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In vitro culture of musa species (CV. Gaja Bantala) using different phytohormones

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Abstract
The effects of different plant growth hormones on shoot proliferation and root development during induction, multiplication and rooting stagewas studied for meristem culture of banana cv. Gaja Bantala. MS medium supplied with 6 mg/l BAP in addition to 2 mg/l IAA and 100 mg/l ADS showed remarkable results for initial culture. During multiplication culture the explants cultured on MS medium along with 3 mg/l BAP + 1 mg/l IAA + 0.25 mg/l NAA developed highest number of shoot buds. For root induction MS medium with Activated charcoal (50 mg/l) and 1 mg/l NAA gave most suitable result.

Keywords: *Musa*, Gaja Bantala, *In vitro*, propagation, phytohormones,

Introduction
Banana and Plantain which belongs to Musaceae, is among one of the six families of the order Zingiberales originating from the tropical Malay region and the Northern Indian region, respectively (Simmonds, 1962) [23]. Archaeological evidence was also been found in support of earlier cultivation in Papua New Guinea, at least 7,000 years ago and possibly as far back as10, 000 years ago. It is world's second largest fruit crop and fourth most important global food crop which is produced over 150 million metric tons per year (Banana Market ReviewandBanana Statistics 2012-2013, FAO, 2014) [10]. Banana and plantain are mostly cultivated within 30◦ latitude north and south of the equator (Stover and Simmonds, 1987) [25] in the tropical region. They require an average temperature of about 30◦C and a minimal rainfall of 100 mm per month (Swennen and Rosales, 1994) [27]. Cultivated bananas and plantains are predominantly triploid (2*n* = 3*x* = 33) varieties that evolved from two diploid (2*n* = 2*x* = 22) species, *Musa acuminate* (genome AA) and *Musa balbisiana*(genome BB). The cultivated bananas and plantains differ from their wild relatives by being seedless and parthenocarpic. Although there is no straight forward botanical distinction between bananas and plantains and, both belong to *Musa* species, but bananas (referred to as desert banana) are soft, sweet (with more sugar content) and can be eaten raw whereas plantains are a starchy, low in sugar variety that is cooked before serving as it is unsuitable raw. The plantain averages about 65% moisture content and the banana averages about 83%. It is well known that fruits like banana contain various antioxidants, such as vitamin C, vitamin E and β-carotene (Kanazawa and Sakakibara, 2000). The fruit has been reportedly used as anti-scorbutic, aphrodisiac and diuretic (Salawu *et al.*, 2010) [22]. Along with other fruits and vegetables, consumption of bananas is associated with a reduced risk of colorectal cancer (Deneo-Pellegrini *et al.*, 1996) [8], renal cell carcinoma (Rashidkhani *et al.*, 2005) [21] and breast cancer in women (Zhang *et al.*, 2009) [32]. Banana contains low protein levels and can be used to produce large amount of recombinant proteins (i.e. vaccines) (Arvanitoyannis *et al.*, 2008). Conventional vegetative means for banana propagation has been found to express several negative impacts which include transmission of diseases, low production, very slow as the rate of multiplication of suckers and poor preservation of original plant genetic material (Hussein, 2012) [13]. The problem of emerging from conventional breeding process can be solved by propagating banana through tissue culture (Ali *et al.*, 2011) [3] which offers mass propagation and clean planting material. Several scientist and researchers had reported various techniques for in vitro propagation of banana (Madhulatha *et al.*, 2004; Strosse *et al.*, 2006; Wong *et al.*, 2006; Venkatachalam *et al.*, 2007) [15 24, 30, 29]. Effect of plant growth regulators for production of multiple shoots through *in vitro* culture of different varieties of banana (*Musa* spp.) was studied (Keshari and Deo,2020) [14].
The objective of present experiment was to study the effects of plant growth hormones during initial, multiplication and rooting cultures *Musa* spp. cv. Gaja Bantala. The study will be helpful in developing an efficient protocol for mass propagation of this local banana variety.

Material and Methods
Plant Material
The *Musa* species taken into consideration for the following studies is *Musa paradisiaca* cv. Gaja Bantala a plantain variety which is widely cultivated in India. Gaja Bantala and Patakpura varieties are confined to very specific pockets of Odisha, although due to its nutritive qualities, good taste and size it is highly appreciated by the people of Odisha and outside Odisha. The suckers of Gaja Bantala were collected during January, 2019 from the banana mother block of RPRC. Healthy suckers with 40-50 cm in length and 7-8 cm in diameter were obtained from elite disease free mother plants.

Sucker Sterilization
Sucker collected from the mother block were trimmed and washed in liquid detergent (Labolene) for 2-3minute. Explants were then dipped in bavistin solution (1 %) for 30 minutes. After 30 minutes the suckers were washed with autoclaved double distilled water and transferred to mercuric chloride solution (0.5 %) for 45 minutes.Finally the suckers were washed 3- 4 times with autoclaved double distilled water to remove excess chemicals from the sucker surface.

Culture Medium
The most commonly use medium for banana tissue culture is Murashige & Skoog Medium (MS). Plant growth hormone (6-benzylaminopurine (BAP), Indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA), and adenine

sulphate (ADS)) in different concentration and combination were used in MS medium for initial, multiplication and rooting cultures. All the media were autoclaved at 15 PSI and 121°C for 20 minutes.

Data Analysis

All the data of each experiment was observed, noted and analyzed at appropriate time interval. For initial culture the data were collected at date of inoculation and up to 15 days. Similarly for multiplication culture and rooting culture the data were noted from inoculation to 21 days. All the statistical data analysis was conducted in EXCEL. In multiplication culture growth value and dry matter percentage of explants were calculated.

$$\text{Growth value} = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Initial fresh weight}}$$

$$\text{Dry matter (\%)} = \frac{\text{Dry weight} \times 100}{\text{Fresh weight}}$$

Results

Effect of hormones on growth during initial culture

The explants for initial culture were inoculated in MS medium supplied with different combination of plant growth regulators. Out of different initial cultures it was observed that the explants grown on medium with 1 mg/l BAP content had the least final fresh weight (2.9 ± 0.22 gm) among all the explants cultured on other mediums. The browning of explants started after 2-3 days of inoculation. After 4-5 days slight light green color appeared on the leaf sheaths of explants. Remarkable results were observed in medium containing 6 mg/l BAP + 2 mg/l IAA + 100 mg/l ADS where the explants showed better growth and proliferations in comparison to other medium.

In an average the percentage of response was 100 % and the days of response was 6 ± 0.81 days which is the least recorded days of response among all the experiments. Highest final fresh weight (4.5 ± 0.19 gm) of explants was marked. Nearly similar results were detected in explants cultured on medium supplied with 6 mg/l BAP + 2 mg/l IAA. The percentage of response was 100 % in both the cases but the days of response was 7 ± 1.03 days and final fresh weight was 4.3 ± 0.18 gm (Fig.1 and Table 1-2).

A **B**

Fig 1: (A) Inoculation of explants and (B)- Growth after 15 days on MS + 6 mg/l BAP + 2 mg/l IAA + 100 mg/l ADS initial medium.

The data obtained from 4 mg/l BAP + 2 mg/l IAA and 6 mg/l BAP + 2 mg/l IAA+ 50 ADS mediums were all most equal.

In both the cases the percentage of response was 100 %, final fresh weight of explants was nearly 3.9 gm and the days of response was7 days (Table 1 -2).

Table 1: Growth of explants during initial culture of Gaja Bantala.

Hormones Conc. (mg/l)			Final fresh weight (gm)		
BAP	**IAA**	**ADS**	**0th DAI**	**7th DAI**	**15th DAI**
2	0		1.5 ± 0.09	2.0 ± 0.12	2.9 ± 0.22
	1.0		1.5 ± 0.07	2.3 ± 0.16	3.2 ± 0.21
	2.0		1.5 ± 0.07	2.4 ± 0.18	3.4 ± 0.17
4	0		1.5 ± 0.08	2.8 ± 0.21	3.7 ± 0.16
	1.0		1.5 ± 0.06	2.6 ± 0.20	3.5 ± 0.15
	2.0		1.5 ± 0.09	2.5 ± 0.19	3.9 ± 0.15
6	0		1.5 ± 0.08	2.6 ± 0.19	3.4 ± 0.17
	1.0		1.5 ± 0.13	2.7 ± 0.18	3.6 ± 0.16
	2.0		1.5 ± 0.06	3.1 ± 0.20	4.3 ± 0.18
6	2.0	50	1.5 ± 0.09	2.9 ± 0.22	3.9 ± 0.19
	2.0	100	1.5 ± 0.06	3.4 ± 0.23	4.5 ± 0.19

Table 2: Observation after 15 days of inoculation during initial culture.

Hormones Conc. (mg/l)			No. of explants	Days of response	% of response
BAP	IAA	ADS			
2	0		10	8 ± 0.91	100
	1.0		10	7 ± 1.03	100
	2.0		10	8 ± 0.91	90
4	0		10	8 ± 1.13	80
	1.0		10	8 ± 0.99	100
	2.0		10	7 ± 1.03	100
6	0		10	8 ± 1.13	90
	1.0		10	7 ± 0.88	100
	2.0		10	6 ± 0.95	100
6	2.0	50	10	7 ± 0.81	90
	2.0	100	10	6 ± 0.81	100

Effect of hormones on shoot proliferation during multiplication culture

It was observed that medium containing only BAP induced shoot bud proliferation whereas medium containing only kinetin developed individual shoots. The explants developed leaf instead of developing shoots. The lowest shoot buds number (80.2 ± 9.74) as well as no. of shoots (2) was marked in medium which supplied with 1 mg/L BAP. With the increase in BAP concentration the percentage of response and shoot buds number increased. Better results were obtained when explants were cultured on medium containing BAP along with IAA and NAA.

The percentage of response was 100 % in all mediums used for multiplication culture but the days of response varied slightly. The lowest day of response was 6 days after inoculation in medium 3 mg/l BAP except the medium containing 3 mg/l BAP + 1 mg/lIAA.Highest number of shoot buds (143.8 ± 12.40) and the number of individual shoots (6)wereobserved in medium supplied with 3 mg/l BAP + 1 mg/l IAA + 0.25 mg/l NAA (Fig.2 and Chart.1-4).

Fig 2: Shoot buds proliferation (a) and shoot development (b) in explants cultured on medium supplied with 3 mg/l BAP + 1 mg/l IAA + 0.25 mg/l NAA after 21 days during 3rd sub-culture.

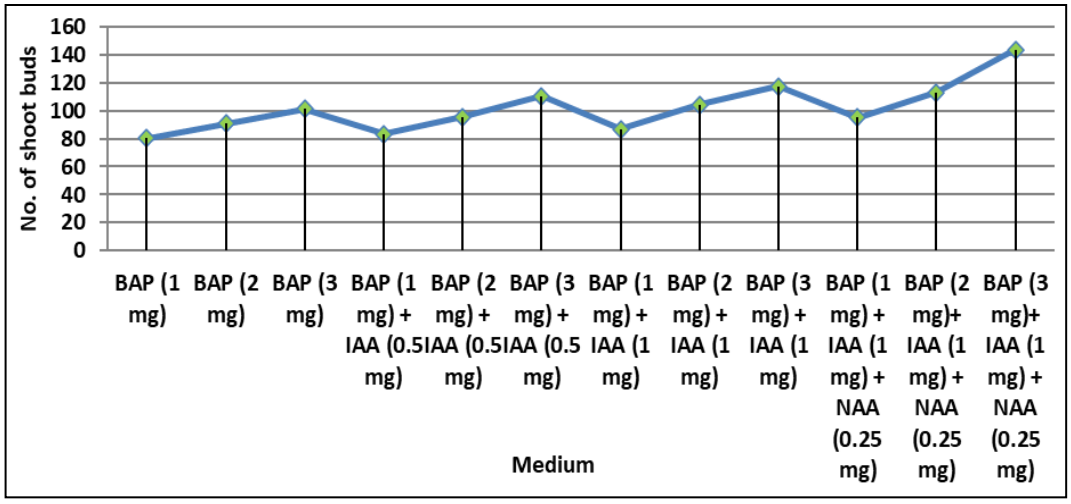

Chart 1: Numbers of shoot buds developed during multiplication culture of Gaja Bantala explants is different mediums.

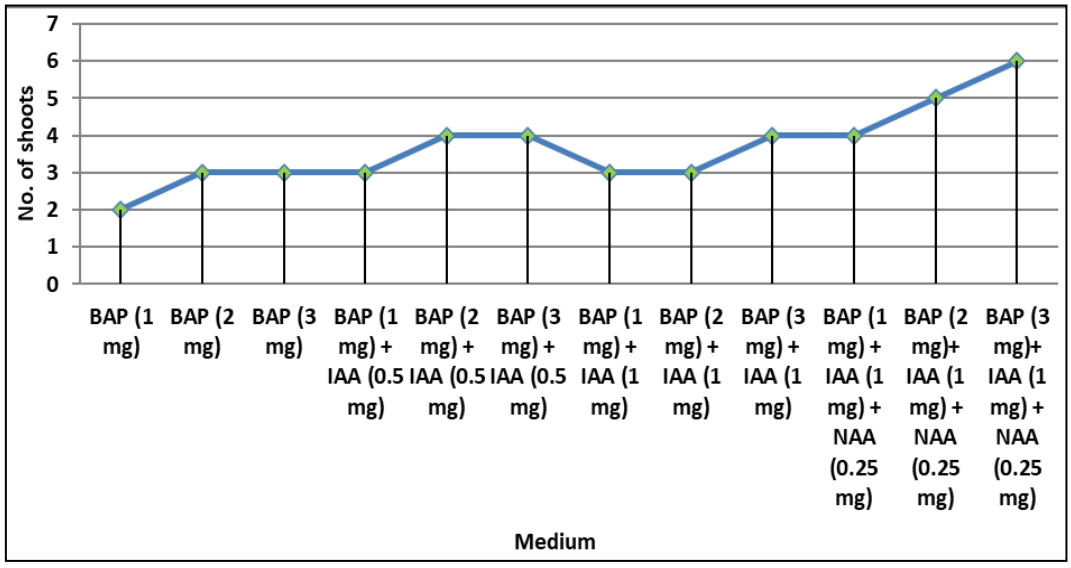

Chart 2: Number of shoots developed in Gaja Bantala explants cultured on different multiplication medium.

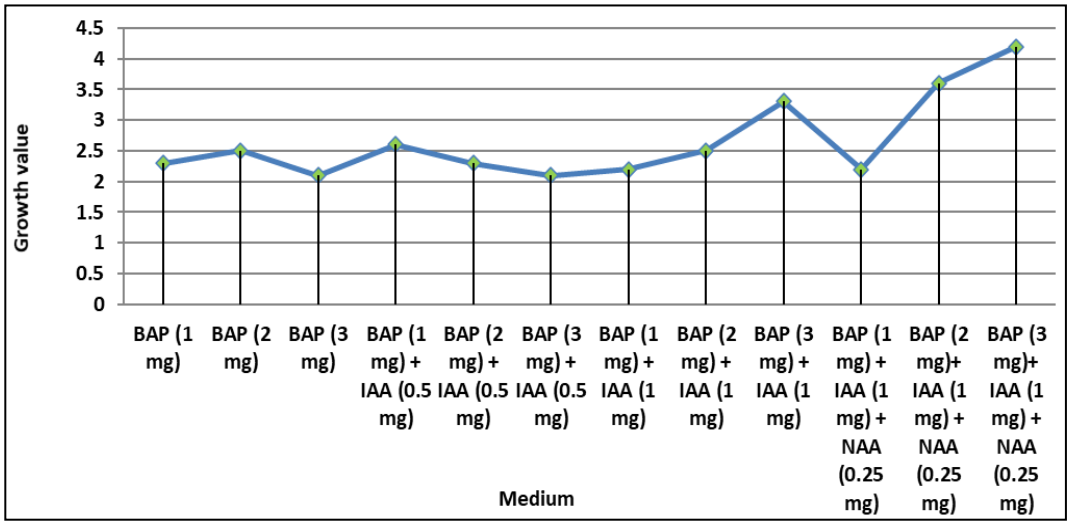

Chart 3: Growth values of Gaja Bantal explants observed during multiplication phase.

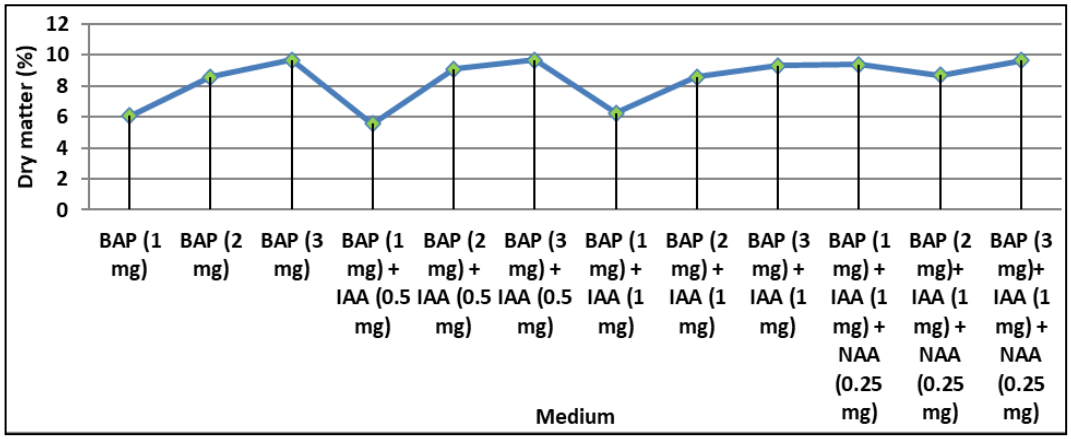

Chart 4: Dry matter percentage observed during multiplication culture of Gaja Bantala.

Effect of hormones on growth and proliferation of root

The developed shoots of *Musa* sp. cv. Gaja Bantala were cultured in MS medium with different auxins concentration for root induction and growth. The best results were marked in shoots grown in MS medium containing Agar and Activate charcoal supplied with 1 mg/l NAA. In an average 9 ± 0.94 numbers of roots per shoot was observed with short days of response (7 ± 0.88 days). MS medium containing 1 mg/l IAA had also shown better results but in comparison to 1 mg/l NAA medium the numbers of roots per shoot was less (8 ± 1.13) and days of response was 8 ± 0.92 days. In both mediums (1 mg/l NAA and 1 mg/l IAA) the average length of roots was 5 ±1.66 cm.

Shoots grown in MS medium with Agar only showed better percentage of response, numbers of roots per shoot, and root lengths but the days of response was more. Effect of auxins (IAA and NAA) used for root induction was less on growth and elongation of shoots and leaves. The length of shoots varied from 4 – 6cm and number of leaves varied from 3 – 4 (Fig.3 and Table 3-4).

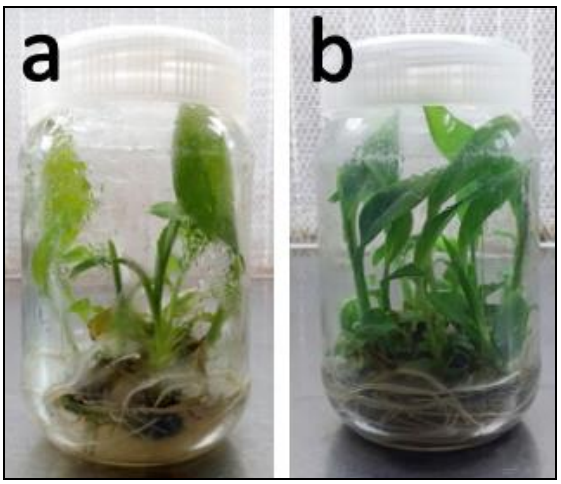

Fig 3: (A)- Root induction in NAA (1 mg/l) medium without activated charcoal and (B) Root induction in medium with activated charcoal (50 mg/l) and NAA (1 mg/l).

Table 3: Root induction during rooting culture in medium without activated charcoal-

Hormones Conc. (mg/l)		No. of explants (Shoots)	Days of response	% of response	Average no. of roots per shoot	Average root length (cm)
IAA	0.5	10	9 ± 0.88	100	4 ± 1.29	5 ± 1.61
	1.0	10	8 ± 1.13	100	7 ± 0.88	4 ± 1.41
	1.5	10	11 ± 1.05	90	6 ± 0.95	5 ± 1.76
	2.0	10	10 ± 1.20	90	8 ± 0.91	3 ± 1.63
NAA	0.5	10	8 ± 1.13	100	5 ± 1.32	4 ± 1.37
	1.0	10	9 ± 0.94	100	6 ± 0.95	4 ± 1.41
	1.5	10	10 ± 0.99	100	6 ± 1.41	4 ± 1.41
	2.0	10	12 ± 1.20	90	5 ± 1.20	5 ± 1.61

Table 4: Observation after 21 days in rooting culture in medium with activated charcoal-

Hormones Conc. (mg/l)		No. of explants (Shoots)	Days of response	% of response	Average no. of roots per shoot	Average root length (cm)
IAA	0.5	10	8 ± 0.92	100	7 ± 0.88	4 ± 1.41
	1.0	10	8 ± 0.92	100	8 ± 1.13	5 ± 1.66
	1.5	10	11 ± 1.47	100	7 ± 0.88	3 ± 0.81
	2.0	10	10 ± 1.05	100	6 ± 1.41	4 ± 1.37
NAA	0.5	10	8 ± 1.13	100	5 ± 1.20	4 ± 1.41
	1.0	10	7 ± 0.88	100	9 ± 0.94	5 ± 1.66
	1.5	10	10 ± 0.97	100	4 ± 0.95	4 ± 1.37
	2.0	10	12 ±1.25	100	7 ± 0.81	5 ± 1.29

Discussion

Cytokinins such as benzyl aminopurine (BAP) and kinetin are known to reduce the apical meristem dominance and induce both auxiliary and adventitious shoot formation from meristematic explants in banana (Khalid, 2011). However, the application of higher BAP concentrations inhibits elongation of adventitious meristems and the conversion into complete plants (Buising *et al.*, 1994) [7]. Higher concentration of BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during *in vitro* multiplication of banana cv. Nendran (Madhulatha *et al.*, 2004) [15].

In the present study for Gaja Bantala *in vitro* culture among different combination of phytohormones used for initial culture, MS medium supplied with 6 mg/l BAP in addition with 2 mg/l IAA and 100 mg/l ADS showed remarkable results. During multiplication culture the explants cultured on MS medium along with 3 mg/l BAP + 1 mg/l IAA + 0.25 mg/l NAA had highest number of shoot buds (143.8 ± 12.40).

The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas (Rahman *et al.*, 2006; Farahani *et al.*, 2008; Buah *et al.*, 2010) [18, 11, 6]. BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Buah *et al.*, 2010) [6]. Abeyaretne and Lathiff (2002) [1] states that BAP is widely used to increase the multiplication rate for plantlets and concurrently control its mutagenic effect so as to decrease the percentage of morphologically abnormal plantlet formation and at the same time high concentration of BAP may lead to produce off type plantlets. They reported that 2-3 mg/l of BAP with basal media is an advisable concentration for banana shoot tip culture.

Meanwhile, combinations of BAP with auxins such as indole acetic acid (IAA) or indole-3-butyric acid (IBA) were also used for *in vitro* multiplication of bananas (Dhed'a *et al.*, 1991; Resmi and Nair, 2007) [9, 19]. The importance of the application of high BAP concentration to initiate bud formation from explants was reported by Zaffari *et al.* (2000) [31] and Subramaniam *et al.* (2008) [26] in Cavendish banana cultivar Brasilian (AAA). Dhed'a*et al.* (1991) [9]. Reported that combinations of BAP with IAA or IBA were effective for *in vitro* multiplication of bananas and plantains. Resmi and Nair (2007) [19] reported high shoot multiplication but a reduction in the length of shoots in media with a combination of BAP and IAA in triploid cultivar by using inflorescence explants. Anbazhagan *et al.* (2014) [4] observed MS medium supplemented with BAP+IAA at the concentration of 3.0mg/l and 0.5mg/L was good for shoot inductions respectively. Robert *et al.* (2013) [20] revealed that the highest multiple shoot induction was found in MS+5 mg/l BAP at 2.17 shoots while MS+1 mg/l NAA+0.2 mg/l BAP gave the longest regenerated shoots after 45 days of incubation.

Auxins and other growth regulator such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues (Bohidar *et al.*, 2008; Alexandrova *et al.*, 1996) [5, 2]. Auxins such as Naphtalene acetic acid (NAA) have been reported to promote plant rooting *in vitro* (Hussein *et al.*, 2012) [13].

In the present experiment for root induction two different types of MS medium such as without Activated charcoal and with Activated charcoal (50 mg/l) mediums along with IAA and NAA are utilized. Out of various treatments the shoots grown on MS medium (solid + Activated charcoal) along with 1 mg/l NAA showed optimum results.

Activated charcoal (AC) has a very fine network of pores with large inner surface area on which many substances can be adsorbed (Pan and van Staden, 1998) [17]. Therefore (AC) is often used in tissue culture to improve cell growth and development. The main benefit is its adsorption of inhibitory substances in culture media. The phenolic oxidation or brown exudates accumulation can be significantly reduced by activated charcoal (Fridborg *et al.*, 1978; Thomas, 2008) [12]. However, AC can also adsorb vitamins, cytokinins and auxins, thus changing the ratios of medium components and subsequently influencing plant regeneration (Teng and Ngai, 1999) [28]. This makes the researchers using AC unaware of the actual quantity available to the plant tissues.

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