

The *in Vitro* Propagation Techniques for Producing Banana Using Shoot Tip Cultures

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Abstract

Banana is an important food crop and the second most important fruit crop. Despite the significant commercial value of the crop, the main production constrain is the availability of reliable and safe planting material. The planting materials obtained through conventional methods (suckers) do not meet the increasing demand for planting and they are of poor quality. Tissue culture is the approach which can solve these problems. Micro propagation of the crop is also faced with challenges which need to be addressed in order to improve its production. Some of the problems which hinder the success of the crop include oxidative browning of the wounded tissues and low number of shoots produce per explant. This review highlights the challenges encountered in tissue culture of banana and explores the *in vitro* propagation techniques by using shoot tip cultures of banana as the possibilities to overcome these problems.

Keywords

Antioxidants, Auxins, Bud Splitting, Cytokinins, Media Composition, Oxidative Browning

1. Introduction

Banana (*Musa accuminata* L.) belongs to the Musaceae family. Banana provides millions of people throughout the tropics and subtropics with staple food and accounts for one of the most widely exported fruits in the world.

Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha, producing 95.6 million tonnes [1]. It accounts for approximately 22% of the fresh fruit production and as the second most important fruit crop; Africa accounts for 35% of the total world production [2]. It is one of the oldest fruits known to mankind and is the most delicious fruit used as subsidiary food. It is used in different ways such as for

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table purpose as well as culinary fruit; its leaves and stems are chopped and used as cattle feed. Some species of banana yield fibre, which is used for making ropes. The tip of inflorescence is cooked as a vegetable in some places [3]. The plant is also used for decoration purpose in wedding, festivals and fairs. It is also used as raw material in industries for preparation of banana powder, chips, juices and beer. The juice of banana stem is used in making paper bond, tissue paper etc.

Despite this important use of banana, its production has been decreasing since nineteen seventies. In north western parts of Tanzania in Kagera region, production decreased from 10 t to 4 t per Hectare due to declining soil fertility and emergence of pests and diseases [4]. This is very low production compared with banana production in other parts of the world like India where the production was on an average of 26.7 t per Hectare.

Banana plants are usually propagated by vegetative means by using suckers which grow from lateral buds originating from corms, and suckers are used for production of individual plants. In some instances, complete or separated corms with one or several buds may be used. This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material [5]. The non-professional cultivation practices, pest epidemic and viral diseases affect the yield and quality of banana crop [6].

The problem of emerging diseases can be solved by propagating banana through tissue culture [7]. Tissue culture (TC) technology offers mass propagation and clean planting material. *In vitro* banana production technology is a superior technology over traditional method (Sucker-propagated) of banana production with respect to optimal yield, uniformity, disease-free planting material and true to type plants. Mass multiplication of tissue culture plants could be done in a short time. They are cheaper to transport than conventional suckers and the coupling with virus indexing allows for safe movement, and exchange and conservation of germplasm. In addition, bananas produced using the TC technology are reported to be more vigorous, higher yielding and produce better quality fruits than those produced by conventional means [8].

For commercialization, it is necessary that consistent supplies of good quality bananas are produced to meet the increasing demand. The high rate of multiplication of genetically uniform, pest and disease free planting material can be achieved through tissue culture. Tissue culture approaches would also allow for off-season production of fast growing plants compared with conventionally propagated ones [9].

The *in vitro* propagation technique for producing banana using shoot tip cultures is a necessary approach in dealing with the problems encountered in tissue culture. These techniques will ensure sustainable production of banana planting materials.

2. Effects of Media Composition on Growth and Development of Banana Shoot Tips

The ingredients of plant tissue culture media can be categorized as inorganic salts, organic compounds, complex natural preparations and inert supportive materials [10]. The successes with plant cell and organ cultures have been depended on use of appropriate nutrient media. By providing the necessary chemicals in good combinations and forms, it has been possible to establish cultures from virtually every plant part [10]. Inorganic macro-nutrient and micronutrient levels used in most plant tissue culture media are based on levels established in the plant tissue culture medium developed by [11] for tobacco tissue culture “MS medium” [12]. As such no single medium can be suggested as being entirely satisfactory for all types of plant tissues and organs [13]. Murashige and Skoog [11], is the most widely used plant culture medium [14]. Several media formulations have been reported for banana shoot tip culture with slight modifications of MS media [15]. Other popular media include B5 [16], SH (Schenk and Hildebrandt [17], N6 [18] and Linsmaier and Skoog (LS) [19] media [20] [21]. The MS medium of Murashige and Skoog (1962) is a salt composition that supplies the needed macro and micronutrients. In order to achieve growth and differentiation of cells or tissues, concentrations of inorganic nutrients must be optimized such that the medium meets the requirements of the cells or tissues used [22].

The active factor in the medium is the ions of different types rather than the compounds. One type of ion can be contributed by more than one compound, example NO_3^- ions may be contributed by NH_4NO_3 as well as KNO_3 [13]. Micro propagation of bananas in East Africa with the aim of improved or suitable growth media started recently [23]. However, the success in these efforts is limited. For example, Maerere *et al.* (2003) successfully managed to (develop a protocol for cv. Bukoba and Uganda) in Tanzania but the field performance of these varieties is not yet evaluated. Optimal growth and morphogenesis of tissues may vary from plant to plant

based on their nutritional requirements. The source of explants from different parts of same plants may also have different requirements for satisfactory growth [21]. Therefore a need for developing a media with suitable composition for a specific banana cultivar is important in ensuring successful *in vitro* growth and development of each cultivar.

3. The Role of Vitamins in Banana Tissue Culture

Although the basis of all nutrient media is a composition of essential nutrients [24], vitamins are required in trace amounts to serve the catalytic functions in enzyme systems [22]. Some plants can synthesize the essential vitamins for their growth. However when plants are grown *in vitro*, vitamins may act as limiting factors for cell growth and differentiation [25]. Other studies have confirmed that the vitamins thiamine and nicotinic acid affected cellular division in the pea root meristem [26] [27]. These vitamins worked in combination, *i.e.* both the presence of thiamine and nicotinic acid promoted the root growth. The four vitamins; myo-inositol, thiamine, nicotinic acid, and pyridoxine are ingredients of Murashige and Skoog (1962) medium and have been used in varying proportions for the culture of tissues of many plant species [28]. The requirements of cells for added vitamins vary according to the nature of the plant and the type of culture [29]. This makes it impossible to conclude that all the vitamins which have been used in a particular experiment were essential or they will work for another experiment.

In banana, vitamins supplementation to the tissue culture media has been scarcely studied and often research workers tend to adopt a “belt and braces” attitude to minor media components, such as vitamins and add unusual supplements just to ensure that there is no missing factor which will limit the success of their experiment. Sometimes complex mixtures of as many as nine or ten vitamins have been employed [29] [30]. Therefore, there is a need to investigate the optimum amount of commonly used vitamins for each banana variety in tissue culture.

4. The Use of Ant Oxidant and Activated Charcoal in Reducing Oxidative Browning in Banana Tissue Culture

Banana contains constituents of phenolic enzymes principally polyphenoloxidase enzymes. The polyphenoloxidase enzymes serve as a very important phyto auxine in banana and help to defend the plant against infection from fungi, viruses and bacteria when injured [31]. The constituent of phenols in *Musa spp.* are principally dopamine, catechin, chlorogenic acid, cinnamic acid, hydroxybenzoic, resorcinol, progallic acid, salicylic acid, ferulic acid, vanillin coumarin, P-coumaric acid and phenol [32]. Browning reactions and astringency of the fruit are caused by phenolic compounds, and are responsible for high mortality rate (lethal browning) in tissue culture [30]. This process is initiated by browning of the surface of plant tissues due to the oxidation of phenolic compounds resulting in the formation of quinines which are highly reactive and toxic to plant tissue [30] [33].

Apart from being an important group of secondary metabolites, phenolics may act as modulators of plant development by regulating indole acetic acid (IAA) catabolism [34]. They also play effective role in plant growth regulation, cell differentiation and organogenesis [35]. Their concentration is often affected by several internal and external factors example nutrients [36] [37]. Other factors include stress factors such as drought, water, radiation and pathogen infection from injured surfaces, which directly affects the concentrations of phenolics in plants [38].

Activated charcoal is used in control of lethal browning. Its use in micro propagation was first reported by Lu *et al.* (1990) and it was suggested that charcoal was responsible for adsorption and desorption which controlled the release of nutrients in the production of synthetic seeds [39]. Activated charcoal has a very fine network of pores with large inner surface area on which many substances can be adsorbed [40]. Activated charcoal (AC) is often used in tissue culture to improve cell growth and development. The main benefit is its adsorption of inhibitory substances in culture media. The phenolic oxidation or brown exudates accumulation can be significantly reduced by activated charcoal [41] [42]. However, AC can also adsorb vitamins, cytokinins and auxins, thus changing the ratios of medium components and subsequently influencing plant regeneration [43]. This makes the researchers using AC unaware of the actual quantity available to the plant tissues. Obtaining target levels of adequate free exogenous hormone may be difficult [42].

Control of lethal browning in tissue culture of banana has been reported by different studies. Onuoha *et al.* [31], reported the use of ant oxidant potassium citrate and citrate (K-C: C) in prevention of browning in plantain culture. As an antioxidant, potassium citrate-citrate reduced browning within two hours before culturing the

tissues.

Ascorbic acid is also an antioxidant used to control oxidation of phenols [44]-[46]. It is able to scavenge oxygen radicals produced when plant tissue is wounded, thus protecting the cells from the damage resulting from the injury. The detoxification of the free radicals by AA produced through oxidation of the phenolic compounds reduces the extent of browning [33]. In controlling of lethal browning in faba beans, [47] reported that the use of activated charcoal, ascorbic acid, cysteine and silver nitrate had a significant effect on the number of shoots and the length of shoots regenerated per explants. [48] reported that addition of cysteine to the growth media reduced explants blackening in banana tissue culture. Understanding the processes contributing to the oxidation of phenols and how these can be minimized when initiating banana tissue culture is critical for successful *in vitro* culture, not only of banana but also of other crops.

Among the banana varieties susceptible to tissue browning, elimination or reduction of this process is necessary requirement for successful culture establishment. Development of suitable and efficient treatment to minimize tissue browning of these varieties by focusing on suitable antioxidant, the optimum concentration and method of application during explants preparation is of paramount importance.

5. The Role of Growth Regulators (Auxins and Cytokinin) on Growth and Development of Banana Shoot Tips

Growth regulators play a key role for developing a specific mode of growth in the cultured cells or tissues, which may be due to accumulation of specific biochemical contents in them. The single or combination of different hormones in the medium causes maintenance of specific and balanced inorganic and organic contents in the growing tissue. This leads the cells or tissues to develop either into shoots/or roots or even death [49].

In tissue culture, plant growth regulators are important media components in determining the development and developmental pathway of the plant cells. Growth regulators are used in different proportions to break dormancy and enhance shoot formation as the apical dormancy is under control of these growth regulators [50]. The cytokinins and auxins are of importance in *in vitro* culture as the later are concerned with root formation, the former is mainly required in the media for shoot formation and growth of buds [30]. These growth regulators are required in combination in the media as it is always the manipulation and variation of auxins and cytokinins levels that can successfully change the growth behavior of plant cultures [51].

Cytokinins such as benzyl aminopurine (BAP) and kinetin are known to reduce the apical meristem dominance and induce both auxiliary and adventitious shoot formation from meristematic explants in banana [52]. However, the application of higher BAP concentrations inhibits elongation of adventitious meristems and the conversion into complete plants [53].

Auxins and other growth regulator such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues [54] [55]. Auxins such as Naphtalene acetic acid (NAA) have been reported to promote plant rooting *in vitro* [5] [14].

The use of plant growth regulators (PGRs) in plant nutrient media for *in vitro* culture depends on plant tissue growth stage and expected end product. Cytokinins play an important role in buds formation *in vitro*. However buds proliferation *in vitro* is influenced by apical dominance which is controlled by various growth regulators [56] [57]. Genotype of given plant species determines its buds proliferation *in vitro*. Apart from the influence of genotypes, shoot proliferation rate and elongation are influenced by cytokinin types and their concentration. Adenine-based cytokinins are used in several *Musa spp.* for *in-vitro* propagation [58]. N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin [14]. The others are isopentyladenine (2-ip), zeatin and kinetin [59]. The concentration of exogenous cytokinin appears to be the main factor affecting multiplication.

Many studies have reported the use of auxins and cytokinin in tissue culture. Gubbuk and Pekmzci [58], reported that moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation [58]. Also they reported higher shoot proliferation and elongation with Thidiazuron (TDZ) than with BAP. However, BAP above 20 μM and TDZ over 2 μM decreased shoot elongation. The use of TDZ is known to inhibit shoot elongation. In a study conducted by Lee (2004), it was found that TDZ at 0.91 μM induced the largest number of shoots, but at higher concentration of TDZ (9.1 μM), elongation of shoots was inhibited and clumps of small globular buds appeared at the base of shoots [60].

In a study on effects of auxin/cytokinin combination on shoot proliferation on banana cultivars, [61], reported

that incorporation of a strong auxin in the media suppressed the shoot proliferation rates of the banana cultivars. On media modified with low cytokinin/auxin ratios, for example 16.8/1.0 and 16.8/1.2 ZN/NAA combinations, the East African Highland banana (AAA-EA) cultivars showed single shoot development and callus induction due to apical dominance resulting from increased level of auxin concentration [61]. In another study, Buah *et al.* [62], demonstrated that differences existed in the relative strengths of different cytokinin types in inducing shoots. This differential ability of different hormones in inducing shoots *in vitro* was attributed to factors such as stability, mobility and the rate of conjugation and oxidation of hormones [62]. The concentration and combination of auxins and cytokinins in the nutrient mediums is an important factor which determines successful plant regeneration [22]. Thus for efficient *in vitro* propagation of banana the establishment of optimum combination of cytokinins and auxins and their interaction in a tissue culture medium for a specific cultivar is necessary.

6. The Effect of Bud Splitting on Suppression of Apical Dominance and to Induce Multiple Buds Development

The demand for banana planting material is a major constraint to the expansion of banana production. The long time required for conventional planting material to mature and the prevalence of pests and diseases contributes to these problems.

In the application of bud splitting technique, the banana plantlets of a younger physiological age (single leaf) are longitudinally split to increase the number of explants during tissue culture [5]. This is thought to promote buds proliferation by breaking the apical dominance [63].

It is established that lateral bud development in stems is under the control of the apical bud and that auxin application can sufficiently replace the stem apex in this function in as much as it has also been found that auxin stimulates C_2H_4 production in stems [64] [65]. The development of auxiliary meristems is inhibited by apical dominance [66]. These auxiliary meristems are commonly the source of buds formation in nature especially when the apical shoots are damaged or injured [67].

Poor ratooning of plantains results from the strong apical dominance exhibited by the main plant and the competition between suckers [68]. The apical dominance is controlled by growth substances released by the terminal bud, which inhibit growth of lateral buds [69]. Apical dominance is also under the control of several plant growth regulators [56] [57] [63]. Thus, sword suckers of plantains remain small until after flowering of the mother plant because their demand for assimilates (sinks) is too weak under the influence of apical dominance [70].

Bud splitting is not a common practice in banana tissue culture. Some reports on the role of apical dominance in shoot production from explants of banana are conflicting. For example, [71] confirmed that the arrest of apical dominance by removing the shoot tips was essential for the production of multiple shoot initials in cv. Cavendish. However, other studies reported that multiple initials were produced in presence of apical domes in cv. Robusta [72]. [73] reported that removal of apical dome was not essential for multiple shoots formation. On different approach to the same treatment *i.e.* splitting of the shoot tip; [72] reported a strongly increase in growth rate and shoot proliferation as compared with solid media on subculture of split shoot tips in liquid media. [74] found that temporary immersion of split shoot tips in liquid media for 20 minutes produced the highest multiplication rate after 24 hours [74]. On another study, [75] noticed that longitudinal cuts of buds induced a threefold increase in multiplication. The multiplication rate was also found to depend on the origin of sucker bud, where by lateral buds doubled in size within three weeks while the apical buds reached three times their size.

In general, different cultivars shows variation in the degree of their shoot bud proliferation tendency and the type of multiple budding can be distinguished. This is because the multiple budding appears to be linked to genome configuration of a given cultivar [73] and apical dominance which is under control of PGRs. Thus appropriate concentration of PGRs and bud splitting technique in effort to break dormancy and induce multiple buds development is a necessary study to ensure large numbers of shoots are produced *in vitro*.

7. Conclusion

Banana is a staple food and it accounts for one of the most widely exported fruits in the world. Demand for planting material of banana is very high throughout the tropics and sub tropics. Despite this high demand, availability of safe and reliable planting material is a challenge facing small-scale and large-scale farmers. Propagation of banana through tissue culture is reliable solution to the problems facing farmers. The success in producing

banana planting material through tissue culture is limited by the technical difficulties associated with our public laboratories among other things. From this background efficient *in vitro* propagation techniques are necessary to overcome these challenges. Through control of lethal brown, optimum concentration of auxins and cytokinin and arrest of apical dominance through bud splitting technique, these constraints would be solved and greatly contribute to the safe and reliable planting material of banana.

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