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An Efficient and Reproducible Multiplication System for different Banana Varieties

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Abstract: *Banana is the second most significant fruit crop and also is important food crop. Banana production is affected by many biotic and abiotic factors. The banana production is also affected by the viruses and among these one is banana bunchy virus (BBTV). Throughout the world and also in Pakistan Banana bunchy virus is a major threat to the cultivation of banana. For Virus free multiplication and propagation of banana tissue culture is regarded as the main method. The main aim of this study was to develop the effective and reproducible banana culture with the help of tissue culture technique. Two new verities i-e 8818-william and Brazilian were tested through two different types of treatment in the culture. One MS media was provided with MS salts which contained sucrose 30g/L, vitamins 4.3g/L, kinetin 1mg/L, BAP 5mg/L, IAA 0.5mg/L, Gellum gum 1.8 g/L, silver nitrate 50µM and cacl2 0.1g/L. while the second media was supplemented with same ingredients used in the first media but the exception was that in place of Gellum gum 0.2 gm cotton was used for support. Liquid media was more efficient in the multiplication of shoots with the number of 13.6 in comparison to the solid media which scored 9.5 number of shoots. The maximum efficiency of the liquid media in proliferation and multiplication of shoots was probably due to the even spreading of the nutrients in the media. Along with that liquid media is also advantageous as the explants can easily obtain the nutrients as compared to solid media.*

Key words: *Solid and Liquid Ms Media, Shoot Multiplication, proliferation, Tissue Culture*

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I. Introduction

Banana is in the family Musaceae which has two genera. The genus Musa includes the banana and order of the banana is Zingiberales. Banana is herbaceous plant (Purseglove, 1976). It is regarded as one of the main of source of income in the national and international market. It is one of the significant horticulture crops in the world and also it is major staple food (Adane et al., 2015). Banana contains different kinds of vitamins but is rich in vitamin C. banana is rich in minerals especially phosphorus, potassium and calcium. Moreover, it is having sufficient amount of carbohydrates (NIBAP, 1987). After mango banana is the most important fruit crop and is available round the year. Banana production has main role in the economy of any country (Robert et al., 2012). Currently banana has 95.6 million tons production around the world. It is grown in 150 different countries and occupies the area of almost 4.84 hectares (Singh et al., 2011). In Pakistan banana is one of the basic fruit crops. In Pakistan the production of banana is estimated to be 154,800 tons and is grown on the 34,800 hectares (SEDF, 2013). In Pakistan the environment of Sindh is very much favourable for the banana cultivation. It is mostly grown in Sindh and the figures shows that total share of Sindh is around 87% of the whole nations. Many natural environmental disasters results in the loss of banana. Some of the viruses are also responsible for the destruction of banana crop. In 1998, banana crop was observed to be affected by the new kind of disease which was not previously observed. This disease was in epidemic form and in some places, it resulted in the 100% loss of the banana crop (Soomro et al., 1992). Since 1913, it was first time that Pakistan agriculture crop was attacked and damaged on such large scale. Shortening, extreme brittleness of leaves and bunching are the signs of the disease.

The leaves contained dark green streaking on leaves, dot-dash, stalk and petioles of the stems was also seemed to be affected. Seeing the symptoms, serology and virus particle morphology, the disease was said the banana bunchy disease (BBTD), which was caused by the virus named banana bunchy top virus (BBTV) (Khalid et al., 1993). The incidence and prevalence were evaluated during July and October 1991, and was again measured in the 1992 (Soomro et al., 1992).

According to 1991 report all the southern banana-growing regions in the Sindh province was affected by this disease. As a result of this disease, the banana crop was almost disappeared from the Badin and

Hyderabad and was replaced by the sugarcane. According to the reports of Extension Department of Sindh, by the end of 1992 the banana plants were remained on the area of 8000 hectares. This report excluded the plantings in the kachacha lands of the Sindh. This loss was accounted to be the 60 percent since 1987 in that areas of the Sindh which was tax payable. Based on the reports in 1992 the local market was suffered loss of 915 million rupees. Banana bunchy top disease (BBTD) is the disease of the major concern to the banana cultivation throughout the world (Dale et al., 1998). Previously efforts have been made to improve the rates of propagation and made the production virus free with the help of traditional techniques (Berg andBustomante, 1974). But to encounter all these problems we need modern techniques. Among these modern techniques tissue culture is one of the most widely used techniques. Now a day the techniques of molecular genetic engineering are also available to improve the crop production. Meristem is a culture which is very efficient for the conservation of germplasm in plants, virus free propagation and also it supports rapid propagation (CronuerandKrikorian, 1984). Regarding the performance of the tissue culture and conventional method, tissue culture has 39% better yield than conventional techniques (Pradeep et al., 1992). Thousands of plants can be produced in comparatively very less time through invitro culture techniques, either by using apex explants which will need culture media in order to differentiate their roots and multiply their shoots or using somatic embryos (CronauerandKrikorian, 1985). In both developed and developing countries, the use of tissue culture and other rapid propagation techniques are getting more attention. Tissue culture has the advantage that it can be used not only to increase the propagation but also to improve the germplasm (Bonga, 1882). For the in vitro regeneration of the plants in the artificial medium, plant growth regulators are the prime need of the media. Normallyauxin is used for rooting, while auxin is used for the for the proliferation of shoots. The use of the growth regulators will depend on the stage of tissue growth, conditions of the culture, genotype of the banana and the expected end product. So, depending on these factors the growth regulators will be used (CronauerandKrikorian, 1984). In Pakistannaine (G-9) variety is freshly introduced and work is in progress to introduce the other new varieties like Brazilian, 8818-william and pisang variety.

Brazilian and 8818-williams are usual commercial varieties which are grown in Pakistan. These verities are exported from china (Muhammad et al., 2004). These both varieties are best suited for the desert areas and both have more sweetness, apple tinted flavour. The Brazilian variety is dwarf type variety and grows short as compared to 8818-william which tall (Smith and Drew, 1990).The yield of these two verities in Pakistan is 25-30 tons/acre. These two varieties have the characteristics to combat the abiotic factors, has well bunches and also shows good resistant to the BBTV (Shiragiet al., 2008). The fruit of these plants has more uniform colour and has better quality and shelf life than other cultivars (Gallez et al., 2004). The present study is focused on these two varieties which are general cultivars in Sindh province. The environment of Sindh is suitable for these two verities (SEDF, 2013). To get healthy and disease-free plants, there will be need of well-organised proliferation and multiplication protocol. This study is aimed at developing and efficient and virus free culture for these two newly introduced banana varieties. Different nutrient supplements and hormonal combination was checked on it.

II. Material And Methods

This study was performed at Gomal centre of Biochemistry and Biotechnology (GCBB), GomalUniversity, Deraismail khan, in 2018.

Biological Material

Two varieties, 8818-William and Brazilian imported from china was used in this experiment for invitro multiplication system. The previous sword suckers from thatta, Karachi were also used.

Nutrient Media

To make invitro multiplication system two type of media were used in this experiment. The composition of hormones and nutrients in these two medias were same. The two kind of media differed inthat; one was solid media while other was liquid media. The composition of first media (Media-1) was sucrose 30g/L, vitamins 4.3g/L, Kinetin 1mg/L, BAP 5mg/L, silver nitrate 50µM, IAA 0.5 mg/L, gellen gum 1.8 g/L and cacl2 0.1 g/L. The composition of other type of media (Media-II) was MS Salts with Sucrose 30g/L, Vitamins 4.3g/L, BAP 5mg/L, KIN 1mg/L, IAA 1mg/L, Silver Nitrate 50µM, CaCl2 0.1g/L.

Pouring of media in Jars

The media was poured into the clean jars. 6 jars of media were prepared of media 1 and in each jar, there was poured 25 ml of media. The jar was covered with polyethylene bags. To pour the media II into the jars, 200 mg of cotton was placed at the base of each jar and then 15 ml liquid media was poured into each jar. These 6 jars of media II were also covered with polyethylene bags. All the 12 jars of media and II were placed in autoclave for 20 minutes at 121oC under the 15 PSI.

Sterilization

The room was enlightened with UV light for 15 minutes to sterilize it. Laminar air flow cabinet was also sterilized with 70% ethanol before placing the explants in it. The hands and arms were also surface sterilized with 70% ethanol. Surgical blades and forceps were sterilized with flame.

Excision of Shoots from Explant

Shoot explants were obtained from already *invitro* grown culture. Those explants which contained three shoots were taken from the invitro culture in the laminar flow hood. These explants were inoculated in the jars which contained either media I or II. Three explants of 8818-william variety were inoculated in media I and three in media II. Similarly, 6 explants of Brazilian variety were also taken and three/three were inoculated in each media (media I and media II).

Growth Room

These jars were transferred to the growth room which was set under the controlled environment. Room temperature was maintained at 25±2°C. A light period of 16-hour was provided and light intensity was 2000 lux of the growth and development.

Statistical Analysis

The data was recorded after 30 days of inoculation. Completely randomized design was used for all experiment. The data was analysed through statistic 8.1 and LSD was also determined by same software.

III. Results And Discussion

Statistically analysis indicated that both varieties of Banana i.e. 8818-William and Brazilian are non-significantly different from each other with respect to number of shoot production as P value is more than 0.05 (P> 0.05). Average number of shoots of 8818-William was found 11.5 which is statistically not different from 11.6 shoots of Brazilian.

No specific interaction was observed between varieties and media (P > 0.05) however the two types of media were significantly different from each other with respect to the number of shoot production after 30 days (P<0.05). Liquid media supported more multiplication of shoots than solid media. The average number of shoots on liquid media was found 13.6 compared to 9.5 on solid media (See Table No. 1).

The results of this study are in agreement with Muhammad et al., (2007). They observed maximum number of shoots (5.4) on liquid MS medium supplemented with 4.0mg/l BAP compared to 4.8 average numbers of shoots on solid MS medium. Similar results were also obtained when they replaced BAP with another cytokine i.e. Kinetin. Using kinetin at the rate of 6 mg/l, they obtained 4.7 average numbers of shoots on liquid media compared to 4.4 on solid media. In our study we used both type of cytokinin which give the synergistic effect and increased the multiplication rate. Azad and Amin (2001) developed a medium for regeneration of banana from excised floral apices in which they used IAA in multiplication media beside Cytokinin. In the present study we also used 1mg/ l IAA for shoot elongation to produce more multiplying shoots in short time for further inoculation. The combination of cytokine and Auxin was also found best to regenerate shoots from suckers (Akbar and Stark, 2003). FarahaniandMajd (2012) compared four different types of liquid media as well as solid culture media. They found maximum average number of shoots (3.5) on liquid MS medium containing cotton compared to 2.7 average numbers of shoots on solid MS medium. In our experiment we also used cotton with liquid media and that favoured a greater number of shoots compared to the solid MS media hence our results are strongly in agreement with the result of Farhani and Majid 2012. More shoot multiplication on liquid media may be due to the easy uptake of nutrients by explant. The finding of present study also agreement with Alvard*et al.* (2015). They compared five different types of liquid culture medium with solid culture medium for meristem propagation of bananas and founddifferent multiplication rate at different medium. They observed highest multiplication rates on liquid medium compared to solid medium.

In summary, an efficient and reproducible multiplication system has been established for both 8818-William and Brazilian varieties of Banana by investigating different parameters. Modifications in the cultural condition remained successful for enhanced shoot multiplication. Our study can be efficiently utilized for multiplication of other banana varieties as well.

IV. Conclusion

In the present investigation, the effect of physical state of media was observed on average number of shoot production. The result indicated that the two different varieties were non-significantly different from each other with respect to average number of shoot production but the two physical states of media (Media I and Media II) were significantly different from each other. Liquid media supported maximum multiplication of shoots and proliferation than solid media probably due to the uniform distribution of nutrients in liquid media.

Moreover, it is easier for explant to get nutrition more easily and rapidly from liquid media compared to solid media.

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Table 1: Effect of different media on number of shoots after 30 days of Inoculation.

Variety	Media		Mean
	T1	T2	
	Solid	Liquid	
8818-William	**9.6 b**	**13.3a**	**11.5A**
Brazilian	**9.3b**	**14.0a**	**11.6A**
Mean	**9.5B**	**13.6A**	
LSD	**1.15**		**1.15**

Means followed by same letters are not significantly different at 5% probability level.

Data followed by capital alphabets represent media and cultivars means while data followed by small alphabets denote the individual values as an average of three replicates.

Comparison of No. of shoots with respect to two different Ms Media (Solid and Liquid) and varieties were also checked by Bar graph (See Figure 1& 2).

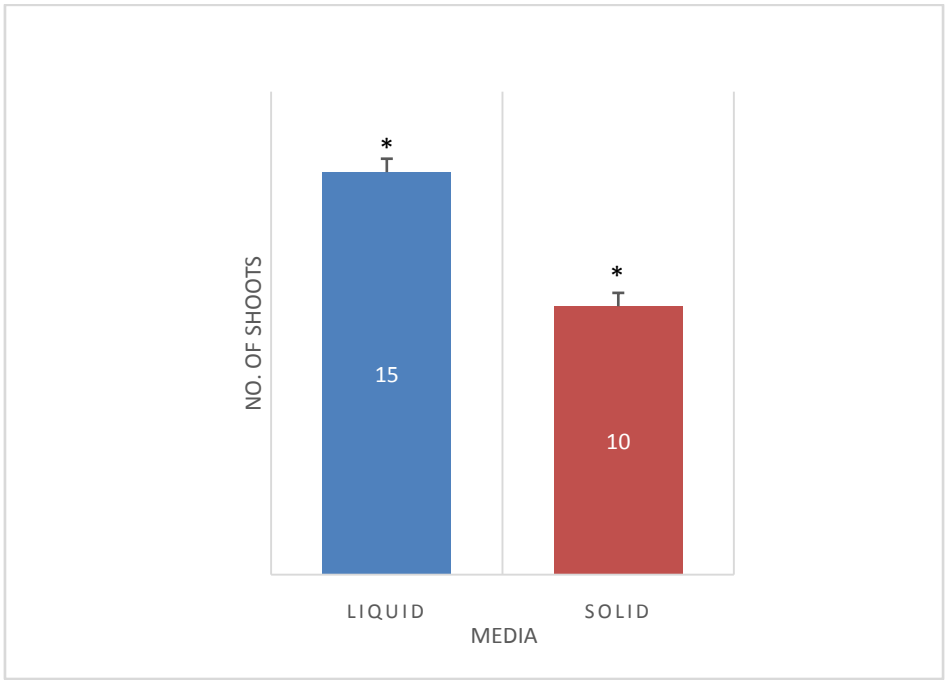

Figure 1: Comparison between No. of shoots and types of media after culturing two varieties of banana i.e. William-8818 and Brazilian in MS. Solid and liquid medium.

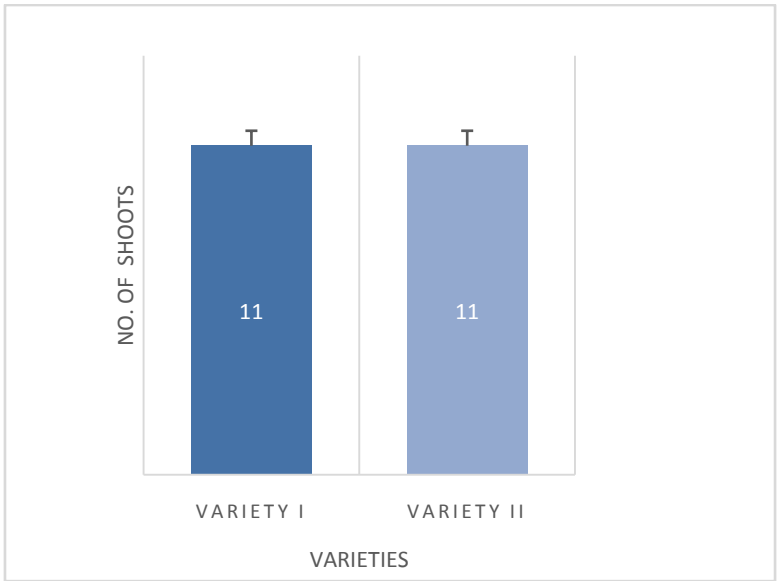

Figure 2: Comparison between No. of shoots and varieties after culturing in MS. Solid and liquid medium.

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