Advancement in tissue culture techniques for fruit crops

Abstract:

Tissue culture is a highly promising approach that enables the efficient propagation of many plants from tiny fragments of the parent plant within a relatively brief timeframe and confined area. Tissue culture, a contemporary approach, is primarily employed for the efficient and extensive replication of many commercially significant plant species, such as the date palm. Utilizing the tissue culture technique presents a potential approach for generating a substantial quantity of genetically homogeneous palm plants that resemble other plants and yield typical fruit within four years from initial planting. Furthermore, this technique allows for the production of date palm plants devoid of diseases, exhibiting an exceptionally high survival rate of nearly 100% when compared to the vegetative propagation of shoots, owing to the robustness of their root system. The process of surface sterilization holds significant importance in the production of explants for in vitro studies, as it effectively addresses the issue of bacterial and fungal contamination originating from field sources, which might vary considerably across different fruit plant species. The efficacy of tissue culture techniques for date palm acclimatization in vitro is contingent upon the observation of leaf count prior to transplantation in the greenhouse. Hence, the primary objective of this study was to investigate the determinants that govern the tissue culture of fruit trees. India is known for being the native land of various fruit crops that are both significant and minor in terms of their importance. These crops include Indian gooseberry (*Emblicaofficinalis*Gaertn.), Karonda (*Carissa carandas* L.), Bael (*Aeglemarmelos* Corr.), Jamun (*Syzygiumcuminii* L.), and jackfruit (*Artocarpusheterophyllus* L.), etc. These fruits possess considerable nutritional, medicinal, and therapeutic value, making them highly valuable in commercial sectors such as medicine, food, and cosmetics. The limited availability of suitable planting materials imposes constraints on the commercial production process for these crops. Using plant tissue culture techniques holds promise in substantially augmenting the number of novel cultivars or genotypes inside fruit crops. The primary aim of this review study is to consolidate and synthesize the extant body of knowledge about the tissue culture techniques employed in cultivating various fruit crops.

Keywords: Tissue culture; In vitro; Somaclonal variation; Embryo rescue; Molecular marker

Comment [M1]: traditional vegetative

Introduction:

Comment [M2]: Introduction

Fruits hold significant significance in human existence owing to their consumable, therapeutic, and cultural worth. Global fruit production has reached a substantial level of 896.45 million tons. This notable output is mostly attributed to five key fruit crops: bananas and plantains, watermelons, grapes, oranges, and apples (FAO, 2021). Significant output has been observed in bananas and plantains, which are both classified as fruits. As primary suppliers of essential nutrients and secondary metabolites, fruits play a crucial role in the human diet and possess significant nutritional and therapeutic attributes (Cornara et al., 2020). In contemporary times, several fruits have emerged as functional foods due to their capacity to offer antioxidants and therapeutic phytochemicals, as evidenced by the research conducted by Sabbadini et al. (2021). The demand for fresh fruits and fruit-based goods remains consistent, leading to increased

momentum in fruit production across various fruit species. As a result, breeders are placing greater emphasis on achieving higher productivity levels. Post-harvest losses are a significant challenge in obtaining increased output and long-term economic benefits. These losses are mostly attributed to the perishable nature of the produce, accelerated fruit ripening, and the deterioration of nutritional quality. Furthermore, other issues necessitate ongoing breeding efforts, including an extended juvenile phase, diminished fruit quality, increased seed quantities, and rootstock/scion incompatibility. Within this particular framework, the utilization of traditional breeding techniques has substantially impacted the advancement of novel and enhanced cultivars regarding fruit quality, scent, antioxidants, yield, and nutritional characteristics (Sun-Waterhouse, 2011). Nevertheless, given the current challenges posed by climate change and the need for nutritional security, there is a growing need for extensive research efforts and innovative breeding strategies focused on enhancing several traits connected to fruit crops. These qualities include tolerance to biotic and abiotic stresses and an increase in nutritional quality. Several tropical fruits, such as banana, citrus, avocado, dragon fruit, papaya, mango, and guava, have recently garnered increasing interest in applying integrated omics methods (Sabbadini et al., 2021).

In the present context, several breeding techniques, including polyploidy, in vitro culture, mutagenesis, somaclonal variation, molecular markers, transgenics, and genome editing, are recognized as significant instruments for enhancing traits. Polyploidy in crop plants is frequently linked to an augmentation in cell size, commonly called the gigas effect. This phenomenon has been effectively utilized in ornamental species, as highlighted by Blasco et al. (2015). The phenomenon of polyploidy has been found to have a notable impact on increased heterozygosity, the emergence of new genotypes, and enhanced vigour, as documented by Sattler et al. (2016). According to Paterson (2005), polyploid plants frequently exhibit unique biochemical, physiological, morphological, and ecological characteristics that facilitate environmental adaptation. Multiple instances of polyploid fruit species have been documented in scientific literature, showcasing their advantageous characteristics such as enhanced quality (Wu et al., 2013), larger fruit size (Wu et al., 2012), improved disease resistance in Actinidia sp. (Wu et al., 2011), increased productivity (Predieri, 2001), as well as augmented biomass, fruit and flower size, pigment content, and secondary metabolite production (Touchell et al., 2020). The utilization of induced mutations has been vital in advancing favourable mutants, which have been approved for cultivation as novel crop types worldwide (Sarsu, 2020). Several noteworthy examples encompass rice, barley, cotton, groundnut, legumes, ornamentals, rapeseed, and Japanese pear. The field of biotechnological research in fruit crops, namely focusing on bananas, strawberries, papaya, pineapple, apples, citrus, and grapes, has seen significant advancements compared to other perennial fruit trees. Research in the field of plant cell and tissue culture has made significant contributions towards the establishment of methodologies for the efficient propagation of superior clones on a wide scale and the production of planting material free from viral contamination. Furthermore, this research has yielded valuable insights into the phenomenon of somaclonal variation, the process of somatic embryogenesis, and the techniques involved in genetic transformation.

Tissue culture is a highly prevalent method utilized for expeditious asexual multiplication in vitro. This technique demonstrates efficiency in terms of time and space use, resulting in increased productivity and producing pathogen-free and superior propagules. Additionally, it enables the secure and controlled transportation of germplasm between countries. When conventional approaches prove insufficient in meeting the demand for propagation material, this technology can generate a vast number of evenly flowering and producing plants. Micropropagation technology plays a crucial role in the efficient and

Comment [M3]: aroma,

Comment [M4]: toward

Comment [M5]: area

Comment [M6]: traditional

large-scale multiplication of vegetatively propagated plant species, ensuring the preservation of their genetic integrity, quick propagation, and maintenance of their unique characteristics. The global expansion of this technology has been substantial. Various fundamental techniques of tissue culture, including anther/microspore culture, somaclonal variation, embryo culture, and somatic hybridization, are currently being utilized to harness valuable genetic diversity to achieve gradual enhancements in commercial cultivars.

Comment [M7]: cultivarsorcommercial varieties

Tissue culture techniques in fruit crops:

The utilization of plant cell and tissue culture techniques has substantially impacted various aspects, including the propagation of economically valuable fruit species, preservation of genetic resources, synthesis of bioactive substances, and manipulation of desirable features through genetic modification. The commercial cultivation of various fruit species has been made feasible by implementing improved techniques for in vitro multiplication. This has been successfully achieved in peach, apple, cherry, apricot, citrus spp., mango, banana, and date palm. Tissue culture technologies have made significant contributions to various aspects of plant growth, including the establishment of techniques for the production of plants free from viral infections, the efficient propagation of superior clones, the induction of somatic embryogenesis, the generation of somaclonal variation, the creation of transgenic plants, and the preservation of genetic resources. When dealing with vegetatively propagated plants, the utilization of multicellular meristems is a common practice for doing in vitro mutagenesis (Wang et al., 2021). Nevertheless, the presence of chimeras and the potential for phenotypic instability pose constraints in this approach.

Comment [M8]: the transgenic plants,

Comment [M9]: vegetative

1. **Micropropagation**

Micropropagation has become a viable method for propagating nearly all fruit crops. Using meristem culture has facilitated the production of virus-free planting material in several horticultural crops. Notably, strawberry is one of the initial fruit crops in which micropropagation technology has been established as a standardized practice. Plants reproduced by in vitro techniques exhibit enhanced uniformity, a greater propensity for runner production, improved field survival rates, and a 24% increase in fruit yield compared to plants propagated through traditional methods. Plant tissue culture offers significant opportunities for the micropropagation of several fruit and horticultural crops, including strawberries, papaya, banana, grapes, pineapple, Citrus, tomato, cucumber, and watermelon (Anuradha and Malik, 2017). Tissue-based shoot tip culture techniques have significantly contributed to the micropropagation industry in commercial crops like bananas. These techniques have facilitated high-volume in vitro multiplication and the production of superior planting material. Over the past ten years, researchers have successfully achieved mass propagation using somatic embryogenesis and embryogenic cell suspension (ECS) techniques (Uma et al., 2021). These findings indicate that these methods have promise for the micropropagation sector. The maintenance of genetic homogeneity in clonally propagated plant populations has emerged as a significant challenge for the micropropagation business, as the occurrence of genetic variation in the offspring of these plants is considered undesirable (Sahijram et al., 2015). One prominent illustration can be seen in the case of bananas, where off-types originating from tissue-cultivated plantlets varied from 6 to 38% in Cavendish cultivars (Sahijram et al., 2003). Nevertheless, older reports have indicated that this percentage could reach as high as 90% (Smith, 1988). From a business perspective, any variation, especially genetic variation, is seen as detrimental and of little

value in micropropagation for commercial purposes. This is because such variations can result in a loss of genetic integrity. In recent decades, genetic variants have been noticeable in in vitro cultivated tissues, including undifferentiated cells, isolated protoplasts, and calli tissues (Krishna et al., 2016). The commercial propagation of bananas is achieved by utilizing tissue culture techniques. The future of the banana business in the country is contingent upon effective management and control of Banana Bunchy Top Virus (BBTV) illness. The optimal solution is using in vitro technology to produce disease-free banana plants to replace fields that have been afflicted. Several fruit crops utilize sexual propagation methods. Certain species exhibit dioecy, wherein male and female flowers are borne on separate individuals.

From a commercial perspective, there is a preference for the production of female plants, as these are the ones that give fruits. This objective may be achieved exclusively through the utilization of tissue culture techniques. The process involves grafting a minute shoot tip that has been carefully removed from a superior mother tree onto a rootstock seedling that has been previously decapitated. This rootstock seedling has been cultivated in a sterile environment to ensure optimal conditions for growth. The initial endeavour in Citrus was undertaken by Murashige et al., which was subsequently improved upon by Navarro et al. Embryo rescue refers to a technique employed in plant breeding and biotechnology that involves rescuing and cultivating embryos from sexually incompatible embryos. Embryo rescue is a distinct domain wherein plant breeders possess the capacity to salvage their hybridizations that would otherwise undergo abortion. Using cultured embryos at appropriate phases of development can effectively address challenges associated with post-zygotic incompatibility. This technique holds great significance in cultivating horticultural species characterized by their intractability and extended endurance. The utilization of ovule culture in grape cultivation facilitates the advancement of hybrid varieties.

Comment [M10]: in vitro

Comment [M11]: endeavor

Comment [M12]: hardiness and extended tolerance.

Comment [M13]: cultivars

2. Somaclonal variation

Somaclonal variation is a valuable mechanism for generating novel plant genotypes in breeding. Recent advancements in tissue culture techniques have significantly expanded this phenomenon's potential applications in viticulture. The field of tissue culture techniques has witnessed significant progress, enabling the regeneration of diverse horticultural species by in vitro methods. Micropropagation protocols, designed for large-scale multiplication, have been developed for various crops, facilitating their commercial cultivation. Plant tissue culture can potentially induce genetic variability, specifically somaclonal variants, through gene mutation or alterations in epigenetic markings. Conspicuous somaclonal variation is a disadvantage for both in vitro cloning and germplasm preservation. Somaclonal variation has emerged as a valuable tool for breeders in horticultural crops, particularly those challenging to breed or possessing limited genetic diversity. This phenomenon offers a means to quickly and efficiently introduce genetic variability without needing advanced technological interventions. Somaclonal variants confer advantageous attributes in generating diversity and enhancing resilience to environmental pressures. The phrase "somaclonal variation" was coined by Larkin and Scowcroft to describe the occurrence of variation resulting from the cultivation of cells or tissues. Somaclonal variants have become increasingly prevalent, and they now serve as a novel means of creating genetic diversity to acquire desirable features. The phenomenon of somaclonal variation holds significant potential in the context of fruit crops, mainly due to their predominant method of vegetative propagation and other breeding challenges, like limited genetic diversity and an extended period of immaturity. Somaclonal variation in conjunction with in vitro selection has been employed as an in vitro methodology to screen

desirable traits. The resulting soma clones, enhanced through this process, have found application in breeding fruit crops (Jain, 2001; Rai, 2011). Yoo et al. (2017) proposed that the application of selection pressure during in vitro selection, along with accurate identification of somaclonal variation, holds the potential for introducing desirable traits, such as resistance to Phytophthora, herbicide tolerance, and heat tolerance, into strawberry breeding programs.

Somatic embryogenesis, temporary immersion, and plant cell cultures exhibit considerable promise for fruit trees' in vitro propagation and genetic modification, including mango, banana, pistachio, apple, papaya, coffee, and date palm. Somatic embryogenesis presents several advantages in comparison to organogenesis. These advantages encompass its notable rates of multiplication, the potential for scaling up through the utilization of bioreactors, and the ability to supply synthetic seeds. Furthermore, somatic embryogenesis serves as a viable gene transfer target, as Egertsdotter et al. (2019) highlighted. Saraswathi et al. (2020) have successfully constructed somatic embryogenic systems in bananas, which have proven to be effective in facilitating high-frequency and large-scale propagation systems and in the creation of mutants by in vitro mutagenesis. Embryogenic cell cultures offer several advantages, including acquiring non-chimeric offspring and efficiently separating chimeric sectors. Figure 1 illustrates a scheme outlining the effective establishment of embryogenic cell suspension cultures derived from male floral apices in bananas. This system has become a standard practice in numerous prospective applications, such as developing synthetic seeds, mutants, transgenic plants, and genome-edited plants (Suprasanna et al., 2019; Ganapathi, 2021).

Comment [M14]: artificial

Comment [M15]: artificial

Factors affecting tissue culture of fruit crops

1. **Effect of mother plant on tissue culture:**Tang et al. (2008) investigated the impact of explant type, genotype, and age on shoot regeneration in pyrus communism. The researchers found that the most significant number of shoots and optimal shoot regeneration occurred when leaf sections were cultured on a Murashige and Skoog (MS) medium supplemented with 6.0 mg L^{-1} benzyl adenine (BA) and 0.1 mg L^{-1}naphthaleneacetic acid (NAA). The issue of phenolic exudation and contamination in guava c.v. "Banaras" was shown to be absent when utilizing somatic embryo-produced young and aseptic plantlets as the source of explants (Rai et al., 2009). In a study by Mustafa et al. (2016), shoot tips from three different fig cultivars, namely Aboudi, Gizy, and Sultany, cultivated plantlets. These shoot tips were cultured individually on Murashige and Skoog (MS) medium, supplemented with 0.5 mg L^{-1} 6-Benzylaminopurine. The addition of this growth regulator was found to enhance the development of explants while simultaneously mitigating issues such as necrosis and browning. Simultaneously, Emam (2006) discovered that the successful development of shoot tips of pear rootstock was achieved through cultivation on MS media, compared to using one-nodal cutting. According to the findings of Strosse et al. (2006), shoot tips derived from young suckers in banana plants were effectively cultivated on an MS media during the establishing phase. In a study conducted by Baiea (2009), it was discovered that leaf disc explants from two peach rootstocks exhibited good development when cultivated on a medium containing Murashige and Skoog (MS) nutrients. In a study conducted by Sumalatha in 2016, it was demonstrated that the shoot tip, when utilized as the explant, proved to be an effective method for the micropropagation of banana plants. A study conducted by Virk et al. (2010) found that nodal segments were more effective than leaf and root segments of citrus trees for inducing callus. Simultaneously, utilizing somatic embryo-produced young and aseptic

plantlets as explants source proved effective in micropropagationthe guava cultivar "Banarasi" (Rai et al., 2009). An in vitro study was conducted on papaya (*Carica papaya* L.) using five sources of explants (Shoot tip, nodule stem, internode segment, young leaf, and petiole) for shoot proliferation. It was found that all explants formed calli when grown on MS medium + 1 mg L^{-1} BA + 0.5 mg L^{-1} NAA four weeks after culturing. When re-culturing callus on the same components of the nutrient medium, it led to indirect shoot regeneration from callus induced from the shoot tips and nodule stem only (Al-Drisi et al., 2023).

Comment [M16]: of the guava

2. **Effect of surface sterilization on tissue culture:**The explants of banana plants underwent surface sterilization using a solution of 0.1% $HgCl_2$ containing tween 20 for 5 minutes. Following this, the explants were meticulously rinsed with sterile, deionized water (Sumalatha, 2016). In the study conducted by Singh et al. (2010), it was shown that the survival rate of explants in pomegranate was significantly improved by subjecting the shoot tip and nodal bud to a 20-minute treatment with sodium hypochlorite (NaOCl), commonly known as Clorox. In a separate investigation about the in vitro propagation of mangoes, it was observed that the utilization of a mixture of 10% sodium hypochlorite (specifically, "Clorox" containing 5.25% sodium hypochlorite, NaOCl) and 0.05% mercuric chloride ($HgCl_2$) for dipping periods of 7 and 10 minutes resulted in the most favourable outcomes in terms of survival percentages and contamination levels. Furthermore, using a 10% sodium hypochlorite solution yielded favourable outcomes regarding surface sterilization, resulting in minimal visible contaminants on the rootstocks. The species referred to as Mariana (Prunusmariana) was studied by Amiri et al. in 2013. Furthermore, Sim (2006) provided a comprehensive account of the successful surface sterilization of grape tissue (Vitisvinifera) by utilizing a 10% sodium hypochlorite solution combined with surfactant drops, with an exposure time of 10 minutes. According to Elbotaty (2012), immersing explants in a 15% sodium hypochlorite solution for 15 minutes proved an efficacious method for sterilizing grape rootstocks.

Comment [M17]: *Prunusmariana*

3. **Effect of phenol exudation on tissue culture**: The utilization of activated charcoal in the culture media resulted in the stimulation of initiation and proliferation of woody plants. However, it is essential to note that activated charcoal might have adverse effects, such as the adsorption of growth regulators and the reduction of the medium's pH (Feyissa et al., 2005). Simultaneously, subjecting newly sprouted apple shoots to a prolonged period of darkness effectively diminishes the excretion of phenolic chemicals from those shoots. Singh et al. (2007) conducted a study wherein they tried to develop pomegranate c.v. Mridula in vitro. They observed that using sterile wax on nodal segments reduced phenol exudation and led to a higher proportion of successful establishment of the explants. Various techniques were employed in this study, including maintaining explants in a dark environment following culture. Additionally, antioxidants such as citric acid (100 mg L^{-1}) and ascorbic acid (150 mg L^{-1}) were introduced to the medium for 30 minutes. Furthermore, activated charcoal ($3gL^{-1}$) was incorporated into the media. According to Ahmed et al. (2012), applying these treatments leads to a decrease in the production of phenolic compounds and the occurrence of browning in olive explants.
4. **Effect of media strength and type on tissue culture:**The study conducted by Mustafa et al. (2013) indicated that the medium with half MS strength exhibited the highest shoot number value. Furthermore, in their study, Mukherjee et al. (2010) reported that the induction of shoot proliferation from nodal explants of grape rootstock was observed when cultured on a medium with half-strength Murashige and Skoog formulation. According to Mazri (2012), the date palm

exhibited the maximum survival rate (ranging from 70% to 86%) when subjected to a medium strength half medium following a pre-acclimatization stage of one month. In contrast, the survival rate was much lower (12% to 28%) when the date palm was directly transferred to the greenhouse. In a study conducted by Yehia et al. (2012), it was observed that the most substantial enhancement in greening and explant growth per microflower bud was observed when using full and one-half medium strength compared to one-quarter medium strength in the case of pear "Le Conte" flower buds. Several parameters have been shown to influence the in vitro root production of date palm c.v. Boufeggous plantlets, as discussed by Othmani et al. (2009). These factors include the use of solid or liquid medium prior to acclimatization. According to Fki et al. (2011), using a liquid medium is considered more appropriate for the intermediate stage of plant growth (solid or semisolid media) prior to the transplantation of Barhee date palm plantlets into a greenhouse. According to Youssef (205), the survival rate of in vitro acclimatized date palm c.v. Barhee was found to be highest among plantlets cultivated on liquid Ms medium, compared to other medium conditions.

5. **Effect of carbon source on tissue culture:** In a study conducted on the effect of different concentrations of sucrose (10, 20, 30, 40, and 50 g L^{-1}) added to the MS medium with the aim of multiplication of papaya (*Carica papaya* L.) shoots. It was noted that the concentration of 30 gm L^{-1} sucrose was significantly superior to the rest of the sucrose concentrations in terms of the high response to shoot multiplication. It recorded the highest rate of number of branches, reaching 4.1. shoots per culture (Al-Drisi et al., 2022a).The study by Kabir et al. (2007) demonstrated that adding 30 g L^{-1} sucrose to the growth medium yielded the most favourable outcome for shoot tip explants of papaya. In a study conducted by Ahmed et al. (2014), it was demonstrated that a concentration of 30 g L^{-1} of sucrose, used as a carbon source, exhibited the most favourable outcomes for the in vitro rooting of banana (Musaspp) cultivar Grand naine plantlets. Simultaneously, adding 30-60 g L^{-1} sucrose to the culture medium resulted in optimal explant development and shoot growth of date palms. Fructose, on the other hand, exhibited the highest dry weight values compared to other carbon sources such as glucose, sucrose, and maltose (Al-Khateeb, 2008). According to Yehia et al. (2012), including a medium strength treatment resulted in increased proliferation % and shoot quantity compared to the other treatments. According to Sumalatha (2016), sucrose concentrations ranging from 30-40 g L^{-1} as carbon sources yielded optimal outcomes in the micropropagation of banana plants.
6. **Effect of light, temperature and pH on tissue culture:** The factors that influence the growth and development of organisms include light availability, temperature conditions, and pH requirements. Culturing date palm plantlets of the Barhi cultivar in vitro typically involves subjecting them to a light intensity of 2000 lux. This is achieved by utilizing varying quantities of white cool fluorescent lamps, which provide 16 hours of light followed by 8 hours of darkness. This particular light intensity has been found to enhance several critical parameters under investigation, including root length, root number, shoot length, and greening, compared to alternative light intensities. In a study conducted by Hagagy et al. (2015), it was shown that the shoot thickness of the cultures increased when exposed to a light intensity of 3000 lux. According to Chandra et al. (2010), exposure of in vitro plantlets to elevated levels of light intensity (ranging from 4000-12000 lux) and temperature (ranging from 26-36EC) can potentially result in leaf charring and plantlet wilting. Furthermore, the optimal shoot thickness for in vitro root production of date palm cultivar Barhi was observed when the cultures were exposed to a light

Comment [M18]: before

Comment [M19]: before

Comment [M20]: *Musa*

Comment [M21]: Naine

intensity of 3000 lux. Youssef (2015) documented the number of roots, root length, number of leaves, and greening in the cultures under a light intensity of 2000 lux. Furthermore, subjecting the explant to cold pretreatment at a temperature of 5EC for three days resulted in the most favourable outcomes regarding the reactions exhibited by peach rootstocks. Ahmed et al. (2014) achieved optimal root development. They minimized root initiation time while maximizing root length at a pH of 5.5 during the medium preparation for in vitro rooting of Banana plantlets, namely the Grand Naine cultivar.

7. **Effect of plant hormones on tissue culture**: Adding 3 mg L^{-1} BA and 0.5 mg L^{-1} NAA to the culture media resulted in a regeneration response of 71.89% from model segments in citrus plants. According to Savita et al. (2010), the highest rooting percentage (71%) was achieved in certain instances by cultivating callus produced from modal segments on Ms medium enriched with 0.5 mg L^{-1} NAA and 3 mg L^{-1} BA. Adding 1.0 mg L^{-1} BA to the Msmedium resulted in the most effective proliferation of almond plantlets of the Nonpareil cultivar. In another study, papaya shoots were multiplied in vitro from axillary bud explant by using different combinations of plant growth regulators. It was found that culturing the axillary buds of papaya plants in MS medium + 1 mg L^{-1} BA and 0.5 mg L^{-1} NAA led to a high shoot multiplication response (100%) and the highest shoot rate reached 4.7 shoots per culture (Al-Drisi et al., 2022b).It was found in a study that culturing the shoot tips of the Citrus japonica Thumb. plant in MS medium + 2 mg L^{-1} BA and 15 mg L^{-1} chitosan led to a high response to shoot proliferation and recorded the highest average number of shoots, reaching 5.2 shoots per explant. When the shoots produced from the multiplication were grown on MS medium + 2 mg L^{-1} NAA and 15 mg L^{-1} chitosan, it led to a high response to root induction (Safana et al., 2022).Nodule stem explants of moringa (*Moringaoleifera* L.) plant that were grown in MS medium + 1 mg L^{-1} BA and 0.2 mg L^{-1} NAA recorded the highest response rate to shoot proliferation and the number of shoots reached 68.33% and 4.91 shoots, respectively (Ewhayid et al., 2023).According to Isikalan et al. (2008), the highest number of roots and longest root length/plantlets were seen when the concentration of indole acetic acid was 8.0 mg L^{-1}. In a study conducted by Guadi (2011), it was observed that the addition of 1.0 and 1.5 mg L^{-1} of benzylaminopurine (BAP) in combination with 1.0 mg L^{-1} of indole-3-butyric acid (IBA) resulted in a survival rate of 96.7% for MM 106 apple plantlets and 93.3% for Anna apple plantlets. Furthermore, adding 0.5 mg L^{-1}BAP to the Ms medium stimulated shoot proliferation, with an average of 5.37 shoots per explant seen in a study conducted on a dwarfing cherry rootstock (Mahdavian et al., 2011). The findings of this study indicate that the addition of 2.22 mg L^{-1} BAP to the Olive medium resulted in the highest proliferation rates, with an average of 3.4 additional explants observed after 30 days. Peixe et al. (2007) reported that OM treated with 3 g L^{-1}IBA achieved a rooting rate of 85% in similar circumstances. Simultaneously, applying a high cytokinin concentration resulted in jojoba's most effective shoot proliferation. Fayek et al. (2007) observed the highest callus induction when using Auxins alone or combined with cytokinin.

Secondary metabolite production through tissue culture

The term "secondary metabolite" pertains to a molecule synthesized by plants that is not essential for their primary growth and development (Pickens et al., 2011; Ibrahim, 2022). Throughout history, humans have extensively utilized the derivatives of plant secondary metabolism to fulfil a diverse range of requirements (Cragg and Newman 2013). The predominant application of these substances has

Comment [M22]: S: Capital letter

Comment [M23]: fulfill

historically been in medicine, initially by empirical methods and then, in the 19th century, through a more systematic approach facilitated by the isolation of molecules (Cragg and Newman, 2013). Secondary metabolites are commonly identified by their intricate and varied chemical composition, often consisting of several chiral centres and labile bonds, which presents difficulties in their chemical production (Pickens et al., 2011; Ibrahim et al., 2022). Hence, the extraction of biologically active compounds is predominantly derived from their natural sources. Nevertheless, due to the prevalence of wild plants as opposed to cultivated varieties, there is a potential hazard of over-utilization and a subsequent constraint in synthesizing these substances when sourcing from their native environments. The regulated cultivation of plant cells and tissues in a laboratory setting, known as in vitro culture, provides a robust technological framework for synthesizing plant-derived natural compounds. In vitro propagation, called micropropagation, involves cultivating plants or plant organs (often roots) or calluses in a controlled laboratory environment. This technique has been shown to yield plant material capable of creating secondary metabolites, as demonstrated by studies conducted by Ochoa-Villarreal et al. (2016) and Espinosa-Leal et al. (2017). Micropropagation has emerged as a financially profitable venture, offering significant benefits compared to traditional horticultural propagation methods. It enables the consistent production of a large quantity of genetically identical plants throughout the year, the creation of pathogen-free plant materials, and a notable increase in multiplication rates (Debnarh et al., 2006). According to Fischer et al. (2015), using plant cell culture has emerged as a highly effective method for producing several valuable natural products. The array of economically significant products encompasses colours such as anthocyanins and betacyanins, anti-inflammatory drugs like berberine and rosmarinic acid, and anti-cancer compounds such as paclitaxel and podophyllotoxin.Culturing moringa plant callus in MS medium + 5 mg L^{-1} chitosan led to the induction of the largest amount of the bioactive compound myricetin, which amounted to 79.572 mol L^{-1}myricetin compared to other concentrations of chitosan. When the callus was grown in MS medium + 10 mg L^{-1}nanosilver, it led to the induction of the largest amount of the bioactive compound myricetin, reaching 26.387 mol L^{-1}myricetin compared to other nanosilver concentrations (Ewhayid et al., 2022).

Conclusion

The propagation of new plants by plant tissue culture (PTC) exhibits promising commercial potential across various plant categories, encompassing crops, fruits, vegetables, and ornamental species. Over 100 species in India have undergone reengineering through contemporary PTC methods. According to current estimates, India can generate about 350 million cultured plants annually. The utilization of PTC in plant biology offers potential solutions to various challenges encountered in experimental biology, particularly laborious when using traditional methods. PTC currently assumes a substantial role in preserving plant well-being, encompassing several aspects such as genetic engineering, breeding, and afforestation. The agriculture and horticulture industries have greatly benefited from its various advantages, including cultivating plants resistant to pests, diseases, and viruses. Creating plants that exhibit resistance to abiotic stressors and possess biofortified traits has significantly influenced the trajectory of contemporary agriculture and food production. The commercial cultivation of plants through PTC industries holds significant promise and potential, provided that production facilities can overcome many limitations, including the availability of advanced research resources, financial considerations, and effective marketing strategies. India possesses a range of agroclimatic zones and benefits from cost-effective labour availability. These advantageous conditions, along with the implementation of government initiatives, have the potential to facilitate India's sustained self-sufficiency in agricultural production. Moreover,

digital technologies can bring about a transformative impact on the PTC (Public Transportation Company) market. The research and implementation of novel software for the purpose of monitoring plant growth, alongside the introduction of mobile applications designed to assist farmers and industrialists in various stages of the plant production process, ranging from the collecting of explants to the final sale of fully developed plants in the market. Taking into account these various considerations, it can be said that the Indian tissue culture business, despite its relatively late start, has the potential to have a significant impact on the global stage.

Comment [M24]: Plant Tissue Culture

References:

Ahmed, A.R. and R.S. Ali, 2012.In vitro microporpagation of olive (Aeaeuropaea L.)Mission by nodal segments. J. Bol. Environ., 6: 155-159.

Ahmed, S., A. Sharma, B. Bhushan, A.K. Singh and V.K. Wali, 2014.Effect of carbohydrate source, pH and supporting media on in vitro rooting of banana (Musa spp.) cv. Grand naine plantlets. Afr. J. Agric. Res., 9: 1135-1140.

Al-Drisi, E.E., Ibrahim, M.A. and Jasim, A.M., 2022a.Impact of Different Sucrose Concentrations on Shoot Multiplication of Papaya (*Carica papaya* L.)Cultured in vitro. Basrah Journal of Agricultural Sciences, 35(2):240-247.

Al-Drisi, E.E., Ibrahim, M.A. and Jasim, A.M., 2022b.Effect of different combinations of benzyl adenine and naphthalene acetic acid on micropropagation of *Carica papaya*L. hybrid plant in vitro. Journal of Agricultural and Statistical Sciences, 18(2):681-688.

Al-Drisi, E.E., Ibrahim, M. and Jasim, A.M., 2023. Explant type influences callus induction and shoots organogenesis in papaya under in vitro conditions. DYSONA-Applied Science, 4(2023):15-20.

Al-Khateeb, A.A., 2008.Regulation of in vitro bud formation of date palm (Phoenix dactylifera L.) cv. Khanezi by different carbon sources.Bioresour. Technol., 99: 6550-6555.

Amiri, S., S. Ashtari, H.B. Abdullah, S.A. Nazari, E. Khodadadi, E. Khodadadi and M. Sabzi, 2013. Control contamination during micropropagation process of rootstock Mariana (Prunusmariana). Ann. Biol. Res., 4: 149-151.

Anuradha, S.; Malik, A. 2017.Biotechnology a Modern Tool for Fruits Production—A Review. Int. J. Curr. Microbiol. App. Sci., 6, 1902–1912.

Baiea, M.H., 2009. Physiological and cytological studies on tolerance of some stone fruits root stocks to salinity and Drought by using micro-propagation. Ph.D. Thesis, Zagazig University, Egypt

Blasco, M.; Badenes, M.L.; Naval, M.M.2015.Colchicine-induced polyploidy in loquat (Eriobotrya japonica (Thunb.)Lindl.). Plant Cell Tissue Organ, 120, 453–461.

Chandra, S.V.B., O.V. Kumar and R. Chandra, 2010. Acclimation of tissue cultured plants from laboratory to land. Biotec Letts., 32: 1199-1205

Cornara, L.; Xiao, J.; Smeriglio, A.; Trombetta, D.; Burlando, B.2020.Emerging Exotic Fruits: New Functional Foods in the European Market. Food, 1, 126–139.

Cragg GM, Newman DJ 2013 Natural products: a continuing source of novel drug leads. BiochimBiophysActa 1830:3670–3695.

Debnarh M, Malik CP, Baisen PS 2006 Micropropagation:a tool for the production of high quality plant based medicines. Curr Pharm Biotechnol 7:33–49

Egertsdotter, U.; Ahmad, I.; Clapham, D. 2019.Automation and Scale Up of Somatic Embryogenesis for Commercial Plant Production, With Emphasis on Conifers. Front. Plant Sci., 10, 109.

Elbotaty, E.M.A., 2012. Production of developed grape rootstocks using in vitro mutations.Ph.D. Thesis, Cairo University, Egypt.

Emam, H.E., 2006. Physiological studies on in vitro propagation of pyruscommunis root stock. Ph.D. Thesis, Cairo University, Egypt.

Espinosa-Leal CA, Garza-Padrón RA, Morales-Rubio ME 2017 Cultivo in vitro comoalternativapara la producción de metabolitos. Editorial Académica Española, Saarbrücken

Ewhayid, B.M., Ibrahim, M.A. and Abdulzahra, E.M., 2022. Effect of Growth Regulators, Chitosan, and Silver Nanoparticles on Callus Induction and Stimulating the Myricetin Production in Moringa (*MoringaOleifera* Lam.)Tree. Texas Journal of Agriculture and Biological Sciences, 10:85-94.

Ewhayid, B.M., Ibrahim, M.A. and Abdulzahra, E.M., 2023. Chitosan as a growth stimulator of moringa (*Moringaoleifera* L.) under in vitro conditions. DYSONA-Applied Science, DAS 4 (2023):28-34.

FAO. World Food and Agriculture—Statistical Yearbook 2021; FAO: Rome, Italy, 2021.

Feyissa, T., M. Welander and L. Negash, 2005.Micropropagation of Hageniaabyssinica: A multipurpose tree. Plant Cell Tissue Organ Culture, 80: 119-127

Fischer R, Vasilev N, Twyman RM, Schillberg S 2015 High-value products from plants: the challenges of process optimization. CurrOpinBiotechnol 32:156–162.

Fki, L., N. Bouaziz, W. Kriaa, R. Benjemaa-Masmoudi, R. Gargouri-Bouzid, A. Rival and N. Drira, 2011. Multiple bud cultures of 'Barhee' date palm (Phoenix dactylifera) and physiological status of regenerated plants. J. Plant Physiol., 168: 1694-1700

Ganapathi, T.R.; Negi, S.; Tak, H.; Bapat, V.A. 2021.Transgenic Banana: Current Status, Opportunities and Challenges. In Genetically Modified Crops; KaviKishor, P.B., Rajam, M.V., Pullaiah, T., Eds.; Springer: Singapore,; pp. 111–128.

Guadi, D., 2011. Micropropagation of two apple (MalusdomesticaBorkh) varieties from shoot tip explants. M.Sc. Thesis, Addis Ababa University, Ethiopia.

Hagagy, N.A.A., N.S. Zayed, Y.Y. Abdel Atty and D.A.B. Youssef, 2015. Trials on acclimatization of tissue culture derived date palm plants (Phoenix dactylifera L.). Ann. Agric. Sci. Moshtohor, 53: 243-248.

Ibrahim M (2022) Role of Endogenous and Exogenous Hormones in Bioactive Compounds Production in Medicinal Plants via In Vitro Culture Technique.Plant Hormones - Recent Advances, New Perspectives and Applications.IntechOpen, London, UK. Available at: http://dx.doi.org/10.5772/intechopen.102814

Ibrahim, M.A., Al-Jabir, H.S.S. and Lafta, A.Y., 2022.Protocols of micropropagation of some medicinal plants. Multidisciplinary Reviews, 5(3):1-15.

Isikalan, C., F.A. Akbas, S. Namli, E. Tilkat and D. Basaran, 2008.In vitro micropropagation of almond (Amygdaluscommunis L. cv. Nonpareil). Afr. J. Biotechnol., 7: 1875-1880.

Jain, S.M. 2001. Tissue culture-derived variation in crop improvement.Euphytica, 118, 153–166.

Kabir, A.H., M.A. Bari, A.K.M.N. Huda, M.A. Rezvy and I. Mahfuz, 2007.Effect of growth regulators and carbon sources on axillary shoot proliferation from shoot-tip explant and successful transplantation of papaya (Carica papaya L.). Biotechnology, 6: 268-272.

Krishna, H.; Alizadeh, M.; Singh, D.; Singh, U.; Chauhan, N.; Eftekhari, M.; Sadh, R.K. 2016. Somaclonal variations and their applications in horticultural crops improvement.3 Biotech, 6, 54.

Mahdavian, M., N. Bouzari and H. Abdollahi, 2011.Effects of media and plant growth regulators on micropropagation of a dwarfing cherry rootstock (PHL-A).Biharean Biol., 5: 86-90

Mazri, M.A., 2012. Effect of liquid media and in vitro pre-acclimatization stage on shoot elongation and acclimatization of date palm (Phoenix dactylifera L.) cv. Najda. J. Ornament Hortic. Plants, 2: 225-231.

Mukherjee, P., N. Husain, S.C. Misra and V.S. Rao, 2010. In vitro propagation of a grape rootstock, deGrasset (Vitischampinii Planch.): Effects of medium compositions and plant growth regulators. Scient.Hortic., 126: 13-19.

Mustafa, N.S., R.A. Taha, S.A.M. Hassan and N.S.M. Zaid, 2013.Effect of medium strength and carbon source on in vitro shoot multiplication of two Ficuscarica cultivars. J. Applied Sci. Res., 9: 3068-3074.

Mustafa, N.S., S.A.M. Hassan and R.A. Taha, 2016.In vitro studies on growth and rooting of some fig cultivars. Res. J. Pharm. Biol. Chem. Sci., 7: 124-130.

Ochoa-Villarreal M, Howat S, Hong S, Jang MO, Jin YW, Lee EK, Loake GJ 2016 Plant cell culture strategies for the production of natural products. BMB Rep 49:149–158

Othmani, A., C. Bayoudh, N. Drira, M. Marrakchi and M. Trifi, 2009. Somatic embryogenesis and plant regeneration in date palm Phoenix dactylifera L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant Cell Tissue Organ Culture, 97: 71-79.

Paterson, A.H. 2005. Polyploidy, evolutionary opportunity, and crop adaptation. Genetica, 123, 191–196.

Peixe, A., A. Raposo, R. Lourenco, H. Cardoso and E. Macedo, 2007. Coconut water and BAP successfully replaced zeatin in olive (Oleaeuropaea L.) micropropagation. Scient.Hortic., 113: 1-7.

Pickens LB, Tang Y, Chooi Y-H 2011 Metabolic engineering for the production of natural products. Annu Rev ChemBiomolEng 2:211–236.

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. Plant Cell Tissue Organ, 64, 185–210.

Rai, M.; Kalia, R.; Singh, R.; Gangola, M.P.; Dhawan, A. 2011.Developing stress tolerant plants through in vitro selection—An overview of the recent progress. Environ. Exp. Bot., 71, 89–98.

Rai, M.K., V.S. Jaiswal and U. Jaiswal, 2009. Shoot multiplication and plant regeneration of guava (Psidiumguajava L.) from nodal explants of in vitro raised plantlets. J. Fruit Ornamental Plant Res., 17: 29-38.

Sabbadini, S.; Capocasa, F.; Battino, M.; Mazzoni, L.; Mezzetti, B2021.Improved nutritional quality in fruit tree species through traditional and biotechnological approaches. Trends Food Sci. Technol., 117, 125–138.

Safana, H.S., Ibrahim, M.A. and Abd, A.M., 2022. Impact of chitosan and benzyl adenine on shoot multiplication of kumquat plant (*Citrus japonica* Thumb.) in vitro. Int. J. Agricult. Stat. Sci. Vol, 18(1):359-365.

Sahijram, L. 2015. Soma clonal Variation in Micropropagated Plants. In Plant Biology and Biotechnology; Bahadur, B., VenkatRajam, M., Sahijram, L., Krishnamurthy, K., Eds.; Springer: New Delhi, India,; pp. 407–416.

Sahijram, L.; Soneji, J.R.; Bollamma, K.T. 2003.Analyzing soma clonal variation in micropropagated bananas (Musa spp.).Vitr.Cell. Dev. Biol.-Plant, 39, 551–556.

Saraswathi, M.S.; Kannan, G.; Uma, S.; Kalaiponman, K. Improvement in Banana through Mutation Breeding: Status and Prospect. In Bananas and Plantains: Leading-Edge Research and Development. Vol. 1: Diversity, Improvement and Protection; Uma, S., Vaganan, M.M., Agrawal, A., Eds.; ICAR-National Research Centre for Banana: Tiruchirappalli, India,; pp. 288–308.

Sarsu, F. 2020. Contribution of induced mutation in crops to global food security.ACI Av. Cienc.Ing., 12, 10.

Sattler, M.C.; Carvalho, C.R.; Clarindo, W.R. 2016.The polyploidy and its key role in plant breeding.Planta, 243, 281–296

Savita, V., G. Virk and A. Nagpal, 2010.Effect of explant type and different plant growth regulators on callus induction and plantlet regeneration in Citrus jambhiri Lush. Environ. We Int. J. Sci. Technol., 5: 97-106.

Sim, S.T., 2006. Virus elimination from grape selections using tissue culture. FPS Grape Program Newsletter, November 2006, pp: 30-31.

Singh, N.V., S.K. Singh and V.B. Patel, 2007. In-vitro axillary shoot proliferation and clonal propagation of 'G 137' pomegranate (Punicagranatum). Indian J. Agric. Sci., 77: 505-508.

Singh, S.K., A. Singh, N.V. Singh and D. Ramajayam, 2010.Pomegranate tissue culture and biotechnology. Fruit Vegetable Cereal Sci. Biotechnol., 4: 35-44.

Smith, M.K. 1988. A review of factors influencing the genetic stability of micropropagated bananas. Fruits, 43, 219–223

Strosse, H., H. Schoofs, B. Panis, E. Andre, K. Reyniers and R. Swennen, 2006.Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (Musa spp.). Plant Sci., 170: 104-112.

Sumalatha, A., 2016. Plant tissue culture of Banana in laboratory. J. Bot. Sci., 3: 54-62.

Sun-Waterhouse, D.2011. The development of fruit-based functional foods targeting the health and wellness market: A review. Int. J. Food Sci. Technol., 46, 899–920

Suprasanna, P.; Ghag, S.; Ganapathi, T.R.; Jain, S.M. 2020.Induced genetic diversity in banana. In Genetic Diversity in Horticultural Plants; Nandwani, D., Ed.; Sustainable Development and Biodiversity; Springer: Heidelberg, Germany, 2019; Volume 22, pp. 273–297.

Tang, H., Y. Luo and C. Liu, 2008.Plant regeneration from in vitro leaves of four commercial Pyrus species. Plant Soil Environ., 54: 140-148

Touchell, D.H.; Palmer, I.E.; Ranney, T.2020. In vitro Ploidy Manipulation for Crop Improvement. Front. Plant Sci., 11, 722.

Uma, S.; Kumaravel, M.; Backiyarani, S.; Saraswathi, M.S.; Durai, P.; Karthic, R. 2021.Somatic embryogenesis as a tool for reproduction of genetically stable plants in banana and confirmatory field trials. Plant Cell Tissue Organ Cult., 147, 181–188.

Wang, X.; Wang, A.; Li, Y.; Xu, Y.; Wei, Q.; Wang, J.; Lin, F.; Gong, D.; Liu, F.; Wang, Y.; et al. 2021. A Novel Banana Mutant "RF 1" (Musa spp. ABB, PisangAwak Subgroup) for Improved Agronomic Traits and Enhanced Cold Tolerance and Disease Resistance. Front. Plant Sci., 12, 730718.

Wu, J.-H.; Ferguson, A.; Murray, B.; Duffy, A.M.; Jia, Y.; Cheng, C.; Martin, P. 2013. Fruit Quality in Induced Polyploids of Actinidiachinensis.HortScience, 48, 701–707.

Wu, J.H.; Ferguson, A.R.; Murray, B.G. 2011.Manipulation of ploidy for kiwifruit breeding: In vitro chromosome doubling in diploid Actinidiachinensis Planch. Plant Cell Tissue Organ Cult., 106, 503–511.

Wu, J.H.; Ferguson, A.R.; Murray, B.G.; Jia, Y.; Datson, P.M.; Zhang, J. 2012. Induced polyploidy dramatically increases the size and alters the shape of fruit in Actinidiachinensis. Ann. Bot., 109, 169–179.

Yehia, T.A., A.A. Hegazi, M.A. Saleh, D.M. Ahmed, N.S. Zaied and S.A. Hassan, 2012. Physiological studies on development flower bud in vitro of Le Conte pear trees. Middle-East J. Sci. Res., 12: 864-869.

Yoo, C.M.; Dalid, C.; Moyer, C.; Whitaker, V.; Lee, S. 2011, Improving Strawberry Varieties by Somaclonal Variation. UF-IFS Extension.x, 1–5.

Youssef, D.A.B., 2015. Physiological studies on acclimatization and growth behavior of date palm plants resultant from tissue culture. M.Sc. Thesis, Zagazig University, Egypt