STANDARDIZATION THE EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF BANANA (*MUSA SPP*) EXPLANTS: A REVIEW

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ABSTRACT

Banana (*Musa spp.*) is a large herbaceous perennial monocotyledonous and monocarpic plant. It belongs to the family *Musaceae*. Banana is one of the popular berry fruit which is available to every common person. Banana crop is mostly cultivated through sword suckers, for ease production some *in vitro* propagation methods came into existence like tissue culture technology. Some of them are micro propagation, clonal propagation etc, For *in vitro* growth of the excited tissues, plant growth regulators have been considered as one of the most significant factor. Most commonly, auxins and cytokinins are used in the growth and development of the banana explants. The plant growth regulators are required in different concentrations along with Murashige and Skoog media for the best shooting and rooting of the explants.

Keywords: Plant growth regulators, Concentrations, Media, Sword suckers, Tissue culture.

INTRODUCTION

Banana (*Musa spp.*) is one of the tropical fruit belonging to the family *Musaceae*, genus *Musa*. Banana is the second most important fruit crop in India next to mango. The fruit is valued worldwide due to its flavour, nutritional value, and availability throughout the year. Bananas are mostly derived from the wild ancestors: *Musa accuminata* and *Musa balbisiana* (Lehmann *et al.*, 2002). Its origin is

South-east Asia and also native to India and Western Pacific (Carreel *et al.*, 2002). The most edible banana cultivars are derived from inter/ intra specific hybridization of diploid ($2n = 2x = 22$; AA; BB or AB), triploid ($2n = 3x = 33$; AAA; AAB; or ABB) and tetraploid ($2n = 4x = 44$; AAAA; AAAB; or ABBB) from subspecies of *Musa accuminata* (A genome) and *Musa balbisiana* (B genome) (Onildo *et al.*, 2013; Pillay *et al.*, 2006; Simmonds and Shepherd., 1995).

Examples of some common cultivars (with emphasis on cultivars grown in India) (Venkatachalam, 2008):

Genomic Group	Sub-group	Common cultivars
AA	Sucrier	Matti, Kadali, Anai koomban, Chingan
AB	Ney Poovan	Ney poovan, safed velchi, Soneri, Devabale, Elakki bale
	Dwarf Cavendish	Basrai, Loton, Kabuli, Mauritius, Bhusaval, Pachabale
AAA	Giant Cavendish	Bombay green, Pedda pacha arati, Harichal
	Mysore	Poovan, Lal velchi, Champa, Karpura chakkarakeli
	Silk	Rasthali, Malbhog, Sabari, Rasabale, Amrithapani, Mutheli

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AAB	French Plantain	Nendran, Rajeli, Ethakai
	Pome	Virupakshi, Sirumalai, Vannan, Dacca martaban
ABB	Bluggoe	Monthan, Kanchkala, Madhurangabale, Peyan, Khasadia
	Pisangawak	Kostha bontha, Pey kunnan, Manuva kola
AABB		CO-1 (A new hybrid)

India leads the world in banana production with an annual output of about 14.2 million tonnes. Banana ranks first in production and third in area among all other fruit crops in India. It accounts 13% of total area and 33% of its total production. Maharashtra (3924.1 thousand tonnes) is the state which is highest in production followed by Tamilnadu (3543.8 thousand tonnes). Banana grows well in deep loamy soil with pH 6.5-7.5 along with temperature range of 15 – 35°C and relative humidity of 75 – 85%. Suckers (Sword suckers) are used as the planting material for cultivation. Tissue culture seedlings are also used by 30% of farmers as propagating material (IHD, 2014).

Bananas are a healthy source of fibre, potassium, vitamin B6, vitamin C and various antioxidants and phytonutrients. Bananas are mostly categorized into two types. They are 1) Dessert banana; and 2) cooking banana. Sweet dessert bananas are generally eaten raw (fruit), while cooking bananas and plantain are boiled, steamed, roasted and fried. In the ripening process, the starch is converted to sugar; a fully ripened banana contains only 1-2% starch (Forsyth, 1980).

Plant growth regulators are the chemical substances which are responsible for the growth and development within the plants. These are also referred with other names like phytohormones and plant growth hormones. The maintenance of specific and balanced organic and inorganic contents in growing tissues is caused by the single or combination of different hormones in the medium. This leads the cells or tissues to develop either into shoots or roots or even death (Ikram-ul-Haq and Dahot, 2007, Raghavendar *et al.*, 2019).

Plant growth regulators play a vital role in determining the development and development pathway of the plant cells as they act as the media components. Different proportions of growth regulators are used to break the dormancy and also to enhance the formation of shoots as it is well demonstrated that the apical dormancy is under the control of these plant growth regulators (Madhulatha *et al.*, 2004). Cytokinins such as Benzylaminopurine (BAP) and Kinetin are very well-known to reduce the apical dominance and induce both axillary and adventitious shoot formation from

meristematic explants in banana (Jafari *et al.*, 2011). The growth and differentiation of culture cells and tissues is seen by the application of auxins and other growth regulators like gibberellins (Alexandrova *et al.*, 1996; Bohidar *et al.*, 2008).

Auxins like Naphthalene acetic acid (NAA) have been reported to promote the best plant rooting *in vitro* (Vuylsteke, 1989; Hussein, 2012). The most commonly preferred cytokinin is N⁶-benzylaminopurine (BAP) (Vuylsteke, 1989). Shoot proliferation rate and elongation are influenced by the cytokinin types and their concentrations due to the influence of genotypes. In several *Musa* spp. for *in vitro* propagation, adenine-based cytokinins were used (Gubbuk and Pekmezci, 2004).

ROLE OF AUXINS AND CYTOKININS ON SHOOT TIP CULTURE OF BANANA (*MUSA* SPP) EXPLANTS

W. C. Wong. (1986) reported about the *in vitro* propagation of banana (*Musa* spp.): initiation; proliferation and development of shoot tips using culture media with explants. Murashige and Skoog medium containing 6 – benzylaminopurine (BAP) and indolebutyric acid (IBA) was taken to culture a total of 22 banana cultivars successfully. These cultivars showed a wide variation in their multiplication rates in response to cytokinins, the BAP showed consistent effectiveness than kinetin (Kn).

A micro propagation protocol for banana (*Musa* spp) cv. Agnishwar was done by using shoot tip cultures. Shoot tips were obtained from suckers removing the leaf sheaths and cultured aseptically on MS medium supplemented with the different concentrations of cytokinins i.e. 6-benzylaminopurine (BAP), Kinetin (Kn), N⁶ – (2-isopentyl) adenine for multiplication of shoots and auxins such as Indole-3- butyric acid (IBA), a Naphthalene acetic acid (NAA) for rooting. MS medium containing 4mg/l BAP showed maximum multiplication (95%). The highest average no. of shoots per each explant (5.9) was found in MS medium having 4mg/l BAP while maximum elongation of shoots (4.9) was observed with 5mg/l BAP. The concentration of 1.0mg/l of IBA was found suitable for the rooting of shoots (Rahman *et al.*, 2013).

Shahnawaz Ahmed *et al.* (2014) *In vitro* multiplication of banana (*Musa spp.*) cv. Grand Naine reported that Murashige and Skoogs (MS) medium with BAP 4.0mg/l and with IAA 2.0mg/l resulted in more number of cultures in less time along with more multiple shoots. Maximum rooting was attained with the concentrations of 1mg/l IBA and 200mg/l activated charcoal on MS medium. Many other studies have reported the use of auxins and cytokinins in tissue culture. Gubbuk and Pekmezci (2004) reported that the shoot proliferation rate was increased by the moderate concentrations of cytokinins, but very high concentrations decreased multiplication and mostly shoot elongation. Thidiazuron (TDZ) resulted in high shoot proliferation and elongation than with BAP. However, BAP above 20 μ M and TDZ over 2 μ M decreased the shoot elongation. Thidiazuron inhibits the shoot elongation. In another study, TDZ at 0.91 μ M induced the largest number of shoots, but elongation of shoots and clumps of small globular buds appeared at the base of shoots with higher concentrations of TDZ at 9.1 μ M (Shirani *et al.*, 2011).

M. H. Ferdous *et al.* (2015) studied on BAP and IBA pulsing for *in vitro* multiplication of banana cultivars through shoot tip culture. MS medium along with different concentrations of BAP (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg/l) and IBA (0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/l) was used to assess the influence on *in vitro* shoot regeneration and subsequent root formation of Amritsagar and Sabri banana cultivars. Under *ex vitro* conditions the survival rate of the plantlets of both cultivars was more than 82%. MS media along with the concentrations of 0.5mg/l BAP and 0.3mg/l IBA can be used for shoot and root formation of Amritsagar and Sabri banana cultivars through shoot tip culture.

Another study was carried out at the Tissue Culture Laboratory of Melkassa Agriculture Research Centre, Ethiopian Institute of Agricultural Research (EIAR) to investigate the effects of different types and concentrations of cytokinins and auxins on shoot initiation and multiplication, and *in vitro* rooting of shoots of three banana varieties using shoot tip explants. The combination of BAP and indole-3-acetic acid with MS medium at 3+0.4, 4+0.4, and 3+0.2 mg/l for Dwarf, Giant and Poyo respectively, were best combinations for higher shoot proliferation rate and elongation. When MS medium with the concentrations of 2.12mg/l α -naphthalene acetic acid (NAA) for Dwarf and Giant while 1.74mg/l indole-3-butyric acid (IBA) for Poyo showed the better rooting when the shoots were cultured (Asmare *et al.*, 2012).

In a study on effects of cytokinin/auxin combination on shoot proliferation on banana cultivars, Arinaitwe *et al.* (2000) reported that the shoot proliferation rate of the banana cultivars was suppressed due to the incorporation of a strong auxin in the media. For example, the East African Highland banana (AAA-EA) showed single shoot development and callus induction on media modified with low (cytokinin/auxin ratios, 16.8/1.0 and 16.8/1.2 ZN/NAA) due to apical dominance resulted from increased level of auxin concentration. In other study, Buah *et al.* (2010) that the differences existed in the relative strengths of different cytokinin types in shoot induction. The factors such as stability, mobility and the rate of conjugation and oxidation of hormones are attributed due to the differential ability of different hormones in inducing shoots *in vitro*.

Zaffari *et al.* (2000) reported that 2ip is a major cytokinin occurred naturally in banana tissues. In other way, the application of 2ip to shoot-tip cultures of banana (Arinaitwe *et al.* 2000), *Canna indica* (Kromer and Kukulczanka, 1985) and Manila hemp (Mante and Tepper, 1983) didn't prove effective when compared to BA and Kn applications. Arinaitwe *et al.* (2000) attributed the lacking in growth promotion of explants by 2ip due fact that is easily decomposed by cytokinin oxidase.

ROLE OF CARBON SOURCES ON IN VITRO ROOT AND SHOOT MULTIPLICATION OF BANANA EXPLANTS

Sugar acts as a major transport metabolite and involves in the osmotic process for the uptake of nutrients from the medium (Lalonde *et al.*, 1999). In the study, four carbon sources at three different concentrations were involved. In these, mannitol found to be an inferior carbon source, irrespective of the concentration in nutrient pool. Due to this, shoot buds sprouting and multiplication is not seen (Bhojwani and Razdan, 1996). Cultures grown on mannitol-enriched medium showed poor multiplication with shortest shoot length in 'Nendran' banana (Madhulatha *et al.*, 2006).

Fructose which is followed by sucrose is found to be the most suitable carbon source for shoot multiplication response. And fructose also found to be superior for other growth parameters like shoot thickness. The process of organogenesis is in great demand in terms of energy, so energy source is more accessible for plant cultures. The reports have been proved that fructose is more accessible energy source than sucrose (Torpe, 1980). The most commonly used carbon source for large number of plant

species including banana is considered as sucrose (Hussein, 2012; Madhulatha *et al.*, 2006). The conversion of sucrose into glucose and fructose is seen in cultures. A particular amount of sucrose is hydrolyzed into its component sugars during sterilization of media (Son *et al.*, 2000).

Vora and Jasrai. (2011) reported that the fructose is most suitable sugar for inducing highest number of shoots (12.5 per explants) statistically in culture followed by tissue culture grade sucrose (10.8). The fructose-containing media showed superiority in 'Grand Naine' has reported by (Vora and Jasrai, 2011; Khafagy, 2007).

The studies also showed the effects of different concentrations of carbon sources on *in vitro* root induction. In 100% plantlets, root induction was favoured by the low concentrations of sugars. The cuttings which are rooted under *ex vitro* (field) conditions reduced nutrient strength and in order to the survival of plant these nutrients send signals to the cuttings for root induction (Hanumantharaya *et al.*, 2011).

ROLE OF DIFFERENT CONCENTRATIONS OF GROWTH HORMONES ON ROOTING

Al-Amin *et al.* (2009) reported root initiation in banana cv. BARI-1 by using the half strength MS medium supplemented with different concentrations of IBA (0, 0.5, 1.0 and 1.5mg/l) and IAA (0, 0.5, and 1.0mg/l). The combination of 0.5mg/l IAA and 0.5mg/l IBA showed the highest number of roots (6.50) and the longest length of root (5.88cm). The multiplied shoots of banana cv. Grand Naine were inoculated on rooting media incorporated with either IBA or NAA (0.25, 0.5, 1.0, 2.0, 2.5 and 3.0mg/l) and charcoal (2g/l) for root induction. These IBA (2mg/l) and charcoal (2g/l) produced maximum number of roots (8.5) with a lot of root hairs (Rai *et al.*, 2012).

Anbazhagan *et al.* (2014) found that the half strength MS medium supplemented with IBA at 1.0mg/l resulted in best root formation (96%) and root number / explants (11.80) of *in vitro* developed shoots of Musa spp. The rooting on MS medium (half strength) combined with IBA 1.0mg/l and activated charcoal 200mg/l obtained best rooting (Ahmed *et al.*, 2014). The rooting experiments involving the use of IAA, IBA, NAA, smoke-water (SW) and karrikinolide (KAR1) were conducted. SW and KAR1 showed the significant increase in the number and length of roots. As compared to BA, the usage of topolin resulted higher mean shoot number per explants at lower cytokinin

concentrations and the ease of rooting during the shoot proliferation phase (Aremu *et al.*, 2012).

Jamir and Maiti (2014) studied the effect of IBA and NAA on rooting of banana cultivars Grand Naine and Jahaji. NAA and IBA at the concentrations of (0, 0.1, 0.2, and 1mg/l) were used individually for rooting purpose. The medium with 1mg/l NAA showed the cent percent rooting and highest no. of functional roots (6.33 and 5.2) with moderate root length (2-4cm).

CONCLUSION

Plant growth regulators play a prominent role in the tissue culture technology. Mostly the generalisations about the plant growth regulators and their use in the plant cell culture media have been developed from the initial observations which are made in the 1950s. Some considerable difficulty in predicting the effect of plant growth regulators because of the great difference in the culture response between the species, cultivars and even some plants of the same cultivar are grown under different conditions. However, some principles do hold true and have become the paradigm on which most plant tissue culture regimes are based.

Out of these, auxins and cytokinins are the most widely used growth hormones in the plant tissue culture. In most of the cases, these both hormones are used together for the proper growth and development. The optimum concentrations of BAP resulted in better shooting of the explants and the optimum concentrations of IBA showed better rooting as compared to the other growth hormones. Some carbon sources like glucose, sucrose and fructose are also involved in the rooting and shooting of the banana explants. Among these, fructose is considered to be the most suitable sugar for shooting.

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