modeling can be

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applied to quite accurately predict and simulate the growth kinetics of the culture and also predict the resulting biomass (37-57).

Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in plant tissue cultures (37-57). First of all, Artificial neural networks (ANNs) can simulate the growth of plants under different in vitro conditions (37-57). Their usefulness has been confirmed in the estimation of biomass in plant cell cultures and the length of shoots in vitro, in the classification of somatic embryos, evaluation of the physical conditions of an in vitro environment, and in the prediction of optimal conditions for in vitro culture to achieve maximum efficiency and productivity (37-57). Secondly, with the help of various types of neural models, in vitro -regenerated plants are sorted, respectively, to their quality and likeliness of further development (37-57). Thirdly, Artificial neural networks (ANNs) are capable of predicting plant behaviour during in vitro rhizogenesis and subsequent acclimatization to ex vitro conditions (37-57).

V. Artificial intelligence (AI): Agriculture

Development in the agricultural sector with the application of artificial intelligence (AI) will boost the rural development, rural transformation and eventually resulting in the structural transformation (22-37). Surprisingly, agriculture particularly hemp cultivation though being the least digitized, has seen momentum for the development and commercialization of agricultural technologies (22-37). Artificial Intelligence (AI) has begun to play a major role in daily lives, extending our perceptions and ability to modify the environment around us (22-37). Agricultural robots targeted at diverse aspects in agricultural industry have been developed and improved greatly in the past years, and although pointing out the hardship of applying machines and algorithms tested in experimental environment to real environments (22-37).

Because of their versatility as well as amazing imaging technology which covers from delivery to photography, the ability to be piloted with a remote controller (22-37-57). This enables to do a lot with these devices, drones or UAVs (Unpiloted Aerial Vehicle) are becoming increasingly popular to reach great heights and distances and carrying out several applications (22-37). Therefore, image recognition and perception plays an important role in agriculture (22-37). Artificial Intelligence (AI) in agriculture can be applied which would automate several processes, reduce risks and provide farmers with a comparatively easy and efficient farming (22-37). Due to artificial intelligence, farming will be found to be a mixture of technological as well as biological skills in the near future which will not only serve as a better outcome in the matter of quality for all the farmers but also minimizes their losses and workloads (22-37). The emerging Artificial Intelligence (AI) technologies have helped the best selection of the crops and even have improved the selection of hybrid seed choices which are best suited for farmer's needs (22-37). It has been implemented by understanding how the seeds react to various weather conditions, different soil types (22-37). By collecting this information, the chances of plant diseases are reduced. Therefore, farmers are able to meet the market trends, yearly outcomes, consumer needs, thus farmers are efficiently able to maximize the return on crops. Artificial intelligence powered Chatbots, along with machine learning techniques has enabled farmers to understand natural language and interact with users in a way more personalized way (22-56). They are mainly equipped for retail, travel, media, and agriculture has used this facility by assisting the farmers to receive answers to their unanswered questions, for giving advice to them and providing various recommendations (23-56).

Robotics has played a substantial role in the agricultural production and management. The main purpose of coming up with this technology is to replace human labour and produce effective benefits on small as well as large scale productions (23-37). The robots are performing various agricultural operations autonomously such as weeding, irrigation, guarding the farms for delivering effective reports, ensuring that the adverse environmental conditions do not affect the production, increase precision, and manage individual plants in various unfamiliar ways (22-37). Therefore, robotic technologies has amplified productivity immensely. The manual irrigation which was based on soil water measurement was replaced by automatic irrigation scheduling techniques (22-37). Automatic plant irrigators are planted on the field through wireless technology for drip irrigation. This method ensures the fertility of the soil and ensures the effective use of water resource (22-37). The technology of smart irrigation is developed to increase the production without the involvement of large number of man power by detecting the level of water, temperature of the soil, nutrient content and weather forecasting (22-37). The actuation is performed according to the microcontroller by turning ON/OF the irrigator pump (22-37).

Recently one of the study developed the idea of efficient and automated irrigation system by developing remote sensors using the technology of Arduino which can increase the production up to 40% (22-37). In another parallel approach, different sensors were built for different purposes like the soil moisture sensor to detect the moisture content in the soil, the temperature sensor to detect the temperature, the pressure regulator sensor to maintain pressure and the molecular sensor for better crop growth. The installation of digital cameras, the output of all these devices is converted to digital signal and it is sent to the multiplexer through wireless network such as Zigbee and hotspot (22-37).

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Later researchers came with different sensors which were used to detect the need of water supply to the fields as soil moisture sensor and rain drop sensor, which were instructed through wireless broadband network and powered by solar panels (22-37). The rain drop sensor and soil moisture sensor informs the farmer about the moisture content in the soil through SMS in their cell phone using GSM module (22-37). Accordingly the farmer can give commands using SMS to ON and OFF the water supply. Hence this system will detect part or area in the fields which required more water and could hold off the farmer from watering when it's raining (22-37). The amount of irrigation needed can also be determined on the basis of the dielectric constant. Further, dielectric soil moisture sensors for real time irrigation control have been developed (22-37). The measurement method based on the dielectric properties is considered to be the most potential one. The installation of sensors plays an important role in the efficient implementation of irrigation robotics (22-37). One can use a single sensor to control the irrigation of multiple zones in the fields. And one can also set multiple sensors to irrigate individual zones (22-37). Before developing a weed control automated system is in need to differentiate between the crop seedlings and the weeds (22-37). The vision based technologies which were used to guide the robots along the row structure to remove weeds and to differentiate the single crop among the weed plants (22-37). Digital yield mapping and monitoring is also applied in agriculture. With the utilization of a Geographic Information System (GIS) programming, the yield determined at each field area can be shown (22-37).

The artificial intelligence (AI) technology will be useful to predict the weather and other conditions related to agriculture like land quality, round water, crop cycle, and pest attack, etc (22-37). The accurate projection or prediction with the help of the artificial intelligence (AI) technology will reduce most of the concerns of the farmers (22-37). Artificial intelligence (AI)-driven sensors are very useful to extract important data related to agriculture (22-37). The data will be useful in enhancing production. In agriculture, there is a huge scope for these sensors (22-37). The biggest challenge to farming is the crop damage due to any kind of disasters including the pest attack. Most of the time due to lack of the proper information farmers lost their crops. In this cyber age, the technology would be useful for the farmers to protect their cultivation from any kind of attacks (22-37).

Artificial intelligence (AI)-enabled image recognition will be useful in this direction (22-57). Many companies have implemented drones to monitor the production and to identify any kind of pest attacks (22-37). Artificial intelligence (AI) is likely to transform agriculture and the market in the next few years (22-37). The proper implementation of artificial intelligence (AI) in agriculture will help the cultivation process and to create an ambiance for the market (22-37). The autonomous robots improve efficiency, and also reduced the need for unnecessary pesticides and herbicides. Besides this, farmers can spray pesticides and herbicides effectively in their farms with the aid of drones, and plant monitoring is also no longer a burden (22-37).

VI. Plant Tissue Culture: Importance and Applications

Plant tissue culture is an in vitro aseptic culture of cells, tissues, organs or whole plant under controlled defined nutritional medium conditions often to produce the clones of plants (57-80). The resulting clones are true to type of selected genotypes and used for the large scale plant multiplication (57-135). The beauty of plant tissue culture lies in the culture of small piece of plant material (explant) on a defined nutrient medium to produce large number of plantlets or clones within a limited time in a continuous process, irrespective of season, and weather on year round basis (57-170). Plant tissue culture can be used for a wide range of purposes with various applications in research and industry. Tissue culture technique offers several advantages over plant propagation under natural conditions. 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 (57-167). Plant in vitro propagation using tissue culture techniques have been exploited for the commercialization of ornamental plants (orchids), vegetable and fruit plants (papaya, mango, and grape), medicinal, woody, and conifers with economically important products (57-170). Plant tissue culture is an important biotechnological technique solving the problems of modern agricultural providing solutions to major food security issues (57-160). Large-scale plant tissue culture has been shown to be an appealing alternative approach to traditional plantation methods since it provides a regulated supply of defined nutrients (including carbohydrate) independent of plant availability (57-168).

Tissue culture can be used to propagate perennial woody species, orchids, endangered and threatened plant species irrespective of weather or season (57-125). This also helps to accelerate the production process of new crop varieties with superior traits as tissue culture experiments required less time and space compared to in-vivo plant growth (57-145). It also helps in the development of pathogen-free micro-plants saved from various diseases and the new plants produced by tissue culture under aseptic conditions are also disease free plants (57-160). In vitro micro-propagation is a valuable technology since many secondary plant metabolites cannot be manufactured chemically. Various plant tissue culture systems have been extensively studied to improve and enhance the production and quality of plant metabolites produced by the medicinal plants. In vitro plant micropropagation is a powerful technique in order to acquire plant extracts with various commercial applications than using whole plants (57-165).

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Tissue culture technique depends mainly on the concept of totipotentiality of plant cells, which refers to the ability of single cell to express the full genome by cell division. The totipotency of somatic plant cells is a specific and scientifically exciting phenomenon, which is based on the developmental program of plants (57-175). It can be best demonstrated in an *in vitro* system where somatic plant cells can regain their totipotency and are capable of forming embryos through the developmental pathway of somatic embryogenesis (57-175). Under *in vitro* conditions, one or a few somatic cells of the plant or explants have to be competent to receive a signal (endogenous or exogenous). This then triggers the reprogramming of plant cells into the pathway of embryogenic development (commitment) leading to somatic embryo formation (57-168). The controlled conditions provide the culture of explants on a defined nutrient medium with the source of carbohydrate in an environment conducive for their growth and multiplication (57-160). These conditions include proper supply of nutrients, source of carbohydrate, pH of the medium, adequate temperature and proper gaseous and liquid environment (57-168).

Plant tissue culture is a technique in which fragments of tissues from a plant (explants) are developed in vitro in an artificial medium under aseptic conditions (57-160). It involves culturing explants (such as shoot tip, root tip, callus, seed, embryo, pollen grain, ovule or even a single cell) isolated from mother plant on a sterile defined nutrient medium which leads to cell multiplication and plant regeneration (57-165). Several methods are available for plant tissue culture. In organogenesis, the commonly used method, organ formation can occur directly from meristems, or indirectly from dedifferentiated cells (callus). The resultant cultures can then be utilized to mass produce plants (micro propagation) or to develop specific organs (e.g., roots in hairy root culture) (57-160).

There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy (57-180). Plant tissue culture plays a significant role in basic research in the areas of plant pathology, plant physiology, plant metabolites and conservation (57-179). Due to advancement in contemporary techniques, several protocols have been developed for the production of a wide variety of plants secondary metabolites on a commercial scale (57-168). Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become the major commercial importance in the area of plant propagation system, disease elimination, plant improvement, secondary metabolite production and genetic transformation (57-170).

Plant tissue culture coupled with molecular biological approaches leads towards sustainable agricultural development providing solutions to major food security issues (57-156). Plant tissue culture plays an important role in plant biotechnology due to its potential for massive production of improved crop varieties and high yield of important secondary metabolites. Several efforts have been made to ameliorate the effectiveness and production of plant tissue culture using biotic and abiotic factors. Several endangered, rare, threatened, and commercially important plant species have been tissue cultured and successfully micropropagated for large scale production (57-160). In addition to this plant tissue culture is an efficient technology for the production of somaclonal and gametoclonal vatiants (57-168). The micropropagation technology has a vast potential to produce plants of disease resistant and plants of superior quality, well adapted high yielding genotypes with better stress tolerance capacities (57-179).

Micropropagation via in vitro culture technique has several advantages over the traditional methods of propagation through seed, cuttings, grafting and air-layering etc. Plant micropropagation, also known as plant tissue culture, is a technique that isolates, sterilizes, and incubates cells, tissues, or organs of chosen plants in a growth-promoting aseptic environment to create a large number of plantlets (57-180). The isolated cloning technique revealed that, given the right conditions, somatic cells may develop into a complete plant (57-178). The sterile or endangered flora can also be conserved by plant micropropagation methods. Hence, plant tissue culture is an extremely efficient and cost-effective technique for biosynthetic studies and bio-production, biotransformation, or bioconversion of plant derived compounds (57-178). However, there are certain limitations of in-vitro plant regeneration system including difficulties with continuous operation, product removal, and aseptic conditions. For sustainable industrial applications of in vitro regenerated plants on a large scale, these constraints need to be addressed in future studies (57-180).

In vitro micropropagation process is a rapid process that can lead to the production of plants of virus free. In plant cell culture, plant tissues or organs are grown under in vitro fully defined nutrient medium under aseptic conditions and controlled environment (57-178). Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic compounds and plant growth regulators, carbon source and some gelling agents of solid medium (57-170). There are many mediums used for the in vitro culture and one common medium is the MS medium (57-170). The Murashigeand Skoog (MS) basal medium supplemented with the required amounts of plant hormones which include auxins, cytokinins, abscisic acid, gibberellins, smoke saturated water, ethylene, and growth regulators with similar metabolic effects (57-178). Plant micropropagation with different explants like seeds, embryos,

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calli, anthers, protoplasts, and meristemetic tissues of root/shoot tips is used for large-scale production of industrial products (57-179).

The pH of the nutrint medium (5.8) is also important that affects both the growth and activity of plant growth regulators (57-170). Plant growth regulators (PGRs) plays an important role as signal molecules and regulators of growth and development in plants. An endogenous auxin pulse is one of the first signals leading to the induction of somatic embryogenesis (57-150). Auxin (IAA, IBA, NAA and 2,4-D) and cytokinins (BA, TDZ, 24-Epibrassinolide, Melatonin, Smoke saturated water, BAP, Kinetin) are the main PGRs in plants involved in the regulation of cell division and differentiation (57-160). The higher concentrations of auxins generally favours root formation, where as higher concentration of cytokinin favours shoot formation (57-170). Endogenous PGR levels, however, can be considered as major factors in determining the specificity of cellular responses to these rather general stress stimuli. In addition to the absolute requirement of exogenous auxins for sustained growth in *in vitro* cultures, plant cells may produce substantial amounts of the native auxin, indole-3- acetic acid (IAA) (57-175). A balance of both auxin and cytokinin some times leads to the formation of unorganized mass of cells known as callus (57-170).

Plant tissue culture has many commercial applications and can be applied for the production of disease resistant plants either via organogenesis or somatic embryogenesis. In case of organogenesis, the explants of selected plant produces plants via callus formation. Organogenesis is the production of plant organs from a specific tissue in order to develop complete plants (57-180). It is characterized by being polar, which means that just one aerial organ or root is released and a new complete plant is generated from this. Simultaneously, organogenesis can be direct in which the organogenic shoot is produced directly from the explants, or indirect, in which the organogenic process happens from previously created callus in the original explants (57-166). Callus is an undifferentiated mass of tissue that forms explants after a few weeks on growth medium with appropriate hormones. Callus development is the result of a well-known process known as de-differentiation or re-differentiation. To stimulate callus induction and development, several growth hormones are employed. Callus induction and development were increased by 2,4-D, NAA, and kinetin. Organogenesis allows for the effective regeneration of new plants from callus (57-178).

Somatic embryogenesis is the process of producing embryos from somatic plant cells (any non-sexual cell) in order to produce a whole plant. In contrast to organogenesis, this is a polar process in which the aerial structures and roots of plants develop from the somatic embryo (57-140). It can either be direct or indirect, depending on whether the process begins with the original explants or with previously produced callus. On the other hand, in case of somatic embryogenesis, the explants cultured under in vitro conditions produces callus tissue (57-146). The cells of callus tissue are programmed towards somatic embryogenesis and produce somatic embryos. There are few specific examples where explants produced somatic embryos directly without callus formation. The somatic embryos on germination medium germinate to produce shoot and roots. This can be applied in the conservation of endangered/rare threatened plant species (57-170). Clonal propagation of high-value forest trees, medicinal plants, endangered /threatened plant species through somatic embryogenesis has the potential to rapidly capture the benefits of breeding or genetic engineering programs and to improve the uniformity and quality of the nursery stock (57-160). The greatest value of plant cell/ tissue culture rests not so much on their application to mass clonal propagation (micro propagation), but also in their involvement in plant improvement and bio-processing (57-170).

Embryo culture has fertilized or unfertilized zygotic (seed) embryos dissected from maturing seeds or fruits and cultivated in vitro until seedling formation (57-178). Embryo culture is not the same as somatic embryogenesis. In case of conifers, the use of zygotic embryos as explants has several disadvantages including heterogeneity as a result of cross pollination, which may result in the new generation having characteristics inferior to those of the parent (seed-bearing) tree (57-139). The disadvantages associated with zygotic explants may be overcome if mass propagation of elite, mature trees can be achieved from vegetative tissue explants, such as secondary needles or apical shoots, because the regenerated plantlets will be uniform and possess elite characteristics from clearly defined parents. The induction of somatic embryogenesis using shoot apical thin layers has been successful in few conifers (*Pinus roxburghii, Pinus kesiya, Pinus patula,* Scots pine and *Pinus pinea)* (57-140).

In some cases, inter-specific and inter-generic hybrids can be obtained using embryo rescue technique which is not possible through conventional methods. Plant tissue culture coupled with biotechnological approaches is applicable to the development of genetically modified plants as well as embryo rescue procedures (57-140). Tissue culture has been extensively utilized in breeding programs and the hybrids of such crosses are often sterile due to embryo abortion but can be 'rescued' by means of culturing or transplanting the embryos (57-150). The medium utilized for cell culture can be optimized for the production of desirable products. As a result, plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants. Plant tissue culture is also used to help save endangered species, as many therapeutic plants are on the verge of extinction due to overuse. The development of plant tissue culture techniques will expand the long-term use of therapeutic plants in the future (57-150).

Shoot tip culture is developed from excised shoot tips/buds larger than the shoot apices (used for meristems cultures), and had several leaf primordia. These shoot apices are often cultivated such that each one generates several shoots. Lateral bud

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node culture is carried out on a short piece of stem tissue where stem portions carrying single or many nodes may be cultivated. Each bud is cultivated to produce a single shoot. In isolated root culture, a branching root system can be generated by growing roots that are not attached to shoots (57-140).

The second application of plant tissue culture is the cryopreservation (107-108). Cryopreservation is the successful storage of biological materials at ultra low termperatures -196^0 C (107-108). The risk of loosing the plant material due to human or accidental error or undesired genetic changes is always present. To reduce the costs and risk, cryopreservation is a valuable method for long term preservation of plant material (107-108). Cryopreservation of embryogenic tissue has generally been considered as a means of avoiding the loss of embryo maturation potential during long-term *in vitro* culture of evading possible somaclonal variation caused by the long-term maintenance of actively growing embryogenic cultures, and of storing a large number of genotypes. Similarly, cryopreservation provides a means of storing genetically altered material while field tests are conducted (107-108). It may also limit the amount of contamination and somaclonal variation resulting from routine subculturing of callus tissue. Furthermore, several plant species generate resistant seeds that cannot be retained for extended periods of time. Hence in this situation, tissue culture can be utilized for plant conservation in vegetative state, generally under slow growth conditions or for cryopreservation (107-108).

A third powerful tool in plant biotechnology is genetic transformation in order to transfer relevant genes from bacteria, fungi, animals or plants into plants of interest (120-139, 141). The most recent feature of plant cell and tissue culture is genetic transformation, which allows the transfer of genes with desirable traits into host plants and the recovery of transgenic plants (120-139, 141) This approach offers a high potential for the development of agricultural plants with novel traits. The genetically modified plants exhibit agronomically significant features such as greater yield, improved nutritional quality, improved pest and disease resistance (120-139).

Plant tissue culture plays a pivotal role in vector mediated or vector-independent gene-delivery into plant genome for the production of transgenic plants with improved traits (120-139, 141). One important aim of genetic manipulation of plants is the increase of resistance against various fungal pathogens. The recent development of molecular tools for genomic analysis of plant species makes it possible to transfer or identify genes controlling agronomic traits (120-139, 141). However, both the mechanisms for DNA transfer to a plant cell and targeting of the DNA to a complex tissue or organ competent for regeneration is another major issue to be considered for effective and successful transformation (120-139, 141). Now-a-days there are many genes available for use in plant transformation experiments. However, most of these genes have been used as reporter genes for establishing a model transformation system, and very few have been used for novel phenotypes or for tolerance to various stresses (120-139, 141). Selectable marker genes have been pivotal to the development of plant transformation technologies because the marker genes allow scientists to identify or isolate the cells that are expressing the cloned DNA, and to select for the transformed progeny (120-139, 141). Different transcription factors which regulate nutrient assimilation pathways have been over expressed in staple crops that may improve crop yield. There are three methods of genetic transformation, 1) Biolistic method and 2) Agrobacterium method, 3) Protoplast method (120-139, 141).

Biolistic or particle bombardment is used as a direct gene transfer method without using a vector for plant transformation, and relies entirely on physical or chemical principles to deliver foreign DNA into the plant cells. In this method, there is no dependence on bacteria, so the limitations inherent in organisms such as *A. tumefaciens* do no apply (120-139, 141). Plant transformation method by *A. tumefaciens*, soil pathogenic bacterium, has become the most used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants under in vitro conditions. Various transgenic plants including Arabidopsis, wheat and tobacco are developed through genetic engineering and plant tissue culture conferring resistance against different environmental stresses (120-139, 141).

In the last two decades, the technique based on *Agrobacterium rhizogenes* inoculation has gained popularity as a means of creating secondary metabolites generated in plant roots (120-139, 141). Organized root cultures can contribute significantly to the generation of secondary metabolites. Hairy root disease is caused by *Agrobacterium rhizogenes* in plants (120-139, 141). The neoplastic (malignant) roots, generated at rapid growth rate, by *A. rhizogenes* infection are genetically stable and are developed in hormone-free conditions (120-139, 141). Hairy roots high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites. Hairy roots have high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites (120-139, 141).

Protoplasts are plant cells with cell walls removed by enzymatic or mechanical methods (106). Protoplasts are obtained by immersing plant cells in a hypertonic solution, which causes the plasma membrane to shrink off the cell wall due to water efflux (106). Cell wall may be removed using either enzymatic digestion with pectinase and cellulose, or by mechanical techniques (106). Plant protoplasts are the important ideal tools for genetic manipulations such as gene transfer, mutation breeding and somatic hybridization (106). Protoplast culture when coupled with an efficient protocol for gene delivery and plantlet regeneration serves as an excellent system for the recovery of transgenic plants. Various crops with superior traits have

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been developed using this technology with enhanced nutritional value and biotic/ abiotic stress resistance that leads to increased crop yield (106). Conventionally, somatic hybridization, which produces interspecific and intergeneric hybrids, was commonly used as an essential method for plant breeding (106). The procedure entails the fusion of two somatic protoplasts followed by the selection of desirable hybrid cells and then regeneration of hybrid plants (106). Protoplast fusion is an effective method of transferring genes with desirable traits from one species to another with a great impact on crop development (106). Somatic hybrids created from rice and ditch reed by electrofusion exhibited better results against salt stress (106).

The fourth application of plant tissue culture is the synthesis of synthetic seeds under in vitro conditions (103-105). Synthetic seed production is a well documented applied technology that capitalizes on the capacity for rapid plant multiplication via somatic embryogenesis (103-105). Synthetic seed is also defined as a somatic embryo, or any other vegetative propagule, with an artificial, biodegradable coating entrapped in a nutrient medium supplying carbon sources, mineral nutrients, vitamins and growth regulators (103-105). The entrapping matrix or coating should not have any adverse effect on the embryo or propagule and should allow germination under both in vitro or ex vitro conditions (103-105). The term therefore, reflects the artificial nature of the seed coat as well as somatic origin of the encapsulated propagule (103-105).

The fifth application of plant tissue culture is the anther culture. Anther culture technique is the most viable and efficient method of producing homozygous doubled haploid plants within a short period (227-228). Agricultural activity demands improved crop varieties with desirable traits such as quality, crop yield, and resistance to environmental stresses. However, the practical application of this technology in plant improvement is still limited by various factors that influence culture efficiency (227-228). Therefore, the improved anther culture method can produce doubled haploid plants for which can be useful in different breeding programs that will enable the speedy development of commercially important plant varieties for resource-poor farmers (227-228). Although conventional breeding approaches have substantially enhanced cereal productivity, and their progress rate is slow. Thus, there is a need to respond quickly and make rapid changes to drastically reduce breeding cycles to sustain the crop under unpredictable environmental conditions (227-228). The application of anther culture along with advanced biotechnological approaches could hasten the process to develop superior breeding materials or varieties by decreasing the number of breeding cycles dramatically. Anther culture technique is a biotechnology tool that has found importance and a niche in expediting many crop breeding processes (227-228). Therefore, in the future, the prospects for anther culture and the integration of genomics tools provide novel opportunities for improving selection efficiency, maximizing genetic grain, and improving varietal development, leading to the early release of rice varieties with desirable traits (227-228).

Use of haploids has emerged as a key strategy for crop improvement. Haploids having a single set of chromosomes in the sporophytic phase have become a valuable source to screen for desired traits or to introduce a mutation in their genetic content (227-228). Furthermore, doubled haploids (DHs) can be obtained by spontaneous or induced chromosome doubling (25-190). Double haploids are homozygous at all loci, and they can be propagated through seed. Doubled haploids (DHs) achieve complete homozygosity in a single generation (227-228). On the contrary, the conventional breeding method requires six to seven generations of self-crossing. In vitro production of haploids for crop improvement has been successfully achieved in many crops such as rice, wheat, barley, maize, tomato, potato, brassicas, grapes, sunflower and so on (227-228). In addition to crop improvement, doubled haploids (DHs) are an excellent source for gene mapping, cytogenetic research, and evolutionary studies. Various agronomic crops i.e., cereals, fruits, vegetables, ornamental plants and forest trees are currently being used for in vitro propagation (227-228).

The sixth application of plant tissue culture is the production of nanoparticles (157-162). Nowadays, the addition of nanoparticles as elicitors has, for instance, gained worldwide interest because of its success in microbial decontamination and enhancement of secondary metabolites (157-162). Nanoparticles are entities in the nanometric dimension range. 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 (157-162). Therefore, the evaluation of the integration of nanotechnology with plant tissue culture is a new advancement of plant biotechnology (157-162). The highlight is especially conveyed on secondary metabolite enhancement, effects on plant growth and biomass accumulation as well as their possible mechanism of action. In addition, the use of nanomaterials as potential therapeutic agents is gaining interest worldwide. 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 (157-162).

The seventh application of plant tissue culture is the mass in vitro propagation of medicinal plants for the isolation of secondary metabolites of pharmaceutical interest (120-139, 141). In vitro cell culture has the inherent advantage of producing therapeutic proteins such as monoclonal antibodies, antigenic proteins that act as immunogens, human serum albumin, interferon, immuno-contraceptive proteins, antihypertensive drug angiotensins, and human haemoglobin in certain situations (120-139, 141). The manufacturing of pharmaceuticals using culture systems of plants can provide remarkable benefits including cost reduction,

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quick production, and scalability. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites (120-139, 141).

Tissue culture technology advancements showed that transcription factors are effective new molecular tools for plant metabolic engineering to boost the synthesis of important chemicals. The in vitro plant propagation has not only made a significant contribution in the knowledge of basic research, but it also offers potential applications as it guarantees a sustainable industry that relies on commercial production of plant-derived compounds. Alkaloids are the structurally diverse group of secondary metabolites which possess significant biological activities (120-139, 141). Plants make them as a defence mechanism in response to biotic and abiotic stressors. Plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants. Plant tissue culture is also used to help and save the endangered species, as many therapeutic plants are on the verge of extinction due to overuse (120-139, 141).

The culture of plant tissues is an effective instrument for the isolation and processing of active compounds, including secondary substances and engineered molecules, from economically important plants. Plant tissue culture technique is also used for the micro propagation of medicinal plants in pharmaceutical industry for the isolation of bioactive phytocompounds which has numerous industrial applications. It provides potential benefits for different industries which include food, pharmaceutical and cosmetics (120-139, 141). Plants are the rich source of phytochemicals with medicinal properties rendering them useful for the industrial production of pharmaceuticals and nutraceuticals. Furthermore, there are numerous plant compounds with application in the cosmetics industry. Cosmetic extracts derived from plant cell cultures suit the markets increasingly stringent safety requirements. Plant cell culture cosmetic production is not dependent on appropriate seasonal conditions. Hence, it requires less time and energy. In addition to being free of pathogens, pollutants, and pesticide residues, plant cells generated under aseptic laboratory conditions rarely include any malignant compound or potential allergen, which would otherwise destroy the majority of the plant extracts obtained. Plant tissue cultures are a perfect source of safe and pure components for cosmetic goods since they can be cultivated under controlled conditions with minimum possibility of pathogen or environmental contamination. Utilizing various extraction techniques and solvents while taking advantage of the chemical makeup of plant cell components, plant tissue culture technology allows the isolation of many active ingredients from a single cell culture. Plant cell cultures are now being used for the production of cosmeceuticals, products having cosmetic as well as therapeutic (medical or drug-like) effects that exert beneficial effects on skin health. Plant cell culture extracts with several particular actions for skin care, make-up, and hair care as supplement components are gaining popularity in the cosmetics sector(120-139, 141).

In addition to having moisturizing, anti-ageing, anti-wrinkle effects; plant-derived compounds also possess pharmacological properties such as antiviral, antimicrobial, antifungal, anticancer, antioxidant, anti-inflammatory, and anti-allergy characteristics (120-139, 141). The in vitro propagation of industrially significant flora is gaining attention because of its several advantages over conventional plant propagation methods. One of the major advantages of this technique is the quick availability of food throughout the year, irrespective of the growing season, thus opening new opportunities to the producers and farmers (120-139, 141). The extensive research on plant cell culture has caused a surge in the use of this technique in the pharmaceutical industry. Plants are abundant sources of pharmaceutically significant compounds. However, there is a need to manufacture these compounds within stringent laboratory conditions (120-139, 141). Various secondary metabolites having medicinal values can be obtained from plant cell culture. An extensive investigation is being done to investigate the plant sources producing active ingredients, such as antioxidants, ingredients with antimicrobial, anti-viral, anti-cancerous, antifungal, anti-inflammatory, and anti-allergy properties along with moisturizing, anti-ageing, anti-wrinkle and UV protective properties, which are crucial to cosmetics industry (120-139, 141). Most of the phytochemicals such as polyphenols, phenolics acids, triterpenes, flavonoids, stilbenes, steroids, carotenoids, steroidal saponins, sterols, fatty acids, polysaccharides, sugars, and peptides are extracted with relevant solvents and utilized as an active constituents in cosmetic preparations (120-139, 141).

In vitro suspension cultures are created when friable calli are grown on liquid medium in a suitable container and regularly agitated to provide free cell suspension. Conical flasks are utilized because of its enormous surface area, which aids in the retention of liquid medium and the constant exchange of gases. Suspension cultures are classified as batch or continuous cultures (120-139, 141). At regular intervals, a part of the original cell suspension is collected and sub-cultured on to fresh medium in batch cultures. In continuous cultures, new media is introduced to the same culture on regular basis, and surplus cell suspensions are discarded. Suspension cultures are commonly utilized in large-scale synthesis of secondary metabolites (120-139, 141). Cell suspension culture methods are currently being employed for large-scale plant cell culture from which secondary metabolites are extracted (120-139, 141). A suspension culture is created by moving the comparatively friable component of the callus into liquid media and maintaining it under appropriate physical conditions of aeration, agitation, light, temperature, and other physical factors. Cell cultures not only produce defined standard phytochemicals in huge quantities, but they also reduce the presence of interfering substances found in field-grown plants (120-139, 141). The primary benefit of cell cultures is the production of bioactive secondary metabolites in a controlled environment that is independent of climate and soil conditions (120-139, 141).

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Chemostat bioreactors are the devices particularly built for large-scale continuous culturing (120-139, 141). Recent breakthroughs in plant cell culture, molecular biology, enzymology, and fermentation technology indicated that these systems are a viable source of synthesis of important secondary metabolites (120-139, 141). Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention. Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention (120-139, 141). Plants are used to treat and prevent particular illnesses and diseases in human beings since long time ago. Plants which possess healing metabolites with useful pharmacological effects are referred to as medicinal plants. They are rich in phytochemicals which have the spectacular capability to treat diseases and may be used for the industrial production of pharmaceuticals and nutraceuticals. Applications of plants/flowers extracts in cosmetics are significant which include skin moisturizing, whitening or tanning products, sunscreens, radical-scavenging antioxidants, immune stimulants, and skin thickeners etc (120-139, 141).

Plant micro-propagation has extensively been used to have an insight into plant pathology studies which include factors influencing penetration, infection and multiplication of pathogens, the nature of irregular cell division or growth, and the morphogenetic potential of the diseased cell (57-167). The employment of plant tissue culture in plant pathology is not only restricted to use plant tissues as a substrate for the pathogens, but it provides the basic understanding of various characteristics of pathological growth, pathogens' attack weapons, and the host response to an infection caused by the invading organism (57-175). Plant physiology and plant morphogenesis requires the capability to grow plants in vitro that might be best accomplished with plant tissue culture procedures (57-170). Furthermore, the conservation of plant biodiversity is indispensable for future crops safety due to increasing challenges of biotic and abiotic stresses. In this regard, in vitro techniques permit improvement in various traits associated with plant growth and yield that can later be used for ex-situ conservation (57-170). The active plant compounds obtained from rare or endangered species can be manufactured by in vitro techniques without adverse environmental effects and in agreement with the bio- sustainability matters that the market demands (57-170).

Plant tissue culture is a powerful tool of agricultural improvement and offers tangible solutions to major crop problems that arise due to constant threat of biotic and abiotic stresses minimizing the crop yield (57-160). Since plant tissue culture is simple, low-cost and environment friendly, it is imperative to employ this technique for the development of sustainable agriculture in order to meet the food demand of increasing human population. Agricultural diversification to satisfy our future demands necessitates the implementation of innovative agricultural technology. The finest cultural methods, excessive fertilizers, and pest control procedures will not yield the desired results unless the best planting material is used. Tissue culture is now widely being used as a viable horticultural propagation. technology, and it has changed the horticultural business (57-170). This approach is used to achieve mass proliferation and the creation of disease-free stock material. Commercial laboratories are now using tissue culture protocols that minimize somaclonal variations, and working on creating accurate screening and selection approaches for early detection of off-types (57-175). Although somaclonal variation is deleterious to quick clonal multiplication, some off-types have been discovered that have significant agronomic utility (59-170). In this regard, plant tissue culture methods hold enormous promise. In the future, plant tissue cultures might not only be a source of novel chemicals with uncharted biological actions, but they might also work as alternative recombinant protein bio-factories, particularly for those whose expression might be problematic or constrained in fermenting microbes. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites (57-170).

In vitro culture is a method applied for the growth and development of plant cells, tissues, and organs that uses a nutritive culture medium under controlled sterilized conditions (209). This method is considered as one of the most promising and environmentally friendly biotechnological practices for the sustainable supply of biofuels (57-190, 209). There are three main in vitro culture systems including organogenesis (e.g., embryogenesis, direct and indirect shoot regeneration), rhizogenesis, and callogenesis (57-178, 209). Among these methods, callogenesis can be considered as a robust method for biofuel production. The main advantages of callus culture are- The callus is generally defined as an irregular bulk of parenchymatous tissue with meristematic cells that are broadly used for the production of different bioactive plant molecules (57-176, 209). Genetic manipulation of callus for lignin engineering through transient gene transformation is much easier than other methods due to the lack of need for transgenic plant regeneration (60-175, 209). Somaclonal variations, which are usually seen in callus culture, can result in changes to metabolic pathways and even allows the production of new metabolites (209). Any phenotypic variation during callus culture is referred to as somaclonal variation that can be a result of RNA interference, histone modification, chromatin remodeling, DNA methylation, and spontaneous mutation (209). Callus culture can be easily scaled up in different bioreactor systems. Callus culture is considered a sustainable and eco-friendly process (57-189, 209). These callus cultures of many medicinal plants were also used for the production of thin films as biodegradable **biopolymer composite** in food packaging industries (215-226).

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The limitations of plant tissue culture methods include difficulties with continuous operation, product removal, and aseptic conditions (57-200). 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 (57-180). 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 (57-167). This is attributed to somaclonal variation in production of clones and low secondary metabolite titers. In contrast to these detrimental consequences, its use in crop improvement through the development of new variations is widely recognized. The expense of culture material, electricity, and labour are other issues with in vitro tissue culture cultivation (60-190). Somaclonal differences that occur during in vitro propagation, commercial phytochemical synthesis, or genetically modified plants can have significant economic consequences are the major impediment to the practical application of plant tissue culture techniques for the production of active metabolites (60-190).

VII. Cannabis sativa: Plant Tissue Culture Updates

Plant tissue culture techniques are the most frequently used biotechnology tools ranging from basic to applied investigation purposes in plant sciences (57-211). Chemical and hormonal control of regeneration, basic and applied aspects of organogenesis and somatic embryogenesis, micropropagation and production of virus-free plants, haploid plants, production of secondary metabolites, and large-scale cell cultures in bioreactors are few landmarks and notable discoveries in plant tissue culture research (57-211). There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy (57-211).

According to the limited literature survey, Cannabis is considered as recalcitrant under in vitro conditions and available protocols are inconsistent (163-211). There are no reproducible in vitro protocols available for Cannabis (163-211). A detailed in vitro study is lacking due to strict prohibition surrounding Cannabis plant (163-211). Another point is that even the in vitro micropropagation protocol is owned by the private companies and not available for public (163-211). Most experienced Cannabis companies have developed tissue culture and micropropagation techniques over the last two decades (163-211). Most achievements in this in vitro field are held as a trade secret because of the competitive advantage provided within the industry (163-211). Although there are multiple reports on shoot proliferation via micropropagation, there are fewer scientific reports showing regeneration of a full plant through de novo regeneration (163-211). The majority of regenerated strains and cultivars were monoecious, with few dioecious lines (163-211). Recently, the optimization of a micropropagation and callogenesis protocol was reported for a few medical Cannabis genotypes (163-211). Limited study has been reported since the first report of in vitro cell culture in Cannabis, the available protocols are inconsistent. In vitro regeneration of a Cannabis plant from a single cell is still a challenge (163-211). Thus, the multi-billion dollar Cannabis industry needs an optimized tissue regeneration protocol for both industrial and medical Cannabis. Cannabis spp. have gained a wide reputation for being recalcitrant to tissue culture (163-211). At the beginning of the 1970s, along with the conventional propagation system, in vitro cultures of Cannabis were initiated (163-211). The majority of the earlier in vitro studies were focused on Cannabis callus culture to produce cannabinoids (163-211).

Recently one of the study reported the propagation of axillary shoots of Cannabis sativa L. using liquid medium in temporary immersion bioreactors (210). The effect of immersion frequency (3 or 6 immersions per day), explant type (apical or basal sections), explant number (8, 10, and 16 explants), mineral medium (Murashige and Skoog half-strength nitrates, β-A and β-H, all supplemented with 2-μM metatopoline), sucrose supplementation (2, 0.5, and 0% sucrose), culture duration (4 and 6 weeks), and bioreactor type (RITA® and PlantformTM) were investigated (210). This protocol has been standardised for the proliferation of Cannabis apical segments in RITA® or PlantformTM bioreactors (210). The explants (8 per RITA® and 24 per PlantformTM) are immersed for 1min, 3 times per day in β-A medium supplemented with 2-μM metatopoline and 0.5% of sucrose and subcultured every 4 weeks (210). This is the first study using temporary immersion systems in *Cannabis sativa* production, and results have provided new opportunities for the mass propagation of this species (210). This method provides a simple and efficient in vitro propagation system for the large-scale multiplication of Cannabis, which shortens the culture period and reduces the use of sucrose. Beatriz, Moniek, and Mati genotypes were successfully cultured in liquid medium using commercial RITAR temporary immersion bioreactors and PlantformTM, which showed promising results in the two genotypes in which was tested (Beatriz and Moniek) (210).

In vitro regeneration of Cannabis callus tissue has been used in the production of biofuel (209). The novel use of industrial hemp callus resulted in a high-quality biocrude mainly comprised of ketones and alkenes (209). This is mainly due to somaclonal variations that occurred throughout the callogenesis process (209). The yield of light gasoline, heavy gasoline, and biodiesel for PL, under optimal conditions, were 3.17%,11.1%, and 36.03%, respectively (209). HTL biocrude production from industrial hemp using this plant tissue culture method offers the opportunity to integrate advanced plant agriculture and bioenergy

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to create a circular economy (209). The fifth generation of biofuel has the following advantages: First, genetic manipulation of callus enables us to engineer the lignin content (209). Second, the engineered calli samples produce high-quality biofuel (209). Finally, callus cultures have no known negative impact on ecosystems and the environment, by avoiding competition for arable land and the food and feed industry (209).

Hemp (Cannabis sativa) growers are interested in micropropagation as an alternative to propagation by stem cuttings from stock mother plants, which has several limitations (169). The development of a reliable micropropagation protocol for hemp has been problematic as a result of the occurrence of hyperhydricity of shoots during stage 1 and culture decline or the inability of shoot cultures to maintain high-quality growth for an extended period of time during stage 2 (169). Micropropagation of hemp (Cannabis sativa) is constrained by problems with hyperhydricity and culture decline of microshoots (169). These problems can be reduced by increasing agar and nutrients in the media during micropropagation stages 1 and 2, respectfully (169). Performance of microshoots of 'Abacus' and 'Wife' hemp cultured in Driver and Kuniyuki Walnut medium (DKW) for 15 weeks (6 weeks of stage 1 + 9 weeks of stage 2), with subculturing every 3 weeks during both stages 1 and 2, or in Murashige and Skoog with vitamins medium (MS) for 6 weeks (stage 1) followed by Lubell-Brand Cannabis medium (LBC) for 9 weeks (stage 2), with subculturing every 3 weeks during both stages 1 and 2, was evaluated (169). In a separate study, microshoot performance of 'Abacus' and 'Wife' in MS for 3 weeks (stage 1) followed by LBC for 6 weeks (stage 2), with subculturing every 3 weeks, using boxes (Magenta GA-7) with lids featuring a vent with a diameter of 10 mm and a pore size of 0.2 mM or using microboxes (Sac O2 O95/114 + OD95) with lids featuring a filter (Sac O2 #10) were evaluated (169). Shoot multiplication rate (SMR) and explant height were greater for 'Abacus' in LBC than DKW. For 'Wife', SMR at 9 weeks was greater in LBC, as LBC provided more nutrients and water than cultures had received in MS initially during stage 1 (169). Culture medium did not influence ex vitro rooting success, which was 75% for 'Abacus' and $ 90% for 'Wife' (169). Microboxes resulted in greater hyperhydricity of shoots and a lower ex vitro rooting percentage than boxes (169). For cultivars that are highly prone to developing hyperhydricity, like 'Abacus', the microboxes were not adequate to control this condition (169).

Tissue culture procedures for shoot development in drug varieties of *Cannabis sativa* L. (marijuana or cannabis) from meristems or nodes followed by rooting and acclimatization were investigated in seven strains (189-195). **Sodium metasilicate** and **silver nitrate** are novel compounds to tissue culture of Cannabis (189-195). Sodium metasilicate improved the visual appearance of the leaflets and both sodium metasilicate and silver nitrate improved the rate of rooting (189-195). Contamination of nodal explants by bacteria, fungi and yeasts was high but shoots derived from meristems had no contaminants (189-195). Explants from leaves and petioles were tested for callus induction using various medium formulations (189-195). Callus induction was most successful on multiplication medium without charcoal (189-195). No regeneration or somatic embryos from callus were observed in this study (189-195). Meristems and Nodal explants were able to produce shoots on multiplication media but propagation of Cannabis through direct or indirect regeneration is strain-dependent and influenced by microbial contaminants and frequency of rooting (189-195).

Cannabis companies always monitor the THC and CBD content of their products. Issues with somaclonal variation affecting the cannabinoid content of plants arising from tissue culture will be caught but unless there is long term research done on variation arising over time, there is a chance changes will not be caught until the batch has been harvested (189-195). Follow up research on somaclonal variation over time especially from tissue cultures arising from callus would benefit a commercial tissue culture system (189-195). While Tissue culture is possible in Cannabis and has been demonstrated to produce healthy plants, it may be some time before it plays a major role in the industry (189-195).

In vitro cultivation and multiplication are becoming an essential part of the rocketing Cannabis industry (211). More methods and approaches are being introduced to increase the multiplication coefficient and speed up the process of in vitro propagation (211). The in vitro shoot propagation of Cannabis sativa L. is an emerging research area for large scale plant material production (211). However, how in vitro conditions influence the genetic stability of maintained material, as well as whether changes in the concentration and composition of secondary metabolites can be expected are aspects that need to be better understood (211). These features are essential for the standardised production of medicinal Cannabis. One of the study reported to find out whether the presence of the auxin antagonist α-(2-oxo-2-phenylethyl)-1H-indole-3-acetic acid (PEOIAA) in the culture media influenced the relative gene expression (RGE) of the genes of interest (OAC, CBCA, CBDA, THCA) and the concentrations of studied cannabinoids (CBCA, CBDA, CBC, D9-THCA, and D9-THC) (211). Two *Cannabis sativa* cultivars, 'USO-31' and 'Tatanka Pure CBD', were cultivated by in vitro conditions with PEO-IAA presence and then analysed (211).

The RT-qPCR results indicated that even though some changes in the RGE profiles could be observed, no differences were statistically significant compared with the control variant (211). The results of the phytochemical analyses demonstrated that although there were some differences from the control variant, only the cultivar 'Tatanka Pure CBD' showed a statistically significant increase (at a statistical significance level $\alpha = 0.05$) in the concentration of the cannabinoid CBDA (211). This experimental study concluded that using PEO-IAA in the culture medium is a suitable approach to improve in vitro Cannabis

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multiplication (211). Therefore, this study assumed that the negative effect of the presence of one micromolar concentration of PEO-IAA in the culture media should not be a significant negative factor for other cultivars as well (211). However, further research on other genotypes is needed to understand the issue comprehensively (211). Moreover, additional monitoring of plants after the transfer to ex vitro conditions and reaching the generative flowering stage may also add valuable information to the effect of PEO-IAA on the main qualitative factors of the produced plant material (211).

Although tissue culture techniques for plant regeneration and micropropagation have been reported for different Cannabis genotypes and explant sources, there are significant variations in the response of cultures and the morphogenic pathway (57-211). Methods for many high-yielding elite strains are still rudimentary, and protocols are not yet established (57-211). With a recent focus on sequencing and genomics in Cannabis, genetic transformation systems are applied to medical Cannabis and hemp for functional gene annotation via traditional and transient transformation methods to create novel phenotypes by gene expression modulation and to validate gene function (57-211).

The process of developing new varieties through conventional breeding can take 7–12 years, depending on crop species (57-211). The progress of Cannabis breeding programs is limited due to the difficulty in maintaining selected high yielding cross-pollinated elite genotypes under field or greenhouse conditions (163-211). Therefore, tissue culture techniques are advantageous for Cannabis improvement because they can facilitate high multiplication rate and production of disease-free elite plants by overcoming the problems of heterozygosity from cross-pollination (163-211). The development of new industrial hemp and medical Cannabis cultivars with improved traits could be further advanced using genome editing and other precision breeding tools, combined with in vitro techniques for regeneration (163-211). Unfortunately, hemp and Cannabis plants' dioecious nature complicates the efforts toward the improvement of specific traits, such as resistance to pests and diseases. Therefore, with the recent legalization, calls for serious targeted efforts are required to advance the regeneration and transformation (163-211).

VIII. Conclusion

Cannabis which is a native of **Indian origin** is renowned for its secondary metabolites, including cannabinoids and terpenes. Cannabinoids are a class of compounds that can interact with the endocannabinoid system (ECS) and many have medicinal or psychoactive properties. This versatile and phenotypically diverse plant has been used for a wide variety of commercial and medicinal purposes. Cannabinoid levels have been used both in setting legal definitions for different categories of Cannabis products and for 'chemotaxonomic' purposes to classify different Cannabis cultivars based on THC:CBD ratios. Currently machine learning algorithms are in the process of revolutionizing health. Several studies investigated the existence of links between cannabis use and psychotic disorders. A refined Machine Learning framework for understanding the links between Cannabis use and 1st episode psychosis has been reported.

In vitro micropropagation techniques offer opportunity to proliferate, maintain, and study dynamic plant responses in a highly controlled environments. With medicinal and recreational interests for Cannabis sativa L. growing, the optimization of in vitro practices is needed to improve the current methods. Unfortunately, due to the large array of factors influencing tissue culture, existing approaches to optimize in vitro methods are tedious and time-consuming. Therefore, there is great potential to use new computational methodologies for analyzing data to develop improved plant in vitro protocols more efficiently.

Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in plant tissue cultures. The Artificial neural networks (ANNs) can simulate the growth of plants under different in vitro conditions. Their usefulness has been confirmed in the estimation of biomass in plant cell cultures and the length of shoots in vitro, in the classification of somatic embryos, evaluation of the physical conditions of an in vitro environment, and in the prediction of optimal conditions for in vitro culture to achieve maximum efficiency and productivity. Secondly, with the help of various types of neural models, in vitro -regenerated plants are sorted, respectively, to their quality and likeliness of further development. Thirdly, ANNs are capable of predicting plant behaviour during in vitro rhizogenesis and subsequent acclimatization to ex vitro conditions.

Finally, there are only limited in vitro studies of Cannabis has been reported. These in vitro regeneration protocols are owned by private Cannabis industries. As per the literature survey, most of the protocols confirmed the in vitro recalcitrance nature of the Cannabis. Currently only few in vitro shoot regeneration protocols have been reported in Cannabis. Therefore, there is a golden opportunity to develop new reproducible consistent in vitro micropropation protocols for Cannabis. Therefore, somatic embryogenesis, protoplast culture, genetic transformation, anther culture, cryopreservation, production of synthetic seeds reproducible protocols should be developed for Cannabis.

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References

1. **Malabadi RB**, Kolkar KP, Chalannavar RK. Cannabis sativa: Ethnobotany and phytochemistry. International Journal of Innovation Scienfic Research and Review. 2023; 5(2): 3990-3998.
2. **Malabadi RB**, Kolkar KP, Acharya M, Chalannavar RK. Cannabis sativa:Medicinal plant with 1000 molecules of pharmaceutical interest. International Journal of Innovation Scientific Research and Review. 2023;5 (2):3999-4005.
3. **Malabadi RB**, Kolkar KP, Chalannavar RK. Cannabis sativa: Industrial hemp (fiber type)- An Ayurvedic traditional herbal medicine. International Journal of Innovation Scientific Research and Review. 2023;5 (2): 4040-4046.
4. **Malabadi RB**, Kolkar KP, Chalannavar RK. Medical Cannabis sativa (Marijuana or Drug type); The story of discovery of Δ9-Tetrahydrocannabinol (THC). International Journal of Innovation Scientific Research and Review. 2023; 5: (3):4134-4143.
5. **Malabadi RB**, Kolkar KP, Chalannavar RK. Δ9-Tetrahydrocannabinol (THC): The major psychoactive component is of botanical origin. International Journal of Innovation Scientific Research and Review. 2023;5(3): 4177-4184.
6. **Malabadi RB,** Kolkar KP, Chalannavar RK**.** Cannabis sativa**:** Industrial Hemp **(**fibre-type**)-** An emerging opportunity for **India.** International Journal of Research and Scientific Innovations (IJRSI). 2023; X (3):01-9.
7. **Malabadi RB,** Kolkar KP, Chalannavar RK**.** Industrial Cannabis sativa (Hemp fiber type):Hempcrete-A plant based eco-friendly building construction material. International Journal of Research and Innovations in Applied Sciences(IJRIAS). 2023; 8(3): 67-78.
8. **Malabadi RB**, Kolkar KP, Chalannavar RK, Lavanya L**, Abdi G.** Cannabis sativa: The difference between Δ8-THC and Δ9-Tetrahydrocannabinol (THC). International Journal of Innovation Scientific Research and Review. 2023; 5(4): 4315-4318.
9. **Malabadi RB**, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. **Hemp Helps Human Health**: Role of phytocannabinoids. International Journal of Innovation Scientific Research and Review. 2023; 5 (4): 4340-4349.
10. **Malabadi RB**, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. Cannabis sativa: Botany, cross pollination and plant breeding problems. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8 (4): 174-190.
11. **Malabadi RB**, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G, Baijnath H. Cannabis products contamination problem: A major quality issue. International Journal of Innovation Scientific Research and Review**.** 2023;5(4): 4402-4405.
12. **Malabadi RB**, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G**.** Medical Cannabis sativa (Marijuana or drug type): Psychoactive molecule, Δ9-Tetrahydrocannabinol (Δ9-THC). International Journal of Research and Innovations in Applied Science. 2023; 8(4): 236-249.
13. **Malabadi RB**, Kolkar KP, Chalannavar RK, Mondal M, Lavanya L, Abdi G, Baijnath H**.** Cannabis sativa: Release of volatile organic compounds (VOCs) affecting air quality. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(5): 23-35.
14. Smith CJ, Vergara D, Keegan B, Jikomes N. The phytochemical diversity of commercial *Cannabis* in the United States. PLoS ONE. 2022; 17(5): e0267498.
15. Innes PA, Daniela Vergara D. Genomic description of critical upstream cannabinoid 1 biosynthesis genes. bioRxiv preprint. 2022.
16. Hussain T, Jeena G, Pitakbut T, Vasilev N, Kayser O. Cannabis sativa research trends, challenges, and new-age perspectives. IScience. 2021; 24: 103391.
17. Singh S, Balhara YPS. A review of Indian research on co-occurring cannabis use disorders & psychiatric disorders. Indian J. Med Res. 2017; 146: 186-195.
18. Varma L. Cannabis psychosis. Indian J. Psychiatry. 1972;14: 241.
19. Parakh P, Basu D. Cannabis and Psychosis: Have we found the missing links? Asian J. Psychiatr. 2013; 6 : 281-7.
20. Shrivastava A, Johnston M, Terpstra K, Bureau Y. Cannabis and psychosis: Neurobiology. Indian J. Psychiatry. 2014;56: 8-16.
21. Ghosh A, Basu D. Cannabis and psychopathology: The meandering journey of the last decade. Indian J. Psychiatry. 2015; 57:140-9.
22. **Talaviya** T, Shah D, Patel N, Yagnik H, Shah M. Implementation of artificial intelligence in agriculture for optimisation of irrigation and application of pesticides and herbicides. Artificial Intelligence in Agriculture. 2020; 4: 58–73.
23. Negriff S, Dilkina B, Matai L, Rice E. Using machine learning to determine the shared and unique risk factors for marijuana use among child-welfare versus community adolescents. PLoS ONE. 2022; 17(9): e0274998.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

24. Zha J. Artificial Intelligence in Agriculture. Journal of Physics: Conference Series. 2020; 1693**.** 012058 IOP Publishing doi:10.1088/1742-6596/1693/1/012058.
25. Rai N, Zhang Y, Ram BG, Schumacher L, Yellavajjala RK, Bajwa S, Sun X. Applications of deep learning in precision weed management: A review. Computers and Electronics in Agriculture. 2023; 206: 107698.
26. Albiero D. Agricultural robotics: A promising challenge. Current Agriculture Research Journal. 2019; *7*(1): 1.
27. Ryan M, Bedir I, Tekinerdogan B. An interdisciplinary approach to artificial intelligence in agriculture, NJAS: Impact in Agricultural and Life Sciences. 2023; 95:1, 2168568.
28. **Ilan Y**. Second-generation digital health platforms: placing the patient at the center and focusing on clinically meaningful endpoints title: second generation artificial intelligence algorithms. Front Digit Health. 2020; 2:569178.
29. **Ilan Y**. Digital Medical Cannabis as Market Differentiator: Second-Generation Artificial Intelligence Systems to Improve Response. Front. Med. 2022; 8:788777. doi: 10.3389/fmed.2021.788777.
30. Buch VH, Ahmed I. Maruthappu M. Artificial intelligence in medicine: Current trends and future possibilities Br J. Gen Pract. 2018; 68:143–4.
31. Kelly CJ, Karthikesalingam A, Suleyman M, Corrado G. King D. Key challenges for delivering clinical impact with artificial intelligence. BMC Med. 2019; 17:195.
32. Briganti G, Le Moine O. Artificial intelligence in medicine: Today and tomorrow. Front Med (Lausanne). 2020; 7:27.
33. Banerjee G, Sarkar U, Das S, Ghosh I. Artificial Intelligence in Agriculture: A literature Survey[J]. International Journal of Scientific Research in Computer Science Applications and Management Studies. 2018, 7(3):1-6.
34. Patrícioa DI, Riederb R. Computer vision and artificial intelligence in precision agriculture for grain crops: A systematic review. Computers and Electronics in Agriculture. 2018; 153:69-81.
35. Itkenhead MJ, Dalgetty IA, Mullins CE et al. Weed and crop discrimination using image analysis and artificial intelligence methods[J]. Computers & Electronics in Agriculture. 2003; 39(3):157-171.
36. Aasim M, Katırc R, Akgur O, Yildirim B, Mustafa Z, Nadeem MA, Baloch FS, Karakoy T, Yılmaz G. Machine learning (ML) algorithms and artificial neural network for optimizing in vitro germination and growth indices of industrial hemp (*Cannabis sativa* L.). Industrial Crops and Products. 2022; 181: 114801 https://doi.org/10.1016/j.indcrop.2022.114801.
37. Zielinska S, Kepczynska E. Neural modeling of plant tissue cultures: A review. BioTechnologia. Journal of Biotechnology, Computational Biology and Bionanotechnology. 2013; 94(3): 253-268.
38. Albiol J, Campmajó C, Casas C, Poch M. Biomass estimation in plant cell cultures: a neural network appro ach. Biotechnol. Prog. 1995;11: 88-92**.**
39. 9. Gago J, Landin M, Gallego PP. Artificial neural net works modeling the in vitro rhizogenesis and acclimatization of Vitis vinifera L. J. Plant Physiol. 2010a; 167: 1226-1231.
40. Gago J, Landin M, Gallego PP. A neurofuzzy logic approach for modeling plant processes: A practical case of in vitro direct rooting and acclimatization of Vitis vinifera L. 2010b; Plant Sci. 179: 241-249.
41. Gago J, Pérez-Tornero O, Landin M, Burgos L, Gallego PP. Improving knowledge of plant tissue culture and media formulation by neurofuzzy logic: A practical case of data mining using apricot databases. J. Plant Physiol. 2011; 168: 1858-1865.
42. Gallego PP, Gago J, Landin M. Artificial neural networks technology to model and predict plant biology process. In: Artificial neural networks – methodological advances and biomedical applications, ed. Suzuki K., InTech, Rijeka. 2011;197-216.
43. Honda H., Ito T., Yamada J., Hanai T., Matsuoka M., Kobayashi T. Selection of embryogenic sugarcane callus by image analysis. J. Biosci. Bioeng. 1999; 87: 700-702.
44. Honda H, Liu C, Kobayashi T. Selection of embryos. In: Advances in Biochemical Engineering/ Biotechnology, ed. Sheper T., vol. 72. Springer-Verlag, Berlin, Heidelerg: 2001; 177-178.
45. Honda H, Takikawa N, Noguchi H, Hanai T, Kobayashi T. Image analysis associated with a fuzzy neural net work and estimation of shoot length of regenerated rice callus. J. Ferment. Bioeng. 1997; 84: 342-347.
46. Mehrotra S, Prakash O, Mishra BN, Dwevedi B. Efficiency of neural networks for prediction of in vitro culture conditions and inoculum properties for optimum productivity. Plant Cell Tiss. Organ Cult. 2008; 95: 29-35.
47. Mehrotra S, Prakash O, Khan F, Kukreja AK. Efficiency of neural network-based combinatorial model predicting optimal culture conditions for maximum biomass yield in hairy root cultures. Plant Cell Rep. 2013; 32: 309-317.
48. Murase H, Okayama T, Suroso S. Intelligence inverse analysis for temperature distribution in a plant culture vessel. In: Plant Tissue Culture Engineering, eds. Dutta Gupta S., Ibaraki Y. Focus on Biotechnology, series eds. Hofman M., Anné J., vol. 6. Springer-Verlag, Berlin: 2008; 373- 39.
49. Prakash O, Mehrotra S, Krishna A, Mishra BN. A neural network approach for the prediction of in vitro culture parameters for maximum biomass yields in hairy root cultures. J. Theoretical Biol. 2010; 265: 579-585.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

50. Prasad VSS, Dutta A, Gupta S. Applications and potentials of artificial neural networks in plant tissue culture. In: Plant Tissue Culture Engineering, eds. Dutta Gupta S., Ibaraki Y. Focus on Biotechnology, series eds. Hofman M., Anné J., vol. 6. Springer-Verlag, Berlin: 2008a; 47-67.
51. Uozumi N, Yoshino T, Shiotani S, Suehara K, Arai F, Fukuda T, Kobayashi T. Application of image analysis with neural network for plant somatic embryo culture. J. Ferment. Bioeng. 1993; 76: 505-50.
52. Zhang C, Timmis R, Hu WS. A neural network based pattern recognition system for somatic embryos of Douglas fir. Plant Cell Tiss. Organ Cult. 1999; 56: 25-35
53. **Prasad VSS**, Dutta A, Gupta S. Photometric clustering of regenerated plants of gladiolus by neural networks and its biological validation. Comput. Electron. Agr. 60: 8-17. Ruan R., Xu J., Zhang C., Chi C.-M., Hu W.-S. (1997) Classification of plant somatic embryos by using neural network classifiers. Biotechnol. Prog. 2008b; 13: 741-746.
54. **Pepe** M, Hesami M, Small F, Jones AMP. Comparative Analysis of Machine Learning and Evolutionary Optimization Algorithms for Precision Micropropagation of Cannabis sativa: Prediction and Validation of in vitro Shoot Growth and Development Based on the Optimization of Light and Carbohydrate Sources. Front. Plant Sci. 2021; 12:757869. doi: 10.3389/fpls.2021.757869.
55. Hesami M, Jones AMP. Application of artificial intelligence models and optimization algorithms in plant cell tissue culture. Appl. Microbial Biotechnol. 2020; 104: 9449-9485.
56. Jafari M, Daneshvar MH, Jafari S, Hesami M. Machine Learning-Assisted In Vitro Rooting Optimization in Passiflora caerulea. Forests. 2022; 13: https://doi.org/10.3390/f1312202.
57. **Malabadi RB**, Mulgund GS, Nataraja K, Vijayakumar S. Induction of somatic embryogenesis and plant regeneration in different varieties of **Sugarcane** (*Saccharam officinarum* L.). Research in Plant Biology. 2011; 1(4):39-41.
58. **Malabadi RB,** Mulgund GS, Nataraja K, Vijayakumar S. Induction of somatic embryogenesis in **Papaya** (*Carica papaya* L.). Research in Biotechnology. 2011; 2(5):40-55.
59. **Malabadi RB**, Teixeira da Silva JA, Nataraja K, Vijayakumar S, Mulgund GS Induction of somatic embryogenesis in **Mango** (*Mangifera indica*). International Journal of Biological Technology. 2011; 2(2):12-18.
60. **Malabadi RB**, Vijayakumar S, Nataraja K, Mulgund GS. Induction of somatic embryogenesis and plant regeneration in **Grapes** (*Vitis vinifera* L.). Botany Research International. 2010; 3 (2):48-55.
61. Ramarosandratana AV, **Malabadi** RB, Van Staden J. Gain and loss of embryogenic competence in Norway spruce (*Picea abies*) embryo segments. South African Journal of Botany. 2004; 70(2):365.
62. Hasnain A, Naqvi SAH, Ayesha SI, Khalid F, Ellahi M, Iqbal S, Hassan MZ, Abbas A, Adamski R, Markowska D, Baazeem A, Mustafa G, Moustafa M, Hasan ME and Abdelhamid MMA. Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. Front. Plant Sci. 2022; 13:1009395.
63. Norouzi O, Hesami M, Pepe M, Dutta **A, Jones AMP.** In vitro plant tissue culture as the fifth generation of bioenergy. Scientific Reports**.** 2022; 12:5038 https://doi.org/10.1038/s41598-022-09066-3.
64. Ramarosandratana AV, **Malabadi RB**, Van Staden J. Triiodobenzoic-acid mimics the effect of supraoptimal dose of auxin by inhibiting somatic embryo initiation in Norway spruce. South African Journal of Botany. 2004; 70 (2):365.
65. **Malabadi RB**, Choudhary H, Tandon P. Effect of gelling agent, carbon sources and sterilization methods on initiation and establishment of embryogenic cultures in Khasi pine (*Pinus kesiya* Royle ex. Gord). Applied Biological Research. 2003; 8(1&2): 1-8.
66. **Malabadi RB**, Mulgund GS, Nataraja K. Plant regeneration *via* somatic embryogenesis in *Pinus kesiya* (Royle ex. Gord.) influenced by triacontanol. **Acta Physiologiae Plantarum**. 2005; 27 (4A): 531-537.
67. **Malabadi RB**, van Staden J. Cold-enhanced somatic embryogenesis in *Pinus patula* is mediated by calcium. **South African Journal of Botany**. 2006; 72(4): 613-618.
68. **Malabadi RB**, van Staden J. Somatic embryogenesis from vegetative shoot apices of mature trees of *Pinus patula.* **Tree Physiology**. 2005; 25: 11-16.
69. **Malabadi RB**, Mulgund GS, Vijaykumar S. How somatic cells follows embryogenic pathway during cloning mature trees of conifers? Journal of Phytological Research. 2009; 22 (1): 53-56.
70. **Malabadi RB,** Nataraja K. 24-epibrassinolide induces somatic embryogenesis in *Pinus wallichiana* A. B. Jacks. Journal of Plant Sciences. 2007; 2(2):171-178.
71. **Malabadi RB**, Nataraja K. Plant regeneration *via* somatic embryogenesis using secondary needles of mature trees of *Pinus roxburghii* Sarg. International Journal of Botany. 2007; 3(1):40-47.
72. **Malabadi RB**, Teixeira da Silva JA, Nataraja K, Vijayakumar S, Mulgund GS. Induction of somatic embryogenesis in mature coniferous forest trees. Research in Biotechnology. 2011; 2(5):08-33.
73. **Malabadi RB**, van Staden J. Breakthrough in Forest Biotechnology. University of KwaZulu Natal, Pietermaritzburg, South Africa, News paper. Vol-2 (3) March 2005 page no-3.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

74. **Malabadi RB** et al., Induction of Somatic Embryogenesis using shoot apex in Maritime Pine (**Pinus pinaster**): 2007. ITQB-Progress Report-Page No-96. **Portugal.** 2007.
75. Park SY, Klimaszewska KK, **Malabadi RB**, Mansfield SD. Embryogenic cultures of Lodgepole pine originating from mature trees and from immature seed explants. IUFRO Tree Biotechnology Conference, June 28th- July 2nd 2009,**Whistler, BC**, **Canada**, p 60 (abstract). 2009.
76. Aronen T, Pehkonen T, **Malabadi RB**, Ryynänen L. Somatic embryogenesis of Scots pine –Advances in pine tissue culture at Metla. Vegetative propagation of conifers for enhancing landscaping and tree breeding Proceedings of the Nordic meeting held in September 10th-11th 2008 at Punkaharju, Finland.
77. Aronen TS, Pehkonen T, **Malabadi RB**, Ryynanen L. Somatic embryogenesis of Scots pine-advances in pine tissue culture at Metla. Vegetative propagation of conifers for enhancing landscaping and tree breeding. Proceedings of the Nordic meeting held in September 10-11th 2008 at Punkaharju, Finland. Working Papers of the Finnish Forest Research Institute. 2008; 114; 68-71.
78. Aronen TS, Ryynanen L, **Malabadi RB**. Somatic embryogenesis of Scots pine: Initiation of cultures from mature tree explants and enhancement of culture system [Abstract]. In: IUFRO Tree Biotechnology Conference , June 3-8, 2007,Ponta Delgada, Azores, Portugal, No.SIX. 2. 2007.
79. **Malabadi RB**, Mulgund GS, Nataraja K. Triacontanol induced somatic embryogenesis and plantlet regeneration in *Catharanthus roseus*. Journal of Medicinal and Aromatic Plant Sciences. 2009; 31: 147-151.
80. Teixeira da Silva JA, **Malabadi RB**. Factors affecting somatic embryogenesis in conifers. **Journal of Forestry Research**. 2012; 23(4):503-515.
81. **Malabadi RB**, Mulgund GS, Meti NT, Nataraja K, Vijayakumar S. Influence of bud break and apical meristematic tissue competence during cloning mature trees of conifers. Research in Plant Biology. 2012; 2(2): 43-47.
82. **Malabadi RB**, Mulgund GS, Vijaykumar S. Smoke induced seed germination and somatic embryogenesis. Journal of Phytological Research. 2009; 22 (2):205-209.
83. **Malabadi RB,** Meti NT, Vijayakumar S, Mulgund GS, Nataraja K. Activation of cambial layer influences cloning of mature trees of conifers. Research in Biotechnology. 2012; 3(2): 78-82.
84. Mulgund GS, Meti NT, **Malabadi RB**, Nataraja K, Vijayakumar S. Factors influencing cloning mature trees of conifers. Research in Plant Biology. 2012; 2(2): 38-42.
85. **Malabadi RB**, van Staden J. Somatic embryos can be induced from the vegetative shoot apex of mature *Pinus patula* trees. **South African Journal of Botany**. 2003; :450-451.
86. **Malabadi RB**, Teixeira da Silva JA, Nataraja K. Salicylic acid induces somatic embryogenesis from mature trees of *Pinus roxburghii* (Chir pine) using TCL Technology. Tree and Forestry Science and Biotechnology. 2008; 2(1): 34-39.
87. Mulgund GS, Meti NT, **Malabadi RB**, Nataraja K, Vijayakumar S. Role of salicyclic acid on conifer somatic embryogenesis. Research in Biotechnology. 2012; 3(2): 57-61.
88. **Malabadi RB**. Effect of **glutathione** on maturation of somatic embryos derived from vegetative shoot apices of mature trees of *Pinus roxburghii*. Journal of Phytological Research. 2006; 19 (1): 35-38.
89. **Aronen** TS, Ryynanen L, **Malabadi** RB. Somatic embryogenesis of Scots pine: Initiation of cultures from mature tree explants and enhancement of culture system. 2007 IUFRO tree biotechnology conference held on 3-8th June in Ponta delgada, Azores islands, Portugal. SIX-2. 2007; (Abstract).
90. **Malabadi RB**, Choudhary H, Tandon P. Plant regeneration *via* somatic embryogenesis in *Pinus kesiya* (Royle ex. Gord). Applied Biological Research. 2002; 4: 1-10.
91. **Malabadi RB**, Nataraja K. Putrescine influences somatic embryogenesis and plant regeneration in *Pinus gerardiana* Wall. American Journal of Plant Physiology. 2007; 2(2):107-114.
92. **Malabadi RB**, Nataraja K. **Smoke-saturated** water influences somatic embryogenesis using vegetative shoot apices of mature trees of *Pinus wallichiana* A. B. Jacks. Journal of Plant Sciences. 2007; 2 (1): 45- 53.
93. **Malabadi RB**, Teixeira da Silva JA, Nataraja K. A new approach involving salicyclic acid and thin cell layers for cloning mature trees of *Pinus roxburghii* (Chir Pine). The Americas Journal of Plant Science and Biotechnology. 2008; 2(2):56-59.
94. **Malabadi RB**, van Staden J. Optimized somatic embryogenesis in *Pinus patula*. Sixth Annual Meeting Conference of the Research Centre for Plant Growth and Development, Department of Botany, University of Natal, Pietermaritzburg, South Africa. 2004; Pp-20.
95. **Malabadi RB**, Nataraja K. Somatic embryogenesis and biochemical analysis of *in vitro* derived plants in mothbean (*Vigna aconitifolia* Jacq.). Plant Cell Biotechnology and Molecular Biology. 2003; 4: 69- 74.
96. **Malabadi RB**, Teixeira da Silva JA. Thin cell layers: Application to forestry biotechnology. Tree and Forestry Science and Biotechnology. 2011; 5(1): 14-18.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

97. **Malabadi RB**, Choudhury H, Tandon P. Initiation, maintenance and maturation of somatic embryos from thin apical dome sections in *Pinus kesiya* (Royle ex. Gord) promoted by partial desiccation and gellan gum. **Scientia Horticulturae**. 2004; 102: 449-459.
98. **Malabadi RB**, Teixeira da Silva JA, Mulgund GS. Induction of somatic embryogenesis in *Pinus caribaea*. Tree and Forestry Science and Biotechnology. 2011; 5(1): 27-32.
99. **Malabadi RB**, Nataraja K. Influence of triacontanol on somatic embryogenesis of *Pinus roxburghii* Sarg. **Baltic Forestry**. 2007; 13(1): 39-44.
100. **Malabadi RB**, van Staden J. Recent developments of clonal forestry in South Africa. Seventh Annual Meeting Conference of the Research Centre for Plant Growth and Development, Department of Botany, University of KwaZulu-Natal, Pietermaritzburg, South Africa. 2005; 2.
101. **Malabadi RB**, Nataraja K, Vijaykumar S, Mulgund GS. Evidence of ***WUSCHEL*** (*WOX2*) gene expression during induction of somatic embryogenesis from apical shoot buds of mature trees of *P. roxburghii*. Research in Plant Biology. 2011; 1(4):77-85.
102. **Malabadi RB**, Nataraja K, Vijayakumar S, Mulgund GS. Journey of a single cell to a plantlet *via in vitro* cloning mature trees of conifers. Research in Biotechnology. 2011; 2(6):01-07.
103. **Malabadi RB**, van Staden J. Storability and germination of sodium alginate **encapsulated** somatic embryos derived from the vegetative shoot apices of mature *Pinus patula* trees. **Plant Cell Tissue and Organ Culture**. 2005; 82:259-265.
104. **Malabadi RB**, Nataraja K. Large scale production and storability of **encapsulated s**omatic embryos of Mothbean (*Vigna aconitifolia* Jacq.). **Journal of Plant Biochemistry and Biotechnology**. 2002; 11:61-64.
105. **Malabadi RB**, Nataraja K. In vitro storage of **synthetic seeds** in *Clitoria ternatea* (Linn.). Phytomorphology. 2002; 52 (2&3): 231-237.
106. **Malabadi RB**. **Protoplast isolation**, culture and plant regeneration in Butterfly pea (*Clitoria ternatea* Linn.). Indian Journal of Genetics and Plant breeding. 2003; 243-246.
107. **Malabadi RB**, Nataraja K. **Cryopreservation** and plant regeneration *via* somatic embryogenesis in *Clitoria ternatea*. Phytomorphology. 2004; 54 (1&2):7-17.
108. **Malabadi RB**, Nataraja K. **Cryopreservation** and plant regeneration *via* somatic embryogenesis using shoot apical domes of mature *Pinus roxburghii* Sarg. Trees. ***In vitro* Cellular and Developmental Biology-Plant**. 2006; 42 (2): 152-159.
109. **Malabadi RB**, Lokare-Naik S, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. Synthesis of **silver nanoparticles** from *in vitro* derived plants and callus cultures of *Clitoria ternatea*; Evaluation of antimicrobial activity. Research in Biotechnology. 2012; 3(5): 26-38
110. **Malabadi RB**, Chalannavar RK, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. Synthesis of antimicrobial **silver nanoparticles** by callus cultures and *in vitro* derived plants of ***Catharanthus roseus***. Research in Pharmacy. 2012; 2(6):18- 31.
111. **Malabadi RB**, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. **Synthesis of silver nanoparticles** from *in vitro* derived plants and callus cultures of ***Costus speciosus*** (Koen.): Assessment of antibacterial activity. Research in Plant Biology. 2012; 2(4): 32-42.
112. **Malabadi RB**, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. **Smoke saturated water p**romoted *in vitro* seed germination of an epiphytic orchid *Oberonia ensiformis* (Rees) Lindl. Research in Plant Biology. 2012; 2(5): 32-40.
113. Mulgund GS, Meti NT, **Malabadi RB**, Nataraja K, Vijayakumar S. Smoke promoted *in vitro* seed germination of *Pholidota pallida*. Research in Plant Biology. 2012; 2(2): 24-29.
114. Mulgund GS, Nataraja K, **Malabadi RB,** Vijayakumar S. TDZ induced *in vitro* propagation of an epiphytic orchid *Xenikophyton smeeanum* (Reichb. f.). Research in Plant Biology. 2011; 1(4):07-15.
115. **Malabadi RB**, Teixeira da Silva JA, Nataraja K, Vijayakumar S, Mulgund GS. *In vitro* seed germination of an epiphytic orchid *Xenikophyton smeeanum* (Reichb. f.) by using smoke-saturated-water as a natural growth promoter. International Journal of Biological Technology. 2011; 2(2):35-41.
116. **Malabadi RB**, Teixeira da Silva JA, Mulgund GS. *In vitro* shoot regeneration by culture of *Liparis elliptica* (Rees) Lindl., shoot tip-derived transverse thin cell layers induced by 24-epi Brassinolide. International Journal of Plant Developmental Biology. 2009; 3(1): 47-51.
117. **Malabadi RB**, Teixeira da Silva JA, Mulgund GS. TDZ induced *in vitro* shoot regeneration of *Aerides maculosum* Lindl., from shoot tip thin cell layers. Floriculture and Ornamental Biotechnology. 2009; 3(1): 35-39.
118. **Malabadi RB**, Teixeira da Silva JA, Mulgund GS. Micropropagation of *Eria dalzelli* (Dalz.) Lindl. through TCL *in vitro* culture. Floriculture and Ornamental Biotechnology. 2008; 2(2):77-80.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

119. **Malabadi RB**, Teixeira da Silva JA, Nataraja K, Mulgund GS. Shoot tip transverse thin cell layers and 24-epibrassinolide in the micropropagation of *Cymbidium bicolor* Lindl. Floriculture and Ornamental Biotechnology. 2008; 2(2): 44-48.
120. **Malabadi RB,** Parashar A, Ganguly A, Mavanur SR. **Expression of Dengue virus envelope protein in a different plant system**. Faculty Research and Development day, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada, 19th November 2010. Abstract No-69, page no-31. (Poster presentation).
121. **Malabadi RB,** Chalannavar RK, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S, Narayanaswamy VK, Odhav B. Detection of ***Glutathione S-Transferase*** gene (*GST2* and *GST3*) during induction of somatic embryogenesis in grape. Research in Biotechnology. 2013; 4(1):01-11.
122. **Malabadi RB**, Mulgund GS, Vijaykumar S. Expression of ***WUSCHEL***-gene promoting somatic embryogenesis in plants. Journal of Phytological Research. 2009; 22 (1): 103-106.
123. **Malabadi RB**, Teixeira da Silva JA, Nataraja K. Stable and consistent ***Agrobacterium***-mediated genetic transformation in *Pinus roxburghi* (Chir Pine). Tree and Forestry Science and Biotechnology. 2008; 2(1):7-13.
124. **Malabadi RB**, Nataraja K. **Alkaloid biosynthesis** influenced by *Agrobacterium- rhizogenesis* mediated genetic transformation and bioreactor in *Clitoria ternatea* (Linn.). Plant Cell Biotechnology and Molecular Biology. 2003; 4: 169-178.
125. **Malabadi RB,** Mulgund GS, Vijaykmar S. Tree biotechnology: Recent updates on **genetic transformation** of conifers. Journal of Phytological Research. 2009; 22 (2):177-181.
126. **Malabadi RB**. Production of edible vaccines for oral immunization in **transgenic plants:** Current and future prospective. Journal of Phytological Research. 2008; 21(1):1-10.
127. **Malabadi RB**, Nataraja K. A **biolistic** approach for the production of transgenic plants using embryogenic tissue in *Pinus kesiya* Royle Ex. Gord (Khasi pine). Biotechnology. 2007; 6(1): 87-93.
128. **Malabadi RB**, Nataraja K. **Genetic transformation** of *Vanilla planifolia* by *Agrobacterium tumefaciens* using shoot tip sections. Research Journal of Botany. 2007; 2(2): 86-94.
129. **Malabadi RB**, Vijaykmar S. Role of transgenic plants in phytoremediation: Applications, present status and future prospectives. Journal of Phytological Research. 2009; 22 (1):1-12.
130. **Malabadi RB**. *Agrobacterium*-mediated genetic transformation of *Vigna unguiculata.* Journal of Phytological Research. 2006; 19 (1): 1-4.
131. **Malabadi RB,** Teixeira da Silva JA, Nataraja K. *Agrobacterium*-mediated genetic transformation of *Pinus kesiya* Royle ex Gord (Khasi Pine). The Asian and Australasian Journal of Plant Science and Biotechnolog. 2008; 2(1): 7-14
132. **Malabadi RB** Teixeira da Silva JA, Nataraja K. **Green fluorescent protein** in the genetic transformation of plants. Transgenic Plant Journal. 2008; 2(2):86-109.
133. **Malabadi RB**, Nataraja K. **Genetic transformation** of conifers: Applications in and impacts on commercial forestry. Transgenic Plant Journal. 2007; 1(2): 289-313.
134. **Malabadi RB**, Nataraja K. Stable transformation and recovery of transgenic plants by particle bombardment in *Pinus wallichiana* A. B. Jacks (Himalayan blue pine). Biotechnology. 2007; 6(1): 105-111.
135. **Malabadi RB**, Nataraja K.Production of **transgenic** plants *via Agrobacterium- tumefaciens* mediated genetic transformation in *Pinus wallichiana* (Himalayan blue pine). Transgenic Plant Journal. 2007;1(2): 376- 383.
136. **Malabadi RB**, Nataraja K. **Isolation of cDNA clones** of genes differentially expressed during somatic embryogenesis of *Pinus roxburghii.* American Journal of Plant Physiology. 2007; 2(6):333-343.
137. **Malabadi RB**, Nataraja K. Gene transfer by particle bombardment of embryogenic tissue derived from the shoot apices of mature trees of *Pinus roxburghii* (Chir pine). American Journal of Plant Physiology. 2007; 2(2):90-98.
138. **Malabadi RB**, Nataraja K. *Agrobacterium tumefaciens* mediated genetic transformation in *Vigna aconitifolia* and stable transmission of genes to somatic seedlings. International Journal of Agricultural Research. 2007; 2(5): 450- 458.
139. **Malabadi RB**, Nataraja K. RAPD detect no somaclonal variation in cryopreserved cultures of *Pinus roxburghii*. SARG. Propagation of Ornamental Plants. 2006; 6(3): 114-120.
140. **Malabadi RB**, Teixeira da Silva JA, Mulgund GS. Smoke-saturated water influences *in vitro* seed germination of *Vanda parviflora* Lindl. Seed Science and Biotechnology. 2008; 2(2):65-69.
141. **Malabadi RB**, Hills PN, van Staden J. RAPD assessment of clonal identity of somatic seedlings derived from vegetative shoot apices of mature *Pinus patula* trees. **South African Journal of Botany**. 2006; 72:181-183.
142. **Malabadi RB**, Mulgund GS, Nataraja K. Micropropagation of *Dendrobium nobile* from shoot tip sections. **Journal of Plant Physiology**. 2005; 162 (4) 473-478.
143. **Malabadi RB**, Van Staden J. Role of antioxidants and amino acids on somatic embryogenesis of *Pinus patula*. ***In Vitro*** **Cellular and Developmental Biology-Plant.** 2005; 41 (2):181-186.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

144. **Malabadi RB**, Mulgund GS, Nataraja K. Effect of triacontanol on the micropropagation of *Costus speciosus* (Koen.) Sm. Using rhizome thin sections. ***In Vitro* Cellular and Developmental Biology-Plant**. 2005; 41 (2): 129-132.
145. **Malabadi RB** *In vitro* plant regeneration of Cowpea (*Vigna unguiculata* (L.) Walp. Using distal half of cotyledon. Journal of Phytological Research. 2005; 18 (1):71-75.
146. **Malabadi RB**, Mulgund GS, Nataraja K. Efficient regeneration of *Vanda coerulea*, an endangered orchid using thidiazuron. **Plant Cell Tissue and Organ Culture**. 2004; 76: 289-293.
147. **Malabadi RB**, Mulgund GS, Nataraja K. Thidiazuron induced shoot regeneration of *Costus speciosus* (Koen.) Sm using thin rhizome sections. **South African Journal of Botany**. 2004; 70(2):255-258.
148. **Malabadi RB**, van Staden J Regeneration of Ornithogalum in vitro. **South African Journal of Botany**. 2004; 70 (4):618-621.
149. **Malabadi RB**. Histological changes associated with shoot regeneration in the leaf explants of *Clitoria ternatea* (Linn) cultured *in vitro*. Journal of Phytological Research. 2002; 15(2):169-172.
150. **Malabadi RB**, Nataraja K. Shoot regeneration in leaf explants of *Clitoria ternatea* L. cultured *in vitro*. Phytomorphology. 2001; 51 (2):169-171.
151. **Malabadi RB**, Nataraja K. Peroxidase activity as a marker of xylogenesis in the cultured cells of Guava (*Psidium guajava* L.). **Indian Journal of Forestry.** 2002; 25(2): 196-200.
152. **Malabadi RB**. *In vitro* propagation of **spiral ginger** (*Costus speciosus*) (Koen.) Sm. **Indian Journal of Genetics and Plant breeding**. 2002; 62(3): 277-278.
153. **Malabadi RB**. Plant regeneration from *in vitro* cultured leaf in mothbean. Journal of Phytological Research. 2002; 15(2): 137-140.
154. **Malabadi RB**, Van Staden J Plant regeneration from *in vitro* cultured cotyledon in *Clitoria ternatea* (Linn.). Abstract and Poster presented in the Global Summit on Medicinal Plants, Mauritius Island, 25-30th September 2003; Page 117 (Abstract).
155. **Malabadi RB**, Nataraja K. *In vitro* plant regeneration in *Clitoria ternatea*. Journal of Medicinal and Aromatic Plant Sciences. 2002; 24: 733-737.
156. **Malabadi RB**, Nataraja K. Brassinosteroids influences *in vitro* regeneration of *Cymbidium elegans*, Lindl, an endangered orchid using shoot tip sections. Asian Journal of Plant Sciences. 2007; 6 (2):308-313.
157. Rahmawati M, Mahfud C, Risuleo G, Jadid N. Nanotechnology in Plant Metabolite Improvement and in Animal Welfare. Applied Sciences. 2022; 12(2). 838.
158. Nityasree BR, Chalannavar RK, Kouser S, Divakar MS, Gani RS, Sowmyashree K, **Malabadi RB**. Bioinspired synthesis of zinc oxide nanoparticles by using leaf extract of *Solanum lycopersicum* L. for larvicidal activity of *Aedes aegypti* L. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2021; 12(1):1-8. (https://doi.org/10.1088/2043-6262/abeaae).
159. **Malabadi RB**, Chalannavar RK, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. Synthesis of antimicrobial silver nanoparticles by callus cultures and *in vitro* derived plants of *Catharanthus roseus.* Research in Pharmacy. 2012; 2(6):18- 31.
160. **Malabadi RB,** Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. Synthesis of **silver nanoparticles** from *in vitro* derived plants and callus cultures of *Costus speciosus* (Koen.): Assessment of antibacterial activity. Research in Plant Biology. 2012; 2(4): 32-42.
161. **Malabadi RB,** Lokare-Naik S, Meti NT, Mulgund GS, Nataraja K, Vijayakumar S. Synthesis of **silver nanoparticles** from *in vitro* derived plants and callus cultures of *Clitoria ternatea*; Evaluation of antimicrobial activity. Research in Biotechnology. 2012; 3(5): 26-38
162. **Malabadi RB**, Mulgund GS, Meti NT, Nataraja K, Vijayakumar S. Antibacterial activity of **silver nanoparticles** synthesized from whole plant extracts of *Clitoria ternatea*. Research in Pharmacy. 2012; (4):11-21.
163. **Chandra S**, Lata H, Mehmedic Z, Khan IA, ElSohly MA. Assessment of cannabinoids content in micropropagated plants of Cannabis sativa L., and their comparison with conventionally propagated plants and mother plant during developmental stages of growth. Planta Med. 2010; 76: 743–750.
164. **Lata H**, Chandra S, Khan IA, ElSohly MA. Thidiazuron induced high frequency direct shoot organogenesis of Cannabis sativa L. In Vitro Cell. Dev. Biol.- Plant. 2009a; 45: 12–19.
165. **Lata H**, Chandra S, Techen N, Khan IA, ElSohly MA. Assessment of genetic stability of micropropagated plants of Cannabis sativa L. by ISSR markers. Planta Med. 2009b; 76: 97–100.
166. **Lata H**, Chandra S, Khan IA, ElSohly MA. High frequency plant regeneration from leaf derived callus of high D9-tetrahydrocannabinol yielding Cannabis sativa L. Planta Med. 2010; 76: 1629–1633.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

167. **Lata H**, Chandra S, Techen N, Khan IA, ElSohly MA. In vitro mass propagation of Cannabis sativa: A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants. J. Appl. Res. Med. Aromat. Plants. 2016; 3: 18–26.
168. **Chandra S**, Lata H, ElSohly MA. Propagation of Cannabis for Clinical Research: An Approach Towards a Modern Herbal Medicinal Products Development. Front. Plant Sci. 2020; 11:958.
169. Borbas LN, Kurtz LE, Lubell-Brand JD. A Comparison of Two Media Formulations and Two Vented Culture Vessels for Shoot Multiplication and Rooting of Hemp Shoot Tip Cultures. HortTechnology. 2023; 33(2): 233-238.
170. Kurtz LE, Borbas LN, Brand MH, Lubell- Brand JD. 2022. Ex vitro rooting of Cannabis sativa microcuttings and their performance compared to re tip and stem cuttings. HortScience. 2022; 57:1576–1579.
171. Lubell-Brand JD, Kurtz LE, Brand MH. An in vitro-ex vitro micropropagation system for hemp. HortTechnology. 2021; 31:199–207.
172. Monthony AS, Bagheri S, Zheng Y, Jones AMP. Flower power: Floral reversion as a viable alternative to nodal micropropagation in Cannabis sativa. In Vitro Cell Dev Biol Plant. 2021a; 57:1018–1030.
173. Monthony AS, Kyne ST, Grainger CM, Jones AMP. Recalcitrance of Cannabis sativa to de novo regeneration: A multi-genotype replication study. PLoS One. 2021b; 16:30235525. https://doi.org/10.1371/ journal.pone.0235525.
174. Monthony AS, Page SR, Hesami M, **Jones AMP**. The past, present and future of Cannabis sativa tissue culture. Plants. 2021c; 10:185. https://doi.org/10.3390/ plants10010185.
175. Page SRG, Monthony AS, **Jones AMP**. DKW basal salts improve micropropagation and callogenesis compared with MS basal salts in multiple commercial cultivars of Cannabis sativa. Botany. 2021; 99:269–279. https://doi.org/10.1139/ cjb-2020-0179.
176. Rosslee J. 2020. The future of cannabis cloning: Tissue culture. https://www plantcelltechnology.com/pct-blog/the-future of-cannabis-cloning-tissue-culture/.
177. Wrobel T, Dreger M, Wielgus K, Słomski R. Modified nodal cuttings and shoot tips protocol for rapid regeneration of Cannabis sativa L. J. Nat Fibers. 2020; 19:536–545.
178. Wielgus K, Luwanska A, Lassocinski W, Kaczmarek, Z. Estimation of Cannabis sativa L. tissue culture conditions essential for callus induction and plant regeneration. J. Nat. Fib. 2008; 5: 199–207.
179. Wang R, He L.-S, Xia B, Tong J.-F, Li N, Peng F. A micropropagation system for cloning of hemp (Cannabis sativa L.) by shoot tip culture. Pak. J. Bot. 2009; 41(2): 603-608.
180. Wahby I, Caba J, Ligero F. Agrobacterium infection of hemp (Cannabis sativa L.): Establishment of hairy root cultures. J. Plant Interact. 2013; 8(4): 312-320.
181. Smýkalová I, Vrbová M, Cvečková M, Plačková L, Žukauskaitė A, Zatloukal M, Hrdlička J, Plíhalová L, Doležal K, Griga M. The effects of novel synthetic cytokinin derivatives and endogenous cytokinins on the in vitro growth responses of hemp (Cannabis sativa L.) explants. Plant Cell Tiss Organ Cult. 2019; 139: 381–394.
182. Ślusarkiewicz-Jarzina A, Ponitka A, Kaczmarek Z. Influence of cultivar, explant source and plant growth regulator on callus induction and plant regeneration of Cannabis sativa L. Acta Biol. Cracov. Bot. 2005; 47(2): 145–151.
183. Braemer R, Paris, M. Biotransformation of cannabinoids by a cell suspension culture of *Cannabis sativa* L. Plant Cell Rep. 1987; 6: 150–152.
184. Chandra S, Lata H, ElSohly MA, Walker LA, Potter D. Cannabis cultivation: methodological issues for obtaining medical-grade product. Epilep Behav. 2017; 70: 302-312.
185. Chandra SH, Lata I, Khan A, ElSohly MA. Propagation of elite *Cannabis* sativa for the production of Δ9-Tetrahydrocannabinol (THC) using biotechnological tools. In: Rajesh A (ed) Medicinal Plant Biotechnology. CABI, UK, 2010; pages 98-114.
186. Farag S. Cannabinoids production in Cannabis sativa *L*.: An in vitro approach. [dissertation] Dortmund: Technical University Dortmund. 2014.
187. Feeney M, Punja ZK. Tissue culture and *Agrobacterium*-mediated transformation of hemp (*Cannabis sativa* L.). In Vitro Cell Dev. Biol. -Plant. 2003; 39: 578–585.
188. Kodym A, Leeb C. Back to the roots: Protocol for the photoautotrophic micropropagation of medicinal Cannabis. Plant Cell, Tiss Org Cult. 2019; 13*8*(2): 399-402.
189. **Collyer D**. A Tissue Culture Method for Propagation of Plantlets of *Cannabis sativa* L. Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Biological Sciences Faculty of Science. Simon Fraser University, 2016. BC, Canada. **(**Zamir Punja Supervisor Professor, Biological Sciences). 2016.
190. Holmes J, **Punja**, **ZK**. Development of a Tissue Culture Based Agrobacterium-mediated Transformation System for Cannabis sativa L. (marijuana). In Vitro Cell. Dev. Biol.-Anim. 2021; 57: S27-S28.
191. Collyer D, Lung S, **Punja ZK**. Propagation of Marijuana (Cannabis sativa L.) Plantlets from Meristems and Nodal Explants and Identification of Fungal Contaminants in Tissue Culture Using a PCR-based Assay. In Vitro Cell. Dev. Biol.-Anim. 2020; 56: S44-S45.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VI June 2023

192. Collyer D, Lung S, **Punja ZK**. Propagation of Marijuana (Cannabis sativa L.) Plantlets from Meristems and Nodal Explants and Identification of Fungal Contaminants in Tissue Culture Using a PCR-based Assay. In Vitro Cell. Dev. Biol.-Anim. 2020; 56: 44-4529.
193. **Punja ZK**, Holmes J, Collyer D, Lung S. Development of Tissue Culture Methods for Marijuana (Cannabis sativa L.) Strains to Achieve Agrobacterium-mediated Transformation to Enhance Disease Resistance. In Vitro Cell. Dev. Biol.-Anim. 2019; 55: S23-S23.
194. **Punja ZK**, Collyer D, Lung S, Feeney M. Tissue Culture of Cannabis sativa and Approaches to Genetic Engineering. In Vitro Cell. Dev. Biol.-Anim. 2018;54.
195. Feeney M, **Punja ZK**. Tissue culture and Agrobacterium-mediated transformation of hemp (Cannabis sativa L.). In Vitro Cellular & Developmental Biology-Plant. 2003; 39: 578-585.
196. Adhikary D, Kulkarni M, El-Mezawy A, Mobini S, Elhiti M, Gjuric R, Ray A, Polowick P, Slaski JJ, **Jones MP**, Bhowmik P. Medical Cannabis and Industrial Hemp Tissue Culture: Present Status and Future Potential. Front. Plant Sci. 2021; 12:627240.
197. Wahby I, Arráez-Román D, Segura-Carretero A, Ligero F, Caba JM, Fernández-Gutiérrez A. (2006). Analysis of choline and atropine in hairy root cultures of Cannabis sativa L. by capillary electrophoresis-electrospray mass spectrometry. Electrophoresis. 2006; 27: 2208–2215.
198. Veliky IA, Genest K. Growth and metabolites of Cannabis sativa cell suspension cultures. Lloydia. 1972; 35: 450–456.
199. Uchendu E, Lata H, Chandra S, Khan I, ElSohly MA. Cryopreservation of shoot tips of elite cultivars of Cannabis sativa L. by droplet vitrification. Med. Cannabis cannabinoids. 2019; 2: 29–34.
200. Movahedi M, Ghasemi-Omran VO, Torabi S. The effect of different concentrations of TDZ and BA on in vitro regeneration of Iranian Cannabis (Cannabis sativa) using cotyledon and epicotyl explants. J. Plant Mol. Breed. 2015; 3: 20–27.
201. Piunno KF, Golenia G, Boudka EA, Downey C, Jones AM. Regeneration of shoots from immature and mature inflorescences of Cannabis sativa. Can. J. Plant Sci. 2019; 99: 556–559.
202. Plawuszewski M, Lassocinski W, Wieglus K. "Regeneration of polish cultivars of monoecious hemp (Cannabis sativa L.) grown in vitro," in Renewable resources and plant biotechnology, eds R. Kozlowski, E. Gennady, and F. Pudel (New York, NY: Nova Science Publishers Inc.). 2005; 149–154.
203. **Potter DJ**. The propagation, characterisation and optimisation of Cannabis sativa L. as a phytopharmaceutical. Ph.D. thesis, London: King's College London, 2009; 255.
204. Richez-Dumanois C, Braut-Boucher F, Cosson L, Paris M. Multiplication vegetative in vitro du chanvre (Cannabis sativa L.) application a la conservation des clones selectionnes. Agronomie. 1986; 6: 487–495.
205. Chaohua C, Gonggu Z, Lining Z, Chunsheng G, Qing T, Jianhua C et al. A rapid shoot regeneration protocol from the cotyledons of hemp (Cannabis sativa L.). Ind. Crop Prod. 2016; 83: 61–65.
206. Braemer R, Paris M. Biotransformation of cannabinoids by a cell suspension culture of Cannabis sativa L. Plant Cell Rep. 1987; 6 :150–152.
207. Prohens J, Herraiz FJ. Development of a Direct in vitro Plant Regeneration Protocol From Cannabis sativa L. Seedling Explants: Developmental Morphology of Shoot Regeneration and Ploidy Level of Regenerated Plants. Front. Plant Sci. 2020; 11:645. doi: 10.3389/fpls.2020.00645.
208. Feeney M, Punja ZK. "The role of Agrobacterium-mediated and other gene-transfer technologies in cannabis research and product development," in Cannabis sativa L. – Botany and Biotechnology, eds S. Chandra, H. Lata, and M. A. ElSohly (Berlin: Springer), 2017.
209. Norouzi O, Hesami M, Pepe M, Dutta A, **Jones AMP**. In vitro plant tissue culture as the fifth generation of bioenergy. Scientific Reports. 2022; 12:5038
210. Rico S, Garrido J, Sánchez C, Ferreiro-Vera C, Codesido V, Vidal N. A Temporary Immersion System to Improve Cannabis sativa Micropropagation. Front. Plant Sci. 2022; 13:895971.
211. Šenkyˇrík JB, Kˇrivánková T, Kaczorová D, Štefelová N. Investigation of the Effect of the Auxin Antagonist PEO-IAA on Cannabinoid Gene Expression and Content in Cannabis sativa L. Plants under In Vitro Conditions. Plants. **2023**; 12, 1664.
212. Ji B, Xuan L, Zhang Y, Mu W, Paek KY, Park SY, Wang J, Gao W. Application of Data Modeling, Instrument Engineering and Nanomaterials in Selected Medid the Scientific Recinal Plant Tissue Culture. Plants. 2023; 12:1505.
213. **Nethravathi TL**, Akshay KA, Sanman A, Gautam J, Praveen AY. TRAFFIC RECOGNITION SYSTEM USING MACHINE LEARNING. International Research Journal of Modernization in Engineering Technology and Science. 2022; 4(2): 550-552.
214. **Nethravathi TL**, Patil RL, Bhavana S, Choudhury SR, Monisha S. VIRTUAL PAINTER USING ARTIFICIAL INTELLIGENCE AND OPENCV. International Research Journal of Modernization in Engineering Technology and Science. 2022; 4(6): 3617-3620.

215. Goudar N, Vanjeri VN, Kasai D, Gouripur G, **Malabadi RB**, Masti SP, Chougale RB. ZnO NPs doped PVA/*Spathodea campanulata* thin films for food packaging. **Journal of Polymers and the Environment.** 2021. (https://doi.org/10.1007/s10924-021-02070-0).
216. Narasagoudr SS, Hegde VG, Chougale RB, Masti SP, Vootla S, **Malabadi RB.** Physico-chemical and functional properties of rutin induced chitosan/poly (vinyl alcohol) bioactive films for food packaging applications**. Food Hydrocolloids.** 2020; 109 ; 106096.
217. Gasti T, Dixit S, D'souza OJ, Hiremani VD, Vootla SK, Masti SP, Chougale RB, **Malabadi RB**. Smart biodegradable films based on chitosan/methylcellulose containing *Phyllanthus reticulatus* anthocyanin for monitoring the freshness of fish fillet. **International Journal of Biological Macromolecules**. 2021;187:451-467.
218. Hiremani VD, Goudar N, Gasti T, Khanapure S, Vanjeri VN, Sataraddi S, D'souza JO, Vootla SK, Masti SP, **Malabadi RB**, Chougale RB. Exploration of multifunctional properties of *Piper betel* leaves extract incorporated polyvinyl alcohol oxidized maize starch blend films for active packaging applications. **Journal of Polymers and the Environment.** 2021. (https://doi.org/10.1007/s10924-021-02277-1).
219. Kasai D, Chougale R, Masti SP, Gouripur G, **Malabadi RB**, Chalannavar RK, Raghu AV, Radhika D, Shanavaz H, Dhanavant S. Preparation, characterization and antimicrobial activity of betel-leaf-extract-doped polysaccharide blend films. **Green Materials.** 2021; 9(2): 49–68. (https://doi.org/10.1680/jgrma.20.00014).
220. Gasti T, Hiremani VD, Sataraddi SP, Vanjeri VN, Goudar N, Masti SP, Chougale RB, **Malabadi RB**. UV screening, swelling and in-vitro cytotoxicity study of novel chitosan/poly (1-vinylpyrrolidone-co-vinyl acetate)blend films. **Chemical Data Collections.** 2021; 33: 100684.
221. Gasti T, Dixit S, Sataraddi SP, Hiremani VD, Masti SP, Chougale RB, **Malabadi RB.** Physicochemical and Biological Evaluation of Different Extracts of Edible *Solanum nigrum* L. Leaves Incorporated Chitosan/Poly (Vinyl Alcohol) composite Films. **Journal of Polymers and the Environment.** 2020; **(**https://doi.org/10.1007/s10924-020-01832-6).
222. Kasai D, Chougale R, Masti S, Chalannavar R, **Malabadi** RB, Gani RS, Gouripur G. An investigation into the influence of Filler *Piper nigrum* leaves extract on physicochemical and antimicrobial properties of chitosan/poly (Vinyl Alcohol) blend films. **Journal of Polymers and the Environment.** 2019; 27(3): 472-488.
223. D'souza OJ, Hiremani VD, Gasti T, Goudar N, Varsha SL, Masti SP, Mudigoudra BS, **Malabadi RB**, Chougale RB. Fabrication and Study of Poly (vinyl alcohol) Film Functionalized with *Basella alba* Stem Extract. **Journal of Polymers and the Environment**. 2022; (**Doi.org/10.1007/s10924-022-02395-4**).
224. Hiremani VD, Gasti T, Masti SP, **Malabadi RB,** Chougale RB. Polysaccharide-based blend films as a promising material for food packaging applications: Physicochemical properties. **Iranian Polymer Journal. 2022.** (https://Doi.org/10.1007/s13726-021-01014).
225. Hiremani VD, Khanapure S, Gasti T, Goudar N, Vootla SK, Masti SP, **Malabadi RB**, Mudigoudra BS, Chougale RB. Preparation and physicochemical assessment of bioactive films based on chitosan and starchy powder of white turmeric rhizomes (*Curcuma zedoaria*) for green packaging applications. **International Journal of Biological Macromolecules**. 2021; 193(Part-B):2192-2201. Doi.org/10.1016/j.ijbiomac.2021.11.050.
226. Kasai D, Chougale RB, Masti S, Chalannavar KR, **Malabadi RB**, Gani RS. Influence of *Syzygium cumini* leaves extract on morphological, thermal, mechanical, and antimicrobial properties of PVA and PVA/chitosan blend films. **Journal of Applied Polymer Science**. 2018;(DOI: 10.1002/APP.46188; 1-17).
227. Ali J, Nicolas KLC, Akther S, Torabi A, Ebadi AA, Marfori-Nazarea CM, Mahender A. Improved Anther Culture Media for Enhanced Callus Formation and Plant Regeneration in Rice (Oryza sativa L.). Plants. 2021, 10, 839.
228. Chen QF, Wang CL, Lu YM, Shen M, Afza R, Duren, MV, Brunner H. Anther culture in connection with induced mutations for rice improvement. Euphytica. 2001; 120: 401–408.