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In Vitro Production Of Multiple Shoots Of Banana G-9 Through Suckers

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ABSTRACT:
The present investigation was undertaken to study the effect of different concentrations of BAP on shooting in G-9 species of Banana. Suckers small part used as explants were inoculated on MS medium supplemented with different concentrations of BAP (3mg/l,5mg/l, 7mg/l 8mg/l) cultures were incubated at 25±1^0 C with 16 hr photoperiod provided and 8 hr dark period provided. Second concentrations were used BAP with combination of IAA (1.5 mg/l). Third concentrations were used BAP with combination of NAA (0.5 mg/l). The highest average number of shoots for each explant (3.8) was found in MS medium fortified with 7mg/l BAP. The cultured material were observed at 25days interval in same medium to produce multiple shoots while MS medium with 7mg/l BAP combination with 0.5mg/l NAA was found most suitable for rooting of shoot. The rooted shoots were acclimatized and successfully transferred to plastic cup. After hardening they were transferred to the plastic pot and the survival rate was around 90%. This protocol might be used for the *in vitro* production of the plantlets of banana cv.G-9.
Key words: BAP, Banana, fresh, sucker, medium

INTRODUCTION:

Banana is a perennial, herbaceous monocot which belongs to musa genus of musaceae family. It can be cultivated under subtropical conditions if planting time regulated, but it can be best grown in tropical region. It accounts for approximately 22 percent of the fresh food production and ranked as second most important fruit crops. Banana contributes to the fruit security of millions of peoples in developing world. The origin of banana is from south-east Asian region, where greater diversity of edible banana found. It is believed to be one of the oldest fruit which have originated from Malaysia through a complex hybridization process *(Novak,* 1992). Near about 70 species of musa were recognized by the world check list of selected plant families as of January 2013. G9 bananas are cultivars of *musa acuminate*. It is one of the most commonly cultivated bananas and a source of commercial *Cavendish* bananas. The G9 refers to its relative height compared to the *Giant Cavendish* and Dwarf Cavendish cultivars. It could be distinguished from another cultivar by growing side by side and comparing heights. Being and angiosperm, the G9 produces large inflorescence which develops in to the edible fruit. G9 has become one of the most popular varieties for commercial plantations. Its characteristic medium height and large fruit yield make it ideal for commercial agriculture. It is high yielding Cavendish variety introduces to India from Israel.

Bananas are rich source of carbohydrates, mainly starch in unripe fruits and sugars in ripe fruits of Banana. Unripe banana fruits contain 70-80 percent starch Banana is healthy source of fibre, potassium, vit. A and vit.C (Chandler 1985), antioxidants, phytonutrients and catechin.

Banana is generally propagated vegetative through suckers. But the traditional method is laborious, time consuming and not very efficient as far as production homogenous plant is concerted (Banearjee and D Langhe, 1985) Only 5 to 10 suckers can be obtained from a plant per year. Banana production sometimes became seriously affected by different disease (Rahman et al. 2004) As a result, the productivity of banana decreases and the yield become very poor.

To overcome this problem, production of saplings using in vita culture techniques could be an effective for production of planting materials of bananas.

G9 is an important banana cultivar. Throughout the investigation multiple shoot obtained from sword suckers of Banana CV G-9 (Grand naine) were cultured on in s medium supplement with (3 mg/L, 5mg/L, 7 mg/L, 8mg/L) BAP and (1.5 mg/L) IAD and (0.5mg/L) NAA for induction of multiple. MS medium with 7 mg/L BAP combination with o.5mg/L NAA was found most suitable for rooting of shoot.

MATERIALS AND METHODS :

The explant materials of G-9 cultivar of banana were obtained from plants grown at the field of shri shivaji college of agril biotechnology Amravati.The fresh suckers were used as a explants. The suckers were treated with bavistin for 30min and then chopped off about 3-5 cm length and washed thoroughly under running tap water for 10-15 min. All traces were removed by repeated washings under running tap water for 4-5 times and finally with distilled water. Shoot tips were prepared by trimming corm and outer leaf sheath from the suckers . These shoot tips were treated with 0.1%$HgCl_2$ solution for 6 minutes. The shoot tips were rinsed with sterile distilled water 3-4 times under aseptic conditions. All

explants were cultured on MS medium(Murashige and Skoog.1962) with different concentration of BAP (3mg/l, 5mg/l 7mg/l, 8mg/l) and IAA (1.5mg/l), NAA 0.5mg/l).The pH of medium was adjusted to 5.8 prior to autoclaving. All culture bottles were incubated at 25^0 C ± 2^0 C with a 16 hr light and 8hr dark photo period(approximately 2000 lux)provided by cool white fluorescent tubes. The materials were sub- cultured at regular 25 days interval in same medium to produce multiple shoots.

The plantlets were carefully removed from the culture bottles.The roots of the plantlets were gently washed under running tap water to remove agar attached to the roots then treated with bavistin for 5 min then immediately after washing they were transferred to small plastic cup containing a mixture of soil,sand,cocopeat in 1:1:1 ratio.The plantlets with cup were covered with polythene bags to check sudden desiccation. The polythene Bags were gradually perforated to expose the plantlets to the outer normal environment and subsequently removed after 10 days. They were transferred to earthen pot and finally into the field. The data were taken at 60 day intervals after subculture. There were 10 bottles for each treatment. The data were measuredstatistical analysis based on average value and standard error (±) (Panse and Sukhatme 1958)

RESULT AND DISSCUSION :

Inthis study the results showed that multiple shoot obtained from sword suckers of banana cv G-9(grand naine) were cultured onto MS medium supplement with (3mg/l ,5mg/l, 7mg/l ,8mg/l)BAP and (1.5 mg/l) IAA and (0.5mg/l)NAA for induction of Multiple .The results of multiple shoot development , mean number of multiple shoot development,and effect of different concentration ofsingle BAPin cv grand naineare presented intable no.1. Similarly the effect of diferent concentration of BAP with (1.5 mg/l IAA and with (0.5 mg/l) NAA on multiple shoot induction in cv. grand naine are present in table no. 2 and table no. 3 respectively

The effect of different concentration of BAP and BAP with (1.5 mg/l) IAA and BAP with (0.5 mg/l)on shoot initiation and shoot multiplication were investigated after the initial shoot cut out from sucker and remaning sucker subculture on same medium multiple shoot were produced from the base of explant after 25 days table no.1 shows the number of multiple shoot development at different levels of BAP in variety grand naine

During the culture period the number of shoot increased gradually in all the media supplemented with different concentration of BAP, BAP with IAA and BAP with NAA .The highest average number of shoot per explant was found (...3.6±1.6.....) at 7mg/l BAP and the lowest was found (1.4 ±0.6.....) at 3mg/l BAP (FIGURE 1) The number of multiple shoot development concentration BAPwith combination 0f (1.5 mg 1l IAA) are presented in table no.2 The highest multiple shoot was found (3.2± 1.4.....)at 7mg/l BAP and 1.5mg /l IAA and the lowest multiple shoot was recorded at (0.8± 0.35.....) 3mg/l BAP and 1.5 mg/l IAA (FIGURE 2) In Table no. 3 shows the number of multiple shoot development at different concentration of BAP with0.5mg/ Thehighest number(2.8±1.2)of multiple shoot was found at 7mg/lBAP and 0.5mg/ LNAAand the lowest number (0.6± 0.26)of multiple shoot was found at 3mg/l BAP and 0.5mg/l NAA(figure3).In present investigation we studied effect of different concentration of BAP on grand naine cultivar of banana. Similarly study was also confirmed on different cultivar by various workers. The cultivar Basrai,Shrimati,Ardhapuri optioned (4.5) multiple shoots at 7mg/l and (6.2) multiple shoots obtained at 7mg/l BAP with respectively (U.P.Bhosle 2011)

In 2014 S.Ahmed studied on in vitro multiplication of banana. MS medium with 4mg/l with IAA2.00mg/l gives maximum multiple shoots. Jaisy and Ghai (2011) worked on in vitro propagation of banana also found treatment of explant with HgCl2 ()0.1%) for 6 min most effective surface sterilization procedure registering maximum culture establishment with minimum contamination.

In2013 S.Rahman studied on micro propagation of banana cv. Agnishwar by in vitro shoot tip culture shows that maximum multiple shoot for each explant(5.9) was found in ms medium 4mg/l BAP.

Regenerated shoots were cultured in half strength of MS medium supplemented with 0.5 mg/l NAA.(S.rahman 2013). The media showed vigorous rooting (figure 4). After initiation of roots, plantlets were taken out from the growth room and kept under normal room temperature for about 3-5 days in contact with normal atmospheric temperature and light after five days the plantlets were taken out from culture bottle and washed with running tap water.

Plantlets with vigorous rooting were transferred to plastic cups containing soil,sand,cocopeat, in the ratio 1:1:1 to observation the survivability of transplanted plantlets in the net house. The plastic cup were covered with polythene bags to retain moisture and gradually accalimatized to out door condition (figure5). Efforts were made to establish complete plantlets with sufficient root in plastic cup. The results of this study nearabout 90% plantlets was establish successfully in the field condition (figure 6) However the, the previus studies (Sharma and Thorpe,1990) showed that complete plantlets of Morusalba were successfully establish (100%) in the field condition (S.Rahman and Biswas,2013)showed that (90%) planlets successfully establish of cv. Agnishwar

Table No.1. Effect of different conc. Of BAP on multiple shoots of Banana

Sr. No.	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	**MS + Without hormone**	**No Response**	**No Response**
1.	MS+BAP (3mg/l)	1.4 ± o.6278	1.5 ± o.6726
2.	MS+BAP (5mg/l)	1.6 ± o.7174	0 2 ± o.8968
3.	**MS+BAP(7mg/l)**	**3.6 ± 1.6143**	**3.8 ± 1.7040**
4.	MS+BAP (8mg/l)	01 ± 0.4484	O.8 ± 0.3587

Table No 2: Effect of different conc. of BAP and IAA on multiple shoots of banana-

Sr. No	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction Observed after 50 days.
Control	**MS +Without hormone**	**No Response**	**No Response**
1.	MS+BAP(3mg/l)+IAA(1.5mg/l)	0.8 ± 0.3587	1 ± 0.4484
2.	MS+BAP(5mg/l)+IAA(1.5mg/l)	1.6 ± 0.7174	2 ± 0.8968
3.	MS+BAP(7mg/l)+IAA(1.5mg/l)	3.2 ± 1.4349	3.4 ± 1.5246
4.	MS+BAP(8mg/l)+IAA(1.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587

Table No 3: Effect of different concentration of BAP and NAA on multiple shoots of banana-

Sr. No.	Concentration of hormones (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	**MS + Without hormone**	**No Response**	**No Response**
1.	MS+BAP(3mg/l)+NAA(0.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587
2.	MS+BAP(5mg/l)+NAA(0.5mg/l)	1.2 ± 0.5351	1.4 ± 0.6278
3.	MS+BAP(7mg/l)+NAA(0.5mg/l)	2.8 ± 1.2556	3 ± 1.3452
4.	MS+BAP(8mg/l)+NAA(0.5mg/l)	0.2 ± 0.0896	0.4 ± 0.1793

Fig. 2.a Effect of MS + BAP(7mg/l) + IAA (1.4mg/l) on shoot multiplication

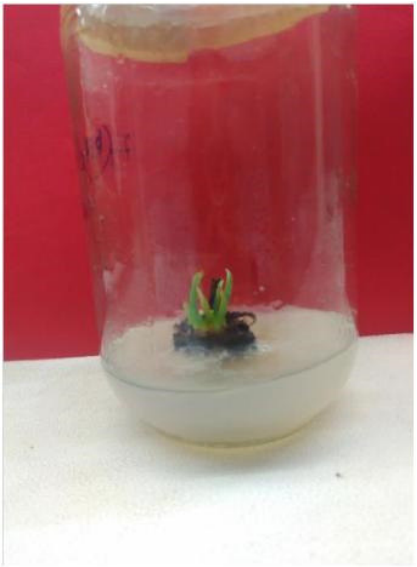

Fig. 2b Effect of MS + BAP(3mg/l)+ IAA(1.5mg/l) on shoot multiplication

Fig. 1 a Effect of MS+ BAP (7mg/l) on shoot multiplication.

Fig. 1 b Effect of MS + BAP(3mg/l) on shoot multiplication

Fig. 4 Rooting observed on ½ MS + Hormone (NAA-0.5mg/l)

Fig. 5 a **b**

Plant transferred in small pot for primary hardening

C **d**

Plants kept for hardening

Fig. 6 a

b

Plants ready to plant in field.

CONCLUSION :

We established suitable media with different concentration of growth hormons for multiple shoots formation of banana cv. Grand naine (S.Ahmed,2014). Multiple shoot regeneration directly from explant (sucker), the suitable media was MS+7mg/lBAP(U.P. Bhosale 2011) for multiple shoot, the suitable medium was ½ MS+ 0.5mg/l NAA for rooting (Gupta et al 1981). Results of this study indicates that from the commercial point of view the media composition application for large scale propagation of healthy and disease free banana cv. Grand Naine.

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