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Studies on *in vitro* Micropropagation in Banana

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A B S T R A C T

Banana is one of the important fruit crops and food source for million people in developing countries. In the study explants (Suckers) of two banana cultivars were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP and Kinetin sole and in combination for shoot initiation and multiplication. The concentration of BAP alone tested was (0.5mg/l and 1.0mg/l) and kinetin (0.5mg/l and 1.0mg/l) whereas, the combination of 0.5mg/l BAP+0.5mg/l kin and 1.0mg/l BAP +0.5mg/l kin were used for shoot initiation. For multiplication, concentration of BAP(2.0,2.5 and 2.5mg/l) alone and in combination BAP+ Kin (1.5mg/l +1.5mg/l, 2.0mg/l +2.0mg/l and 2.5mg/l +2.5mg/l) were used. The rapid shoot initiation obtained from MS medium supplemented with the combination of 1.0mg/l BAP with 0.5mg/l kin (8 and 10 days) in both Poyo and Giant Cavendish respectively. The highest multiple shoot (6.0 and 4.5/explants), in Poyo and Giant Cavendish were observed on the MS medium fortified with 2.5mg/ l BAP+2.5mg/l kin and 2.0mg/l BAP +2.0mg/l Kin respectively. For root induction 1.5mg/l IBA and 1.5mg/l IAA each tested separately on MS medium. IBA showed best performance with 5.12 and 4.69 root/ plantlet after four weeks of inoculation in Poyo and Giant Cavendish respectively. After 12 weeks in vitro plantlets were transferred to green house for acclimatization where 82% and 88% survival rate was recorded in Poyo and Giant Cavendish respectively. The two cultivars studied exhibited variation in shoot initiation, shoot multiplication and rooting. Among the two cultivars tested Poyo found to be more responsive for in-vitro techniques. It had highest rate of shoot initiation and multiplication.

Introduction

Ethiopia is one of country which has highly diverse agro-ecology that can grow different fruit crops with a vast potential for banana production (Seifu, 1999; Kahessay *et al.*, 2010). In Ethiopia the major banana (Musa *paradisiaca var. sapiertum*) producing regions are Southern, Oromia and Amhara (MoA, 2011). Researcher reported that the different banana cultivars include *Giant cavendish, poyo* and other grown in South Ethiopia. Despite of these potentials, the overall production amount includes its productivity and quality is still low. According to CSA (2014), banana crop in Ethiopia in terms of area coverage is highest compare to other fruit crops i.e. 53,956.16 ha with production of

4,782,510 quintals. The global share of Ethiopia in banana export was only 0.02% during 2011 which could be indicated to problems linked with low production and poor quality to meet standards of the export international market. By considering this produce rapid production of banana plantlets through tissue culture is need of hour.

In 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 material of banana in shorter time, lesser space, disease-free plants and adequate germplasm preservation.

Based upon earlier scientific reports, only 5 to 10 suckers can be obtained from a banana plant per year by conventional method. Furthermore, banana production becomes seriously affected by different bacterial, fungal and viral diseases (Rahman *et al.,* 2004). The major cause of low banana production in Ethiopia is the complex of foliar diseases, nematodes, viruses, and insect pests (Seifu, 2003). The insect pests (fruit flies) and diseases (Banana *xanthomonas* wilt and *Fusarium oxysporum*) are major challenges for banana production in Ethiopia (Mekonin, 2014). The considerable yield gap of banana is the widespread use of infected planting materials (sucker) by different pests and diseases. Additional using only traditional propagation (suckers) method of banana is a major barrier to get enough plant materials and very difficult to carry bulks volume of suckers from one place to other. These conditions have been ended the banana production below actual potential per hectare and along with the quality very poor. This resulting yield loss makes banana an expensive commodity for consumers which reduce the cash earnings of producers as well as the potential of the crop to contribute to the food security of rural households (Qaim, 1999). To solve these challenges tissue culture technique is better alternative. Plant tissue culture (PTC) technique also has great potential as a means of vegetative propagation of economically important species, especially for those difficult to propagate by conventional methods like seeds or cuttings (Mohamed, 2007). The numbers of countries in the world like France (Cote *et al.,* 1990), Australia (Drew and Smith, 1990), Israel (Israeli *et al.,* 1995), Cuba and many East African countries like Uganda, Tanzania and Kenya (Abraham, 2009) are using *in vitro* multiplication of banana. To ensure an extremely rapid rate of multiplication, tissue culture technique has definite and indispensable advantage over the conventional method. This technique is independent of season due to controlled conditions and requires limited quantity of plant tissue as the explant source (Arinaitwe C *et al.,* 2000).The production of plantlets using *in-vitro* plant tissues culture techniques could be an effective for providing disease free and enough planting materials of banana. Therefore applying tissue culture technique widely in Ethiopia is an excellent alternative to overcome the obstacles, which reduce the production and quality of banana. By keeping in view the aforementioned prelude the present investigation is designed to development of the efficient *in vitro* regeneration protocol in popular Ethiopian

banana varieties i.e. *Poyo* and *Giant Cavendish.*

Materials and Methods

Study area

The study was conducted at Areka Agricultural Research Center's unit of plant tissue culture. Areka is one of reform town in wolaita zone. It located south to capital city of Ethiopia and about 299 km far from Addis Ababa, Ethiopia.

Preparation of explants

The explants were selected from the field of Areka agricultural research center. Very young sucker about three months old was used as explant in this experiment. The selected suckers carefully removed from mother plants. The suckers' size reduced up to 2-3cm length and 1-2cm width at base.

Surface sterilization

The prepared suckers were dipped in 70% ethanol for 60 second. After this it was washed with distilled water and surface sterilized with 2% sodium hypochlorite solution for 20 minutes with few drop of Tween 20. The Tween 20 used to aid the surface sterilization more effective because of its wetting characteristic. Then the explants (sucker) washed with double distilled water to remove the trace of chemicals. These surface sterilized explants trimmed to 1-2cm size and inoculated under clean conditions.

Experimental treatment and design

In this study, MS medium (Murashige and Skoog, 1961) was used for shoot initiation as well as shoot multiplication. For shoot initiation BAP (MS + 0.5 and 1.0mg/l) and Kinetin (MS + 0.5 and 1.0mg/l) tested separately and in combination of BAP and Kinetin (0.5+0.5 and 1.0+0.5). Whereas, for multiplication higher concentration of BAP (MS + 2.0, 2.5 and 3mg/l) in sole and combination of BAP and kinetin (1.5 +1.5, 2.0+2.0 and 2.5+2.5mg/l) was used. Multiple shoots were transferred on MS medium supplemented with two auxins (IBA and IAA) with the concentration of 1.5 mg/l each for root induction. All the cultures were maintained at 22 ^{o}C with a light/dark cycle of 16/8 h. White fluorescent light with an intensity of 120 μmol m^{-2} s^{-1} was used for illumination. After six weeks of inoculation the rooted plantlets were acclimatized under green house. The experiment for each treatment replicated three times. The experiment was conducted during April 2015 to April 2016.

Data collection and analysis

The data such as number of day required for shoot initiation, shoot numbers per explant, shoot length, leaves number per explant, root number per plantlet and survival rate were recorded after specific interval of time. The experiment was carried out with three replications. The collected data also was analyzed by using statistical significance two ways analysis of variance (ANOVA).

Results and Discussion

Effect of different concentration of BAP and kinetin on shoot initiation

In present investigation, the effects of different concentrations BAP and Kinetin (0.5 and1.0mg/l), BAP (0.5 and 1.0mg/l) and in combination BAP + Kin (0.5 +0.5 and 1.0+0.5mg/l) on MS medium were tested for effective *in-vitro* shoot initiation in two varieties of banana (*Giant Cavendish* and *Poyo*). Through all the hormones responded for shoot initiation all their duration of time

for initiation varied (Table 1). Shoot initiation started after first week of inoculation on the all medium tested. But the MS medium fortified with 1.0mg/l + 0.5mg/l (BAP +Kin) found highly reactive as shoot initiation within week compare to rest treatments. For the shoot initiation, the MS medium fortified with 1.0mg/l BAP + Kin 0.5mg/l proved better as shoots started to initiates within a week compared to the rest of the treatments (8 and 10.2 days) in both *Poyo* and *Giant Cavendish*, with higher number of shoots respectively. These result at par with the findings of Shagufta *et al*, (2011) who observed shoot initiation10.6 days after of inoculation. On other hand, MS medium with 0.5mg/l kinetin took almost double time for shoot initiation, i.e., 19.6 and 20.8 days in *Poyo* and *Giant Cavendish* respectively with low number of shoots (Table 1). In the present study only the plant growth regulator (cytokinin) shown significant effect at alpha ($p \leq 0.05$) level on number of day needed for shoots initiation in both varieties of banana. The BAP proved its superiority over Kinetin for shoot initiation as well as shoot length which support the findings of Khalid *et al* (2011) who proved the marked effects of BAP on shoot formation compared to Kinetin tested in his study. These results also support the findings of Gilmar *et al* (2000), who received more number of shoots when BAP applied alone compared to Kinetin. In present study, furthermore, using BAP combined with kinetin proved best for shoot initiation (Fig. 1).

Influence of various growth regulators on shoot multiplication

Among all the treatments, BAP in sole with the high concentration i.e., MS + 3.0mg/l produced high number of shoots compare to other treatment in *Poyo and Giant Cavendish* (Table 2 and Fig. 2). Many researchers recorded multiple shoots on MS medium with high concentration of BAP (Khanam *et al.,* 1996; Rabbani *et al.,* 1996; Gebeyehu, 2015). Response of shoots towards multiplication varied significantly when growth hormones supplied in combination. The results indicate that mean number of shoots was proportional with concentration of kinetin and observed highest when both the hormones used in same highest concentration (2.5 mg/l BAP + 2.5 mg/l kinetin). The maximum mean numbers of shoots/shoot were 6.0 in *Poyo*. Whereas, the same concentration failed to multiply the highest shoot in another variety i.e. *G. cavedish*. In the *G. cavendish* the MS media fortified 2mg/l BAP combined with 2mg/l Kin proved best for multiplication (4.5 shoots/explants). The source of these variations may be due to genotype difference between two cultivars. These results are in line with the finding of Arinative *et al.,* (2000) who stated that shoot initiation and multiplication is cultivar dependent.

MS medium supplemented with BAP produces multiple shoots in both the cultivars. Whereas, When BAP used in combination with kinetin, the rate of shoot multiplication recorded higher.

In the present investigation, 2.5mg/l BAP combined with 2.5mg/l kinetin proved best result (6.0shoot per explants) compare to rest of treatments for the multiplication in *Poyo* after 5 weeks of inoculation (Table 2). These results are in line with the earlier findings of Davendra Kumar *et al.,* (2013) who reported BAP in combination with kinetin is effective in shoot multiplication. They also recorded highest numbers of shoots/shoot (5.2shoots) of *Cavendish dwarf* variety when inoculated on MS medium fortified with combination of BAP and kinetin at concentration (2mg/l and 1mg/l) respectively.

These results are also coinciding with effort of Azam *et al.,* (2010) who received highest number of shoot (5.2 per explants) in cultivar

'BARI-1' on the MS medium supplied with combination of 2mg/l BAP with 1mg/l kinetin.

However, in *Giant Cavendish*, at the same concentration failed to produce significant results (Table 2). This may due to genotype as well as physical difference between *Poyo* and *Giant Cavendish.* Therefore standardization of concentration of plant growth regulator to develop efficient multiplication protocol was very important in these two popular varieties of Ethiopia. This indicated that optimization in concentration of plant growth regular very important in *in-vitro* micro propagation. In the present study, the combination of 2.5mg/l BAP + 2.5mg/l Kin and 2.0mg/l BAP combined with 2.0mg/l Kin proved better for *poyo* and *Giant Cavendish* respectively.

Giant Cavendish produced less mean number of shoots compare to *Poyo*, the highest shoot recorded were (4.5/explants) which are higher than the Gebeyehu (2015) who received (3.20 shoots/explant) in the same cultivar on the MS medium supplemented with media 5mg/l BAP and 0.5mg/l NAA combination. This variation might be due to the combination of BAP with kinetin (Cytokinin) and BAP with NAA (auxin).

The results of current investigation are somewhat contradictory with Rabbani *et al.,* (1996) who recorded highest number of shoots/plant (3.11 ± 0.66) with 5.0 mg/l of BAP and Kin. This may be due to low concentration of BAP and Kinetin. In the present study *poyo* variety was most responsive, produced maximum number of shoots than *Giant Cavendish*. These results are in agreement with the previous reports on *in-vitro* micro propagation on banana cultivars includes *poyo* variety (Dagnew *et al.,* 2012).The cytokinin concentration and combination shown statistical significant effect at alpha ($p \leq 0.05$) level on multiple shoot number per explants.

Effect of IAA and IBA on root induction

The two concentrations of IAA and IBA have been tested on M.S medium for root induction. Root number varied within two concentrations of IAA and IBA. The results of two concentrations of auxin 1.5mg/l IBA and 1.5mg/l IAA on root formation after two and four weeks have been discussed.

Table.1 The effect of cytokinin (BAP and Kin) in sole and combination on number of days took for shoot initiation

Cytokinin	Treatment	*Poyo*		*G. Cavendish*	
	(mg/l)	Day required for shoot initiation	Means No. of shoot	Day required for shoot initiation	Means No. of shoot
Kin	0.5	19.6± 1.16	1.2	21.2± 1.31	1.0
	1.0	16.4± 1.62	1.6	18.6± 1.89	1.5
BAP	0.5	17.6± 1.57	1.4	18.4±1.75	1.2
	1.0	12.2±1.27	1.8	12.6± 1.55	1.5
BAP+ Kin	0.5+0.5	10.4± 1.88	2.5	11.4± 1.55	2.0
	1.0+0.5	8.4± 0.97	3.0	10.2± 1.71	2.4

Result expressed as mean ± Std. deviation

Table.2 The effect of cytokinin(BAP and Kin) in sole and combinations on shoot multiplication

Cytokinin	Treatment BAP and Kin (mg/l)	Mean no of shoots	
		Poyo	*G. Cavendish*
MS + BAP	2.0	2.1±0.54	1.8±0.65
	2.5	2.8±0.48	2.2±0.55
	3.0	3.2±0.61	2.6 ±0.70
MS + BAP + Kin	1.5 +1.5	3.4±0.72	3.2 ±0.39
	2.0+2.0	4.4±0.85	4.5± 0.76
	2.5 + 2.5	6.0±0.55	3.6 ±0.70

Table.3 The effect of IAA and IBA on root induction

Auxin	Plantlets	*Poyo*		*G. Cavendish*	
		Mean root number after two weeks	Mean root number after four weeks	Mean root number after two weeks	Mean root number after four weeks
1.5mg/l IAA	10	3.56	4.00	3.00	3.21
1.5mg IBA	10	4.12	5.12	4.00	4.69

Mean no of roots/shoots

Table.4 Survival percentage of *in vitro* raised plantlets

S. No	Number of regenerations transfer to pots	Number regeneration survived		Percent of Survival	
		Poyo	G. cvendish	*Poyo*	G. cavendish
1	10	09	10	90%	100%
2	10	08	90	80%	90%
3	10	09	80	90%	80%
4	10	07	80	70%	80%
5	10	08	90	80%	90%

Fig.1 Shoot initiation in *Poyo* (A) and *Giant Cavendish (B*)

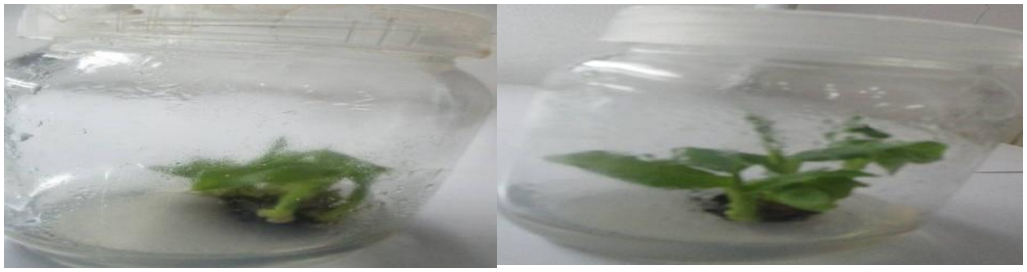

Fig.2 Shoot multiplication in *Poyo*(A) and *Giant cavendish*(B)

A **B**

Elongated shoots were transferred to MS medium containing 1.5mg/l IBA and 1.5mg/l IAA. The cultures were maintained in 16hrs light /8hours dark photoperiod which concluded by biotechnologists show best result in plant *in-vitro* micro propagation. The effect of IAA and IBA on number of roots per explants produced by standardized concentration at 1.5 mg/l was found statistically significant (Table 3). Significantly highest means number of root was produced at 1.5mg/l IBA (5.12 and 4.69) in *Poyo* and *Giant cavedish* respectively. The treatment 1.5mg/l IBA produced 5.12 mean number of roots in *Poyo* and 4.69 mean number of roots in *Giant cavendish* after four weeks of inoculation.

The vigorous rooting of *in-vitro* grown plantlets observed on MS medium fortified with 1.5mg/l IBA. The present results are in line with the findings of Gubbuk and Pekmezci (2004) and Molla, M *et al* (2006) who obtained 5.5 number of root per plantlets on MS medium supplemented with1.5mg/l IBA. They also recorded 3.89 and 3.93 number of roots on 0.2mg/l IBA and 0.3mg/l IBA respectively. The results of present experiment were found similar with findings of Khanam *et al.*, (1996) (Table 3). This result not agree with the other researcher who reported that IBA at the concentration 0.5mg/l the highest percentage of rooting (6 root/plantlet) was observed in *Cavendish dwarf* variety (Davendrakumar *et al*, 2013).The source of variation may difference in cultivars variety and concentration of the auxin.

After four weeks of culture, rooted plantlets were removed from culture jars and were washed in running tap water to remove agar from root surface. Then the rooted plantlets were transplanted to plastic bag containing FYM + Soil with ratio (1:1) and were placed in green house. After 5 weeks the survival percentage was calculated. The survival rate of *in-vitro* regenerated plantlets was about 82% and 88 % for *Poyo* and *Giant Cavendish* (Table 4). These results are in close

conformity with Jasari *et al.,* (1999) and Shiragi *et al.,* (2008) who recorded 83.3% and 92% survival rate respectively.

On the basis of results obtained from present investigation, the following conclusions are drawn.

The two cultivars studied exhibited variation in shoot initiation, shoot multiplication and rooting. This may be due to genetic makeup of two varieties.

Among the two cultivars tested in present study, *Poyo* found to be more responsive for *in-vitro* techniques. It had highest rate of shoot initiation and multiplication.

BAP alone not effective for shoot initiation and multiplication. Whereas, combination of BAP and Kinetin was found most effective for shoot initiation as well as shoot multiplication.

For rooting IBA showed best performance in both cultivars of banana.

In hardening the mixture of FYM+ Soil in ratio (1:1) showed good survival rate in both cultivars varieties of banana.

Plantlets production through *in-vitro* plant tissue culture techniques seems to be very effective for rapid and large scale multiplication of banana cultivars. The Method may also very useful for commercial production of banana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AK, STH, NMC and AB carried out all experimental work, data acquisition and analysis, literature search. STH was responsible for study concept, designing and coordinating the research and supervising the work. Authors GB and AB contributed to writing and manuscript preparation. All authors read and approved the final manuscript.

Competing interests

Authors have declared that no competing interests exist.

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