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Tissue Culture in Banana Cultivation: A Review of its Impact on Disease Management, Yield Improvement, and Sustainable Production

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

In several tropical and subtropical areas, bananas (*Musa* spp.) are an important food source and commercial crop. Disease transmission and production uncertainty are common problems with traditional propagation techniques such as suckers. By supplying consistent, high-quality, and disease-free planting material, tissue culture, known as micro propagation, represents a potential alternative. The application of tissue culture has greatly enhanced disease management in banana cultivation. Reducing the incidence of ailments like Fusarium wilt, bacterial wilt, and Banana Bunchy Top Virus (BBTV), pathogen-free plantlets are grown in controlled conditions. This strategy lessens the demand for artificial fertilizers while also promoting healthier crops and lessening the impact on the environment. Micropropagated banana plants demonstrated consistent growth, early fruiting, and increased yield in contrast to conventionally propagated plants. Tissue-cultured plants exhibit strong development and genetic uniformity, which contribute to these advantages and more consistent and dependable harvests. By promoting efficient land use and reducing the need for chemical inputs, tissue culture enhances sustainability in the production of bananas. The growing demand for bananas may be met without requiring the increase of agricultural land thanks to tissue culture techniques, which enable the large-scale production of enhanced banana cultivars at a quick rate. Furthermore, using planting material free of disease lowers input costs and crop losses, supporting more environmentally friendly farming methods.

Keywords: Tissue culture; micropropagation; composition; disease resistance; sterilization; genetic transformation.

1. INTRODUCTION

Plantains and bananas are herbaceous perennial monocots that are cultivated in more than 130 tropical and subtropical countries. They are members of the Musa genus within the Musaceae family. After rice, wheat, and maize, bananas (*Musa spp.*) rank as the fourth most significant food crop [123]. Because of its inexpensive cost and excellent nutritional content, banana fruit is especially popular in underdeveloped countries. It is a rich source of vitamins A, C, and B6, as well as proteins, carbs, and minerals including calcium, magnesium, manganese, and potassium [102]. Researchers state that in addition to the fruit, other components of the banana plant, such as peels, pseudo-stem, rhizome, leaves, etc., are used in a variety of industries [2,55,4,75]. Traditionally, suckers are used as a beginning material while growing bananas. The plants are not uniform and the banana crops are produced vegetatively in the field [16]. According to the National Horticulture Board (2018), Andhra Pradesh is one of India's top banana-growing states, producing 414,355 t of bananas per hectare and 4.8 t/ha of productivity over an area of 86,320 ha. However, several ailments and pests hinder their production, mostly due to inferior clones [61].

New germplasm can be developed by biotechnology that uses genetic transformation and tissue culture [103]. Using high-tech techniques, tissue culture plants may be cultivated in compact spaces and produce fruits of high quality while remaining consistent, true to type, and free from pests and diseases [41]. Many nations use the excellent alternative of *in vitro* banana plantlet multiplication, including Cuba, Israel [56], France [24], Australia [31], and many African nations [127]. Compared to the traditional method, tissue culture technology

offers a clear and crucial advantage in ensuring a rapid rate of multiplication. Because of the controlled environment and the small amount of plant tissue needed as the transplant source, this procedure is season-independent [8]. Banana micropropagation has been accomplished from male flower apices [33] and shoot tips [26]. Additionally, reports of somatic regeneration and embryogenesis in liquid medium have been published [89].

Nutritional Requirements: An essential component of banana nutrition is nitrogen. Banana plants cannot store nitrogen. But too much nitrogen produces large plants with deep green leaves. Bunch loss can sometimes be caused by the peduncle breaking off inside the pseudo stem. Phosphorus is not as necessary as potassium or nitrogen. Banana plants accumulate the required Phosphorus over an extended period of time. They lose very little Phosphorus through fruit and readily transfer the remainder to the suckers. Three to nine months after planting, banana plants absorb the majority of their phosphorus. When it comes to nutrients, potassium is the most important one for bananas.The growing bunch has a high need for potassium, and if the soil is unable to provide it in sufficient amounts after the blooming stage, the leaf system may collapse as a result of the potassium being taken out of the leaves and supplied to the bunch. The dietary needs of bananas also include traces of calcium, magnesium, sulfur, zinc, manganese, boron, iron, and copper [77].

2. MICROPROPAGATION (SHOOT TIP CULTURE)

Berg and Bustamante [14] were the first authors to publish on the application of banana tissue culture. They found that using thermotherapy in conjunction with banana meristem stem culture could produce virus-free plants. To quickly increase planting material free of Fusarium wilt, Ma and Shi [71] used Cavendish (AAA) shoot tips that had been dissected from suckers. Since then, several features of banana tissue culture have been studied as a catalyst for optimizing banana production [111], and banana micro propagation has been used in a wide range of cultivars [127]. Earlier in the 1980s, tissue culture banana plantlets were planted in large fields for the first time in Taiwan and Jamaica [54,92].

2.1 Source and Selection of an Explant

Small plant fragments, known as explants are used to initiate plant tissue cultures. Explants may be effectively obtained from almost any plant section [3]. Many explants have been used to induce banana micropropagation, such as meristems [29,39], corm tissue [89,85], leaf bases [89], immature zygotic embryos [32], and immature male and female flowers [107,33,25, 40,48]. Nonetheless, Nisyawati and Kariyana [86] state that the most often utilized explants for banana micropropagation are the tips of the banana shoots.

Young suckers' shoot tips, which should range in height from 40 to 100 cm, are used as explants for rapidly reproducing bananas *in vitro*. The apical meristem-containing tissue, ranging in volume from 1 to 2 cubic centimeters, is separated from the banana suckers in these shoots. Any plant portion that has a shoot meristem—that is, the horizontal, small suckers, paternal pseudo stem, and peepers—will usually be the starting point for the creation of shoot cultures [116,7,66].

2.2 Explant Size

The effective initiation and establishment of cultures are significantly influenced by the size of the explants. For the first cultivation in MS medium supplemented with 160 mg/l ADS, 5 mg/l BA, and 5 mg/l IBA, Doreswamy and Sahijram [30], employed a male flower's apical knob, measuring 3 cm. The 0.5 mm floral apex was eliminated after a month and utilized as an explant. According to Darvari [28], when the male inflorescence with a size of 20 mm was employed for micropropagation, the frequency of clusters resembling cauliflower-like entities rose. Decapitated male flower clusters measuring 5.5 to 0.8 cm were utilized to promote inflorescence growth and induce shoot buds [100].

2.3 Surface Sterilization of an Explant

One of the most significant challenges in establishing and keeping an *in vitro* culture functional is microbial contamination. Microorganisms may contaminate field-grown plants; thus, before explants are moved to an *in vitro* setting, they must be cleaned [1]. Goswami and Handique [47] describe surface sterilization of alterations made to the subterranean stem. Many studies conducted *in vitro* have suggested

various methods of sterilizing and chemical disinfectants. *In vitro*, surface sterilization of banana explants is most usually achieved using sodium hypochlorite as the disinfectant [83,112,34,6]. A low concentration of mercuric chloride replaced with sodium hypochlorite is reported by Molla [80]; Titov [118] and Goswami [47]. According to Goswami and Handique [47], the maximum percentage of a contamination-free healthy culture is obtained using 1% sodium hypochlorite for 15 minutes and 0.1% mercuric chloride for 7 minutes. Jafari [57] used the double-disinfection approach, using mercuric chloride followed by Clorox. Ten-day-old suckers of the banana variety "Williams" were considerably less contaminated following double sterilization with NaOCl (3.5%) and Tween 80—first for 15 minutes, followed by five water rinses, and a second time for 5 minutes [50].

2.4 Media Preparation and Composition

Bananas are grown in smaller areas using MS medium. The first step in disinfecting the media is autoclaving it for 30 minutes at 121°C. Agar gelling agent (3-5 g/liter) and sucrose sugar (30–40 g/liter) are added to the media as a carbon source to provide the medium with a semi-strong texture [5,133]. The medium that influences the morphogenesis and development of the tissue transplanted in the media contains auxins and cytokinins. In a glass container, media is poured and then the explants are planted. Their focus and proportion determine the development and morphogenesis of the banana tissue. Banana tissue colonies are often affected by excessive tissue discoloration from the oxidation of polyphenolic mixtures released by damaged tissue [13].

These undesirable explant exudates create a barrier surrounding the tissue that hinders the intake of supplements, inhibits growth, and encourages the sucker to retard growth. In this way, every one to two weeks throughout the first four to six weeks, fresh shot tips are switched out for a new medium. On the other hand, the recently established communities may be maintained in total darkness for a week. To reduce the sucker's darkening, cell reinforcements such as citrus extract or ascorbic corrosive are added to the development medium in concentrations ranging from 10 mg/l to 140 mg/l. Alternatively, the explants are submerged in a solution of cancer prevention agent (cysteine, 50 mg/l) before being placed in the culture medium [108,43,119].

Table 1. Tissue culture media components for the growth of banana shoots (pH 5.7) [27]

Components	Stock solution concentration	The amount required for preparation one liter of the solution
MS macronutrients (0.5x)	25x	20ml
MS micronutrients (0.5x)	100x	5ml
MS vitamins (1x)	100x	10ml
Fe-EDTA (1x)	200x	5ml
Sugar		30g
BAP	100 mg/L	100ml
Agar		6g

**MS= Murashige and Skoog's medium*

Table 2. Rooting medium for Banana explants [27]

Components	Stock Solution concentration	The amount required for preparation one liter of the solution
MS macronutrients (0.5x)	25x	20ml
MS micronutrients (0.5x)	100x	5ml
MS vitamins (1x)	100x	10ml
Fe-EDTA (1x)	200x	5ml
Sugar		30g
Agar		6g
Coconut water (optional)		100ml

**MS= Murashige and Skoog's medium*

3. STAGES OF MICROPROPAGATION

3.1 Stage 1: Shoot Culture Initiation

Banana shoot cultures often begin from any portion of the plant with a shoot meristem, such as the parental pseudostem, tiny suckers, peepers, and lateral buds [127]. To quickly multiply bananas *in vitro,* immature suckers between 40 and 100 cm in height are most commonly utilized as an explants. From the selected sucker, a 1-2 cm3 cube of tissue containing the apical meristem is removed. After 20 minutes, this tissue block is disinfected on the surface with a 2% sodium hypochlorite solution, dipped in 70% ethanol for 10 seconds, and washed three times for 10 minutes in sterile water. There are variations of this decontamination process [81]. The most suitable choice is a meristem-tip culture when eradication of bacteria or viruses is required. The explant is then further cut down to a length of 0.5–1 mm, resulting in a meristematic dome containing one or two leaf initials. The drawback of meristem cultures is that they may have slower initial development and a higher death rate. Direct placement of the explant on a culture medium that promotes multiplication is done. According to Murashige and Skoog [84], MS-based medium are commonly used for banana micropropagation. The explant is placed right into a culture medium that promotes proliferation. Typically, a carbon source supplementation of 30 g/l sucrose is added. The oxidation of polyphenolic chemicals generated from damaged cells is a common cause of excessive blackening in banana tissue cultures [84]. These unwanted exudates envelop the tissue, obstructing its ability to absorb nutrients and promote development. As a result, new shoot tips are shifted to fresh medium every 1-2 weeks for the first 4-6 weeks. Alternatively, fresh cultures can be maintained in total darkness for a week. Antioxidants are added to the growth media in amounts ranging from 10–150 mg/l to minimize blackening or the tissue explants are submerged in an antioxidant solution (50 mg/l cysteine) before being transferred to the culture medium [58].

3.2 Stage 2: Multiplication of Shoot Cultures

It is possible to encourage the growth of many shoots and buds by adding a comparatively high concentration of cytokinins to the medium. The recommended cytokinin in bananas is BA (Benzyl adenine), which is often administered at concentrations ranging from 0.1 to 20 mg/l [12]. The propagules are multiplied using the same medium (p5 medium, which contains 2.25 mg/l BA and 0.175 mg/l IAA) for the initial phase of the shoot cultures. In order to produce highly proliferating meristem cultures, the culture medium (p4 medium, which contains 22.5 mg/l of BA and 0.175 mg/l of IAA) must have ten times more BA. Higher cytokinin concentrations have to be avoided as they frequently have a negative impact on the culture's shape and pace of multiplication. Both the genotype and the cytokinin concentration affect the rate of multiplication. Depending on the original explant size, additional adventitious and axillary shoots may grow straight from the shoot-tip explant about 6–12 weeks afterwards the culture develop. At intervals of 4-6 weeks, clusters can be divided, pruned, and periodically subcultured.

3.3 Stage 3: Rooting of Regenerated Plants

A nutritional media is given to individual shoots or shoot clusters, which encourages the production of roots but does not encourage more shoot proliferation. In the regeneration medium, cytokinin is either significantly reduced or absent entirely. Shoot tips turn into unrooted shoots in two weeks. IAA, NAA (naphthalene acetic acid), or IBA (indole-3-butyric acid) are frequently added to the medium at concentrations ranging from 0.1 to 2 mg/l to initiate rhizogenesis. We adopt a ten times lower BA concentration (0.225 mg/l) but the same auxin concentration (0.175 mg/l IAA) as in the proliferation medium. Activated charcoal (0.1–0.25%) is added to the regeneration/rooting mix for certain genotypes (Musa spp. ABB and BB group) that result in compact proliferating masses of buds to improve shoot elongation and rooting. According to Viehmannová [126], *in vitro* plants of Musa acuminata (AAA) on MS media supplemented with 5 µM NAA developed 14.79 average roots per plant; however, the longest roots were seen on the basal MS and ½ MS medium. Amin [6] found that 0.5 mg· L-1 IAA combined with 0.5 mg· L-1 IBA produced the greatest number of roots and root length. Indolebutyric acid (IBA) was widely utilized for root induction of banana plants cultivated *in vitro* because it performed effectively. Root development on half-strength MS medium with 1.0 and 2.0 mg· L-1 IBA, respectively, was reported by Habiba [49].

3.4 Stage 4: Hardening of *In-vitro* Plantlets

Significant plant losses were observed when rooted plantlets were transferred directly from aseptic culture conditions to the external environment. Micro propagated plants must be given time to acclimate to the outside conditions, which include fluctuating temperatures, reduced humidity, decreased nutrition availability, and the presence of pathogens, after being removed from the tissue culture environment. Since tissue-cultured plants often have thin cuticles, they lose water quickly when they are returned to their natural environment. Furthermore, their photosynthetic activity was not completely active during hardening because of space constraints and the availability of surplus carbon sources, which led to the depletion of carbon sources during hardening. In comparison to the water potential of medium containing sucrose, light is significantly higher and air humidity is significantly lower in the greenhouse, and particularly in the field. Due to this, plantlets often require several weeks of acclimation following ex vitro transplanting, during which the air humidity is gradually lowered [99,60,20]. Organic matter, such as cocopeat, vermiculite, perlite, vermiculite, and vermicompost, is added to the soil to supply the seedlings with an adequate amount of nutrients. The degree of aeration, nutrient availability, and water -holding capacity that these substrates supply to the plants affect the growth and development of the plants. Research has shown that adding cocopeat, vermiculite, and perlite to Grand naine banana plantlets enhances their development as the plant hardens [105].

Bitar [18] reported that incorporating a 1:1 ratio of coco fiber to vermiculite and 100% coco fiber resulted in the greatest transplant growth and chlorophyll content of cv. Grande Naine. The least productive plants were those planted entirely in sand; however, in sand + vermiculite, sand + coco fiber, and sand + rice hull, their development increased. The ratio of substrate components in terms of volume was 1:1. According to Azam [9], 98% of the *in vitro*-raised plantlets cv. Bari-1 with expanded roots survived and developed new leaves two weeks after being transplanted. The plantlets were transferred for seven days for primary hardening and then acclimatized into polythene bags containing garden soil and humus (1:1).

4. EFFECT OF TISSUE CULTURE TECHNIQUES IN CONTAMINATION CONTROL AND DISEASE MANAGEMENT

In tissue cultures, contamination can result from microorganisms introduced during tissue-culture interventions or from indigenous bacteria that evade early disinfection. Both types of pollutants have the ability to persist in plant material for several cultural cycles and for protracted periods of time without showing any symptoms in the tissue or noticeable signs in the media. The lack of sexual reproduction processes in cultivated bananas has made genetic improvement challenging. Although tissue variability is a propagator's biggest adversary, banana breeders may find great use in it [110,78]. Tissue culture may also make it feasible to acquire improved cultivars of common varieties that are resistant to diseases and pests. Physical and chemical mutagenesis have been used to report variability in many banana cultivars [10].

Certain antibiotics effectively reduce the amount of bacteria that contaminate banana tissue cultures. It was shown that rifampicin (100 mg/l) given to liquid cultures for 10–30 days was the most effective way to control Gram-positive bacteria that typically arise in banana shoot tips without interfering with plant development. Nonetheless, it was discovered that any bacterial contamination could be removed by cultivating tiny meristem tips (1 mm) obtained from contaminated *in vitro* plants or from greenhouse plants gained from contaminated *in vitro* plants [125].

4.1 Control of Microbial Contamination During *In- vitro* Culture

One of the main obstacles to plant micro propagation is microbial contamination [117]. Fungi and bacteria are the primary microbial agents that proliferate in plant tissue cultures. The main bacterial contaminants in plant tissue cultures have been characterized as Pseudomonas syringae, Bacillus licheniformis, Bacillus subtilis, Corynebacterium spp., and Erwinia spp. [90], whereas the most frequently detected fungal contaminants are *Alterneria tenius, Aspergillus niger, Aspergillus fumigatus,* and *Fusarium culmorum* [90,91]. These organisms result in significant losses both during the initial phase of the culture and during

subcultures, with each subculture accounting for 5–15% of the total losses [91,95]. Even with the adoption of reliable aseptic methods, the overall losses in bananas and plantains due to microbial contamination are estimated to be between 40 and 60 percent [82]. This means that microbial contamination causes a substantial amount of explants to be lost during culture [49].

4.2 Surface Sterilization of Explants Prior *In-vitro* Culture

The continuing existence of asepsis is an essential component of the in-vitro culture process [69,128]. A certain amount of it is acquired by sanitizing the explants' surface. Requirements for disinfection vary depending on the type of explant. Explants from flowers or inflorescences may be less infected with bacteria which trigger telluric symptoms. Consequently, their disinfection might involve only a quick rinse with ethanol at 70° [130,28]. The mercuric chloride is frequently used at a concentration of 0.1% [63,109,26], whereas, calcium chloride is often used at a concentration of 1.5% [12,73]; [74,67]. Typically, concentrations of sodium hypochlorite (NaOCl) range from 0.25% to 1% [115,113,42]. This disinfectant has demonstrated a very strong anti-contamination action against all types of pollutants [62]. Sodium hypochlorite at 1% concentration would be a more effective disinfectant than mercuric chloride applied at 0.1% concentration for 5 minutes [64]. When a lab NaOCl solution is not available, commercial bleach works just as well. [35].

5. GENETIC TRANSFORMATION OF TISSUE CULTURE RAISED BANANA SAPLINGS FOR DISEASE RESISTANCE

Genetic transformation has been used as a tool for genetic improvement in Musa species in genetic initiatives aimed to create resistance to major diseases like banana bunchy top virus [51] and Fusarium wilt (fungal diseases), which have been identified as the main agronomic issues for plantain and banana production globally [53]. Efficient genetic transformation of bananas was made possible by direct DNA insertion by electroporations [37] into live and highly regenerative protoplasts. Particle bombardment has been done using suspensions of embryogenic cells [11]. Studies on genetic transformation have advanced plant breeding methods and improved knowledge of the fundamental processes behind plant gene regulation [131]. Because most cultivated bananas have long generation periods, high sterility, and triploidy, traditional techniques of breeding for disease resistance have had minimal success in the past 70 years. Agrobacterium-mediated gene introduction was implemented by May [76] to transform bananas, and the regenerants were assessed based on both molecular characterisation and phenotypic observation. Within four weeks of co-cultivating the tissue samples with Agrobacterium, the technique allowed for the recovery of presumptive transformants. They created methods for recovering genetically altered bananas (var. Grande Naine) utilizing kanamycin as a npt-II gene selector.

6. COMPOSITION OF NUTRIENT MEDIUM AND ITS IMPACT ON YIELD AND SUSTAINABLE PRODUCTION OF TISSUE CULTURE RAISED BANANA CULTURE

6.1 Role of Vitamins in nutrient Medium

Vitamins are needed in trace levels to support the catalytic activities in enzyme systems, even though critical nutrients make up the foundation of all nutritional media [101,87]. Certain plants can produce the vitamins needed for development. Vitamins, on the other hand, could restrict cell proliferation and differentiation in plants produced *in vitro* [120]. The effects of thiamine and nicotinic acid on cellular division in the pea root meristem have been verified by additional studies [21,93,94]. These vitamins cooperated, indicating that thiamine and nicotinic acid both encouraged the development of the roots. Vitamin intake to the tissue culture medium in bananas has not yet been extensively investigated and researchers frequently take a "belt and braces" strategy for minor media components like vitamins and add unusual supplements to make sure nothing is missing that could compromise the experiment's success. Complex combinations including nine or 10 vitamins have occasionally been used [44,88]. Investigating the ideal dosage of widely used vitamins for every kind of banana in tissue culture is therefore necessary.To fulfill the needs of the plant, vitamins such as pyridoxine (B6), myo-inositol, nicotinic acid (B3), and thiamine (B1) were mostly added to the artificial plant

growth medium. In the tissue culture medium, thiamine is crucial for cell proliferation and multiplication. It is crucial for the cells' dedifferentiation [93]. Pyridoxine functions as an antioxidant which helps plants to overcome harsh situations. Additionally, it supports the growth of a variety of interior plant tissues (Chen and Xiong, 2005). Specific amounts of these vitamins are utilized in various artificial media formulations [84,22]. A vitamin that belongs to the B-complex family, myo-inositol is essential for the growth and development of plant tissues [45].

6.2 Role of Plant growth regulators (PGRs) in nutrient medium

Auxin and cytokinin are the two types of growth regulators that are often added to the banana growing medium. Their ratio and concentration determine the banana tissue's morphogenesis and development. We regularly supplement the initiation medium with 2.25 mg/l 6-benzyladenine (BA) and 0.175 mg/l indole-3acetic acid (IAA). After two weeks in culture, Punyarani [100] reported direct shoot induction when TDZ (5 µM) and NAA (20 µM) were added to MS media from the base of male inflorescence without callus phase. In approximately five to seven weeks, Hrahsel [52] were able to develop white bud-like structures in MS media supplemented with a lower quantity of BA (0.45 µM) in conjunction with NAA (0.09 µM). Shoot development was thus highest in medium containing NAA (0.09 µM) and kinetin (0.43 µM). It has been discovered that cytokinins inhibit the dominance of apical meristems, promote axillary shoot development, and cause adventitious shoot development from meristematic explants [74]. The cytokinin type, cytokinin concentration, and genotype all affect the creation of "CLB" clusters, or shoot proliferation. It was discovered that the type of cytokinins had an important effect on the male flowers' rate of multiplication. Male flower regrowth requires cytokinins [28]. Unlike any other cytokinin, BA can cause bananas to produce numerous shoots [132,68]. Most banana micropropagation techniques utilize a semi-solid medium. Agar (5–8 g/l) is often added to the culture medium as a gelling agent; however, we prefer Gelrite (2–4 g/l) due to its higher degree of transparency, which enables a more rapid identification of microbial contamination. Although liquid media are better for multiplying shoots, one culture cycle on a semi-solid medium is also required for optimal plant development and survival *in vitro* [15]. Banana shoot-tip cultures are cultured at 28 ± 2 °C, which is the ideal growth temperature, under a 12- to 16-hour light cycle, with a photosynthetic photon flux (PPF) of around 60 µE/m2 s1.

6.3 Role of Minerals in Nutrient Medium

In nature, organisms use several chemical cycles to get all the necessary nutrients from the soil. Different kinds of critical components are needed for the plant to develop effectively. The two main categories of these necessary minerals are macroelements (also known as macronutrients) and microelements (also known as micronutrients) [44]. These micronutrients and macronutrients are important to the plant's physiology. To address the nutritional needs of the plant, any acknowledged optimum plant tissue culture medium is enriched with the appropriate mineral ions. List of macro and micronutrients is given below in Table 3 along with their key roles in tissue culture nutrient media composition.

6.4 Role of Sugar and Amino acids in Nutrient Media

Cells need sugars as a source of energy to survive under adverse conditions. The most popular sugar in the plant tissue culture media is sucrose. These sugars, or carbohydrates, are essential for the growth of organ cultures and for preserving the osmotic potential. Sugars are essential for all energy-intensive activities, including organogenesis and embryogenesis [134]. Although they are not as effective as sucrose, other sugars such as glucose, fructose, maltose, lactose, starch, and galactose can additionally be used. In addition to these, naturally occurring sugar-containing substances such as coconut water and fruit extracts are also utilized [104]. These sugars range in concentration from 2% to 5% in the media.

Proteins are composed mostly of amino acids. Different amino acids combine to form the necessary proteins in plants, which are utilized to carry out physiological functions. Numerous amino acids function in physiological processes as enzymes and catalysts. Considering that they may be assimilated differently than other inorganic sources, amino acids are an adequate source of nitrogen. The plant tissue culture media contains many amino acids, including casein hydrolysate [129,38], l-glutamine, l-asparagine, adenine, glycine, serine, and proline [17].

Table 3. Role of macronutrients and micronutrients in the culture media

Macronutrients	Key role in tissue culture operation	References
Nitrogen	An essential component of plant physiology is nitrogen. It is a major component found in proteins and nucleic acids. It has a significant impact on the plant's morphogenesis. The whole nitrogen content is defined by the ratio of NO3 - and NH4 +, which is entirely dependent upon this morphogenesis activity. In addition, nitrogen is crucial for the growth and development of zygotic embryos.	[46]; [124]
Phosphorus (P)	It has been reported that nucleic acids consist of phosphorus. Since its breakdown in the medium is believed to demonstrate a high quantity of accessible amino acids, it seems to be involved in protein synthesis. Phosphorus has a role in energy transmission as well as respiration and photosynthesis. To finish their physiological tasks, plants must absorb phosphates.	[122]; [72]
Potassium (K)	Potassium is an important cation that is utilized in plant tissue culture media because of its role in plant physiology. It is crucial to control osmotic potential. Excessive moisture, or hyperhydricity, was discovered in tissues grown on a potassium-deficient medium	[97].
Sodium (Na)	Plants need sodium, an essential ion, for a variety of physiological processes. It is recognized as a useful nutrient for plants and is important for the development and proper functioning of plant tissues.	[114].
Calcium (Ca)	One essential element in the formation of membranes and cell walls is calcium. According to reports, calcium is crucial in controlling phytochrome's function, which is involved in physiological activities	[106].
Magnesium (Mg)	agnesium is an essential component of chlorophyll and serves a variety of physiologically important functions as an enzyme cofactor.	
Sulfur (S)	Sulfur is a key component of cysteine and methionine and plays a significant role in the production of amino acids. Low protein synthesis along with slower plant development are the results of sulfur deficiency in plant tissue culture conditions	[65].
Manganese (Mn)	Manganese functions as a cofactor for several enzymes involved in photosynthesis (the oxygen-evolving complex) and respiration, and it may be found in a variety of metalloproteins	[23].
Zinc (Zn)	According to reports, zinc has a role in the biosynthesis of tryptophan, which in turn aids in the creation of indole acetic acid (IAA), a plant growth regulator. It is also a part of several metalloproteins that are connected to different plant physiological processes.	[121];[23]
Iron (Fe)	Iron is the main component of cytochromes and is essential for the transfer of electrons. It serves as a chelating agent; specifically, Fe-EDTA is primarily employed in the medium used for plant tissue culture	[84].
Boron(B)	The plant may get boron as boric acid. By forming cross-linking polymers, it contributes significantly to the integrity of the plasma membrane. According to Blevins and Lukaszewski, boron appears to have a significant role on the quality of product for a variety of fruits and vegetables.	[70]; [19]
Copper (Cu)	According to George, copper is reported to be involved in the oxidation and hydroxylation of dopamine in addition to being present in electron transporters like plastocyanin. Complex polymers like lignin and melanin are formed when	[45]; [23].

Macronutrients	Key role in tissue culture operation	References
	phenolic chemicals undergo posthydroxylation	
Molybdenum (Mo)	Enzymes such as nitrogenase and nitrate reductase contain molybdenum. Moreover, it serves as an enzyme cofactor for several enzymes.	[23]
Cobalt (Co)	According to reports, the pseudo porphyrine ring of vitamin B12 contains cobalt. It also inhibits oxidative processes and is a crucial factor in the toxicity of metal chelates	[36]
Nickel	According to Mishra and Kar, nickel is a crucial element that is essential to the plant's regular growth and development. Additionally, Polacco notes that it is a part of the ureases class of enzymes.	[79]; [98]
Chlorine (Cl)	Numerous physiological processes consist of chlorine. According to Johnson, it is a crucial component of the water-splitting cluster in photosynthesis and a shortage of it causes the plant to wilt.	[96]; [59]

7. SUMMARY AND CONCLUSION

The review of tissue culture in banana cultivation underscores its transformative impact on disease management, yield improvement, and sustainable production. The technique of micropropagation stands out as a pivotal advancement, facilitating the rapid production of disease-free, genetically uniform plantlets. This not only addresses the significant issue of pathogen transmission but also ensures the consistent quality of the crop, which is crucial for commercial viability. The composition of the culture media plays a critical role in the success of tissue culture practices. Key components such as vitamins, sugars, amino acids, and plant growth regulators have been shown to significantly influence the growth and development of banana plantlets. Vitamins act as coenzymes in metabolic pathways, sugars provide essential carbon sources for energy and growth, amino acids contribute to protein synthesis and overall plant vigor, and plant growth regulators, including auxins and cytokinins, are vital for cell division, elongation, and differentiation.

Moreover, the integration of tissue culture techniques in banana cultivation has led to substantial yield improvements. By enabling the mass production of high-yielding and robust cultivars, tissue culture addresses the increasing demand for bananas while enhancing the economic stability of banana farming communities. In terms of disease management, tissue culture offers a robust solution to the pervasive challenge of banana diseases such as Fusarium wilt and Black Sigatoka. The production of pathogen-free planting materials through *in vitro* techniques significantly reduces the incidence of these diseases, leading to healthier plantations and reduced reliance on chemical treatments. This not only minimizes environmental impact but also promotes sustainable agricultural practices. Overall, tissue culture in banana cultivation represents a significant stride towards achieving higher productivity, better disease resistance, and more sustainable production methods. The continuous refinement of tissue culture protocols and the optimization of media composition are essential to further enhance the efficiency and effectiveness of this technology. As such, ongoing research and development in this field will be crucial in meeting the global demand for bananas while ensuring environmental sustainability and economic resilience for growers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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