CHAPTER-2

REVIEW OF LITERATURE

2.1 Tissue culture

The use of micropropagation to obtain large numbers of high-quality planting material has increased in recent years. Behavior in culture, mainly in terms of multiplication rate, varies among genotypes, directly affecting plant production planning. Among the \emph{in vitro} techniques used in agriculture and horticulture, meristem and shoot tip cultures are the most widely used, mainly for clonal multiplication on a large scale (Nehra and Kartha 1994).

The first reports of tissue culture in banana came in early 1970’s from Taiwan when Ma and Shii (1972) produced \emph{in vitro} adventitious buds from banana shoot apex following decapitation. Ma and Shii (1974) and Ma \emph{et al.}, (1978) also did similar kind of activities in the 70’s. These were shortly followed by Berg and Bustamante (1974) in Honduras, who used meristem culture combined with thermotherapy for the production of virus free banana plants. Since then people are working on different aspects of banana tissue culture as enabling tool for maximizing banana production. With regard to banana propagation, protocols have been standardized for \emph{in vitro} propagation of a wide range of \emph{Musa} species and cultivars belonging to various ploidies and genomes (Sathiamoorthy \emph{et al.}, 1998). Shoot tips can be extracted from the pseudostem, suckers, peeplers, lateral buds or even small eyes, which contain a shoot meristem (Jarret \emph{et al.}, 1985; Vuylsteke and De Langhe, 1985). Out of these, peeplers and sword suckers are preferred because of their ease of handling and the minimum damage caused to the parent plants during their removal. It is always better to collect the explants from flowering plants so as to ascertain their trueness to type.
Commercial propagation

Most sweet banana cultivars are sterile and hence are propagated vegetatively from sections of the corm (called ‘bits’) containing unopened buds (or ‘eyes’), or from suckers that are young shoots (Morton 1987; Espino et al., 1992). Micropropagation is used worldwide and more bananas are micropropagated than any other fruit crop (Smith et al., 2005). The efficiency of tissue culture came to light when Hwang et al. (1984) reported the production of one million pathogen free plantlets of banana for commercial planting in Taiwan through meristem culture. However, the cost of micropropagated plants is relatively high and often unaffordable to growers particularly in developing countries (Escalant & Jain 2004). In vitro multiplication of banana plantlets is an excellent alternative with many advantages over field-grown material (Arias 1992; Vuylsteke and Ortiz 1996). The micropropagation procedures used for propagation of bananas have been extensively reviewed by different workers (Vuylsteke 1989; Israeli et al. 1995; Smith et al. 2005).

Josekutty et al., (2003) developed protocol for efficient micropropagation of four local cultivars of bananas (Musa spp.) and found that all the four cultivars studied responded to modified MS medium. They also found that the plant growth regulators, 6-Benzylaminopurine (BAP, 3-7 mg l⁻¹) requirement for different cultivars were substantially distinct in terms of optimal growth and multiplication and longitudinal splitting of the shoot tips improved the multiplication rate in all the cultivars. The techniques of splitting of shoot tips longitudinally though their apex in order to induce multiple shoot formation was described by De Guzman et al., (1980). Krikorian and Cronauer (1984a) obtained rapidly multiplying cultures from excised and split shoot tips of two desert banana clones (Philipian Lacatan and Grand Nain) and two plantain
clones (pelpita and saba). Mateille and Foncelle (1988) also noticed that longitudinal cuts of buds induced a threefold increase in multiplication of *Musa* AAA cv. Poyo.

Somatic embryogenesis in cell suspension cultures has now been scaled up to bioreactor stage for some cultivars (Kosky *et al.* 2002; Kosky *et al.* 2006). Gitonga *et al.*, (2010) evaluated a micropropagation protocol for local banana (*Musa* spp.) (Muunju landrace) in Kenya as an alternative to reduce the unit cost of tissue culture micropropagation and they found that matrices were satisfactory and comparable to the gelling agents (Glass beads were, however, the best matrix in shoot multiplication). Use of support matrices, locally available macronutrients, micronutrients, sugar, equipment and facility reduced the cost of consumable material for banana tissue culturing by about 94%. Shoots were rooted when they were transferred to Murashige and Skoog (MS) medium supplemented with 1 mg l\(^{-1}\) naphthaleneacetic acid (NAA) or 1 mg l\(^{-1}\) Anatone as reported by Gitonga *et al* (2010).

**2.1.1. Explant source for culture initiation**

The plant organ used to initiate a culture is called explant. The choice of the explant depends on the methods of shoot multiplication to be followed, the purpose of culture and the plant species to be initiated. All plant organs viz. nodal segments, internodal segments, shoot tip, root pieces etc. are known to give rise to complete plants. Different explant sources of banana plants are used for initiating cultures in *in vitro* condition. The most commonly used explant sources are shoot apices obtained from parental pseudostem, suckers, peppers, lateral buds and terminal inflorescence. Banana meristem culture is now commonly applied for the clonal multiplication and maintenance of banana cultivars. Regeneration of meristem proceeds through
organogenesis instead of somatic embryogenesis due to which sometimes chimeric plants are obtained (Hwang et al., 1984; Bannerjee and Sharma, 1988; Drew et al., 1989; Blakesley, 1991; Alvard et al., 1993; Mendes et al., 1996; Sagi et al., 1995’ Jasrai et al., 1999; Zaffari et al., 2000; Nandwani et al., 2000, Habiba et al., 2002; Rahman et al., 2004; Molla et al., 2004; Madhulatha et al., 2004; Muhammad et al., 2004).

*In vitro* shoot-tip culture is a suitable alternative to the traditional methods of propagation of banana (*Musa* spp). A study conducted by Hirimburegama and Gamage (1996) with ten banana cultivars of the group AAA, AAB and ABB for *in vitro* multiplication revealed that shoot-tip culture technique can be used for mass propagation of the local cultivars of banana. They observed that variation in multiplication rate was not only among different genomic groups but also among cultivars of the same group. Highest multiplication was observed in Binkehel (AAA) while the lowest was in Alukehel (ABB) and Suwandel (AAB). Thus, multiplication rate appears to be cultivar dependant. The study also showed that sub-culturing enhances shoot multiplication, especially after the second subculture.

Healthy cultures were successfully initiated from shoot primordial meristems when independent dosage of BAP at 4.0 mg/l was given in MS nutrient media (Mukunthakumar et al., 2011). The inflorescence apices responded the most to the *in vitro* culture conditions at 6.0 mg l⁻¹ BAP in MS media itself.

Kanchanapoom and Promsorn (2011) used lateral and apical buds to study the influence of explant on *in vitro* Culture of *Musa balbisiana* ‘Kluai Hin’ (BBB group) and cultured on MS medium supplemented with 22 μM BA and 15% (v/v) coconut water. Comparison of bud orientation showed that the best response of *in vitro* shoot tip
proliferation was obtained with abaxial surface of buds lying down i.e. one side touching the medium (tilt). They obtained mass propagation of shoot tips when cultured buds on MS medium containing 44 μM BA.

Jalil et al., (2003) established culture for plant regeneration from embryonic suspension cultures of *Musa acuminata* cv. Mas (AA) from male inflorescence while Kosky et al., (2002) initiated cell suspensions of the hybrid cultivar FHIA-18 (AAAB) which were established from sections of embryonic tissue derived from male flowers. Navarro et al., (1997) regenerated banana plant through somatic embryogenesis of diploid (*Musa acuminata* spp. *Malaccensis*) and triploid (Grand Nain) bananas from immature zygotic embryos and male flower bud primordia.

Anthers from male flower are another explant source for the production of *in vitro* banana plants and Assani et al., (2003) regenerated haploid banana of *Musa balbisiana* from anthers.

Lee et al., (1997) used sliced rhizome tissue of *Musa* spp. ‘Grand Nain’, for the induction of somatic embryogenesis while mature seed samples of a diploid *Musa acuminata* ssp. Karim et al. (2009) developed an *in vitro* technique for plant regeneration using meristem-derived plantlets of banana cv. BARI-1 (*Musa* sp.) and using this techniques they regenerated highest number of shoot on basal media supplemented with 7.5 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA at 30 days after inoculation (DAI). They also observed that the mean number of shoots significantly reduced when the concentrations of BAP and NAA in the medium was high. Tripathi and Tripathi (2008) established an efficient, simple, and rapid regeneration system for bananas (*Musa* sp.) using sections of corm containing intercalary meristematic tissues as explants. Six different cultivars of banana (‘Mpologoma’, ‘Nakitembe’, ‘Mbawizirume’,
‘Pisang awak’, ‘Sukali ndiizi’, and ‘FHIA-17’) with diverse genetic constitution and ploidy levels were regenerated on Murashige and Skoog (MS) medium supplemented with 5 mg l\(^{-1}\) of 6-benzylaminopurine. About 93–97% of the explants were regenerated with on an average 12–13 shoots from whole section and 16–19 shoots in total from quarter pieces of each section. Dhumale et al., (1997) studied the response of shoot tip explants from banana var. 'Shrimanti' to in vitro culturing and observed that BAP 7 mg/l induced highest number of multiple shoots (6.6), while 2 mg/l concentration of IBA and 2 mg/l IBA fortified with (8.1%) charcoal resulted into profused rooting.

2.1.2. Explant size

Domingues et al. (1995) observed that explant 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.

After initiation the explant base swelled due to the development of leaf primordia and the colour of the explants changed from white cream to green colour. Multiple buds developed from these swollen buds after few days and developed shoots from these buds. The similar kind of results were also reported by Kanchanapoom and Promsorn (2011), where they observed bud swelling, greening and shoots development from the swollen and green buds within forty-nine 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. 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. Amin et al., (2009) reported the development of green globular hard coat mass from where adventitious plantlets were developed. Uddin et al., (2006) reported the swelling of explants and some colour changes from pale white to light/deep green. 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.

### 2.1.3. Disinfection of explants

The use of field grown plants as a direct source of explant material for the production of ‘clean’ in vitro plantlets, presents a major challenge. Microbial contaminations present a major challenge to the initiation and maintenance of viable in vitro cultures. Explant contamination happens due to several plant and environmental related factors such as plant species, age, explant source and prevailing weather condition. Despite the best timing and selection efforts it is difficult to eliminate contamination from in vitro grown plants. 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).

For *in vitro* culture initiation, explants are normally collected from field grown plants, so the plant material is liable to be contaminated by microorganisms which must be disinfected before explants are transferred to *in vitro* conditions. Variations in sterilization procedure have been proposed by several researchers. 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). For the explant disinfected after excision, a shorter treatment time and a lower hypochlorite concentration (0.0525 per cent) is also effective (Vessey and Rivera 1981; Krikorian and Cronauer, 1984).

Some other investigators have replaced sodium hypochlorite with low concentration of mercuric chloride (Banerjee and Sharma 1988; Habiba et al., 2002; Molla et al., 2004, Titov et al., 2006). Habiba et al., (2002) also reported that microbial contamination, mostly bacteria, at the base of the explant observed within 7-15 days after inoculation eventually killed the explants. However, they obtained cent per cent contamination free cultures by soaking the explants in 160 mg/l gentamicin for one hr and 40 min. Double disinfection method has also been applied by some researchers, where first large size explants are disinfected, followed by shoot tip excision and finally disinfection by some other chemical agents (Silva et al., 1998; Nandwani et al., 2000; Rahman et al., 2002; Madhulatha et al., 2004). 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).
Houwe *et al.*, (1998) reported that treatment of shoot tips with rifampicin at 100 mg l$^{-1}$ during 1 month resulted in 100 % bacteria free explants without any phytotoxicity. Ethanol has also been used by a number of research workers for explant disinfection purposes (Silva *et al.*, 1998; Rahman *et al.*, 2002; Jalil *et al.*, 2003). Asif *et al.*, (2001), soaked the banana seeds in 1.4 per cent (v/v) sodium hypochlorite solution for 10 min followed by a quick wash with 70 per cent (v/v) ethanol for generation of *in vitro* culture.

Josekutty *et al.*, (2003) studied different methods of explant sterilization and found that the rate of explant loss due to contamination was 90 % when single step sterilization (15-20 minutes in 25 % v/v Clorox) was done during very wet periods, where as contamination loss was 50 % during drier months. They reported that two steps sterilization procedure reduced explant loss through contamination to 5-8 %. Oyebanji *et al.*, (2009) also evaluated three different surface sterilization methods using seeds and excised embryos of cowpea, rice and sorghum as explants and found that locally produced bleaching solution (JIK ®-Reckitt and Benckiser (Nig) Ltd) containing 3.5 % Sodium hypochlorite at different time intervals resulted in highest reduction in bacterial and fungal contamination (0%) at time intervals between 20-45 minutes.

Uddin *et al.*, (2006) evaluated shoot tips of a traditional table banana [*Musa* sp. cv. Kanthali (Genome AAB)] of Bangladesh for *in vitro* propagation. Initial surface sterilization of shoot tips with 0.1% HgCl$_2$ for 12 min was successful but microbial contamination (mostly bacteria) at the rhizomatous base of the explants were observed within 6-15 days after inoculation which eventually killed 85% inoculated explants. They soaked the explants in two broad spectrum antibiotics namely ampicillin and gentamicin and cent percent contamination free cultures were established by soaking
the explants in 400 mg L$^{-1}$ ampicillin or 200 mg L$^{-1}$ gentamicin for 1 hr. As per their study the antibiotic treated explants were found to be contamination free but failed to regenerate after 3 weeks of culture, but some of them absorbed media for up to 2nd subculture and showed swelling of explants and some colour changes from pale white to light/deep green. Onuoha et al., (2011) achieved the contamination free Plantain culture (100%) in the explants treated with HgCl$_2$ 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) plus bactericide (0.1 g/l) for 10 minutes and rinsed the explants by sterile aquadest 3 times each for 3 minutes. Explants were then sterilized using 70 % alcohol for 1 minute followed by rinsing with sterile aquadest. Finally explants were sterilized by 20% NaOCl solution for 15 minutes and rinsed the treated explants by sterile aquadest 3 times each for 5 minutes and inoculated on initiation media.

2.1.4. Culture initiation

The success of plant tissue culture depends on the correct choice of explant. The external environment is full of microorganisms and pests which infect plants. Usually these pathogens are confined to the outer surface of the plants, while some of these pathogens like viruses may be systemic within the plant tissues. The explant is initiated on a nutritive media, which is also favorable for the growth of most of the types of microorganisms, therefore it is essential to establish and maintain aseptic conditions for plant tissue culture. Since most of the microbial organisms rapidly grow on the same
nutritive media on which plant is growing, it provides a tough competition to the plant material growing in vitro; therefore, it is usually advised to properly sterilize the explant in order to make it free from any kind of microbial contaminants. The method of explant sterilization varies from species to species.

For micropropagation where the aim is to get identical plants, it is advisable to initiate cultures from explants with preformed meristems. The two explants with preformed meristems are shoot tip (terminal/apical meristem) and nodal explants (with axillary meristems). Apical meristem is comparatively soft and thus difficult to sterilize. However, if mother plant is infested with viruses, it is obligatory to initiate cultures from the extreme meristem. While this calls for tedious dissections coupled with poor survival, once a plant is obtained it is very easy to produce multiple copies under aseptic conditions. Usually explants are more responsive if they are collected during the period of active growth.

Explants should preferably be isolated from young, vigorous and healthy looking suckers of 40-100 cm in height with a corm diameter of about 10 cm. These should be collected from a flowering mother plant to guarantee trueness-to-type. Usually only the apical meristem is available from a trimmed sucker. Eventually, smaller buds on the corm can also be used as explants for tissue culture initiation. After the excision of shoot tips, explants are cultured in medium under in vitro condition for shoot initiation. At this stage, the explant consists of shoot apical meristem, covered by 3-6 leaf primordia and supported on a small base of rhizome tissue. This shoot tip is cultured on the medium intact, wounded or fragmented into pieces. Ma and Shii (1972); Hwang et al., (1984) decapitated shoot tips before culture initiation to overcome apical dominance and to encourage axillary bud proliferation. A number of vertical cuts into
the meristematic dome were applied by Vessey and Rivera (1981); Jarret et al., (1985); Cote et al., (1990) and the numbers of incisions ranged from 2-10 and were made in such a way that the base of the explants was kept intact. A number of investigators used fragmentation procedure in which shoot apex was cut longitudinally into halves or more pieces and used as individual explants (Cronauer and Krikorian, 1984; Damasco and Barba, 1984; Jarret et al., 1985; Zamora et al., 1986).

2.1.5. Culture media

Nutritional requirements for optimal growth of a tissue in in vitro condition may vary with the species. Even tissues from different parts of a plant may have different requirements for satisfactory growth (Murashige and Skoog, 1962). No single medium can be suggested as being entirely satisfactory for all types of plant tissues and organs. When starting with a new system, it is essential to work out a medium that would fulfil the specific requirements of that tissue.

The components of a plant tissue culture medium include macronutrients, micronutrients, a separate iron supplement, vitamins, a carbon source, and usually plant growth regulators. Amino acids and various nitrogenous compounds may be present in the vitamin mixture. Macronutrients are nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur. These macronutrients are also called inorganic chemicals, and are essential elements required in relatively large amounts. Micronutrients are traces of certain elements required by all plant cells. Micronutrient elements include iron, manganese, zinc, boron, copper, molybdenum, iodine, cobalt and chlorine.
Meanwhile, vitamins have catalytic functions in enzyme systems and are required only in trace amounts. The plant tissue culture medium comprises of inorganic salts, vitamins, amino acids and sucrose is described as Basal medium. Very often this basal medium is supplemented with growth regulators (auxins, cytokinins etc.), complex organic nutrients (malt extract) and natural plants extracts (coconut milk, potato extract etc.) to meet the growth requirements of specific tissues.

The need to culture diverse tissue and organs has led to the development of several recipes of nutrient medium. White (1943) has created a new medium that is low in salt and free of ammonium ion. White’s medium was the earliest control medium that consists of all needed nutrients, and applied widely for root cultivation. On the other hand, Murashige and Skoog (1962) have developed MS medium that consist of ammonium, nutrient and others mineral includes inorganic nutrient that are needed by plant to establish growth. Until now, the most known mediums that researchers used in plant tissue culture were Murashige and Skoog medium (1962) that is high salt or B5 (Gamborg et al., 1968).

It is often possible to use the same medium for culture initiation and shoot multiplication. In general, tissue culture medium consists of 16 essential elements for plant growth. Some media are simple than others (Knop’s medium for instance contains only four salts). The most important difference among the various media is the overall salt level. Depending on the concentration of salts three categories are identified i.e.

(i) High salt (MS-Murashige and Skoog 1962)
(ii) Intermediate Level (NN-Nitsch and Nitsch 1969)
(iii) Low salt (WM White 1943)
(i) **Carbohydrates:**

These have two principal functions in a tissue culture medium, first are to provide energy source and second is to maintain a minimal osmotic potential around the tissue. Sucrose is the most commonly used carbohydrate source. The concentration used is in the range of 2-4%. In commercial tissue culture, table sugar is routinely used.

(ii) **Growth regulators:**

Few compounds that occur naturally within a plant tissue have a regulatory rather than nutritional function in the growth and development. These compounds are active at very low concentrations and are known as plant hormones. Synthetic chemicals with similar physiological activities to plant growth substances are termed as plant growth regulators. These chemicals are added to basal media and depending on the type and concentration we get either shoot differentiation or root formation. Auxins and cytokinins are two naturally used growth regulators. High cytokinin-auxin ratio favours shoot differentiation and multiplication while high auxin-cytokinin ratio favours rooting. The exogenous requirements for these hormones depend on their endogenous levels in the plants which vary with the plant species explant and phase of development. The naturally used auxins are IAA, IBA, NAA and 2, 4-D. The auxins are usually dissolved in a smaller amount of rectified spirit and final volume is made with water.

The most naturally used cytokinins are benzylamino purine (BAP), kinetin, 2-Zip. Cytokinins are dissolved in small amount of NaOH or HCl and the final volume is made with water. Some times gibrellic acid (GA₃) is added to the media mainly to induce elongation.
(iii) **Activated charcoal:**

It is frequently added to media as it absorbs inhibitory substances produced by the tissue. Activated charcoal may adsorb toxic substances in the medium thereby improving root regeneration and development (Ziv, 1979; Takayama and Misawa, 1980). Activated charcoal is also capable of shading *in vitro* roots from light which in high intensity may inhibit root growth. Activated charcoal can adsorb metabolic inhibitors, improve aeration, adsorb toxic substances and adsorb residual cytokinin and auxin from the previous medium (Hu and Wang, 1983).

### 2.1.5.1. Physical state of culture medium

Medium gelled with 0.6-0.8% agar is satisfactory for large number of species. Semi solid cultures are easy to handle. Even the ‘A’ grade of agar used can give varied responses. Many systems are known to be more responsive to synthetic gelling substance gelrite. Gelrite is used at 0.2% concentration and media is absolutely transparent. Although expensive it is much easier to detect contamination in cultures. Liquid media has been reported to stimulate the establishment and growth of bamboos. Usually the liquid cultures are kept on shaker to provide gaseous exchange. However, if the amount of the media is kept to minimal cultures can be maintained without shaking. The pH of the medium is usually adjusted at a range of 5.5 to 5.8.

Physical state of the medium also played an important role in tissue culture of banana. In most of the laboratories solid medium is used. Role of liquid versus agar-gelled media in mass propagation and *ex vitro* survival in banana was studied by Bhagyalakshmi and Singh (1995) during shoot tip culture of Bluggoe and Silk. Agar-gelled, agitated liquid and static liquid media were assessed for their ability to support
shoot multiplication and *ex vitro* survival. Liquid media was found better for shoot multiplication whereas agar-gelled medium supported maximum *ex vitro* survival. An elegant liquid tissue culture system, based on temporary immersion of explants with liquid for 20 minutes after every 2 hours was described by Alvand *et al.*, (1993). The results indicated that shoots in liquid medium and those on cellulose substrate, proliferated little or not at all, shoots on gelled medium, those subjected to partial immersion, and those in aerated medium displayed multiplication rate of 2.2 to 3.1, and the highest multiplication rate was observed in explants subjected to temporary immersion in the liquid medium.

The effect of liquid pulse treatment of growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA) was studied by Madhulatha *et al.*, (2004). Optimal shoot proliferation rates were achieved due to the pulse treatment of 6-benzylaminopurine (BA) and kinetin combination (1:1) at the concentration of 50 mg/l for 60 min. Similarly high frequency of root induction was obtained due to pulse treatment with combination of NAA and IBA (1:1) at a concentration of 100 mg/l each for 60 min. Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up in a bioreactor was reported by Kosky *et al.*, (2002). Secondary multiplication of somatic embryos was achieved in liquid media on rotary shaker and in bioreactors.

2.1.5.2. Media for culture initiation and shoot multiplication:

Different culture media are used for culture initiation. Some investigators initiate the cultures on the same media as later used for multiplication while other used low concentrations of hormones for culture initiation. Most common salt mixture used for
culture initiation of banana was the MS (1962) with some modification as reported by Hwang et al., 1984; Drew et al., 1989; Cronauer and Krikorian (1984); Hamill et al., (1993); Thomas et al., (1995); Silva et al., (1998); Nandwani et al., (2000); Zaffari et al., (2000); Assani et al., (2003); Molla et al., (2004); Roels et al., (2005).

Assani et al., (2003) initiated cultures from anthers on MS medium containing vitamins of Morel supplemented with 500 mg/l casein hydrolysate, 4.4 μM BAP, and 2.3 μM IAA. When kinetin was used as sole cytokinin, adenine sulphate was also added in the medium as a conducive agent to shoot initiation (Hwang et al., 1984; Drew et al., 1989; Nandwani et al., 2000). Some investigators used only single cytokinins for culture initiation (Cronauer and Krikorian, 1984; Thomas et al., 1995; Silva et al., 1998; Rahman et al., 2004, Molla et al., 2004; Roels et al., 2005), while others used mixtures of cytokinins (Nandwani et al., 2000; Rahman et al., 2002). A combination of cytokinins and auxin was also used for banana culture initiation by a number of researchers (Hwang et al., 1984; Drew et al., 1989; Zaffari et al., 2000; Muhammad et al., 2004).

Buah et al., (2010) conducted a study to determine the appropriate type and level of cytokinin required to achieve shooting response in two cultivars of plantain (Oniaba and Apantu pa). Three cytokinin types, Benzyl aminopurine (BAP), Kinetin and 2ip at two different concentrations (4.5 and 7.5 mg L⁻¹) were used. They found that the media supplemented with 4.5 mg L⁻¹ BAP induced the highest number of shoots after eight weeks of culture. There was also a variation in the ability of the cytokinin types to induce shooting in both the cultivars. BAP had the highest shoot induction response in both the cultivars, followed by Kinetin and 2ip. They found that the degree of efficiency of shooting was dependent on the type of hormone and the plantain cultivar.
Kalimuthu et al., (2007) developed a complete protocol for micropropagation of *Musa sapientum* using shoot meristem. As per their research findings, Murashige and Skoog’s medium supplemented with BAP and NAA (3.0±0.2 mg/l respectively) was the most suitable combination. Crouch et al., (1998) recommended a BAP concentration range of 8.9±22.2 μM for *Musa in vitro* propagation.

Amin et al., (2009) conducted a study 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-1. They observed that 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 and from that ball like structure, adventitious plantlets were developed. Among the different concentrations tried, 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 days after initiation (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. They found the maximum number of leaves (2.50, 3.25 and 7.00 leaves/explant) at 10, 20 and 30 DAI 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. They also found the highest numbers of roots on 0.5 mg/l IAA + 0.5 mg/l IBA medium.

Ernawati et al., (2000) conducted a study to obtain a method of rapid clonal propagation of bananas in cultivars Pisang Mas (AA), Pisang Ambon Kuning (AAA), Pisang Barangan (AAA), and Pisang Rajabulu (AAB). The result of the study showed...
that IAA alone significantly induced shoot multiplication. They found IAA and BAP as the best treatment to induce shoot multiplication and after 8 weeks, the highest number of axillary shoots (12.6 shoots/bottles) was obtained by Pisang Ambon (AAA), followed by Pisang Mas (AA) 8.2 shoot/bottle, Pisang Barangan (AAA) 7.8 shoot/bottle, and Pisang Rajabulu (AAB) 7.6 shoot/bottle.

Gitonga et al., (2010) conducted an experiment to examine the cost economics of conventional tissue culture chemicals, equipment and facility with the alternatives available in Kenya and reported that the substitution of macronutrients and micronutrients with the alternatives reduced the cost by 94.2 and 97.8%, respectively. Habiba et al., (2002) reported that MS + 4.0 mg/l BAP + 1.0 mg/l Kn was the best medium for single shoot development to obtain contamination free culture of the table bananas *Musa sapientum* cv. Chini champa and Sagar and MS + 4.0 mg/l BAP + 2.0 mg/l IAA + 13% CW for shoot multiplication. Khan et al., (2001) reported that commercial grade sugar could replace analytical grade sucrose with no significant change in frequency of shoot formation in banana. Zapata (2001) successfully reduced the cost of banana tissue culture by 90% by replacing the tissue culture sucrose grade with a commercial sugar. Demo et al., (2008) used table sugar as alternative to analytical grade sucrose for *in vitro* micropropagation of potato (*Solanum tuberosum* L) and reported that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 34 to 51% when compared with the analytical grade sucrose.
2.1.6 Control of browning of *in vitro* culture

A serious problem associated with the *in vitro* culture is the oxidation of phenolic substances that are leached out from the cut surface of the explants. Phenols occur in plants in a variety of forms and constitute an exceedingly important group of secondary plant products. The oxidation products of phenols are brown to black and are toxic to the living tissues. Where the problem of browning occurs only at the beginning, a pre-treatment of tissues in anti-oxidant solution or culturing them for a few days in liquid medium has been found to be helpful. Keeping the cultures initially in the dark may also reduce the browning to some extent.

To overcome the browning when it occurs at each subculture, anti-oxidants such as ascorbic acid (50-100 mg l\(^{-1}\)), citric acid (150 mg l\(^{-1}\)), and cysteine HCl (100 mg l\(^{-1}\)) may be incorporated into the medium. Polyvinylpyrrolidone (PVP) has also been used to avoid browning and subsequent death of the cultured tissues. Incorporation of 0.3% activated charcoal in the medium minimized the problem of browning in the cultures of date palm.

Titov *et al.*, (2006) controlled the secretion of phenolic compounds by floral bud explant by pre-soaking them in an antioxidant solution of 0.125 per cent potassium citrate: citrate before culturing. During micropropagation of banana, blackening and necrosis of tissues is commonly observed which interfere with the plant growth. Martin *et al.*, (2007) controlled tissue necrosis of cvs Grand Naine (AAA), Dwarf Cavendish (AAA), Nendran (AAB) and Quintal Nendran (AAB) by the addition of 50-100 mg/l calcium chloride in the MS medium.

Poudyal *et al.* (2008) did a thorough investigation on the browning problem of Yali Aikansui and Abbe Fetel pears and their control measures. According to their
experimental results, Yali was found more severely infected by browning than the other two cultivars and shoot tips of Yali were found more severely infected by browning than the second node and other nodes. This accelerated the mortality rate up to 81% of shoot explants during the summer months of July and August. They recorded that as a curative measures to control browning ascorbic acid at the rate of 100 mg·L⁻¹, 0.02% polyvinyl pyrrolidone (PVP) in the culture medium, 96 hours dark treatment of other nodes, and 12 hours cold treatments of explants at 4°C prior to sterilization of explants, were found to be the best methods to control browning and therefore to increase the survival rate of cultured explants of the Yali pear.

Ko et al., (2009) observed that mass micropropagation of Cavendish banana cv. Formosana has a serious problem of high mortality due to lethal browning of plantlets and found that amendment of culture medium with anion exchange resins, cation exchange resins, polyvinyl pyrrolidone or activated charcoal did not reduce the disease incidence. They reported that addition of ascorbic acid to the surface of culture medium not only prevented the development of lethal browning but also greatly increased the number of plantlets produced and even at 0.005% ascorbic acid was able to reduce the disease incidence by more than 60% and caused over 8-fold increase in number of plantlets produced. In their study, they also found that when cultures raised from 12 different Formosana corms were tested, ascorbic acid was able to reduce disease incidence by an average of 83%, and increase the number of plantlets in each test and when diseased plantlets were transferred to culture medium with ascorbic acid, all of them recovered, and resumed normal growth and multiplication, while all control plantlets on culture medium without ascorbic acid died after one month. Oxidation of
phenolic compounds was controlled by addition of 40 mg/l cystein HCl (Khatri et al., 1997)

Onuoha et al., (2011) used 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 thus inferring that browning in young plantain excised tissue can be greatly reduced by pre-soaking or pre-treatment with antioxidant solution of potassium citrate-citrate before culturing them.

Morfeine (2013) carried out a study for manipulation of problems of oxidation in the initiation phase of Musa species Grand Naine in vitro by using chemical and nonchemical addenda by dipping the explants in vitro. After excision and before disinfection explants were dipped in vitro into the solutions of citric acid (150mg/l) + ascorbic acid (100mg/l), cysteine (100mg/l), ginger (10g/l), activated charcoal (3 g/l) for prevention of browning or blackening. The findings of the study indicated that Cysteine, citric + ascorbic acid and ginger in pre-treatment and use of activated charcoal proved to be effective for preventing browning of medium and reduce browning of explants.

2.1.7. In vitro multiplication

An important factor affecting the efficiency of a micropropagation system is the rate of multiplication (Williams and Taji 1991), which can be calculated as the ratio of shoot number at the end of the subculture to the initial number of shoots (Alvard et al. 1993). It has been observed that banana multiplication rate is genotype dependent (Afza et al. 1996; Hirimburegama and Gamage 1997), and highly variable behaviour has been observed among families of the same banana genotype cultured in vitro (Israeli et al.
1995; Mendes et al. 1996). This suggests that the primary explants should not be considered as replicates and must be analyzed individually.

Plant growth regulators are essential media component for the manipulation of growth and development of explants in vitro. Their concentration and ratio in the medium often determines the pattern of in vitro development of cultures. Cytokinins and auxins are used as growth regulators for Musa tissue culture. The most widely used and the most effective cytokinins for this purpose are adenine-based cytokinins BAP. The concentration of exogenous cytokinin appears to be the main factor affecting multiplication. Wong (1986) stated that when 11.1 μM BAP is supplemented in the medium, each of the explants produces an average of 2.4 shoots, while increasing the BAP concentration to 22.2 μM and 44.4 μM results in 2.6 and 4.3 shoots per explant respectively. However, the optimum recommended BAP concentration is 20 μM for banana micropropagation (Vuylsteke, 1989).

Cronauer, S. S. and Krikorian (1984) achieved 9.1 shoots/explants during in vitro multiplication of Philippine Lacatan’ and ‘Grand Naine’ on a modified Murashige and Skoog (1962) medium supplemented with 5.0 mg/l 6-benzylaminopurine (BAP). On the other hand, Rahman et al., (2002) achieved 4.52 shoots per explant on the same concentration of BAP on MS medium during in vitro multiplication of cv. Bari-1, indicating the genotypic response towards cytokinin. Higher shoot length (3.62 cm) was achieved when MS medium was supplemented with 1.5 mg/l NAA. Mendes et al., (1996) used 4.5 mg/l BAP in MS medium during the study of the behaviour of vegetative apices coming from different rhizome. They reported that shoot tips coming from different rhizomes behave differently under in vitro condition, some being highly productive and other producing a much smaller number of plants under similar culture
conditions. Khatri et al., (1997) used MS medium with 20 µM/l BAP for shoot proliferation of cultures initiated from clones of desert banana (Musa spp.) viz., GN60A, SH3362, William, Highgate and Basrai. At low concentration of BAP (10 µM/l) only one or two shoots regenerated in most of the cultivars used by them. Micropropagation of banana (Musa paradisiaca) through cormlet initiation by in vitro culture of apical meristem slices was achieved by Priyono (2001). He reported cormlet production on medium supplemented with 5-20 mg/l BAP combined with 10-40 per cent sucrose or in the medium supplemented with 5-20 mg/l, BAP combined with 5-20 mg/l ancymidol (ANC). Nor-Aziah and Khalid (2002) used higher concentration of BAP during regeneration of in vitro banana plants from scalps and whole meristem. Scalps were induced on MS supplemented with coconut water and high concentration of BAP (75 µm/l). The average number of regenerated shoots produced from scalp was six-fold higher than that generated from single meristem. Venkatachalam et al., (2006) achieved direct shoot regenerated from leaf sheaths of silk banana (ABB) when cultured on medium containing 22.4 µM BA/l.

Kanchanapoom and Promsorn (2012) achieved the multiple shoot formation of edible bananas (Musa balbisiana, BBB group) ‘Kluai Hin’ through organogenesis in bud culture by culturing excised apical and lateral buds on a modified Murashige and Skoog (MS) medium supplemented with 22 µM BA and 15% (v/v) coconut water (CW). They subcultured the proliferated shoots on MS media containing several concentrations of BA and TDZ for mass multiplication and obtained the highest shoot numbers (21.2 shoots per explant) when subcultured to MS medium supplemented with 44 µM BA.

Jarret (1986) reported in vitro propagation and genetic conservation of bananas and plantain through axillary and adventitious development of plants from shoot tip culture.
Bhalyalakshmi and Singh (1995) used MS medium with 8.9 M benzyl adenine and 0.98 M indole butyric acid during shoot culture of three banana cultivars, Cavendish, Bluggoe and Silk. Similarly Okole and Schulz (1996) used MS medium along with 10 μM BAP and 1 μM IAA for shoot multiplication during culture of leaf segment from banana plants as an alternate approach for the production of adventitious shoots and callus. In their study they obtained an average of 15 shoot buds from micro-cross sections derived from each explant on a shoot-inducing multiplication medium.

Bhosale et al., (2011) studied the effect of different concentration of BAP on bud initiation and shoot multiplication in different species of Banana viz., Ardhapuri, Basrai, and Shrimanti and found increased average numbers of shoots at 7 mg/l BAP and least numbers of shoots at 3 mg/l BAP in all the three varieties.

Srangsam and Kanchanapoom (2007) established in vitro cultures of Musa AA Group ‘Kluai Sa’ and Musa AA Group ‘Kluai Leb Mue Nang’ using shoot tip as explant. The maximum shoot numbers were obtained on the medium containing 5 mg/l BA with 4.9 shoots in ‘Kluai Sa’ and 5.5 shoots in ‘Kluai Leb Mue Nang’. Root and callus formation were achieved when shoot buds were planted on MS media containing various concentrations of NAA (1-Naphthalene acetic acid) in combination with 5 mg/l BA. Adventitious shoots were also regenerated from calli that were cultured on MS medium containing 5 mg/l BA and 0.1 mg/l NAA. All regenerated shoots rooted well on MS medium either supplemented with or without 0.2% activated charcoal. More than 95% of the surviving plantlets were acclimatized and successfully transferred into soil.
Prasad et al., (2011) studied the *in vitro* regeneration of Banana (*Musa* spp) and found the *in vitro* shoot tip formation within 2-3 weeks when meristem were carefully isolated from field grown plants and after proper sterilization implanted in semi solid medium with 2.0 mg/L 6-Benzyl amino purine (BAP). They also found that the frequency of shoot proliferation was increased with increase of subculture in the same medium containing MS+BAP at 2 mg/l.

An efficient medium culture for clonal mass propagation was established by Farahani et al., (2008) for the propagation of two banana (*Musa acuminate* L.) cultivars of Cavendish Dwarf and Valery. They studied the effects of growth regulators on fresh weight, length of shoot and shoot proliferation of the meristem explants in *Musa cv. Cavendish Dwarf* and Valery. The final medium adopted by them included the salt formulation of Murashige and Skoog, 30 g L⁻¹ of sucrose, N-phenyl-N-1, 2, 3-thiadiazol 5-yl Urea (0.5 mg L⁻¹) and Indoleacetic acid (2 mg L⁻¹) and under this condition, a multiplication rate of 25 plantlets per explants was obtained in 120 days.

The effect of different cytokinins viz., BAP, 2ip and Kn each at 5 mg/l alone or in combination on shoot multiplication and auxins viz., IBA, NAA in different concentration (i.e., 0, 1, 2, 3, 4 and 5 mg/l) on rooting of banana cv. Sabri were investigated by Rahman et al., (2002). The highest number of shoots per explant (3.11) was observed in MS medium supplemented with 5 mg/l each of BAP+Kn, while no shoot multiplication was observed in control (i.e., no cytokine). On the other hand, the number of shoots responded for rooting and their survivability were higher with NAA than IBA. MS medium supplemented with 4 mg/l NAA produced the highest number of roots per shoot (20) whereas 1 mg/IBA produced the minimum number of roots per
shoot (2.4) and plantlets grown without any auxin in the medium gave the least number of root (1).

Shiragi et al., (2008) conducted an experiment on virus free in vitro banana plantlets production by the meristem cultured from virus infected four month old banana suckers cv. Amritsagar as explants and four levels of BAP (0, 3, 4 and 5 mg/l), 5 levels of NAA (0.0, 1.0, 1.5, 2.0 and 2.5 mg/l) as treatment for shoot proliferation in experiment-I and four levels of IBA (0, 1, 2 and 3 mg/l), 4 levels of NAA (0, 2, 3 and 4 mg/l) for root formation in experiment-II. ELISA test was conducted for the confirmation of virus eradication rate of regenerated plants and survival rate of regenerated plants in experiment-III. Studies on the different concentrations of BAP + NAA on shoot proliferation and differentiation, revealed that 4 mg/l BAP + 1.5 mg/l NAA produced the greater number of shoots (4.58/ explant), the longest shoot (3.56 cm) and the highest numbers of leaves (3.11/explant). On the other hand highest number of vigorous roots (6.98/explant) and maximum root length (5 cm) were recorded from the treatment combination 2 mg/l IBA+2 mg/l NAA and 3 mg/l IBA+ 3 mg/l NAA, respectively.

Vani and Reddy (1999), cultured shoot tips dissected from healthy suckers of banana (Musa spp) varieties dwarf Cavendish. Amruthapani, Tella Chakkerakeli and Robusta on MS medium supplemented with 6.0 mg L\(^{-1}\) BAP, 2.0 mg L\(^{-1}\) IAA and 200 mg L\(^{-1}\) adenine Sulphate and found that shoot initials developed after 3 weeks and were proliferated on induction medium with BAP concentration reduced to 4.0mg L\(^{-1}\).

Mukunthakumar and Seeni (2005) multiplied 2 primitive diploid Musa cultivars, Matti and Chemmatti from the extreme southern part of the Western Ghats by in vitro culture of sucker-derived shoot apices. They found that after 12 weeks of culture, maximum
numbers of shoots (32) in both the cultivars were produced in approximate 60% of the explants in presence of BAP and IAA each at 1.5 mg l⁻¹ (Matti) and 40% of the explants in 2.5 mg l⁻¹ of BAP and 1.5 mg l⁻¹ of IAA (Chemmatti). Buds were formed from the base of the subcultured shoots and somewhat more number (34) of shoots was obtained in Matti than in Chemmatti (31) after 8 weeks.

Jambhale et al., (2001) reported the production of maximum numbers of multiple shoots per bottle after the 14th subculture in Basrai (12.33) followed by Lal Kela (10.72) and minimum in Nendran and Safed Velchi (8.63). They found the eventual decline in multiple shoot formation rates with the increase in number of subcultures in all four clones. They also found the decline in growth of the clones as measured by stem height, girth, number of leaves and leaf size after the 8th subculture, with some plants exhibiting very stunted growth after the 14th subculture.

Malik et al., (2000) studied the in vitro multiplication protocol of banana cultivars ‘Desi’ and observed that 6-Benzylamino purine (BAP) played a vital role for shoot multiplication and BAP @ 5 mg/l resulted in maximum (4 to 5) plantlets, where as kinetin @ 2 mg/l enhanced shoot elongation.

Akbar and Roy, (2006) achieved a regeneration protocol ensuring a high frequency rooting of micro-shoots derived from apical and axillary buds of suckers of banana cv. Sagar by using liquid medium. Then observed that when the explants were cultured on MS medium supplemented with 0.5 mg/l each of BA, Kn and NAA, a large number of shoots developed. With the progression of the number of subcultures, shoot proliferation was enhanced. Addition of 10 % coconut water to the medium increased the number of shoots per culture and growth of individual shoots.
Arinaitwe et al., (1999) studied the proliferation rate effects of cytokinins on three banana (Musa spp.) cultivars. They cultured the banana cultivars on modified Murashige and Skoog nutrient salts medium which was supplemented with various equimolar concentrations (16.8, 20.8, 24.8 and 28.8 μM) of BAP, TDZ, ZN, 2-iP and Kn to determine suitable concentration ranges of the cytokinins for micropropagation of banana cultivars. Three sub-culture cycles were used and after each subculture, the shoots per explant were counted. They found that shoot proliferation was significantly dependent on cytokinin type, its concentration and the banana cultivar and the responses of cultivars to BAP were significantly better than other adenine-based cytokinins (ZN, Kn and 2-iP).

Uddin et al., (2006) found MS + 4.0 mg L⁻¹ BA + 0.5 mg L⁻¹ Kn + 15% CW as the best medium for single shoot development and average time required for shoot development was 18-21 days, but the regeneration percentage was very low (30%). On the other hand, the best medium for shoot multiplication was MS + 4.0 mg L⁻¹ BA + 2.0 mg L⁻¹ IAA + 15% CW and average time required for production of multiple shoots from single shoot were 40-45 days. They also found that multiplication rate was too low (40%) and only average 3-4 shoots were formed.

Ali et al. (2011) reported that shoot formation response from shoot apical meristem on MS medium containing 1.0 mg/l BAP showed the best response for shoot formation and for that of shoot multiplication, MS medium containing 1.0 mg/l BAP + 0.25 mg/l kin provided the best multiplication response which was 8 shoot per culture vial within 21.6 days after inoculation into shoot multiplication medium.

Hussein (2012) conducted an experiment with the objective to evaluate the effects of nutrient media constituents on growth and development of banana plantlets produced in
vitro and found that during bud proliferation stage, the addition of inositol and white's organics was non-significantly effective. BA concentration at 7.0 mg/l significantly promoted bud proliferation both at complete and splitted plantlets. However, splitted plantlets showed a higher rate in bud proliferation.

Azam et al., (2010) described a method of clonal propagation for one of the elite cultivars of banana BARI-1' (AAA genome, Sapientum subgroup) developed by the Bangladesh Agriculture Research Institute (BARI) and observed that in vitro cormlets were formed within 2–3 weeks when meristems were carefully isolated from field-grown plants and after proper sterilization inoculated in semisolid Murashige and Skoog (MS) media supplemented with 2.0 mg L⁻¹ 6-benzylaminopurine (BAP). They also observed that the regeneration of cormlets was geared up and shoot multiplication took place when MS was enriched with 2.0 mg L⁻¹ BAP and 1.0 mg L⁻¹ Kinetin. With the increase of subculture (up to 9th maximum), frequency of shoot proliferation was enhanced. Addition of 0.1 mg L⁻¹ Indole-3-acetic acid and 10% coconut water to the medium increased shoot elongation and stimulated growth of the shoots, respectively.

Rahman et al., (2005) studied the effect of various Cytokinin (viz., BAP and Kin), auxin (viz., IBA, NAA, and IAA) and coconut water (CW) alone or in combinations on shoot multiplication of banana cv. Anupom and reported that MS medium supplemented with 5.0 mg L⁻¹ each of BAP and kinetin and 13 % coconut water produced the highest number of shoots per explant.

Azad and Amin (1999) cultured banana cv. Sabri using shoot tips (1.0-1.5 cm) of the field grown young suckers on MS medium supplemented with 3.0-5.0 mgL⁻¹ BA+2.0-3.0 mgL⁻¹ IAA+15 % coconut water. They observed the Tuber Like Structure (TLS) on above medium within 15 days of culture and when these TLS were longitudinally
sectioned and sub-cultured on MS medium containing 1.0-2.0 mg L\(^{-1}\) BA, 0.5 mg L\(^{-1}\) IAA and 15% coconut water, those produced shoots with 3 to 5 leaves.

Rabbani *et al.* (1996) reported the effect of BAP and IBA on micropropagation of different banana cultivars viz. Amritsagar, Sabri, Anajee and Mehersagar and observed that BAP at the rate of 5.0 mg/l produced the highest number of shoots in Amritsagar and Mehersagar. In another study, Amritsagar banana (AAA) meristem tip generated highest number of shoots on MS medium supplemented with 30μM BAP (Khanam *et al.*, 1996)

Subramaniam *et al.*, (2008) initiated the Multiple Bud Clumps (Mbc's) from the selected corm slices region of the Cavendish banana cultivar, Brasilian (AAA) by culturing them on Murashige and Skoog medium (1962) supplemented with various concentrations of BAP hormone in both solid and liquid medium. Optimization of Mbc's production was assessed based on the number and various sizes of single buds obtained. They recorded the production of highest number of single buds of 3 different sizes from multiple bud clumps at the concentration of 8 mg/L BAP in both solid and liquid medium, respectively.

Haridasan and Caldas (1989) reported that after 8 to 10 weeks of culturing the banana prata, one or two lateral buds were produced and subsequent subculture of these on the same medium produced up to 10 buds after 6 to 10 weeks. The highest multiplication factor observed in subsequent subcultures was 40 new plantlets per explant; the average multiplication factor was 10 buds per explant in 8 weeks.

Muhammad *et al.*, (2007) investigated the effect of benzylaminopurine (BAP) and kinetin alone and in combination with indole 3-acetic acid (IAA) on shoot proliferation of ‘Basrai’ (*Musa* spp., AAA group) and reported that the multiplication rate was
significantly (P ≤ 0.05) dependent upon cytokinin type, its concentration, and type of medium used. They recorded the maximum number of shoots regenerated from a single shoot tip in liquid MS medium containing 4.0 mg·L⁻¹ BAP and found no significant difference between liquid and solid medium when kinetin was used; however, kinetin at 4.0 mg L⁻¹ or above yielded significant results as compared with the control and lower kinetin concentrations. Their findings demonstrated that 4.0 mg·L⁻¹ BAP with 1.0 mg·L⁻¹ IAA in liquid medium was best for shoot multiplication and shoot height during micropropagation of ‘Basrai’.

Acharjee et al., (2004) used in vitro shoot tip culture technique for clonal propagation of indigenous banana cultivars viz., Bhimkhol, Malbhog, Kanchkhol and Jahaji exclusive to the north-east region of India and found shoot elongation in a medium supplemented with lower (2.5 mg L⁻¹) concentration of BA.

There are reports that more than one cytokinins with or without auxins, were used together to enhance the shoot proliferation during banana tissue culture. Gubbuk and Pekmezci (2004) studied the effects of BAP (5, 10, 20 and 30 μM) and Thidiazuron (0.4, 1, 2 and 3 μM) alone or with 1 μM IAA for shoot multiplication of three banana types and found that in all three types, shoot proliferation and elongation were significantly greater with TDZ than with BAP. They also reported that each cytokinin with IAA increased shoot proliferation and elongation more than their use alone.

Gubbuk and Pekmezci (2006) investigated the rapid clonal propagation potential of banana by using excised shoot tips of Musa spp and reported an increase in plant height, root numbers and average number of roots per explant with activated charcoal (AC) as a component in the medium and also recommended the use of MS medium
with 2.5 μM TDZ with 1 μM IAA for multiplication of ‘Dwarf Cavendish followed by rooting with AC only.

Chew et al. (2008) developed an efficient micropropagation protocol for shoot tips of *Nephelium lappaceum* Linn. Rambutan as a starting point, for *in vitro* storage and cryopreservation and found activated charcoal (AC) crucial to the successful culture of rambutan, which was reflected by high survival (100%), shoot elongation (95%), and considerably less browning (12.5%) than without AC.

Kinetin is another synthetic plant growth regulator used in banana tissue culture and was so named because of its ability to promote cytokinesis (cell division). Hwang et al., (1984) added 2 mg/l kinetin and 2 mg/l indole acetic acid in MS medium during meristem culture of banana and found the increase in population of buds by five times per month. Wong (1986) compared kinetin and 6-benzylaminopurine (BA) along with indole butyric acid (IBA) during *in vitro* multiplication of banana (*Musa* spp.) and found that BA was consistently more effective than kinetin.

Gupta (1986) and Madhulatha et al., (2004) used equal concentration of kinetin and BAP on MS medium while Rahman et al., (2002) used BAP, kinetin and 2iP each at 5 mg/l alone or in combination during shoot multiplication of Sabri banana cultivar and found that MS medium supplemented with 5 mg/l each of BAP and kinetin produced the highest number of shoots/explant (3.11). Balakrishnamurthy and Sreenaggaswamy (1988) obtained a very high rate of multiple shoot induction from different cultivars i.e., Matti (AA), Robusta (AAA), Co-1 (synthetic hybride banana AAB) and Monthan (AAB) in MS medium supplemented with 0.025 % charcoal, 10 % coconut water and 5 mg L⁻¹ BAP.
2.1.8. *In vitro* rooting

*In vitro* multiplication of banana is normally carried in the presence of high cytokinin levels which inhibit root formation and elongation. Moreover during *in vitro* multiplication shoots may lack roots and are growing in the form of bunches which can not be transferred directly to field conditions. Prior to transfer to field conditions, individual shoots are separated from cluster and grown on root induction media. There are reports that roots can be induced without growth regulators (Albany *et al.*, 2005; Silva *et al.*, 1998) but most of the authors agreed with the inclusion of growth regulators for root induction.

The concentration of cytokinin in the rooting medium should be lower than auxins in the multiplication medium, so that cytokinin/auxins ratio becomes low which is favourable for root induction as reported by Gupta (1986) and Wong (1986). However, most of the investigators omit cytokinin entirely from the rooting medium. The most frequently incorporated auxins in rooting medium are NAA, IAA and IBA.

Rooting in micropropagation of *Musa* has been induced both in auxin supplemented and auxin free media. For example, NAA and IAA have been used as root-inducing hormones *in vitro* (Kshanika and Niranjali, 1997). On the contrary, Subramanya and Schwandes (1984), Cronauer-Mitra and Krikorian (1987), Novak (1992) and Bart *et al.* (1993) observed rooting in all *Musa* shoots cultured *in vitro* on auxin-free medium. It is worth noting that in rooting the shoots of *Musa in vitro*, much concern should be given to the formation of lateral roots (feeder roots) because these are primarily responsible for water and mineral uptake by the plants; hence dictating the initial establishment and survival of plantlets after transfer to green house conditions.
Hwang et al., (1984) regenerated roots from in vitro plants of *Musa sapientum* L. on MS medium to which 1 gm/l activated charcoal was added. After four weeks of incubation the plants developed numerous roots and were ready for transfer in the field. Nor-Aziah and Khalid (2002); Jalil et al., (2003); Srangsam and Kanchanapoom (2003) used activated charcoal in MS medium for in vitro rooting of banana plants. A study conducted by Hamide and Mustafa (2004) found Charcoal alone better for rooting than auxin treatments or MS medium alone. They also reported that supplementation of 2 μM TDZ, and 1 μM IAA or 20 μM BAP and 1 μM IAA on MS medium, followed 5 g l⁻¹ charcoal at the rooting stage were the best combinations for the in vitro propagation of banana types.

Devi and Nayar (1993) reported that roots were induced within 4-5 days of culturing single shoots on MS medium containing 0.25 % charcoal and 0.1 μM L⁻¹ IBA.

Buah et al., (1998) studied the effects of auxin added to the culture medium on main and branch root formation of banana (*Musa spp.*) shoots and growth characters of the plantlets rooted on the medium with and without auxin. Banana shoots cultures in vitro on Murashige and Skoog medium supplemented with 2 μM 1-naphthylacetic acid (NAA), rooted earlier also had more adventitious roots than those cultures on the medium without NAA. However, the adventitious roots formed on the medium without NAA showed more lateral branching. Plant height and number of leaves per plantlets in in vitro culture were not influenced by the addition of NAA but under nursery conditions, plantlets rooted without NAA showed better growth in terms of days to the appearance of new leaf, plant height and number of leaves per plant.
Viehmanno et al., (2007) compared the influence of growth regulators on the root induction of the *Musa* genus plants cultivated within *in vitro* conditions. They used different concentrations of growth regulators (naphthaleneacetic acid, indole-3-acetic acid, 6-benzylaminopurine, 2,4-dichlorophenoxyacetic acid) and MS and half concentrated MS media without addition of growth regulators as control ones. The induced roots were evaluated in *in vitro* and *ex vitro* conditions and the amount and length of the roots were also evaluated. They reported that the most of the roots were created by using naphthaleneacetic acid (5.4 μM), but the longest roots provided the control variant (MS medium). After 7 weeks of the transfer to *ex vitro* conditions the plants that were growing on medium with addition of indole-3-acetic acid have the best vitality and root absorption.

Mukunthakumar et al., (2011) tried different organic compounds for the formulation of ideal proliferating media and better results were obtained with the media containing 0.1% activated charcoal (AC) that promotes the development of robust plants. The AC treated plants showed better rate of establishment in the field (87 % for shoot tip derived cultures and 93 % for inflorescence derived cultures and hence suggested AC as a requirement for the generation of healthy plantlets having enhanced field establishment potential.

Cronauer and Krikorian (1984b) reported no differences in the root-inducing effects of NAA, IAA or IBA in presence of 0.025% (w/v) activated charcoal, however they obtained rooted plantlets by treating with NAA (1.0 mg L⁻¹) and activated charcoal (0.02%). Murali and Duncan (1991) observed that basal medium supplemented with 1.0 mg L⁻¹ IBA induced root in micropropagated shoots of banana.
Indole butyric acid (IBA) is frequently used for root induction of *in vitro* raised banana plants. Dore-Swamy *et al.*, (1983) first time used IBA during banana tissue culture. Banerjee and Sharma (1988) achieved rooting on semi-solid medium with 0.2 mg/l IBA during plant regeneration from long-term banana cultures. Muhammad *et al.*, (2000, 2004) and Habiba *et al.*, (2002) regenerated roots on half strength MS medium having 1 and 2 mg/l IBA respectively.

Molla *et al.*, (2004) reported that a good number of healthy roots were produced on half MS medium containing 0.4, 0.5, or 0.6 mg/l IBA. Uddin *et al.*, (2006) also reported that *in vitro* proliferated shoots produced roots with maximum frequency (90%) in half strength of MS medium fortified with 0.5 mg L$^{-1}$ IBA. Nandwani *et al.*, (2000) also found 1.0 mg/l IBA suitable in MS medium during mass propagation of Basrai. Madhulatha *et al.*, (2004, 2006) used IBA and NAA in combination during optimization of liquid pulse treatment for production of *in vitro* rooted plants of cv. Nendran (*Musa* spp. AAA). Khanam *et al.*, (1996) observed root initiation within 3-4 days with 2 μM L$^{-1}$ IBA in banana cv. Amritsagar.

Ali *et al.* (2011) found that rooting of well developed *in vitro* shoots on MS medium supplemented with 1.0 mg/l IBA+ 0.5 mg/l NAA produced 3.6 roots per plant after 6.8 days of inoculation into rooting medium with an average root length of 2.4 cm.

Naphthalene acetic acid (NAA) was another auxin used frequently at lower concentrations for root induction of *in vitro* raised banana plants. Cronauer and Krikorian (1984) found 1 mg/l NAA to be satisfactory in *Musa* textiles and AAA and AAA bananas respectively. Arinaitwe *et al.*, (2000) achieved rooting on MS medium containing 1.2 μM NAA during the study of proliferation rate effects of cytokinins on Kibuzi, Bwara and Ndizwemiti banana cultivars. Rahman *et al.*, (2004) used different
concentrations of NAA for root induction of *Musa sapientum* and found that 2 mg/l was better.

Jasrai *et al.*, (1999) used 0.1 μM IAA on MS medium during the study of *ex vitro* survival of *in vitro* derived banana plants without green house facilities. For the induction of roots in *in vitro* raised shoots of banana, De Langhe (1985) and Novak *et al.* (1990) used half strength MS + 1.0 mg/l IBA, whereas Cronauer and Krikorian (1984a) used auxin-free MS medium for rooting of banana microshoots. On the other hand, Banerjee *et al.* (1986) and Azad and Amin (2001) obtained rooted banana shoots in half strength MS agar-gelled medium supplemented with 0.2 mg/l IBA. Akbar and Roy (2006) reported 1.0 mg/l IBA for best rooting response of *in vitro* cultured plants. Research works of Nandi and Chaudhury (1997) found that MS medium supplemented with 5.0 mg L⁻¹ IBA was the best medium for root differentiation in dwarf cultivars Basrai and Shrimanti.

Kumar *et al.* (2012) studied the effect of IBA, IAA and NAA with different concentrations (0.2 mg/l to 2.0 mg/l) either alone or in combinations on *in vitro* rooting in banana cultivar Malbhog. They found that ½ strength MS + 1.0 mg/l IBA + 0.5 mg/l IAA induced roots among 90% of the explants after 12th days of inoculation. They also reported that increase in the concentrations of the above two auxins had no promoting impact; rather the number of roots were reduced. Similarly IBA (1.5 mg/l) alone induced rooting but the percentage response was reduced (66%) and time of initiation was increased (18th days). They also found that lower concentration of IBA (0.5mg/l) + 0.5mg/l IAA had no promoting impact and about 70% of the explants responded in this after 15th days of incubation. Higher concentration of IBA (1.5mg/l) + 0.5mg/l NAA,
revealed reduced rate of response, similarly IBA at its different concentration revealed no better response with NAA at 0.2 or 0.5mg/l.

2.1.9. Acclimatization /Hardening of in vitro raised banana plants

The special conditions during in vitro culture result in the formation of plantlets of abnormal morphology, anatomy and physiology. After transferring the in vitro raised plantlets to ex vitro condition, these plantlets might easily be damaged by sudden changes in environmental conditions and this necessitate a period of acclimatization to correct the abnormalities. After rooting, the in vitro regenerated plantlets are ready to be transferred from the aseptic containers into pots or polybags. Factors that should be considered in transplantation are infection and desiccation. Sterilizing the soil mixture eliminates serious infection problems. Desiccation is the last major block to be conquered in order to reach the goal of micropropagation. To compound the problem, the xylem tissue in the regenerated plants forms a closed system across the base of the shoot prior to root formation. The de novo formed roots, arising from callus, have poor connection to the main vascular system of the shoot (Grout and Aston, 1977). A period of humidity during acclimatization is required for newly transferred plantlets to adapt the outside environment. During acclimatization, humidity is gradually reduced over a period of 2 to 3 weeks.

Micropropagated plants are delicate in nature because they are produced in a closed, sterile environment and grown on nutrient-rich artificial media under controlled conditions with high humidity and low light intensity. The transfer of rooted plantlets from aseptic culture conditions to the external environment can result in significant losses of plants. When removed from the tissue culture environment, micropropagated
plants must be allowed to adjust to the outside environment with its varying light levels, changing temperature, reduced humidity, lower nutrient availability, and pathogen presence.

Tissue cultured plants are generally poor in cuticle, therefore lose water rapidly upon transfer to natural conditions. Moreover, due to limited space and presence of excess carbon source, their photosynthetic apparatus are not fully developed and their energy demands are met by reserves of starch accumulated during culture. These reserves thus deplete creating emerging crisis during hardening.

Banana plantlet (Musa sp.) acclimatization can be divided into two phases. In the first, *in vitro* plantlets are transferred to controlled environments (greenhouse or box shade, under the conditions of 20 to 28°C temperature, 80 to 90% RH, and 70% shade cloth) for a three to six-week period. In the second phase, plantlets are shifted to trays, pots or polybags, under 50% shade, in a temperature range from 18°C to 34°C, and a relative humidity higher than 75%, for a gradual hardening (Souza et al., 1997; Hoffmann, 2002).

### 2.1.9.1. Primary hardening

Primary hardening will take at least 4 weeks depending upon the climatic conditions. In final week the plants are gradually exposed to 50% shade and the plantlets are sprayed with plant protection chemicals and water soluble nutrient solution.

### 2.1.9.2. Secondary hardening

Primary hardened plants after 4 to 5 weeks are transferred to Poly bags of suitable size. Soil mixture is prepared by mixing sand, soil and farm yard manure in suitable
proportion (1:2:1 or 1:1:1 ratio). The plants are kept in these Poly bags for 6 to 8 weeks under 50% shades. Humidity is maintained around 60% to 70% and regular foliar sprays of plant protection chemicals and water soluble fertilizers are given. Deformed plants if any are discarded at this stage. The plant with 5 to 6 opened leaves and almost 1 foot in height are ready for field plantation.

The effect of triazoles on *in vitro* hardening and acclimatization of banana regenerated from floral apices was studied by Murali and Duncan (1995). Banana shoots were grown on culture media with 0, 1, 2 or 4 mg/l of triazoles (triadimefon or uniconazole) for one month. The resulting plantlets were transferred to a peat moss and sand (1:1, v/v) potting mixture. *In vitro* triadimefon treatment (2 mg/l) acted as a conditioning agent and obviated the need for hardening or weaning the plantlets. Triadimefon treated plants were turgid and healthy as compared with control plants.

Growth and development of *in vitro* raised plants cv. Pioneira (*Musa* sp. AAA) during hardening was studied by Silva *et al.*, (1998). *In vitro* rooted plantlets were transferred to plastic bags containing organic substract. Different parameters of growth were recorded in green house, humid chamber, screen house, under tree canopy, humid chamber and field conditions. All treatments showed 100 per cent plantlet growth, except for the direct field planting (39.7 per cent).

Jasrai *et al.*, (1999) developed protocols for hardening of *in vitro* derived banana plants without greenhouse facilities. *In vitro* raised plants were transferred in polythene bags which were perforated six cm from the base. The bags containing the plants were placed inside a plastic tray. High humidity was maintained by spraying water after every two hours. On an average 92 per cent of the plantlets survived.
A study conducted by Shiragi et al., (2008) recorded the highest plant survival rate 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).

Gitonga et al. (2010) successfully acclimatized the in vitro propagated banana plantlets using rice husks and then transplanted into the potted soil in the shade net. Eighty three percent (83 %) of the plants survived during the acclimatization procedure when rice husks were used compared to 80% using conventional approach (use of vermiculite).

Scaranari et al. (2009) evaluated the development of banana plantlets during acclimatization under a full light condition including covered surfaces with red shade cloth (70%, 50%, and 30% shade) and black shade cloth (50% shade), both under a transparent plastic film of 100 mm under controlled temperature, relative air humidity, irrigation, and nutrition conditions. They recorded the physical and physiological parameters at various stages in the greenhouses after three, six, and nine weeks and also after seven weeks of transplanting to field conditions. Combined results indicated superior outcomes of plantlets maintained under black 50% shade cloth for nine weeks, both in the summer and winter seasons. Similar results, but in a shorter time, were obtained with plantlets cultivated under red 70% shade cloth, for six weeks in the summer.

Molla et al., (2004), reported that plantlets transferred to plastic pots after 15-20 days in vitro culture on 0.4-0.6 mg L⁻¹ IBA concentration with 7 days hardening at room temperature showed 95-100 % survival.

Ali et al., (2011) reported that pure peat moss and pure sand when used alone did not give good result and this might be due to the reason that pure sand has very poor water
holding capacity which affects the relative humidity of the plant, whereas in pure peat moss has very high water holding capacity. This again create problem for the plants.

Gitonga et al., (2010) conducted an experiment to examine the cost economics of conventional tissue culture chemicals, equipment and facility with the alternatives available in Kenya and found that use of a shade net instead of a greenhouse with elaborate controlled conditions for the acclimatization of in vitro regenerants reduced the associated costs by 94%.

Vasane and Kothari (2006) conducted study with regard to optimization of secondary hardening process of banana plantlets and found that plants performed well in bags of 23 x 20 cm size and were almost at par with plants in bags of 20 x 16 cm size. They also highlighted the role of potting mixture in the growth and development of plants in the nursery stage and found press mud cake mixed with soil as the optimal medium for producing sturdy plant during the secondary hardening process of banana plantlets and potting mixture without any organic matter in soil showed poor growth of plants.

Environments with 50 to 60% shading for three to six weeks and with a fine atomization system with water during the first week are required in the first phase, decreasing by 30 to 50% in the second phase (hardening) during acclimatization of micropropagated banana plantlets cv. Grande Naine (Marie, 1995).

Azad and Amin (1999) stated that rooted plantlets of banana cv. Sabri produced in vitro were successfully established on potting mixture containing sand, soil and compost at 1:1:1 ratio. They also mentioned that survival of the plantlets under ex vitro condition was 80%. Cronauer and Krikorian (1984a) mentioned that in vitro induced rooted shoots of four banana cultivars were successfully established on potting mixture
containing soil and vermiculite at 1:1 ratio and also stated that survival from culture vessel to soil was 100%.

2.1.10. Sucker management in banana

One of the major problems of the banana industry is an effective sucker management to sustain high banana production in 4-6 years life span of the plantation. Maintaining two suckers give significantly superior yield of the plant crop. Increasing the number of sucker results in a corresponding reduction in the yield of the plant crop. Desuckering serves three purposes

- Selecting the best follower suckers and preventing competition,
- Conserving the homogeneity of plant layout in the field,
- Maintaining the same number of plants per hectare.

Tissue culture plants grow suckers very soon after planting. Two to five can be seen at the surface of the soil in the second month. The suckers grown by bananas micropropagated in vitro do not grow in a particular direction. Even if they are of different physiological ages, it is impossible in most cases to identify a sucker that is dominant in relation to the others. The first series of suckers grow deeply around the mother plant with their insertion points beneath the mother corm. The connection between mother and daughter is narrow (2 to 4 cm in diameter); it soon reaches its final diameter and does not grow with the sucker. The sucker insertion point does not move when the mother plant grow. The suckers are progressively covered by the mother corms and buried. Desuckering is carried out to ensure that the number of bunch bearing plants is maintained at a level which stops competition for water, light, and nutrients and sustains substantial yield and also to maintain a balance between plants.
vegetative growth and yield. For desuckering purpose a special desuckering spade or knife is used. The sucker pseudostem is cut off near its corm and the sharp point of the knife or spade is twisted in the growing point, thus killing it. During operations care should be taken not to harm the daughter sucker.

The yield per banana plant of the first ratoon crop decrease with increase in the number of suckers maintained. If all of the suckers which arise from the stool are allowed to grow, bunches will be small and of poor quality and some may not bear fruits at all (Seifu, 2003). Therefore, sucker management is very important to get better yield.

Hidoto (2009) conducted an experiment to study the effect of sucker management on banana yield and reported that there was a highly significant growth and bunch weight difference due to sucker management. One sucker per hill out-yielded (42.7 t ha⁻¹) the other treatments of two suckers per hill (35.4 t ha⁻¹) and the un-removed plant yielded the least (26.3 t ha⁻¹). The overall result showed that farmers growing banana could use one to two suckers to get good yields.

2.1.11. Field performance of tissue culture banana plants

Traditionally banana is propagated by suckers, but due to uneven size, weight and age of suckers, the sprouting of suckers is affected which leads to uneven growth, flowering and thus longer flowering span resulting delayed harvesting.

The advantages of in vitro micropropagated banana plants included higher rates of multiplication, production of disease free planting material, small space required to multiply the large numbers of plants, uniform flowering, better yield etc.

In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have shorter and more uniform production periods and produce higher yields
than conventional propagules (Zamora et al., 1989, Robinson et al., 1993) as in vitro micropropagated plantlets are cleaner and already possess an active root and shoot system at planting.

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

Optimum plot size for banana field trials as suggested by Nokoe and Ortiz (1998) was 16±3 plants per plot which were needed to evaluate the growth characteristics and yield potential of the cultivars. The recommended on an average optimum plot size for plant crop and ratoon crop were 13±3 plants per plot and 15±2 plants per plot respectively. Hwang et al., (1984) and Smith and Drew (1990) reported that tissue culture plants had bigger pseudostem and retained more healthy leaves than those originating from suckers.

Robinson et al., (1993) achieved 20.4 per cent higher yield than conventional plants, due to larger bunches and a shorter cycle to harvest. On the other hand in vitro derived plants of plantain (Musa spp. AAB) did not show any higher yield (Vuylsteke and Ortiz 1996) and more phenotypic variation was observed in tissue culture plants.

Vuylsteke et al., (1996) compared the field performance of in vitro raised variants with true-to-type plantains to evaluate their horticultural traits and significant variation was observed for plant and fruit maturity, leaf size, yield and its components, but not for leaf number, plant height, or suckering. Three of the four somaclonal variants were horticulturally inferior to the original clone from which they were derived. Only one variant which resembled an existing cultivar, out yielded the true-to-type clone.
However, its fruit weight and size were lower. Optimum plantlet size for tissue culture banana plants was studied by Fraser and Eckstein (1998). They reported that small plants of 100 mm took three weeks longer to harvest and had six per cent lower yields as compared to 300 mm size. Plants of 500 mm size showed slightly lower yield as compared with 300 mm plants. It was advisable that 200 mm plants should be planted at least 100 mm below soil surface, preferably in a furrow.

Cote et al., (2000) compared banana plants originated from embryogenic cell suspensions and plants produced by the conventional in vitro budding multiplication method. There were no significant differences between the plants produced by either micropropagation techniques for the plants height and circumference, the length of the reference leaf, the number of nodal clusters of the inflorescence and of fruits, the bunch weight, the period of time between planting and flowering, and between planting and harvesting. This study showed that banana plants with agronomical behaviour similar to those produced by the conventional in vitro budding method could be regenerated from embryogenic cell suspension.

Sheela and Nair (2001) conducted experiment to evaluate the growth, flowering and yield potential of tissue culture plants of Nendran banana Musa (AAB group) compared to plants from suckers. They found that the initial establishment and early growth phase of tissue culture plants were comparable with that of the suckers, but their growth rate become faster during the later stages and ultimately they recorded an increase of 6.7 per cent in height, 11.92 per cent in girth and 3.35 leaves more than the plants from suckers. They showed less variability in time taken for flowering and recorded an increase in yield of 25.63 per cent compared to plants from suckers. They reported the
attributes responsible for the increase in yield were length of bunch, number of fingers and length and girth of finger.

Patil et al., (2010) evaluated the field performance of in vitro propagated Grande Naine banana plants and compared the performance from 8th subculture with that of the 15th subculture plants. They observed that 8th subculture plants established and grew faster, taller and bigger than 15th subculture plants. They also found that plants of 8th subculture produced heavier bunches (25.158 kg for bunch) than the plants of 15th subculture (21.137 kg for bunch).

Buah et al., (2000) compared the field performance of in vitro propagated banana (Musa spp) plants with that of the sucker-derived plants and found that in vitro propagated plants established and grew faster, taller and bigger than the conventional sucker-derived plants. They also found that the in vitro propagated plants produced more leaves than the conventional sucker-derived plants and could be harvested earlier. The former also produced heavier bunches and fruit (15.2 kg for bunch and 200 g per finger of fruit) that the later (9.0 kg for bunch and 136.4 g per finger of fruit).

Blomme et al., (2008) did a comparative study of shoot and root development in Micropropagated and sucker-derived banana and plantain (Musa spp.) plants and found that plant height was similar for both types of propagules during the mid-vegetative stage with no significant differences detected at 16 and 20 WAP. However, at flower emergence in vitro-derived plants were significantly taller (12%) than sucker-derived plants. There was also a significant effect of propagule type on plant basal circumference at flower emergence although the corm weight of the in vitro-derived plants remained lower than sucker-derived plants. The superior height of the in vitro-derived plants was associated with a thicker pseudostem. These observations confirm
that Micropropagated plants grow more vigorously and are taller than those derived from conventional propagules. In their investigation they observed that sucker-derived plants have a denser root system, which did not result in better sucker development.

Esendugue et al., (2007) reported that the tissue-cultured plants consistently produced heavier bunch and higher yields than the conventionally-propagated plants. The highest yields (65.92 t·ha⁻¹ for Grand Nain, 64.60 t·ha⁻¹ for Williams and 65.58 t·ha⁻¹ for Zelig) were obtained for the tissue-cultured plants in the third crop cycle compared with 51.43 t·ha⁻¹ and 59.02 t·ha⁻¹ obtained for the traditionally-propagated Grand Nain and Williams plants, respectively. They also reported that there were no significant yield differences between the plants of different origin in the second and fourth cycles, respectively.

Daniells (1988) compared the growth and yield of tissue cultured banana cv. Williams (Lowe) with that of plants derived from suckers planted in north Queensland in October 1986. He reported that the tissue culture plants produced many more suckers than from conventional material and were usually quite uniform in size and these sucker characteristics were related to the greater number of leaves and associated buds that tissue culture plants had. Examination of corms of tissue culture plants near flowering revealed that they have many suckers coming from well underneath the bulkier part of the corm.

Badgujar et al., (2005) conducted an experiment to compare in vitro plants and conventionally produced suckers of Basrai banana. The observations on plant characters consisting growth, duration and yield revealed that, the tissue culture plants were more vigorous in growth and superior in yield compared to banana plants raised from conventional suckers. They recorded the increase in height and girth of
pseudostem by 16.90 and 26.30 per cent respectively in case of plants raised through tissue culture. They also observed that the total number of leaves (36.48) per plant produced by plants raised through tissue culture were significantly more than those raised by using conventional suckers (34.63). Reduction in total crop duration and increase in bunch weight (31.37 per cent) due to increase in number of hands and fingers per bunch was also recorded in plants raised through tissue culture.

Msogoya et al., (2006) conducted a study to evaluate the yield performance of micro-propagated (MPd) East African cooking banana (Musa AAA East Africa) cv. Uganda and cv. Bukoba in the Eastern zone of Tanzania and recorded more uniform fruits in terms of weight, length and girth in case of MPd cv Uganda and uniform fruits in terms of weight and girth in case of MPd cv Bukoba.

A comparative study was conducted by Kawit et al. (1993) on the performance of tissue culture propagated bananas and conventional sucker planting material of 16 banana cultivars in Thailand. The results pointed out that tissue culture plants could be used in commercial production in Thailand with some advantages.

An investigation on field evaluation of tissue cultured banana in South Eastern Queensland was carried out by Drew and Smith (1990). They observed that tissue cultured plants established more quickly were taller and had a shorter time to bunch emergence than the conventional planting materials. They had significantly higher yields in term of bunch weight, which was a function of greater numbers of fingers and hands.

Gupta (1986) reported that the survival of plantlets on transfer from in vitro cultures to soil was more than 95%. He also noted that the meristem derived plants grew faster and facilitated early harvesting compared to conventionally propagated suckers. Moreover,
plants height at maturity and fruit productivity were almost equal among the plants of both origins.

Singh and Bhattacharyya (1992) conducted a study on the phyllochron of the six leading banana cultivars of North East India and 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 'Malbhog' had 9.9 and 10.4 respectively. Their report showed a higher phyllochron of 12.0 and 11.7 respectively in the ABB cultivars 'Manohar' and 'Kachkal'. Their study revealed that the presence of the B genome tended to increase the phyllochron and the development cycle durations were proportional to the phyllochron.

2.1.12. Shoot growth

In an experiment of different numbers of functional leaves on plant height and girth, it was observed that the plant height at shooting was highest on plants with all the leaves retained, and girth was highest on plant with 12-18 leaves (Pillai and Shanmugavelu, 1978). Ahmed et al., (1974) reported that pseudostem base girth was related to plant height, which was in the order ‘Sabri’, ‘Champa’, ‘Amristsagar’ and ‘Basrai’. According to Stover (1979) the average rate of leaf emergence ranged from 8.3 to 12.8 days per leaf depending on season and number of leaves produced.

2.1.13. Flowering

Dumas (1955) reported that a certain energy level is required for flowering and the nourishment of the fruits, the level is quickly attained by few leaved plants, but less active plants have to produces extra leaves before reaching it.
Pillai and Shanmugavelu (1978) observed that plants retained with lower number of leaves (6 to 9) delayed shooting whereas early shooting were recorded in plants with higher number of leaves (12 to 18). For commercial production of banana fruit, a minimum of eight leaves is needed for proper bunch maturation, with nine to 12 leaves being ideal (Fonsah and Chidebelu, 1995; Robinson, 1996; Stover and Simmonds, 1987; Waddick and Stokes, 2000).

Sheela and Nair (2001) conducted experiment to evaluate the growth, flowering and yield potential of tissue culture plants of Nendran banana Musa (AAB group) compared to plants from suckers. Their study revealed that tissue culture plants were superior in performance over the conventional suckers exhibiting vigorous vegetative growth, increased yields and significant uniformity in flowering, compared to plants produced from suckers. The enhanced growth rate exhibited by tissue culture plants did not delay flowering and showed less variability in the time taken for flowering under the same treatment and the plants were able to complete flowering in 9.8 days earlier compared to plants from suckers as recorded in their study.