CHAPTER 5

DISCUSSION

Since banana is being used as food, fiber, medicinal, cultural and industrial purposes and also gives high returns to small holders; it is referred to as “Kalpatharu”- a plant of virtues. In order to cater to the needs of escalating population, banana production needs to be doubled, but the increase in productivity is threatened by the pest and diseases. Among the diseases Fusarium wilt also known as panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* is the major constraint to banana production specially and the disease has been ranked as No.1 fungal disease of banana in India and causes maximum damage to Malbhog banana plantation in Assam. Bunchy top disease caused by Bunchy top virus is another threat to banana plantation. The key is for efficient production of disease free quality planting material through tissue culture (TC). Fusarium wilt is the driving force leading to the development of a meristem culture technique for mass propagation of disease-free banana plantlets for commercial planting in Taiwan, beginning in 1983 (Hwang et al. 1984). Since then, micropropagation of bananas for both the rapid production of planting material and the storage and transfer of germplasm has become common all over the world. Many viruses are known to infect banana, and cause serious losses in many countries, which usually spread from plant to plant in nature by insect vectors, but often are also transmitted over long distances and from one crop cycle to another in vegetative planting material i.e., suckers.

Three banana cultivars viz., Amritsagar, Malbhog and Chenichampa were considered for the present research programme. These cultivars have economic importance to the people of the state of Assam, India. Amritsagar (AAA) is good table banana cultivars
and fairly resembles the internationally reputed banana Gros Michel, which once occupied 63% of the world market. Plants are medium sized, fruit size good, rind medium thick and the flesh when ripe. Malbhog (AAB) a medium tall variety is one of the most popular table banana cultivars indigenous to Assam and has a high demand on market due to its sweet aroma, taste and higher post harvest life. Chenichampa (AAB) is one of the hardiest medium tall banana cultivars and is resistant to Fusarium wilt and fairly resistant to bunchy top disease. Fruits are small in size with thin peel, creamy pulp and sub-acid taste. In general, these cultivars are propagated through suckers, but the rate of multiplication through conventional method is slow and a number of diseases are transmitted to new generation through infected suckers. Therefore, protocol development for commercial production of disease free quality planting material of cultivars Amritsagar, Malbhog and Chenichampa and field performance evaluation of in vitro produced plants were considered very important to increase the productivity. Hence, in the present study, protocol development for commercial production of disease free quality planting material of cultivars Amritsagar, Malbhog and Chenichampa and their field performance evaluation were investigated. The results obtained during the course of investigation were discussed below under the following heads.

5.1. Stress induction and impact on contamination reduction
Fresh weights of banana suckers were found to decrease with increasing storage duration due to loss of moisture from the suckers. The moisture loss was ranged between 17.07 to 39.52 % during 7-15 days of shade drying. The lowest contamination (15 %) was recorded in 15 days of shade drying where the sucker had lost 39.52 and
34.90 % of moisture in cv. Amritsagar and Chenichampa respectively. Increasing moisture loss has negative relation with microbial contamination till 15 days, which could be attributed to the reduction of microbial load present at the periphery of suckers due to induction of moisture stress to the sucker by drying of suckers under shade.

Various techniques to control contamination begin with pre-treatment of mother plants (Holdgate and Zandvoort 1997) with antibiotics and fungicides (Kritzinger et al. 1997) as well as anti-microbial formulations, such as PPM (Guri and Patel 1998). Since plants do not have an immune system to antibiotics and as such many of the antibiotics, that are effective against bacteria, fungi, and phytoplasmas, are also toxic to plants. Use of antibiotics is not foolproof or the desired method to get rid of microbial contamination (Pierik 1989). Islam and Zobayed (2000) reported to control microbial contamination in banana using sugar free medium. But, in the present study an inexpensive and effective method of microbial contamination reduction due to stress induction in suckers were established. Apart from reduction of microbial contamination, this method also facilitated prolonging of inoculation period after collection of explant from field.

5.2. Sterilization of explants

The use of field grown plants as a direct source of explants for the production of clean in vitro plantlets, presents a major challenge with regard to microbial contaminations during the process of initiation and maintenance of viable in vitro cultures. Losses due to contamination in in vitro condition average between 3 and 15% at every subculture in the majority of commercial and scientific plant tissue culture laboratories (Boxus & Terzi, 1987, 1988 ; Leifert et al., 1990), the majority of which is caused by fungal, yeast and bacterial contaminants (Leifert, et al., 1994). In the present study, amongst
the two sterilants i.e. Sodium hypochlorite (NaOCl) and mercuric chloride (HgCl₂). Sodium hypochlorite was found better for controlling the infection and it had not any adverse effect on explants even in long duration (15 minutes) exposure. Sodium hypochlorite at higher concentration (1.0 %) has turned out to be a better sterilant than mercuric chloride alone at 0.1 % for 5 minutes treatment time. However, a treatment combination of NaOCl (1.0%) for 15 minutes followed by HgCl₂ (0.1%) for 7 minutes resulted the highest percentage (85, 75 and 90 %) of aseptic culture establishment in banana cv. Amitsagar, Malbhog and Chenichampa respectively in in vitro condition followed by NaOCl (1.0%) for 10 minutes and HgCl₂ (0.1 %) for 7 minutes and NaOCl (1.0%) for 15 minutes alone. Sodium hypochlorite is the most commonly used disinfectant for surface sterilization of banana explants (Cronauer and Krikorian 1984; Mendes et al., 1996; Muhammad et al., 2004). Some other investigators have replaced NaOCl with low concentration of HgCl₂ (Banerjee and Sharma 1988; Habiba et al., 2002; Molla et al., 2004, Titov et al., 2006). Silva et al., (1998) ; Nandwani et al., (2000) ; Rahman et al., (2002) ; Madhulatha et al., (2004) also adopted the double disinfection method, where first large size explants were disinfected, followed by shoot tip excision and finally disinfection by some other chemical agents. Sometimes explants are treated with fungicides and antibiotics to minimize the contamination load in in vitro cultures (Houwe et al., 1998; Nandwani et al., 2000). Ethanol has also been used by a number of research workers for disinfection purposes (Silva et al., 1998; Rahman et al., 2002; Jalil et al., 2003). Onuoha et al., (2011) achieved the contamination free Plantain culture (100%) in the explants treated with HgCl₂ for 6 min.
Nisyawati and Kariyana, K. (2013) followed a multilevel surface sterilization procedure for sterilization of banana cultivar Barangan (*Musa acuminata* L.). They first used Tween for 5 minutes followed by 3 times washing by sterile aquadest for 3 minutes. After that banana explants were treated with fungicide (0.1 g L⁻¹) plus bactericide (0.1 g L⁻¹) for 10 minutes followed by rinsing with sterile aquadest. Explants were then sterilized using 70 % alcohol for 1 minute and rinsed with sterile aquadest. Final sterilization was done by 20% NaOCl solution for 15 minutes and rinsed the treated explants by sterile aquadest 3 times each for 5 minutes and placed on initiation media. In the present study also fungicide was used to reduce the initial load of microorganism followed by double disinfection method to sterilize the explant to get a clean material for *in vitro* propagation of banana.

5.3. Pretreatment of explants

Pretreatment of explants by different antioxidants (L-ascorbic acid, citric acid and L-cysteine) prior to inoculation into culture media were found to reduce browning and their subsequent mortality. Treatment combination of Ascorbic acid 100 mg L⁻¹ and Citric acid 100 mg L⁻¹ for 1 hour before surface sterilization and 10 minutes after sterilization was found best among the other combination tested as described in the chapter 4 with regard to number of days for initial browning of explants and days required for first subculture. Delay in initial browning of culture delayed the first subculturing period of the cultures by 13.08, 12.08 and 12.67 days for cv. Amrisagar, Malbhog and Chenichampa respectively. Experiment on pretreatment of explants was also conducted by Onuoha *et al.*, (2011) by using potassium citrate and citrate (K-C: C) at various concentrations to prevent browning and had been able to prevent browning.
within 2 hours before culturing the tissues. Morfeine (2013) carried out a study for manipulation of problems of oxidation in the initiation phase of *Musa* species Grand Naine *in vitro* by dipping the explants into the solutions of citric acid (150 mg L\(^{-1}\)) + ascorbic acid (100 mg L\(^{-1}\)), cysteine (100 mg L\(^{-1}\)), ginger (10 g L\(^{-1}\)), activated charcoal (3 g L\(^{-1}\)) *in vitro* after excision and before disinfection of explants *in vitro* for prevention of browning or blackening.

### 5.4 Effect of media on culture initiation

The vertical cuts made on the explants enhanced the emergence of shoot in all the three cultivars studied as reported by Gupta (1986) and Cote *et al.*, (1990). Shoot tip started to grow after one week of culture where the explants grew in size and leaf primordia opened from which the shoot emerged and grew longitudinally thereafter. Among the 4 initiation media tested, MS + BAP 0.3 mg L\(^{-1}\) resulted in good initiation percentage (86.67, 88.33 and 78.33 %) in cv. Amritsagar, Malbhog and Chenichampa respectively. This indicated that in the presence of growth regulators the explant growth was rapid and shoots were healthier as compared to MS Basal medium. In the present study low level of BAP was used considering the secretion of phenolic compounds from the cut ends, as low level of BAP in initiation medium is effective in controlling the phenolic secretions (Vuylsteke, 1998). Use of low level of cytokinins for culture initiations was also reported by Zaffari *et al.* (2000). As reported by Banerjee *et al.*, (1986) and Zaffari *et al.* (2000), in the present study also the growth of epical shoot tip started after 2-3 weeks and explants increased in size. Based on the results achieved from the present study, MS medium containing BAP 0.3 mg L\(^{-1}\) was found suitable for culture initiation and continued for subsequent initiations of the cultures in the study.
5.5. Effect of explants size on days to greening, days to swelling of inoculated explants, on survival percentage, days to emergence of leaf and days to multiple bud initiation

The use of smaller explants resulted in surviving of fewer explants, probably due to tissue damage upon excision and treatment with sterilants during the process of sterilization of the explants. Domingues et al. (1995) observed that explants of 1 cm long and 0.7 cm diameter obtained from banana cv. Maca gave the highest number of buds on nutrient solution containing 5.0 mg L⁻¹ BA for 45 days. Hirimburegama and Gamage (1996) used explants of about 2-3 cm in length and about 2.5 cm in diameter for sterilization and in vitro multiplication of local cultivars of banana (Musa spp.) through shoot-tip culture. Jafari et al. (2011) used the explants of size 3 to 4 cm in length and 2 to 3 cm in diameter after trimming to study the effect of BAP on in vitro multiplication of Musa acuminata (banana) cv. Berangan. Morfeine (2013) also used the banana explants of 1.5-2.0 cm in length after removing the outer leaves to initiate the cultures in in vitro condition. In the present study smaller explants (5 mm) showed slow response to the swelling, greening, survival of explants, emergence of leaf and multiple bud initiation under in vitro condition as compared to the larger explants (20 mm). Survival percentage of 5 mm explants was 50.00%, 41.67% and 58.33 %, whereas, 20 mm explants showed 91.67%, 83.33% and 91.67% survivability for cv. Amritsagar, Malbhog and Chenichampa respectively. Dore Swamy et al. (1983) and Epp (1987) reported that larger explants, consisting of the apical dome with 6-8 overlapping leaf bases, developed into multiple shoots more readily because they contained more lateral buds. However, Sandoval and Muller (1992) reported that
initiating cultures from such large explants increases explants and medium blackening, thereby reducing their survival rate.

In the present study the larger explants (10 mm and 20 mm) responded very well and performed well under \textit{in vitro} condition, which indicate the deviation from the findings of Sandoval and Muller (1992). Larger explants (10 mm and 20 mm) showed better results with regard to days required for greening and swelling of explants base, emergence of leaf from the initiated cultures and multiple bud initiation under \textit{in vitro} condition in all the three banana cultivars studied. The culture meristem first turned brown in colour in 5-6 days, which eventually grew into a green globular hard coat structures after 29-33 days and from that green globular structure adventitious plantlets were developed. The similar results were also observed by Amin \textit{et al.}, (2009). Uddin \textit{et al.}, (2006) reported the swelling of explants and some colour changes from pale white to light/deep green, which substantiate the results of the present investigation. Mukunthakumar and Seeni (2005) noticed the swelling of explants up to 1.5 cm in diameter even while a marginal increase in height (0.7 cm) and greening of the outer leaf sheath surrounding the shoot apex during the first 3 weeks of culturing the explants. Jaisy and Ghai (2011) also found that after few days of initiation, the explants swell and turn green and produce shoots within 4 weeks. All the above findings with regard to swelling of explant base, greening, development of green globular structures and development of shoots in \textit{in vitro} condition for banana micropropagation corroborate the results of the present investigation.
5.6. Standardization of media

5.6.1. Effect of different gelling agents

Gelling agents provide semi-solid supporting matrices for plant growth and therefore, the physical state of the medium affect the diffusion and availability of plant growth regulators and nutrients (Bornman and Vogelmann, 1984) to the growing plant tissues. They also influence gel strength, water potential and contribute to regulation of humidity inside the culture vessel (Debergh, 1983). The concentration and type of gelling agent is also associated with the development of hyperhydricity (Debergh et al., 1992) as there are complex interaction between the plant tissue, gelling agent, the mineral constituents and the gaseous atmosphere in the culture vessels (Cassells and Collins, 2000).

Two gelling agents namely agar (0.8%) and gelrite (0.22%) were studied to understand the role of gelling agents on shoot proliferation and growth of in vitro cultures. The effect of applied gelling agents in vitro was highly pronounced and trends were found similar for all the three cultivars studied. Gelrite was the most suitable gelling agent for all the cultivars, for which the highest proliferation rate was observed on medium solidified by gelrite. Gelrite at 0.22% concentration showed the best shoot multiplication (2.35, 2.36 and 2.09 fold) compared to 1.81, 1.88 and 1.73 fold in media solidified with 0.8 % agar in cv. Amritsagar, Malbhog and Chenichampa respectively. In agar medium, growth was slow and this might be due to the inhibitory compounds and impurities present in agar that are toxic to the plant growth (Nairn et al., 1995; Scholten and Pierik, 1998). On the other hand Huang et al., (1995) considered gelrite to be the most superior gelling agent in micropropagation of many plants because of its high purity, consistent quality, and that it solidifies at lower concentration compare to
agar and easy detection of microbial contaminants due to extra clarity. In the present study the cultures subcultured on gelrite medium were healthier and more vigourous than those cultured on agar media. The present results were supported by finding of Roy et al., (1990) in jackfruit.

**Standardization of the media for in vitro propagation of banana**

Out of the tested 32 combinations of MS media supplemented with BAP (0.5, 1.0, 1.5 and 2.0 mg L⁻¹), Kn (0.5, 1.0, 1.5 and 2.0 mg L⁻¹), 2-4-D (0.5, 1.0, 1.5 and 2.0 mg L⁻¹) with NAA (0.5 mg L⁻¹), AdSO₄ (10 mg L⁻¹) and coconut water (CW) 15 % five media were selected based on the number of days required for bud breaking, bud break response and shoots per cluster at 3rd subculture for subsequent studies. Out of these 5 media, medium MS + BAP 2.0 mg L⁻¹ +NAA 0.5 mg L⁻¹ +2-4-D 1.5 mg L⁻¹ and AdSO₄ 10 mg L⁻¹ with 15 % coconut water found best for multiplication of banana in in vitro condition. The effects of media were discussed under the following heads.

5.6.2. Effect of media on days required for bud breaking in cv. Amritsagar, Malbhog and Chenichampa

The results of media effect on days required for bud breaking indicated that media MSB31, MSB 32, MSB30, MSB 29 and MSB 28 as mentioned in the chapter 4 provided best results out of 32 media tested for the same. Again out of these 5 media, medium MSB31 (MS + BAP 2.0 mg L⁻¹ +NAA 0.5 mg L⁻¹ +2-4-D 1.5 mg L⁻¹ supplemented with and AdSO₄ 10 mg L⁻¹ and 15 % coconut water) was found best with regard to minimum time (11.08, 11.67 and 11.33 days) requirement for bud break in cv. Amritsagar, Malbhog and Chenichampa. Cultivar Amritsagar showed better performance than Malbhog and Chenichampa.
5.6.3. Effect of media on bud breaks response in cv. Amritsagar, Malbhog and Chenichampa

The media effect on bud break percentage were studied for all the three cultivars and found that MSB31 (MS + BAP 2.0 mg L$^{-1}$ + NAA 0.5 mg L$^{-1}$ + 2-4-D 1.5 mg L$^{-1}$ supplemented with and AdSO$_4$ 10 mg L$^{-1}$ and 15 % coconut water) was the best one amongst the best performing 5 media selected from 32 media. This media gave higher bud break percentage of 77.25, 75.08 and 73.92 in cv. Amritsagar, Malbhog and Chenichampa respectively and amongst the three cultivars performance of Amritsagar was better than Malbhog and Chenichampa with respect to bud break response.

5.6.4. Effect of media on shoot per cluster at 3$^{rd}$ subculture in cv. Amritsagar, Malbhog and Chenichampa

The effect of different concentrations of BAP, NAA, 2-4-D with AdSO$_4$ (10 mg L$^{-1}$) and coconut water 15 % on shoot regeneration revealed that media MS + BAP 2.0 mg L$^{-1}$ + NAA 0.5 mg L$^{-1}$ + 2-4-D 1.5 mg L$^{-1}$ supplemented with AdSO$_4$ 10 mg L$^{-1}$ and 15 % coconut water produced highest shoot per cluster (4.58, 4.33 and 4.33) at 3$^{rd}$ subculture in cv. Amritsagar, Malbhog and Chenichampa respectively. The other 4 media (MSB32, MSB 30, MSB 29 and MSB28) also produced a considerably good numbers of shoot at 3$^{rd}$ subculture.

5.7. In vitro Multiplication

The effect of different concentration regimes of BAP (1.5 to 2.0 mg L$^{-1}$), 2-4-D (0.5 to 2.0 mg L$^{-1}$) with NAA (0.5 mg L$^{-1}$), AdSO$_4$ (10 mg L$^{-1}$) and 15 % coconut water on shoot development, shoot length and number of leaves per explant showed
development of multiple adventitious buds from the base of the explants after 30 days, which were found to increase on every sub-culture till 8th subculture. The results of media effect on shoot development, shoot length and numbers of leaves per shoot have been discussed under the following heads.

5.7.1. Effect of multiplication media on shoot number per cluster

Variable number of shoots were produced per cluster in MS media supplemented with different concentrations of BAP and 2-4-D with NAA 0.5 mg L⁻¹, AdSO₄ 10 mg L⁻¹ and coconut water 15 %. In MS media supplemented with BAP 2.0 mg L⁻¹, NAA 0.5 mg L⁻¹, 2-4-D 1.5 mg L⁻¹, AdSO₄ 10 mg L⁻¹ and coconut water 15 % maximum numbers of shoot per cluster were produced in all the three cultivars at the end of 8th subculture. The numbers of shoots per cluster at that stage were 26.58, 25.83 and 25.75 in cv. Amritsagar, Malbhog and Chenichampa respectively, which were significant with other media compositions tested for shoot production. Rahaman et al. (2004) found highest shoot number of 4.52 at 1.5 mg/l BAP + NAA at 30 days after inoculation. This finding also partly support the results of the present investigation, where 3.58, 3.50 and 3.67 shoots were obtained at 42 days after inoculation (DAI) and the number of shoots increased with the increase of sub-culturing cycles.

5.7.2. Effect of multiplication media on shoot length of in vitro raised banana plantlets

The MS medium supplemented with BAP and 2-4-D at various concentration with 0.5 mg L⁻¹ NAA, 10 mg L⁻¹ AdSO₄ and 15 % coconut water showed different results for increasing shoot length which was significantly influenced by different concentrations.
The longest shoot of 6.65, 6.92 and 6.51 cm were produced by the treatment concentrations of BAP 2.0 mg L\(^{-1}\), NAA 0.5 mg L\(^{-1}\), 2-4-D 1.5 mg L\(^{-1}\), ADSO\(_4\) 10 mg L\(^{-1}\) and coconut water 15 % in cv. Amritsagar, Malbhog and Chenichampa respectively at 7\(^{th}\) passage. Amin et al., (2009) reported the production of longest shoot in the medium supplemented with 7.5 mg L\(^{-1}\) BAP + 0.5 mg L\(^{-1}\) NAA (1.03, 2.45 and 3.38 cm) at 10, 20 and 30 DAI, respectively, where as in the present study longest shoots (6.65, 6.92 and 6.51) were observed in the medium supplemented with BAP 2.0 mg L\(^{-1}\), NAA 0.5 mg L\(^{-1}\), and 2-4-D 1.5 mg L\(^{-1}\). 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 \(\mu\) BAP. The MS medium composition BAP 2.0 mg L\(^{-1}\), NAA 0.5 mg L\(^{-1}\), 2-4-D 1.5 mg L\(^{-1}\), ADSO\(_4\) 10 mg L\(^{-1}\) and coconut water 15 % (MSB31) produced shoots with highest length and hence considered the best medium composition for shoot multiplication with desirable shoot length.

5.7.3 **Effect of multiplication media on leaf number per shoot of in vitro raised banana plantlets**

The average number of leaves produced in different concentration of BAP and 2-4-D supplemented with NAA, ADSO\(_4\) and coconut water ranged from 3.17 to 5.83 in cv. Amritsagar, 3.17 to 5.83 in cv. Malbhog and 3.67 to 6.50 in cv. Chenichampa. Maximum numbers of leaves were produced in MS medium with BAP 2.0 mg L\(^{-1}\), NAA 0.5 mg L\(^{-1}\), 2-4-D 1.5 mg L\(^{-1}\), ADSO\(_4\) 10 mg L\(^{-1}\) and coconut water 15 % by *in vitro* cultures of all the cultivars at 7\(^{th}\) passage, where the plantlets were ready for putting in the rooting medium and hence this medium was considered best for production of plantlets with good numbers of leaves in *in vitro* condition. Amin et al.,
(2009) showed the production of maximum number of leaves (2.50, 3.25 and 7.00 leaves per explant at 10, 20 and 30 DAI, respectively) on the medium supplemented with 7.5 mg L\(^{-1}\) BAP and 0.50 mg L\(^{-1}\) NAA. Rahman et al. (2004) and Rabbani et al., (1996) also reported production of maximum number of leaves with 5.0 mg L\(^{-1}\) BAP.

5.8 In vitro rooting

*In vitro* multiplications of banana were carried out in the presence of cytokinin which inhibited the root formation and elongation of roots. Moreover shoots developed during multiplication stage lack sufficient roots and growth took place in the form of clusters and hence as such could not be transferred to potting medium for acclimatization. Prior to transfer to potting media, individual shoots were separated from the clusters and grown on root induction media. Banana shoots after 8\(^{th}\) subcultures were cultured in MS medium supplemented with different concentrations of IAA (0.5 to 1.5 mg L\(^{-1}\)) and IBA (0.5 to 1.5 mg L\(^{-1}\)) in presence of activated charcoal (100 mg L\(^{-1}\)) for root induction. Hwang et al (1984) also regenerated roots from *in vitro* plants of *Musa sapientum* L. on MS medium supplemented with 100 mg L\(^{-1}\) activated charcoal. In the present study 14 media were tested to evaluate the rooting media with various concentrations of IAA and IBA supplemented with activated charcoal for their root induction capacity on the microshoots of *cv.* Amritsagar, Malbhog and Chenichampa.

5.8.1 Effect of IAA and IBA on number of days required to initiate root in *in vitro* banana plantlets of *cv.* Amritsagar, Malbhog and Chenichampa

The response of IAA and IBA either alone or in combination differed markedly with respect to root initiation time. Frequency of root formation was different in all the
media and the best root induction was obtained on MS medium containing 0.5 mg L\(^{-1}\) IAA with 0.5 mg L\(^{-1}\) IBA supplemented with 100 mg L\(^{-1}\) activated charcoal (RT5). In this medium 100% microshoot formed roots within 14-16 days. Kumar et al., (2012) also reported that the medium containing 1.0 mg L\(^{-1}\) IBA + 0.5 mg L\(^{-1}\) IAA showed the best result with respect to initiation of roots on 12th days of inoculation in 90% of the plantlets of cv. Malbhog. As per the study revealed that higher concentration of IAA and IBA took longer time for root induction ranging from 25-27 days, which was found more than that of the control treatment (RT0), where the root induction time was 23-25 days. From the present study it was found that the growth regulators, IAA and IBA when used individually for root induction on microshoots of banana cv. Amritsagar, Malbhog and Chenichampa, they took longer time to initiate roots. However, when these two auxins were used in combinations at lower concentration (0.5 mg L\(^{-1}\) each) with 100 mg L\(^{-1}\) activated charcoal and 3% sucrose, root induction time was found to reduce considerably.

5.8.2 Effect of IAA and IBA on number of roots per plant in in vitro banana plantlets of cv. Amritsagar, Malbhog and Chenichampa

Statistically significant results were obtained with regard to number of roots produced per plant on media containing different concentration of IAA and IBA. The medium MS + 0.5 mg L\(^{-1}\) IAA + 0.5 mg L\(^{-1}\) IBA produced highest number of roots per plant (9.75, 9.25 and 8.42) in cv. Amritsagar, Malbhog and Chenichampa respectively. Similar kind of results were also obtained by Molla et al. (2004) where they got highest 8.28 numbers of roots per plantlet on 0.5 mg L\(^{-1}\) IBA followed by 6.33 roots on 0.6 mg L\(^{-1}\) IBA. Kumar et al., (2012) reported that the medium containing 1.0 mg L\(^{-1}\) IBA +
0.5 mg L\(^{-1}\) IAA was the best rooting medium with 90% of the plantlets producing around 8.5 roots per explant. Culture medium supplemented with 1.0 mg L\(^{-1}\) IBA alone induced rooting only in 66% of explants with 6.5 roots per explants. The findings of the present experiment were found similar with the findings of Amin \textit{et al.}, (2009), where they got the highest number of roots on media supplemented with 0.5 mg L\(^{-1}\) IAA and 0.5 mg L\(^{-1}\) IBA and the findings of Gubbuk and Pekmezci (2001) and Khanam \textit{et al.} (1996).

5.8.3 Effect of IAA and IBA on root length (cm) in \textit{in vitro} banana plantlets of cv. Amritsagar, Malbhog and Chenichampa

Rooting has also been achieved on basal media without growth regulators and the mean root lengths of 1.55, 1.57 and 1.50 cm for cv. Amritsagar, Malbhog and Chenichampa were obtained in the present experiment on the MS medium without hormone. The similar results were also achieved by Cronauer and Krikorian (1984) and Jarret \textit{et al.}, (1985). The root lengths eventually increased on the MS media supplemented with 1.5 mg L\(^{-1}\) IAA and 0.5 mg L\(^{-1}\) IBA for Amritsagar and 0.5 mg L\(^{-1}\) IAA and 0.5 mg L\(^{-1}\) IBA for cv. Malbhog and Chenichampa. The media containing either IAA or IBA at 0.5, 1.0 and 1.5 mg L\(^{-1}\) resulted in the production of roots with average root length in the range of 1.53 to 2.53 cm in cv. Amritsagar, Malbhog and Chenichampa. The present findings indicated that higher concentration of auxin results in lower root length. On the basis of results of the present experiment, it was found that the MS medium containing IAA and IBA at 0.5 mg L\(^{-1}\) each with 100 mg L\(^{-1}\) activated charcoal was better for root induction in \textit{in vitro} raised banana plantlets of cv. Amritsagar, Malbhog and Chenichampa. Similar kind of results were also obtained by Molla \textit{et al.},
(2004) where they got root length in the range of 2.60-5.67 cm in 0.5 mg L⁻¹ IBA. The findings of Amin et al., (2009) also substantiated the results of the present experiment, where they observed highest root length of 5.88 cm at 30 days after inoculation in the medium containing 0.5 mg L⁻¹ IAA and IBA. More of less similar kind of results was also reported by Habiba et al., (2002) and Khanam et al., (1996).

5.9. Acclimatization (Hardening)

The transfer of the plantlets from culture jars to the outside environment is one of the most important steps in the structural and physiological adaptation of micropropagated plants of banana, which is called as acclimatization of *in vitro* raised plantlets. Acclimatization of *in vitro* raised plant is usually done in large number of artificial potting mixes (organic substrates) containing perlite, vermiculite, peat, composted bark, coconut husk, agropeat etc either alone or in combination with soil. Even though, they play an important role in the survival and plant growth during the process of acclimatization of *in vitro* raised plants, there are limited studies describing the role of potting mixes (Dutra *et al.*, 2008). In the present experiment various combinations of river sand, cocopeat and vermicompost at different ratio were tried to optimize the potting medium for acclimatization of banana. Survival of plants and plant growth during the process of acclimatization differed significantly in all the potting media studied. Hardening in pure river sand was unfavourable for survival and subsequent growth of the plants.
5.9.1 Effect of potting media on survival of plantlets during hardening

In the present study, 81.75, 83.75 and 82.17 and 80.25, 80.42 and 80.17 % survival rates in river sand, vermicompost and cocopeat mixture at 2:1:1 ratio at 15 and 30 days after transferring to polybags were obtained in cv. Amritsagar, Malbhog and Chenichampa respectively. These survival percentages were obtained under open hardening condition with intermittent application of water after every one hour. Jasrai et al., (1999) also developed similar kind of protocols for hardening of in vitro derived banana plants without greenhouse facilities. They transferred the in vitro raised plants in polythene bags which were perforated 6 cm from the base maintaining high humidity by spraying water after every two hours. They achieved on an average 92 per cent survival in this method. Another study conducted by Shiragi et al., (2008) recorded the highest plant survival rate of 83.33% in a mixture of sand, soil and cow dung (1:1:1). On the other hand, 67.67 % plant survival rate was found in a mixture of coir and soil (1:2).

5.9.2 Effect of potting media on growth of banana plantlets during hardening after 20 days of transferring

Potting media had an important role in growth of in vitro raised banana plants under acclimatization. In the present study best growth with respect to plant height and girth of plants were observed with river sand, vermicompost and cocopeat at 2:1:1 v/v ratio for initial one month. In this medium increase in plant height and plant girth over control were in the range of 14.02-19.27 and 9.13-16.28 per cent respectively. The growth of the plant was found to vary depending on the potting mixes. The increase in plant height was maximum (6.97, 6.77 and 6.63 cm) in river sand, vermicompost and
cocopeat mixture at 2:1:1; v/v ratio in cv. Malbhog, Amritsagar and Chenichampa respectively. The least increase in plant height was observed in plants grown in river sand alone. Similarly, highest girths of 2.87, 2.73 and 2.71 cm were obtained in cv. Amritsagar, Chenichampa and Malbhog respectively in river sand, vermicompost and cocopeat mixture at 2:1:1; v/v ratio and least being in the river sand alone.

The present experiment indicated that river sand, vermicompost and cocopeat at 2:1:1; v/v ratio was the most effective potting medium for acclimatization of micropropagated banana plants among all the combinations studied. This was due to better aeration and water holding capacity of the potting mixture. This mixture had the advantages of river sand, cocopeat and vermicompost. Coco Peat holds water rather than shedding it like traditional peat does. It retains water 8-9 times of its weight and has the ability to store and release nutrients to plants for extended periods. It also has great oxygenation properties that are important for healthy root development of plants. It improved the texture of the potting mixture when combined with river sand and vermicompost.

5.9.3 Hardening performance of in vitro raised banana plantlets at different stages after transferring to potting media

In vitro raised banana plants showed increased plant height and pseudostem girth over the time during the process of hardening and within 2 months the plant height reached to 26.37, 28.77 and 21.07 cm in cv. Amritsagar, Malbhog and Chenichampa respectively. Pseudostem girth also increased to 7.12, 5.43 and 5.38 cm in cv. Amritsagar, Malbhog and Chenichampa respectively. Similar trend in increase in leaf length, leaf width and petiole length were also observed. The plants attained a height of 21-28 cm with 6-7 healthy leaves at the time of transferring the plants to main field.
The potting medium responded well and produced healthy plants ready for transferring to field within a period of 2 months from the date of transferring the plants to polybags from *in vitro* condition.

### 5.10 Field performance of micropropagated banana plants

There were significant differences between micropropagated and sucker derived plants in all the vegetative growth parameters and the micropropagated plants exhibited higher number of functional leaves with increased pseudostem height and girth and yield than the sucker derived plants.

Field performance of micropropagated vs. conventionally propagated plants (*Musa* spp., AAB group) has been reported by Liu *et al.*, 1989; and Vuylsteke and Ortiz 1996. In both the cases, micropropagated plantains grew vigorously and were taller than conventional plants. In the present experiment also micropropagated plants performed better than sucker derived plants with respect to plant growth and yield.

#### 5.10.1 Morphological characters

The pseudostem height, pseudostem girth, functional leaf and phyllochron at different stages of plant growth were the indicators for evaluating the performance of micropropagated plants under field condition.

#### 5.10.1.1 Pseudostem girth

Pseudostem girth was another parameter which represented the growth of banana plants under field condition. The pseudostem girth recorded at 15 cm height showed an increasing trend of girth over time. Pseudostem girth in micropropagated plants reached
to maximum level of 65.33, 71.01 and 61.61 cm after 180 days against 60.13, 65.13 and 57.09 cm in sucker derived plants of cv. Amristsagar, Malbhog and Chenichampa respectively. No such major differences were found in pseudostem girth at 150 and 180 days after plantation and this period was the stabilization phase of the plant growth.

Initially at planting, the sucker derived plants had more girth than that of micropropagated plants and maintained superiority in the initial stages of plants growth, but later on after 150 days of planting, the girth of micropropagated plants increased over sucker derived plants. The mean stem thickness of in vitro plants was 76.6 cm and for that of sucker derived plants it was 76.0 cm. After 180 days of planting the micropropagated plants recorded an increase of 8.65, 9.02 and 7.91 per cent in girth over the sucker derived plants in cv. Amristsagar, Malbhog and Chenichampa respectively. Sheela and Nair (2001) also reported an increase of 11.92 per cent in girth in tissue culture plants over sucker derived plants.

5.10.1.2 Plant height

At the time of transferring the plants to field condition the height of the plants were around 26-28 cm but with the passage of time, micropropagated plant grew faster as compared to sucker derived plants. From the results it was observed that plant height in all the three cultivars increased over time and after 6 month of planting the plants attained a height of 281.67, 287.91 and 278.58 cm in cv. Amristsagar, Malbhog and Chenichampa respectively. In the present experiment plant height differences of 42.29, 19.41 and 30.84 cm were found in cv. Amristsagar, Malbhog and Chenichampa respectively between micropropagated and sucker derived plants. The results of plant height of the present experiments were in accordance with the findings of Robinson et
al., (1993) and Vuylsteke and Ortiz (1996), where they found plant height differences of 30 and 23 cm in cv. Grand Naine and cv. Aegbagba between tissue culture and sucker derived plants.

5.10.1.3 Number of leaves, leaf length and leaf breadth

The micropropagated plants produced higher number of leaves compared to the sucker derived plants throughout the growing period. In initial stage micropropagated plants had more numbers of leaves as compared to the sucker derived plants and hence micropropagated plants showed faster growth as compared to sucker derived plants. The maximum leaf production was observed at six months after planting. Leaf production in banana is related to increased rate of plant growth (Barker and Steward 1962; Sathyanarayana, 1985). The maximum difference in leaf production was observed just before shooting (5.53 and 3.85 leaves per month). The tissue culture plants produced 3.35 leaves more than the plants from suckers during their growth in the field. Robinson et al. (1993) and Daniells (1988) also reported that the tissue culture plants produced more number of leaves compared to suckers. All these finding support the findings of the present experiments, where more numbers of leaves were found in micropropagated plants than sucker derived plants.

Similarly, the micropropagated plants also recorded higher leaf length and breadth compared to the sucker derived plants throughout the growing period. Micropropagated plant had leaves with 3-16 per cent more in length and 8-15 per cent more in leaf breadth over sucker derived plants in cv. Amritsagar, Malbhog and Chenichampa.
5.10.1.4 Functional leaf and Phyllochron

Number of functional leaves at flowering

The number of functional leaves on a banana plant at flowering stage played a significant role in the yield of the crop. The higher number of functional leaves (about 11) is an indication of a heavy bunch. The high number of leaves at flowering compared favourably with the results of Alvarez (1997). The total number of functional leaves that a plant has at flowering time is a good indicator of its tolerance or susceptibility to diseases, with correlation existing between number of leaves and bunch weight. In the present experiment, the average numbers of functional leaves at flowering stage were 11.17, 11.0 and 10.67 for cv. Amritsagar, Malbhog and Chenichampa respectively for the first cycle micropropagated plants, while the sucker derived plants had functional leaves of 10.58, 10.50 and 10.42 for the cv. Amritsagar, Malbhog and Chenichampa respectively. This indicated the superiority of the micropropagated plants over sucker derived plants. However, the functional leaves decreased in the subsequent crops. Hwang et al., (1984); Smith and Drew (1990) also reported that tissue culture plants retained more healthy leaves than those originating from suckers.

Fast-growing nature of the micropropagated plants emits new leaves at a faster rate (Swennen and De Langhe, 1985). Hwang et al. (1984) and Israeli et al. (1988) noted that micropropagated ‘Cavendish’ bananas retained more numbers of healthy leaves than suckers due to a faster rate of leaf emission. In the present study also micropropagated plants emitted leaves at faster rate as compared to sucker derived plants. A study on the phyllochron of the six leading banana cultivars of North East India conducted by Singh and Bhattacharyya (1992) reported that the cultivars 'Jahaji'
and 'Bar Jahaji' (AAA, Cavendish sub-group) had phyllochrons of 7.5 and 10.0 respectively, whereas the AAB cultivars 'Chenichampa' and 'Mabhog' had 9.9 and 10.4 respectively. Their study supports the findings of the present investigation, where the micropropagated plants of cv. Amristsagar, Mabhog and Chenichampa had a phyllochron of 9.7, 10.3 and 9.8 respectively.

5.11 Crop cycle

There was a significant difference in the days to flowering and days to harvest between sucker-derived and micropropagated plants. The micropropagated plants flowered (shooting) earlier and also matured earlier than the sucker-derived plants.

5.11.1. Planting shooting interval

The planting shooting interval in micropropagated plants was found less than sucker derived plants and in the present study it was found that micropropagated plants flowered 26.34, 30.83 and 45.33 days earlier than sucker derived plants in cv. Amristsagar, Mabhog and Chenichampa respectively. Vigorous and fast growth generally results in earlier flowering and harvest (i.e., earlier maturity and a shorter production cycle) (Swennen and De Langhe, 1985, Vuylsteke and Ortiz, 1996). Indeed, micropropagated plants flowered 1.0-1.5 months earlier than sucker-derived plants. Similar earliness in tissue culture plants for flowering was reported by Hwang et al., (1984), Drew and Smith (1990) in different banana cultivars, which support the findings of the present experiments.
5.11.2. Shooting-harvesting interval

Micropropagated plants matured at 381.42, 395.75 and 395.83 days for the cv. Amritsagar, Malbhog and Chenichampa respectively whereas the sucker derived plants took 31.41, 41.58 and 50.92 days more to harvest. The shooting harvest interval for micropropagated and suckers derived plants in the present investigation were within the range of 118-132 days and 124-138 days respectively. Micropropagated plants flowered about 1.0-1.5 months earlier than the sucker-derived plants and hence this was also reflected in the days to harvesting as the micropropagated plants were harvested about 30-50 days earlier than the sucker-derived plants of cv. Amritsagar, Malbhog and Chenichampa.

5.12 Yield parameters and yield

5.12.1 Number of hands per bunch

Micropropagated plants had more numbers of hands per bunch in main as well as first and second ratoon crop plants than sucker derived main, first and second ratoon crop plants, but these differences were not so much. Mensah (2012) and Osei (1996) also reported that the number of hands per bunch did not show any significant variation. The increase in the average numbers of hands per bunch in micropropagated main crop plants were 14.15, 5.36 and 2.80 per cent for cv. Amritsagar, Malbhog and Chenichampa respectively. The difference in number of hand was in decreasing trend in cv. Amritsagar, whereas in cv. Malbhog it was in increasing trend and cv. Chenichampa showed decreasing trend in 1st ratoon crop and again increased in 2nd ratoon crop.
5.12.2 Number of fingers per hand

The finger numbers per hand in micropropagated plants were slightly more than that of sucker derived plants in all the three crop cycles. The number of fingers per hand in case of micropropagated plants of cv. Amritsagar and Malbhog did not show any distinct variation, but in cv. Chenichampa a decreasing trend was observed. On the other hand sucker derived plants showed a decreasing trend over the crop cycles for all the three cultivars. In cv. Amritsagar there was an linear increase (4.20, 5.76, and 8.35 %) in finger number over sucker derived plants, but in cv. Malbhog and Chenichampa 1st ratoon crop showed highest increase in finger (6.25 and 3.80 %) over sucker derived plants.

5.12.3 Number of fingers per bunch

The finger numbers per bunch in micropropagated plants were more than that of sucker derived plants in all the three crop cycles and found to decrease over the crop cycles in all the three cultivars. The decreased in finger number per bunch were 6.42 and 11.84 % in cv. Amritsagar, 2.09 and 4.09 % in cv. Malbhog and 14.41 and 16.91 % in cv. Chenichampa in first and second ratoon crop plant over the main crop.

In cv. Amritsagar a decreasing trend (18.96, 17.02 and 15.76 %) for main crop, first and second ratoon crop plants were observed, whereas in cv. Malbhog an increasing trend (10.29, 15.73 and 18.21 %) was observed, but in cv. Chenichampa finger number per bunch increased in second ratoon crop plant (6.75 %) and again decreased in first ratoon crop plants (5.49 %). However, the finger number per bunch in micropropagated crop plants were more than that of sucker derived crop plants in all the crop cycles. Badgujar et al., (2005) also reported similar kind of findings, where they highlighted
that increase in number of hands and fingers per bunch contributed to the higher bunch weight in plants raised through tissue culture.

5.12.4 Hand weight

The micropropagated plants produced hands with higher hand weight than the sucker derived plants in all the three crop cycles in cv. Amritsagar, Malbhog and Chenichampa. However, the hand weights were found to decrease over the crop cycles for both micropropagated and sucker derived plants. The increase in hand weights were found in the range of 6-20 % in cv. Amritsagar, 10-13 % in Malbhog and 1-5 % in Chenichampa respectively over sucker derived crop plants.

5.12.5 Bunch weight

The micropropagated plants produced comparatively higher bunch weight than the sucker derived plants in all the three crop cycles, i.e., main crop, first and second ratoon crop plants of cv. Amritsagar, Malbhog and Chenichampa respectively. A decreasing trend in bunch weights over the crop cycles were observed both in micropropagated and sucker derived plants of cv. Amritsagar, Malbhog and Chenichampa. The bunch weight of micropropagated plants increased by 22.01, 20.03 and 8.22 per cent; 8.69, 13.96 and 18.92 per cent and 4.88, 7.99 and 3.85 per cent for the micropropagated main crop, first and second ratoon crop plants of cv. Amritsagar, Malbhog and Chenichampa respectively over sucker derived plants of the same crop cycles. Badgujar et al, (2005) reported an increase in bunch weight (31.37 per cent) in tissue culture plants. Increase in number of hands and fingers per bunch contributed to the higher bunch weight in plants raised through tissue culture. The plants raised
through tissue culture produced significantly higher bunch weight (12.69 kg) than the sucker plants (10.89 kg) and this support the findings of the present investigation. The increases in average bunch weight were observed between planting material and crop cycles.

5.12.6 Yield

The yield of micropropagated plants were more than that of the yield of sucker derived plants in all the crop cycles. The yield of both micropropagated and sucker derived plants were found to decrease over the crop cycles. An increase in yield by 22.01, 20.03 and 8.22 per cent; 8.69, 13.96 and 18.92 per cent and 4.88, 7.99 and 3.84 per cent were recorded for the micropropagated main crop, first and second ratoon crop plants of cv. Amritsagar, Malbhog and Chenichampa respectively over the sucker derived plants of the corresponding crop cycles. Sheela and Nair (2001) reported an increase in yield of 25.63 per cent in tissue culture plants over the plants from suckers. Yield increase in tissue culture plants was reported to 7.0 per cent, 24.6 per cent and 39.0 per cent by Daniells (1988), Robinson and Anderson (1990) and Pradeep et al., (1992) respectively.

5.13 Physical character of finger

The quality attributes viz., finger length and girth were also found slightly higher in plants raised through micropropagation.
5.13.1 Finger length

There were not much different in length of finger. The minimal increase in finger length (%) in micropropagated plants over the sucker derived plants were observed in cv. Amritsagar (1.00, 1.07 and 1.61 per cent) and Malbhog (1.81, 2.20 and -2.84 per cent), where as in cv. Chenichampa a slight increase (4.20, 5.30 and 6.42 per cent) in finger length (%) were observed over the crop cycles.

5.13.2 Finger girth

There were not much different in girth of finger over the crop cycles of both micropropagated and sucker derived crop plants. Although there were slight increased in finger girth in micropropagated plants over the sucker derived plants in all the three crop cycles of cv. Amritsagar, Malbhog and Chenichampa, the increase percentage were not very high and in the range of 1.42-2.93, 2.00-5.42, and 3.04-4.30 per cent in cv. Amritsagar, Malbhog and Chenichampa respectively. Badgujar et al., (2005) also reported an increase in finger length and girth in tissue culture plant by 4.90 and 5.23 per cent over sucker derived plants, which support the results of the present investigation as stated in section 5.13.1 and 5.13.2.

5.13.3 Pulp: peel ratio

The highest pulp: peel ratio (2.37, 3.07 and 3.25) for micropropagated plants were recorded for the main crop for all the three cultivars and in subsequent crop cycles the pulp: peel ration decreased gradually. The highest pulp: peel ratio (2.31, 2.99, and 3.13) for sucker derived plants were recorded for the main crop for cv. Amritsagar, Malbhog and Chenichampa and in subsequent crop cycles the pulp: peel ratio decreased
gradually. The pulp: peel ratio increased in cv. Amritsagar and Malbhog over the crop cycles, whereas cv. Chenichampa showed a decreasing trend over the crop cycles. Vuylsteke et al., (1996) also reported that with fruit weight significantly lower, it is known that the fruit’s pulp/peel ratio is lower. The similar results were also found in the present investigation.