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IN VITRO MICROPROPAGATION OF BANANA (*Musa spp.*)

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Abstract

The present study was conducted at the Biotechnology Laboratory, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during the period from September 2004 to June 2005 to investigate the effect of different concentrations of BAP and NAA on virus free plant regeneration, shoot multiplication and different concentrations of IBA and IAA on *in vitro* root formation of banana cv. BARI Banana-I. The culture meristem first turned brown in colour in 4-5 days which grew into a green globular hard coat mass after 30-35 days. From this ball like structure, adventitious plantlets were developed. Among the different concentrations, 7.5 mg/l BAP + 0.5 mg/l NAA showed highest shoot proliferation of 0.75, 2.75 and 6.25 shoots per explant at 10, 20 and 30 DAI, respectively. The longest shoot (1.03, 2.45 and 3.38 cm) at 10, 20 and 30 DAI, respectively, was produced by the treatment combination of 7.5 mg/l BAP + 0.5 mg/l NAA. The maximum number of leaves (2.50, 3.25 and 7.00 leaves/explant at 10, 20 and 30 DAI) were produced on the medium supplemented with the same treatment and it also produced the longest leaves, 0.85, 2.70 and 4.23 cm at 10, 20 and 30 DAI, respectively. For root initiation half strength MS medium supplemented with different levels of IBA (0, 0.5, 1 .0 and 1.50 mg/l) and IAA (0, 0.5 and 1.0 mg/l) was used. Root numbers varied with different concentrations of IBA and IAA. The highest number of roots were produced by 0.5 mg/l IAA + 0.5 mg/l IBA. The highest length (2.93, 4.63 and 5.88 cm) was recorded at 10, 20 and 30 DAI in the same treatment which was statistically significant. Meristem derived plantlets were transferred to poly bags containing 1:1 (ground soil : cowdung) mixture after 7 days hardening in room temperature (28-30°C) and established plantlet was ready for planting.

Key Words : Banana, regeneration, micropropagation, plantlet.

Introduction

The banana and plantains *(Musa spp.)* belonging to the family *Musaceae* are one of the world's most important subsistence crops. It is originated in Malaysia through a complex hybridization process (Novak, 1992). It is widely grown in the tropics and subtropics in all types of agricultural system, from small, mixed,

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subsistence gardens, to large commercial monocultures. The crop serves in many developing countries as a staple food or the cornerstone of the country's economy. The largest producers are Latin America and Asia, however, much of the South American production is exported to the developed world.

In Bangladesh, banana, which is rich in carbohydrate, minerals, phosphorus, calcium, potassium and vitamin-C is popular for its year round availability, abundant production as well as high acceptability to the consumers. In addition, it has importance for tannin, latex and fiber production.

Banana ranks first in terms of production and second in terms of area among the fruit crops and so has commercial value in Bangladesh. It contributes nearly 42% of the total fruit production of the country. It occupies an area of 43 thousand hectares of land with total production of 606 thousand metric tonnes with an average yield of 14.16 t/ha (BBS, 2003). This yield is quite low compared to that in other banana growing countries of the world like Argentina (34 t/ha) and Costa Rica (33 t/ha) (FAO, 2002).

Banana is also the premier fruit of Asia and the Pacific. It is the most important fruit of Indonesia, Thailand, Bangladesh, Vietnam, the Philippines, the South Pacific island countries and also India, where recently banana has been surpassing mango, traditionally the dominant fruit. Banana also occupies an important position in the agricultural economics of Australia, Malaysia, Taiwan, Srilanka and South China. Taiwan and the Philippines derive substantial earnings form their banana export. The great bulk of bananas produced in our country are traded and consumed in domestic markets.

Many biotic and abiotic factors are responsible for low yield and production of banana in Bangladesh. Virus is one of the major problems. The traditional clonal propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana. The productivity of vegetatively propagated banana and plantain is greatly reduced by virus disease (Lepoivre, 2000). Moreover, 5-10 suckers can be obtained per plant per year which may be of uniform size and virus free.

To minimize the above mentioned problems, micropropagation could be an alternative for propagation of planting materials for banana. In this method, over a million of plant can be grown from a small or even a microscopic piece of plant tissue within a year (Mantell *et al.*, 1985). Moreover, the shoot multiplication cycle is very short (2-6 weeks), each cycle resulting in an exponential increase in the number of shoots and plants multiplication can be continued throughout the year irrespective of the season (Razdan, 1993). Meristem culture offers an efficient method for rapid clonal propagation, production of virus free materials and germplasm preservation in plants (Cronauer and Krikorian, 1984a; Hwang *et al.*, 2000 and Helloit *et al.*, 2002).

As regards yield performance, tissue cultured plants have been reported to produce 39% higher yield than plants from sword suckers (Pradeep *et al.,* 1992). Under Bangladesh conditions, tissue culture derived plantlets of banana performed better than the conventional sword suckers (Faisal *et al.,* 1998).

Plant growth regulators are inevitable for *in vitro* regeneration of crop plants in any artificial medium. Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of banana and culture conditions (Cronauer and Krikorian, 1984a). BARI Banana-I variety plays a vital role in our national economy due to its popularity and acceptability to marginal and commercial farmers. To obtain virus and disease free healthy planting materials, development of a protocol for meristem culture of banana cv. BARI Banana-I are of prime importance. Therefore, considering the above facts, the present study was undertaken with the following objectives:

i) to study the effect of BAP and NAA growth regulators on *in vitro* meristem culture (virus free) and shoot proliferation of banana cv. BARI Banana-I;

ii) to determine the effect of IBA and IAA growth regulators and their concentration required for *in vitro* root development of banana; and

iii) to develop protocol for *in vitro* rapid propagation of banana.

Materials and Method

The present study was carried out in the Biotechnology Laboratory of the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701, Bangladesh during the period from September 2004 to June 2005. The planting materials of BARI Banana-I were collected from Biotechnology Division, BARI, Joydebpur, Gazipur. The meristem was obtained from developing suckers of about four months of age grown under field conditions and was brought to the preparation room. The suckers were washed thoroughly under running tap water. The roots and outer tissues of the suckers were removed with the help of a sharp knife. A number of outer leaves were removed until the shoot measured about 1.0-2.0 cm in length and 1.0 cm width at the base. The meristem used for establishment of culture was prepared through dissection and removal of leaf sheath under the microscope. Then the initial explant was prepared under stereomicroscope by removal of outer tissue of meristem with the help of sterile scalpel, which was about *5x5* mm in size.

Two experiments were conducted to assess the effect of different concentrations of BAP, NAA, IAA and IBA on shoot proliferation and subsequent rooting of the multiplied shoot. In the frist experiment, *in vitro*

derived banana cv. BARI Banana-I plantlets were used as sources of meristem to investigate the effect of BAP, NAA each at different concentrations alone or in combinations on shoot proliferation. Five levels of BAP (0.0, 2.5, 5.0, 7.5, and 10.0 mg/l) and 5 levels of NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l were used. All the combinations of both BAP and NAA were used as treatments. In the second experiment, there were 3 levels of IAA (0.0, 0.5 and 1.0 mg/l) and 4 levels of IBA (0.0, 0.5, 1.0, and 1.5 mg/l) were used as treatments. The experiments were arranged in completely randomized design (CRD) with 4 replications. Each treatment consisted of 10 culture tubes per replication. Data were collected on the effect of different treatments on shoot proliferation and rooting.

Murashige and Skoog (1962) medium supplemented with different phytohormones as per treatments were used as culture medium for shoot induction, shoot multiplication and maintenance and regeneration of roots from multiplied shoot. Hormones were added separately to different media according to the requirements. For the preparation of media, stock solutions were prepared at the beginning and stored at 9±1°C temperature. The respective medium was prepared from the stock solutions. The culture tubes with media were then autoclaved at 1.06 kg/cm^2 pressure at 121°C for 25 minutes. The medium was then cooled at room temperature before use.

The pale white tissue block (l.0 × 2.0 cm) containing meristem and rhizomatous base were taken in a beaker. Surface sterilization was done under laminar Airflow Cabinet with 70% ethyl alcohol, the explants were surface sterilized with 0.1% mercuric chloride and a few drops of Tween 20 for 15 minutes. Finally, the explants were then rinsed three to four times with sterile distilled water.

The isolated and surface sterilized explants were collected carefully under the stereomicroscope through maintaining aseptic condition inside the laminar air flow cabinet to use those as explants. The individual meristems were directly inoculated to each of the culture tube containing 20 ml of MS medium supplemented with different concentrations of hormones as per treatment covered with aluminium foil.

The culture tubes were transferred to growth room and allowed to grow in controlled environment. The temperature of the growth room was maintained within 25±1°C by an air conditioner. A 16-hour light period was maintained with light intensity of 2000 lux for the growth and development of culture.

Some explants become black in colour within 6-7 days after inoculation. To control blackening after about one week, the blackish tissues on the explants were removed and the meristematic tissues were, transferred to similar fresh medium. It was repeated 10 days interval for about one month to minimize further blackening of the tissues.

Initial subculturing was done when the explant produced some shoots. For sub-culturing, the entire samples of *in vitro* shoot were cut into small pieces so that each piece would contain about one shoot. Leaf and blackish or brownish basal tissues were removed to expose the meristems. Each piece was inoculated into a similar fresh medium. It was practiced at the interval of every one month.

When the shoots grew about 3-5 cm in length with 3-6 well developed leaves, they were rescued aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared medium containing different combinations of hormonal supplements for root induction.

Potting mixture containing ground soil and cowdung in the ratio of 1:1 was mixed thoroughly and were placed into a 10 × 15 cm polythene bag for growing *in vitro* grown plantlets under *ex vitro* conditions.

Results and Discussion

Regeneration of shoot from meristem explants

Regeneration of banana plantlets through meristem culture offers a unique scope of developing disease free planting materials against bunchy top, cucumber mosaic virus and panama wilt. *In vitro* culture of meristem results hard meristematic ball like structure in regeneration media containing different concentrations of BAP and NAA. The cultured meristem first turned brown in colour in 4-5 days and after 30-50 days later a green globular hard coat mass grew from which adventitious plantlets were developed. Rahaman *et al*. (2004) observed that hard ball like structure developed from meristem explant in MS media supplemented with 5.0 mg/l BAP. They also noticed that single shoot regeneration from meristem explant was thinner than shoot derived from shoot tip. Similar results were also obtained by Habib (1994) and Ali (1996) in their experiments. They observed that some ball like structures formed at the base of the shoot during shoot multiplication. These ball like structures are suitable for *in vitro* germplasm conservation.

Effect of different concentrations and combination of BAP and NAA on multiple shoot proliferation from meristem derived explant

The results obtained from this experiment have been presented in Table 1-4 and discussed under the following headings:

Number of shoots per explant

Variable number of shoots were produced per explant in MS media supplemented with different concentrations of BAP and NAA. Data were recorded at 10, 20 and 30 days after inoculation (DAI) and results have been presented in Table 1.

Table 1. Effect of different concentrations of BAP and NAA on shoot multiplication of banana plantlet cv. BARI Banana-1 at different days after inoculation.

Treatments		Number of shoots		
BAP (mg/l)	NAA (mg/l)	10 DAI	20 DAI	30 DAI
0	0	0.0 b	1.00 c	1.00 f
	0.5	1.0 a	1.50 bc	2.25 def
	1.0	0.5 ab	1.25 bc	2.50 de
	1.5	1.0 a	1.50 bc	1.50 ef
	2.0	0.75 a	1.25 bc	2.00 def
2.5	0	1.0a	1.00c	l.50ef
	0.5	1.0a	1.25 bc	1.75 ef
	1.0	1.0 a	1.25 bc	2.50 de
	1.5	1.0 a	1.75 abc	1.75 ef
	2.0	1.0 a	1.50 bc	2.25 def
5.0	0	1.0 a	1.25 bc	1.50 ef
	0.5	1.0 a	1.75 abc	2.50 de
	1.0	1.0 a	2.25 ab	2.50 de
	1.5	1.0 a	1.50 bc	2.50 de
	2.0	0.75 a	1.50 bc	1.75 ef
7.5	0	0.75 a	1.75 abc	2.50 de
	0.5	0.75 a	2.75 a	6.25 a
	1.0	0.75 a	2.75 a	5.25 ab
	1.5	1.0 a	1.75 abc	4.25 bc
	2.0	1.0 a	1 .75 abc	2.25 def
10 0	0	1.0 a	1.25 bc	2.25 def
	0.5	0.75 a	2.00 abc	2.50 de
	1.0	0.75 a	2.00 abc	2.50 de
	1.5	0.5ab	1.50 be	3.25cd
	2.0	0.75 a	1.50 be	2.50 de
LSD value (0.01)		0.61	1.05	1.26
CV (%)		38.88	34.55	26.71

The effect of different concentrations of BAP and NAA on shoot regeneration and proliferation were statistically significant at 1% level of significance. Among the different concentrations, 7.5 mg/l BAP + 0.5 mg/l NAA showed highest shoot proliferation of 0.75, 2.75 and 6.25 shoots per explant at 10, 20 and 30 DAI, respectively. But the treatment 7.5 mg/l BAP + 1.0 mg/l

NAA showed (0.75, 2.75 and 5.25 shoots per explant at 10, 20 and 30 DAI, respectively. A good number of shoot proliferations was achieved at 7.5 mg/l BAP + 1.5 mg/l NAA at 30 DAI (4.25) which is superior from the control treatments (1.00). The regeneration and proliferation of shoots was sequentially described in Fig. 1 and 2.

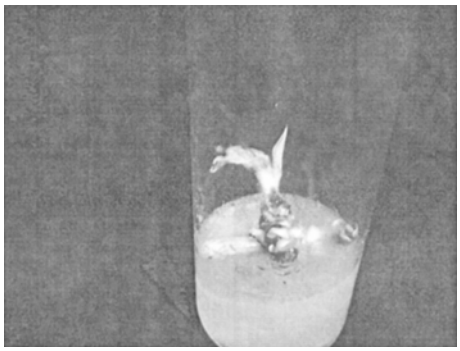

Fig. 1. Multiple shoots produced from meristem explant cultured on MS medium supplemented with 7.5 mg/l BAP + 0.5 mg/l NAA at 30 days after inoculation.

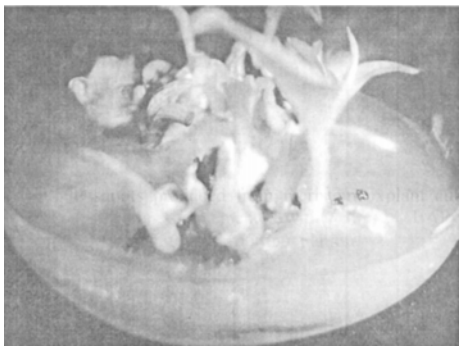

Fig. 2. Multiple shoots produced from meristem explant cultured on MS medium supplemented with 7.5 mg/l BAP + 0.5 mg/l NAA at 2nd subculture.

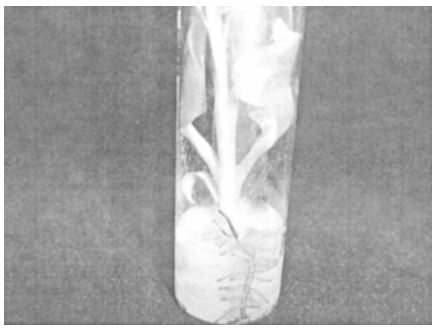

Fig. 3. Vigorous roots of banana cv. BARI Banana-I grown on MS media supplemented with 0.5 mg/l IAA +0.5 mg/l IBA.

Fig. 4. Well established meristern derived plantlets from BARI Banana -I in poly bags.

Rahaman *et al*. (2004) found highest shoot number at 1 .5 mg/l BAP + NAA (4.52 explant) at 30 DAI. The result of current investigation is not fully supported by Rabbani *et al.* (1996) where they found that highest number of shoots per explants at 28 DAI (3.11 ± 0.66) with 5.0 mg/l of BAP and Kn. This variation might be due to the different concentrations of NAA (auxins) and BAP (cytokinin) and their combinations.

Olivia and Barba (1984) obtained 10.10 number of shoots in the optimum concentration of 10.0 mg/l BAP. They also found that increasing results in the proliferation of shoots in the increase of cycles of culture (first cycle 11 .32 and 4th cycle 17.78 number of shoots). Doreswamy *et al.* (1983) obtained 20-25 shoot buds and 35 shoot lets in MS + 10 mg/l + 15% CM.

The explants cultured on MS medium without growth regulator produces only single shoot at 20 and 30 DAI (Table 1). These findings did not agree with the results of Khanam *et al.* (1996), Rabbani *et al.* (1996) and Rehana (1999) where they did not monitor any shoot formation in case of control treatment. It is observed in the study that if the explant in the culture media is not contaminated by fungus or bacteria then the explant develops only a single shoot in the long run. The large variation in number of shoots per explant may be due to the genotype, chemicals and culture environments.

Shoot length

The MS medium supplemented with BAP and NAA showed different results for increasing shoot length which was significantly influenced by different concentrations. The effect of different concentrations of NAA and BAP on the shoot length of banana cv. BARI Banana-I have been presented in Table 2. The longest shoot was produced by the treatment concentrations of 7.5 mg/l BAP + 0.5 mg/l NAA (1.03, 2.45 and 3.38 cm) at 10, 20 and 30 DAI, respectively. Statistically identical shoot length was observed in 5.0 mg/l BAP + 0.5 mg/ NAA at 20 DAI (2.43 cm) and 30 DAT (3.13 cm). Rahaman *et al.* (2004) observed similar results. They obtained longest shoot in the treatment 5.0 mg/l BAP (3.62 cm) followed by 1.5 mg/l NAA and 4.0 mg/l BAP (3.40 cm) using BARI Banana 1. They also found shortest leaves in 2.0 mg/l BAP. The shorter shoot length was produced by the control treatment (1.05 cm) where growth hormones were absent. The treatment 2.5 mg/l BAP + 2.0 mg/l NAA (1.50 cm) and 7.5 mg/l BAP (1.25 cm) produced shorter shoot length closer to control treatments. The result of the present experiment agrees with the findings of Khanam *et al.* (1996) who obtained longest shoot in banana on MS medium supplemented with 25 µ BAP treatments.

Leaf number per explant

The effect of different concentrations of NAA and BAP on number of leaves per explant has been presented in Table 3. The results showed that the maximum number of leaves (2.50, 3.25 and 7.00 leaves/explant at 10, 20 and 30 DAI, respectively) were produced on the medium supplemented with 7.5 mg/l BAP and 0.50 mg/l NAA. The second highest number of leaves (2.75, 4.00 and 6.75 leaves/explant at 10, 20 and 30 DAI, respectively) were produced on the medium

supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. Rahman *et al.* (2004) found in the experiment of BAP and NAA combination that the maximum number of leaves (3.12/plantlets) were produced at 30 DAI with 5.0 mg/l BAP which was identical with the treatment of 4.0 mg/l BAP + 1.50 mg/l NAA. Rabbani *et al.* (1996) obtained similar results from 5.0 mg/l BAP. The lowest number of leaves per explant (0.00. 0.50 and 2.25 at 10, 20 and 30 DAI, respectively) obtained from control treatment (Table 3) which does not agree with the finding of Rabbani *et al.* (1996) and Rahman *et al.* (2004).

Table 2. Effect of different concentrations of BAP and NAA on mean shoot length of banana plantlet cv. BARI Banana-1 at different days after inoculation.

Treatments		Shoot length (cm)		
BAP (mg/l)	NAA (mg/l)	10 DAI	20 DAI	30 DAI
0	0	0.00 i	0.00j	0.00 j
	0.5	0.30gh	1.03 ghi	l.88defgh
	1.0	0.30gh	1.28 defgh	1.95 bcdefg
	1.5	0.23 h	1.68 bcde	2.33 b
	2.0	0.20 h	1.40 cdefg	1.78 fgh
2.5	0	0.23 h	1.23 efgh	1.90 cdefg
	0.5	0.30 gh	1.48 cdefg	2.08 bcdefg
	1.0	0.45 feg	1.28 defgh	1.80 efgh
	1.5	0.3sfgh	1.lofghi	1.83efgh
	2.0	0.45 efg	0.70 i	1.50 hi
5.0	0	0.25 h	0.88 hi	2.28 bc
	0.5	0.48 ef	2.43 a	3.13 a
	1.0	0.88 abc	2.OSab	2.13 bcdef
	1.5	0.58de	1.S3cdef	1.78fgh
	2.0	0.55 e	1.28 defgh	1.70 gh
7.5	0	0.58 de	1.20 efgh	1.25 i
	0.5	1.03 a	2.45 a	3.38 a
	1.0	0.95 ab	1.75 bcd	2.23 bcd
	1.5	0.88 abc	1.88bc	2.10 bcdef
	2.0	0.78 c	1.30 defgh	2.05 bcdefg
10.0	0	0.75 c	1.05 fghi	1.93 cdefg
	0.5	0.73 cd	1.60 bcde	2.18 bcde
	1.0	0.80 bc	1.48 cdefg	2.28 bc
	1.5	0.73cd	1.35 defgh	1.98 bcdefg
	2.0	0.73 cd	1.23 efgh	1.90 cdefg
LSD value (0.01)		0.16	0.50	0.39
CV (%)		16.06	19.27	10.49

Table 3. Effect of different concentrations of BAP and NAA on leaf number of banana plantlet cv. BARI Banana-I at different days after inoculation.

Treatments		Number of leaves		
BAP (mg/l)	NAA (mg/l)	10 DAI	20 DAI	30 DAI
0	0	0.00 e	0.5 c	2.25 d
	0.5	1.50 cd	2.25 b	4.75 c
	1.0	1.75 bcd	2.75 ab	4.50 e
	1.5	1.75 bcd	3.00ab	5.50bc
	2.0	1.50 cd	2.25 b	5.50 be
2.5	0	1.50 ed	2.50 b	4.75 c
	0.5	1.75 bcd	3.00 ab	5.00 c
	1.0	2.25 abc	3.25 ab	5.50 be
	1.5	1.50 cd	2.75 ab	5.75 abc
	2.0	2.00abcd	3.25ab	5.50bc
5.0	0	1.25 d	2.5 b	4.75 e
	0.5	2.50 ab	3.25 ab	5.25 c
	1.0	2.75 a	4.00 a	6.75 ab
	1.5	2.25abc	3.25ab	5.S0bc
	2.0	1.75 bcd	2.75 ab	5.25 c
7.5	0	2.00 abcd	3.25ab	5.25c
	0.5	2.5 ab	3.25 ab	7.00 a
	1.0	1.75 bcd	2.75 ab	5.25 e
	1.5	2.00 abcd	3.00 ab	5.75 abc
	2.0	2.00 abcd	2.5 b	4.50 c
10.0	0	1.50 cd	3.25 ab	4.75 c
	0.5	2.00 abcd	2.5 b	4.50 c
	1.0	1.50cd	3.25 ab	5.25 c
	1.5	2.25 abc	2.75 ab	4.75 c
	2.0	1.75 bcd	2.75 ab	4.75 c
LSD value (0.01)		0.97	1.28	1 .39
CV (%)		28.71	24.22	14.46

Length of largest leaves

The effect of different concentrations of BAP and NAA on the length of largest leaves of banana cv. BARI Banana-I has been presented in Table 4. The MS medium supplemented with BAP and NAA showed different results for increasing shoot length which was significantly influenced by different concentrations. The longest leaves was produced by the treatment concentration 7.5 mg/l BAP + 0.5 mg/l NAA treatment (0.85, 2.70 and 4.23 cm at 10, 20 and 30 DAI, respectively) which was statistically significant. Statistically identical leaf length was observed in 7.5 mg/l BAP + 1.0 mg/l NAA at 20 DAI (2.10 cm) and 30 DAI (3.70 cm). Rahaman *et al.* (2004) obtained longest leaves in the treatment 5.0 mg/l BAP (3.62 cm) followed by 1.5 mg/l NAA and 4.0 mg/l BAP (3.40 cm) using BARI Banana-1. They also found shortest leaves in 2.0 mg/l BAP. The shorter leaf length was produced by the control treatment (0.95 cm)

where growth hormones were absent. The results of present experiment agreed with the findings of Khanam *et al.* (1996) who obtained longest leaves in banana on MS medium supplemented with *25* µM BAP treatments.

Table 4. Effect of different concentrations of BAP and NAA on length of largest leaves of banana plantlet cv. BARI Banana-1 at different days after inoculation.

Treatments		Length of largest leaves (cm)		
BAP (mg/l)	NAA (mg/l)	10 DAI	20 DAI	30 DAI
0	0	0.50 defg	0.55 h	0.95 i
	0.5	0.58 cdef	1.65 fg	2.03 gh
	1.0	0.60 bcde	1.70 efg	1.95 h
	1.5	0.7Oabc	1.80bcdefg	2.00gh
	2.0	0.46 defg	1.60 g	2.08gh
2.5	0	0.50 defg	1.55g	1.95h
	0.5	0.63 bcd	2.03 bcd	2.58 efg
	1 .0	0.600 bcde	1.95 bcdef	2.53 efgh
	1.5	0.45 efg	2.075 bc	2.43 fgh
	2.0	0.45 efg	2.05 bed	2.28 gh
5.0	0	0.35g	1.68fg	2.l5gh
	0.5	0.75 ab	2.60 a	3.60 bc
	1.0	0.70 abc	2.05 bed	3.I0cde
	1.5	0.50 defg	2.03 bcd	2.93_def
	2.0	0.50 defg	2.00 bcde	2.98 def
7.5	0	0.43 fg	1.65 fg	2.23gh
	0.5	0.85 a	2.70 a	4.23 a
	1.0	0.70 abc	2.10 b	3.70 ab
	1.5	0.55 cdef	1.95bcdef	2.58 efg
	2.0	0.55 cdef	1.76 cdefg	2.28 gh
10.0	0	0.S3def	l.53g	2.l5gh
	0.5	0.50 defg	1.75 defg	2.98 def
	1.0	0.43 fg	1.93 bcdef	3.23 bcd
	1.5	0.45 efg	1.95 bcdef	3.23 bcd
	2.0	0.45 efg	1.60 g	3.08 cde
LSD value (0.01)		0.16	0.31	0.58
CV(%)		15.12	8.86	11.86

Effect of IAA and IBA on root proliferation of banana cv. BARI Banana-1

Root numbers varied with different concentrations of IBA and IAA. The results on the effect of different concentration of IBA and IAA on root formation have been discussed with following headings:

Table 5. Effect of different concentrations of IAA and IBA on root number of multiplied shoot of banana cv. BARI Banana-1 at different days after inoculation.

Treatments		Vigour of regenerated root	Number of root		
IAA (mg/L)	IBA (mg/L)		10 DAI	20 DAI	30 DAI
0	0	+	0.00 e	0.00 e	0.00 f
	0.5	+	1.50 cd	2.00 d	3.25 e
	1.0	++	2.25 bc	2.25 d	3.50 de
	1.5	++	2.25 bc	2.50 cd	3.50 de
0.5	0	+++	2.75 ab	2.25 d	3.25 e
	0.5	++ +	3.50 a	4.50 a	6.50 a
	1.0	++	3.25 ab	3.50 abc	6.00 ab
	1.5	++	3.25 ab	4.00 ab	5.00 be
1.0	0	+	1.00 de	2.50 d	3.25 e
	0.5	+	2.75 ab	2.75 cd	3.75 de
	1.0	++	1.50 cd	3.00 bcd	4.00 cde
	1.5	+	1.25 cd	3.50 abc	4.50 cd
LSD value (0.01)			1.03	1.16	1.16
CV(%)			25.36	22.19	15.51

+ Less vigorous growth, ±+= Good *growth* and vigour, +++ Best growth and *vigour*

Number of roots per explant

The effect of IAA and IBA on the number of roots per explant produced by different combinations at 10, 20 and 30 DAI was found statistically significant (Table 5). Significantly highest number of roots was produced by 0.5 mg/l IAA + *0.5* mg/l IBA (3.50, 4.50 and 6.50 per explant, respectively) (Table 5). The treatment, 0.5 mg/l IAA + 1.0 mg/l IBA produced 6.0 roots per explant at 30 DAI but at 20 DAI, 3.50 roots were produced per explant. The lowest number of roots were produced by control treatment. Vigorous roots of *in vitro* grown plantlet on MS media supplemented with 0.5 mg/l IAA + 0.5 mg/l IBA are shown in plate 3. The present results are similar with the findings of Gubbuk and Pekmezci (2001). Molla *et al.* (2004) obtained 8.28 number of roots per plantlet on 0.5 mg/l IBA

followed by 6.33 roots, 0.6 mg/l IBA. They also observed 3.89 and 3.97 number of roots in 0.2 mg/l IBA and 0.3 mg/l IBA, respectively. Molla *et al.* (2004) obtained similar results. The results of the present experiment were found similar with the findings of Khanam *et al.* (1996).

Table 6. Effect of different concentrations of IAA and IBA on root length of multiplied shoot of banana cv. BARI Banana-I at different days after inoculation.

Treatments		Root length (cm)		
IAA (mg/l)	IBA (mg/l)	10 DAI	20 DAI	30 DAI
0	0	0.00 e	2.00 ef	2.00 f
	0.5	1.08 d	1.88 f	2.30 e
	1.0	1.08d	2.30de	3.15d
	1.5	l.13d	2.45d	3.08d
05	0	1.23 d	1.60 f	2.08 e
	0.5	2.93 a	4.63 a	5.88 a
	1.0	2.55 ab	3.88 b	4.83 b
	1.5	3.03 a	3.85 b	4.88 b
1 0	0	1.80 c	2.33 de	3.45 cd
	0.5	1.80 c	2.35 de	3.48 cd
	1.0	2.O8bc	3.15c	3.75c
	1.5	2.l5bc	2.70d	3.70c
LSD value (0.01)		0.49	0.42	0.55
CV(%)		16.28	7.95	7.57

Root length

The length of roots developed by the plantlets was influenced considerably by different concentrations of IAA and IBA used in the experiments and the results have been presented in Table 6.

The results indicated that there was a sharp increasing trends in root length at different DAI (10, 20 and 30) which is significant at 1% level. The root length of plantlets after 30 DAI was remarkably highest indicating that Auxin was essential for successful root induction of banana as also reported by Raut and Lokhand (1989). The highest length was observed at 10, 20 and 30 DAI in the treatment concentration 0.5 mg/l IAA and IBA (2.93, 4.63 and 5,88 cm) which was statistically significant. The second highest result (3.03, 3.85 and 4.88 cm at 10, 20 and 30 DAI respectively) was observed with 0.5 mg/l IAA and 1.5 mg/l IBA and the lowest (0.00, 2.00 and 2.00 cm at 10, 20 and 30 DAI, respectively) value were obtained with control treatment. Similar results were obtained by Molla *et al.* (2004) where they got 2.60-5.67 cm range of root length in 0.5 mg/l IBA. Habiba (2002), Khanam *et al.* (1996) and Ali (1996) also reported more or

less similar results. Therefore, the present result partially agreed with the findings of Gubbuk and Pekmezci (2001).

Established plantlet

Meristem derived plantlets were transferred to poly bags containing 1:1 (ground soil: cowdung) mixture after 7 days hardening in room temperature (28-30°C). A good number of established piantlets were shown in plate 4, which is ready for planting.

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