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DEVELOPMENT OF PROTOCOL IN BANANA FOR MASS PROPAGATION

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Abstract

An efficient protocol is describe for large scale *in vitro* propagation of Banana variety William Hybrid using suckers, Out of cytokinin, BAP and Kn tested alone or in combination for shoot initiation and proliferation BAP(5mg/l) was found most effective in inducing multiple shoot buds in the explants. High frequency of shoot 5-10 shoots/ explants was recorded, shoot cultures were multiplied by subculturing on a fresh medium each time after excising the newly formed shoots. Rooting was induced in 100% of the regenerated shoots on ½ strength MS medium containing 1 mg/l IBA. Rooted plantlets were hardened off and eventually established in soils

Key words: Banana, Micropropagation, Sucker

Introduction

Banana and plantains are among the most important staple food crops for people living in tropical and subtropical countries. Together, they are the fourth most important global agricultural commodity after rice, wheat and milk in term of gross production value. Most are consumed locally, but approximately 10 percent of total world production of 70 million tones is exported. As a result this industry represents a major source of income and employment in many developing countries.

Banana and plantains are herbaceous monocots belonging to the genus Musa, most are seedless, sterile. The rapid rise in consumption of bananas and plantain due to population increase together with the development of new market, especially in Europe (Arias, 1985) has left the traditional clonal propagation methods unable to cope with the demand for new planting material so production of banana plantlets by tissue culture is greatly beneficial for horticulture

Traditionally banana plants are propagated vegetatively by suckers which grow from lateral buds originating from corms and suckers are separated for production of individual plants. In some instances, complete or spitted corms with one or several buds are used, Conventional vegetative multiplication of banana has been found to express several negative impacts including low production transmission of diseases and Poor preservation of original plant genetic material (Ngomuo *et al*. 2014), Therefore the application of various biotechnological approaches has become an integral part of the banana industry. now days (Vuylsteke,1998). Hence, *in vitro* culture technology is proved as best alternative for the production of large numbers of planting materials of banana in shorter time, lesser space, disease free plants and adequate germplasm preservation.

Additional using only traditional propagation (Suckers) method of banana is a major barrier to get enough plant material and very difficult to carry bulks volume of suckers from one place to other. These conditions have ended the banana production below actual potential per hectare and along with the quality very poor, This resulting yield less makes banana an expensive commodity for consumer which reduce the cash earnings of producers as well as the potential of the crop to contribute to the food security of rural household. By keeping in view the afore mentioned prelude the present investigation is designed to development of the efficient *in vitro* regeneration protocol in banana.

A variety of explants such as Meri stem tip, shoot

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apex, floral a pieces have been examined, to establish efficient protocols for adventitious shoot regeneration in banana by Ma and Shil (1972), Cronauer and Krikorian, (1983, 1984, 1985), Banerjee *et al*. (1986) Doreswamy and Shahijram, (1989) and Novak *et al*. 1990.

Material and Methods

In this study the source material used as explants was suckers taken from field grown plants variety William Hybrid. Suckers were washed with soap, rinsed with tap water and then disinfected according to the following scheme, External scales were removed from the suckers, the middle and internal scales were washed again with tap water and disinfected by immersion for 30 min, in a solution of sodium hypochlorite solution (2% active chlorine) containing two drops of surfactant tween 20 followed by treatment for 15 min with 0.1% mercuric chloride and were cut to size of 2 cm x 1 cm followed by three rinses in sterile distilled water for five min. Explants were cultured on Murashige and Skoog (1962) medium with 3% Sucrose and supplemented with different growth regulators at different concentrations giving the following media.

MS1= 4 mg/l BAP

MS2 = 5 mg/l BAP

MS3 = 4 mg/l Kn

MS4 = 5 mg/l Kn

MS5 = 4 mg/ l BAP + 5 mg/l Kn

MS6 = 5 mg/ l BAP + 4 mg/l Kn

½ strength MS media with 3% Sucrose and charcoal 200 mg/l was used for adventious root formation giving the following media

MSR1 = 0.5mg/l IBA

MSR2 = 1mg/l IBA

MSR3 = 0.5mg/l NAA

MSR4 = 1mg/NAA

MSR5= 0.5mg/l IBA + Mg/l NAA

MSR6 = 1mg/l IBA + 0.5 mg/l NAA

The media were solidified with 0.7% agar (Qualegns, Bombay) and autoclaved at 121°C (0.1 MPa) during the time required according to the volume of medium in the vessel. The explants were placed vertically in to the culture media with the basal plate tissue inserted into the medium. For each culture medium 20-30 explants were used. Glass growth tubes each containing 20ml of solid media were used the initial phases of culture and for subsequent proliferation phase glass bottles containing 50ml of medium were. Rooted plantlets were removed from the nutrient medium and treated with fungicide Bavastin 1% for half hours, roots were cut to 3-4cm length and transplanted into pot filled with 1.1 soil + F.Y.M and were transferred to glass house.

Result and Discussion

In the present investigation for surface sterilization both sodium hypochlorite solution (2% active chlorine) and 0.1% mercuric chloride solution was used which prevented contamination. Titov *et al.* (2006) studied that for *in vitro* propagation of banana cv. Kanthali, huge number of escplants die due to microbial contamination, they studied that contamination free culture was established by 1% $HgCl_2$, for 6 min followed by several washes in sterile water and abviated the need to develop extensive and complicated surface sterilization protocol, also Suneeta and Das (2008) studied the investigation on the effect of surface sterilization agent showed variation in respect to their sterilization property, out of sterilizing chemicals, calcium hypochlorite, hydrogen peroxide and bromine water did not show any good response and Jaisy and Ghai (2011) who worked on *in vitro* propagation of banana found treatment of explants with $HgCl_2$ (0.1%) for 6 min most effective surface sterilization procedure registering maximum culture establishment with minimum contamination.

Shoot bud induction and proliferation has been achieved from suckers by addition of BAP in the medium. The proliferation of multiple shoots was best in the medium containing 5 mg/l BAP. By this method a single sucker produced more than 200 well rooted plants after four or five sub culturing. Each passage of subculture was four or five week duration, Cronauer and Krikorian (1984). Khanam *et al*. (1986), Rabbani *et al.*, (1996), Gebeyhu, (2015) Banerjee *et al*. (1986) Have also reported high concentration BAP best for shoot multiplication, However Muhammad *et al*. (2007), Rahman *et al*. (2005) found that in case of shoot multiplication of dessert banana, MS medium supplemented with 4mg/l BAP, 2mg/l NAA and 2mg/l IBA to the be the best, The differences obtained in the requirement of phytohormones as reported by different researcher and also in the present investigation may be attributed to the difference in the levels of endogenous phytohormones, nutrients, metabolities and interaction between various factors. According to Skoog and Miller (1957), quantitative interaction between growth factor may have decisive role in organogenesis in culture are more complex and plant hormones, organic and inorganic nutrients and osmotic concentration exert a performed influence on organogenesis.

In our case after four weeks of culture swelling

Table 1: Shoot initiation on media supplemented with different levels of growth regulators

Media		Shoot Initiation		Shoot proliferation
MS+ 3% Sucrose		After 5 Weeks	After 7 Weeks	Shoots per inoculum
1	BAP - 4 Mg /l	5.2 ± 2.8	17.9 ± 0.14	38±0.5
2	BAP – 5mg/l	9.7±3.2	28.3±0.41	57±1.02
3	Kn – 4mg/l	None	3.1±2.4	nd
4	Kn – 5 mg/l	None	2.8±1.5	Nd
5	BAP – 4 mg /l Kn + 5 Mg/l	None	None	-
6	BAP – 5 mg/l Kn + 4 Mg/l	None	None	-

The data are presented as mean ± S.E. For shoot proliferation, the data presented are for the end of the fourth and fifth sub culture period and are measured across the two culture passage. Nd= not determined.

Fig.: Different stages in the micropropagation of Banana.
A. Proliferation of shoot from sucker after 7 Weeks of culture on MS + BAP, 5 mg/l.
B. Multiple shoot formation from sucker explants on MS+ BAP, 5 mg/ l.
C. Root formation in regenerated shoots of Banana on ½MS+ IBA 1 mg/l+ Charcoal 200 Mg/l. **D.** Complete Plantlet of Banana.

occurred in explants cultured on MS1 and MS2 media, they were cut in to half and cultured on same media and shoot induction occurred after fives of culture. In MS3 and MS4 media swelling appeared after six weeks of culture and MS5 and MS6 media swelling appeared after eight weeks of culture. In MS3 and MS4 media shoot induction occurred after seven weeks of culture. No Shoot induction occurred in MS5 and MS6 media. (Table 1)

The initiation of shoots on MS1 medium was similar to MS2 medium as compared to MS medium , Due to low capacity of the media MS3 and MS4 for shoot induction and shoot proliferation material grown on them was discarded. The shoot clumps produced on both MS1 and MS2 were subsequently sub cultured every 4-6 weeks on a fresh medium.

A steady state in the production on multiple shoot has been achieved by successive subculturing. Damsco and Barba (1984) observed that the production of shoots was higher when field derived explants were used as primary source of explants compared with subculturing shoots, derived *in vitro* in the fifth and sixth generation.

In the present investigation 1mg/l IBA along with 200 mg/l charcoal proved to be best for rooting shoots, *In vitro* multiplication of banana is normally carried in presence of high cytokinin levels, which is inhibit root formation and elongation. Addition of 200 mg/l charcoal enhanced rooting, It was also obvious from the result that incorporation of activated charcoal reduced the time taken for root initiation and further increased the root and shoot length. Reports of Sharma *et al.* (1997), Gubbuk and Pekmezc (2006) and Roy *et al.* (2010) support the results as they obtained rooting only with 1.0 mg/l IBA and 200 mg/l activated charcoal.

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