IN VITRO PROPAGATION OF RED BANANA *Musa acuminata* (AAA) cv. RED DACCA

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IN VITRO PROPAGATION OF RED BANANA

Musa acuminata (AAA) cv. RED DACCA

A thesis presented by

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to

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The thesis attached hereto, entitled **"*In Vitro* Propagation of Red Banana *Musa acuminata* (AAA) cv. Red Dacca"** was prepared under the direction of chairperson of the candidate's supervisory committee and has been approved by all members of that committee and board of examiners as partial fulfilment of the requirements for the degree of **MASTER OF AGRICULTURAL SCIENCE (HORTICULTURE).**

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This thesis represents the original work of the author, except where otherwise stated. It has not been submitted previously for a degree at any University.

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DEDICATED TO MY BELOVED PARENTS,

DR. TIN HTUT AND DAW MAW MAW SOE

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In Vitro Propagation of Red Banana *Musa acuminata* (AAA) cv. Red Dacca

Dr. Khin Thida Myint Htet Aung Htut

ABSTRACT

The experiments were carried out at the Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University from June 2011 to July 2012, in order to investigate the optimum plant growth regulators (PGRs) concentrations for different stages *in vitro* propagation of red banana *Musa acuminata* (AAA) cv. Red Dacca. Two experiments were employed in randomized complete block design (RCBD). Murashige and Skoog (MS) 1962 was used as basal medium throughout the study.

In both initial culture and multiplication stages (experiment 1), the examination of the effect of different cytokinins application on multiple shoot formation was conducted. The treatments were four levels of 6-benzylamino purine (BAP) (2.5, 5.0, 7.5, 10.0 mg· L^{-1}) and three levels of Thidiazuron (TDZ) (0.05, 0.15, 0.25 mg· L^{-1}). In initial culture stage, the explants were inoculated in liquid medium with cotton culture for 2 weeks, then transferred to semi-solid medium for 6 weeks and incubated at 25 ± 2°C under 16 hrs photoperiod of 25 µmol$m^{-2}s^{-1}$ light intensity. In multiplication stage, shoots resulted from initial culture were treated with TDZ 0.15 mg· L^{-1} in two subcultures (S1, S2) and the cultures were incubated in continuous darkness condition throughout the 6 weeks period of each subculture. Survival percent, normal shoot formation percent and days to induce new shoot were collected for both culture stages. The data on number of shoots, shoot length and shoot fresh weight were collected after each subculture. In rooting stage (experiment 2), six PGRs treatments (0.5 and 1.0 mg· L^{-1} each of NAA and IBA, 0.5 and 1.0 mg· L^{-1} of NAA in combination with 0.5 mg· L^{-1} IBA) were given in half MS medium. The PGRs treatments were given in two ways for rooting. One group was cultured on PGRs free medium for 2 weeks prior to transfer to rooting medium (M1) and another group was directly onto rooting medium (M2). Only half MS medium without plant growth regulators was employed as control. The cultures were performed on a culture condition the same to initial culture stage. Number of root, root length, root diameter and plant height were recorded in second experiment. After 8 weeks of culture, plantlets were acclimatized and their survival percent was

collected in *ex vitro* condition. *In vitro* rooted plantlets were planted in wooden tray containing 2:1 compost and sand mixture and maintained under fully shaded condition at 27 ± 2℃ with 80 % relative humidity for 2 weeks followed by net house condition at 32 ± 2℃ and 45 ± 2% relative humidity for 4 weeks.

In experiment 1, 0.15 mg· L^{-1} TDZ produced 100 % survival percent, 70.58 % normal shoot formation percent and 2.25 number of shoot per explant during initial culture. Both BAP and TDZ concentration treated in initial culture increased number of shoots, shoot length and shoot fresh weight by two times in S1. In S2, numbers of shoots and shoot length continuously increased, but shoot fresh weight declined in comparison to S1.

In experiment 2, 1.0 mg· L^{-1} NAA (M1) gave maximum number of roots (17.00), and combination of 1.0 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA (M2) produced the highest number of roots (18.16). While application of auxins resulted in reduced root length and increased root diameter, control treatment provided the longest root length and decreased root diameter. In acclimatization stage, 100 % survival was observed in control treatment, NAA and IBA in M1 and combination of NAA and IBA in M2.

Key words: BAP, TDZ, NAA, IBA, acclimatization

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIX

CHAPTER I

INTRODUCTION

Bananas are native to Southeast Asia and evolved from interspecific and intraspecific hybridization between two wild diploid species of the genus *Musa*, i.e., *sp. acuminata* (AA) and *sp. balbisiana* (BB) (Simonds and Shepherd 1955). *Musa* is one of the two genera in the family Musaceae under the order Zingiberales, the other genus being *Ensete.* Depending on the contribution of *Musa acuminata* and *Musa balbisiana*, the cultivars have been classified into genomic groups (AAA, AAB, ABB, BB, AB, BBB, AAAA and ABBB). The genus *Musa* has about 40 spp. of perennial, stooling or rhizomatous herbs with their center of diversity and probable origin in the Assam-Myanmar-Thailand area. They are mainly plants of the lowland tropics which require high temperatures, high humidities and high light intensities (Rachel 2003).

Bananas and plantains (*Musa* spp.) are two of the world's major fruit crops. They are the staple food for millions of people in the tropics (Bakry et al. 2009). Majority of the edible bananas are triploid and are propagated vegetatively by suckers. The fruits, parthenocarpically produced, are eaten raw, cooked, brewed into an alcoholic beverage or processed into chips. The leaves are used for wrapping food, the terminal bud of the inflorescence cooked as vegetable, fibers used for ropes and corm tissue (*Ensete* spp.) fermented. The top five banana-producing countries are India, Equador, Brazil, Uganda, and the Philippines. Bananas are the world's fourth most important food crop after rice, wheat and maize. Among tropical fruits, banana plays the second most vital role next to mango. They are cultivated in the tropical and sub-tropical areas all over the world; production area is 4,843,000 ha with a yearly production of approximately 95.60 million tons and 19.74 t/ha (FAO 2011).

In Myanmar, banana is important not only as a food crop but also for religious rites and traditional symbol. The total banana production area is 179,946 acres and yield is 1,059 bunches per acre in Myanmar (DOA 2011). It accounts for 11.64% of total sown area in this country. All states and divisions of Myanmar produce banana. Bananas are available year round in Myanmar. The main theme of banana rapid multiplication essentially depends on the improvement of tissue culture technology. (Sein Hla Bo 2003).

Red banana ‘Red Dacca’ possesses large amounts of calories and also more vitamins C and B6 than yellow banana. Many pests and diseases threaten banana production and have resulted in the application of high quantities of pesticides with serious consequences to the environment. Because of its high degree of sterility and polyploidy nature of the edible banana varieties, classical breeding is difficult. In order to argument conventional breeding and to avoid constraints imposed by some pests and pathogens, transgenic and *in vitro* approaches are being considered (Tripathi 2003). Banana shoot-tip cultures were most suitable for micropropagation for large scale production.

Bananas are propagated by means of young "sword sucker"– 50 to 60 cm tall, which bears only long, narrow leaves, or by rhizome pieces cut from old stools. This traditional technique produces a relatively good yield of plants, but cannot match the speed and quality of meristematic culture. Sword suckers are the preferred types of propagule since they produce fruit more quickly than small suckers or pieces of the corm (Simmonds 1966). Consequently meristems are obtained and excised from suckers and rhizomes using the appropriate techniques. Tissue cultured plants perform better than suckers in most of parameters i.e. weight and size of the fingers, weight and number of hands (Faisal et al. 1998).

Banana yield tends to fall from three to five years after planting and declines rapidly after ten to fifteen years. For existing yield to be maintained, a cyclical process of replacement of old for new plants should be undertaken. Plants in a single-cycle plantation have strong vigour and high yield potential because of the juvenile nature of the material and their photosynthetic efficiency. Single cycle consists of the micropropagation of banana shoot tip tissues and the transfer of plantlets from *in vitro* culture to the nursery for acclimatization, and then they are grown out to field planting site. Micropropagation of bananas and nurseries are now present in most banana exporting countries (FAO 2003).

In vitro multiplication of banana plantlets is an excellent alternative and a number of countries in the world, such as Israel (Isaraeli et al. 1995), France (Cote et al. 1990), Australia (Drew and Smith 1990), Cuba, many African countries (Vuylsteke 1998) and the Philippines (Agustin and Molina 2002), are using this technique.

Plant growth regulators are inevitable for *in vitro* regeneration of crop plants in any artificial medium. Generally, cytokinins helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of banana and culture conditions (Cronauer and Krikorian 1984).

The Thidiazuron is a phenylurea, which is very effective even at very low concentrations, basically due to phenylurea's relative tolerance to endogenous cytokinin oxidases (Arinaitweet al. 2000), along with the ability to promote endogenous cytokinin production, through the inhibition of cytokinin oxidases activity (Magioli et al.1998). It is necessary to search for a more active cytokinin than 6-Benzylaminopurine (BAP) to improve the efficiency of the banana micropropagation system due to rapid increase in production cost (Lee 2001).

Micropropagation of plantain using meristem tip culture has shown low level of success, mainly due to oxidation and low proliferation caused by a high degree of apical dominance. *In vitro* propagation through shoot-tip culture has shown low level of success in some banana varieties, mainly due to oxidation caused by phenolic compounds that form a barrier around the tissue, preventing nutrient uptake, hindering growth and highly reactive and toxic to plant tissue (Strosse et al. 2004). The important factor affecting the efficiency of micropropagation system is the rate of multiplication. It has been observed that banana multiplication rate is genotypic dependent as well as variable behaviour has been observed among cultures initiated from same banana genotypes cultured *in vitro* (Israeli et al. 1995 and Mendes et al. 1996).

In vitro multiplication of banana is normally carried out 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 cannot be transferred directly to field conditions. The concentration of cytokinin in the rooting medium should be lower than auxins in the multiplication medium, so that cytokinin/ auxin ratio becomes low, which is favourable for root induction. There are reports which mentioned that roots can be induced without growth regulators but most of the authors agreed to the inclusion of growth regulators for root induction (Rahman et al. 2006; Viehmannova et al. 2007; Al-amin et al. 2009 and Olivia et al. 2010).

The process of acclimatization is not unique to micropropagation as clonally propagated cuttings are also often acclimatized prior to field transfer. However, in the case of *in vitro* raised plantlets, it becomes obligatory to acclimatize because they are not adapted to harsh *in vivo* conditions. Generally, *in vitro* conditions which promote rapid growth, shoot proliferation and plantlet development result in certain abnormal plant characteristics. In fact, ultimate success of micropropagation technology, either for academic or commercial purpose, depends largely upon the successful control over transplanting plantlets with high survival rate. It may again be emphasised that, little attention has been given to standardize the process of acclimatization (or hardening) of micropropagated plantlets. Quite understandably, the process of acclimatization continues to be major bottlenecks for ensuring the successful field transfer (Singh et al. 2002).

Success of clonal propagation may depend largely on several factors such as explants materials, proper basal medium, plant growth regulators, culture conditions, physiological conditions of the donor plants, etc. Therefore, to find out suitable ways for *in vitro* propagation of red banana, this study was carried out with the following objectives:

1. To compare the effect of BAP and TDZ on initial culture of red banana in *in vitro* propagation
2. To observe the effect of TDZ on subsequent multiplication
3. To find out the suitable way for *in vitro* rooting after subsequent multiplication by TDZ
4. To observe the adaptation of plantlets raised by different regime throughout the regeneration procedure

CHAPTER II

LITERATURE REVIEW

Banana tissue culture was first reported by Berg and Bustamante (1974) who studied that banana meristem stem culture combined with thermotherapy for the production of virus free plants. Ma and Shii (1972) used shoot tips of Cavendish (AAA) dissected from suckers as a way to rapidly multiply planting material free of *Fusarium* wilt. Banana micropropagation has since then been applied to a wide range of varieties (Vuylsteke 1998) and worked on different aspects of banana tissue culture as enabling tool for maximizing banana production (Smith et al. 2005).The first major field plantings of tissue culture banana plantlets were undertaken in Taiwan and Jamaica in the early 1980s (Hwang et al. 1984; Ogelsby and Griffis 1986).

2.1 Explant Source for Banana Micropropagation

Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically all parts of a plant have been used successfully as a source of explants (Ahloowalia et al. 2004). Banana micropropagation has been induced from a range of explants, including meristems (Dhed'a et al. 1991; Ganapathi et al. 2001), corm tissue (Novak et al. 1989; Navarro et al. 1997), leaf bases (Novak et al. 1989), immature zygotic embryos (Escalant and Teisson 1989), and immature male and female flowers (Shii et al. 1992; Escalant et al. 1994; Cote et al. 1996; Ganapathi et al. 1999 and Grapin et al. 2000). However, banana shoot tips are the most commonly-used explants for banana micropropagation (Nisyawati and Kariyana 2013). Shoot tips and meristem tips are perhaps the most popular source of explants to initiate tissue cultures (Ahloowalia et al. 2004). Shoot tips and dormant buds taken from independently growing plants survive at a higher frequency and show greater physical stability than shoot tips taken from *in vitro* cultures (Withers 1990).

2.2 Surface Sterilization of Explant

Microbial contaminations are the major hurdle to the initiation and maintenance of viability in *in vitro* culture. Field-grown plants are liable to be contaminated with microorganisms, which must be disinfected before explants are transferred to *in vitro* condition. Surface sterilization of underground modified stem is reported by Goswami and Handique (2013). Different sterilization procedure and chemical disinfectants are proposed by several *in vitro* experiments. Sodium hypochloride is the most commonly, used disinfectant for *in vitro* surface sterilization

of banana explants (Muhamad et al. 2004; Srangsam and Kanchanapoom 2007; Farahani et al. 2008 and AL-amin et al. 2009). Low concentration of mercuric chloride substituted for sodium hypochloride is reported by Molla et al. 2004; Titov et. al 2006 and Goswami and Handique 2013. Goswami and Handique 2013 stated that 1 % sodium hypochloride for 15 minutes and 0.1 % mercuric chloride for 7 minutes give the highest percentage of contamination-free healthy culture. Double disinfection method has been adopted by Jafari et al. 2011, in which Clorox followed by mercuric chloride. Double sterilization with NaOCl (3.5%) and Tween 80- first time for 15 minutes and after five rinsing with water and second time for 5 minutes sterilization- resulted in significantly lower contamination of 10 days old suckers of banana variety 'Williams' (Hamill et al. 1993).

Explants are treated with fungicide and antibiotics to minimize the contamination in *in vitro* cultures (Roy et al. 2010). Ethanol 70% has also been used for disinfection purposes (Farahani et al. 2008; AL-amin et al. 2009 and Chang and Shu 2013).

2.3 Initiation of Shoot Tip Culture

Shoot apical meristem, covered by leaf primordia that are supported on a small base of rhizome tissue, is cultured on medium intact or after incision or fragmentation into pieces (Mbanaso et al. 2006; Srangsam et al. 2007; Tripathi et al. 2008 and Jafari et al. 2011). Kanchanapoom et al. (2011) stated that sucker explant orientation on *in vitro* shoot tip proliferation was obtained with abaxial surface of buds lying down on the medium.

MS (Murashige and Skoog 1962) basal medium is the most widely used for micropagation of banana. Different strength of MS medium, frequently transfer to similar fresh medium, antioxidants and activated charcoal are used to eliminate phenol oxidation in *in vitro* propagation of banana. Chang et al. (2013) reported that browning rate of explants cultured on 1/2 MS or 1/3 MS was lower than that of those cultured on MS basal medium. Ascorbic acid combined with 1/2 MS or 1/3 MS reduced browning. Soaking of explants in an citric acid and ascorbic acid mixture prior to culture was effective in controlling the phenolic exudation (Dayarani et al. 2013). The blackish tissues on the explants were removed and then the meristematic tissues were transferred to similar fresh medium to control blackening after one week culture (Al-amin et al. 2009). Jafari et al. (2011) reported that, during the initial phase, cultures were subcultured three times to reduce browning due to the presence of

phenolic compound. Nisyawati and Kariyana (2013) studied on different concentrations of ascorbic acid and activated charcoal independently to reduce explant browning. Amendment of culture medium with anion exchange resins, cation exchange resins, polyvinyl pyrrolidone or activated charcoal did not reduce the disease incidence. 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 plantlet produced. Ascorbic acid was able to reduce the disease incidence by more than 60% (Ko et al. 2009). Farahani and Majd (2012) stated that liquid medium with cotton culture support was better in multiplication rates, 2.7 to 3.5 times higher than solid medium culture and immersion of explants in liquid medium.

The effect of light incubation conditions on shoot tip explant browning was studied by Nisyawati and Kariyana (2013). MS media supplemented with 1.6 mg· L^{-1} IAA and 4.0 mg· L^{-1} BAP in darkness gave the average of 10.4 shoots per explant. Makara et al. (2003) stated that dark conditions enhanced higher shoot proliferation rates than light conditions in some cultivars, suggesting that banana *in vitro* proliferation is a photomorphogenically responsive process that is enhanced under dark conditions.

2.4 Effects of Plant Growth Regulators on *In Vitro* Propagation of Banana

2.4.1 Shoot tip culture

Different plant growth regulators in various combination and concentrations are used for culture initiation. Some investigators initiated the cultures on the same growth regulators as later used for multiplication while other used high concentrations of hormones for culture initiation. Mendes et al. (1999) studied the dynamics of banana micropropagation rates was available at low concentration of BAP (2.5 mg· L^{-1}) used for in initial culture and then new shoots isolated every 4 weeks to proliferation medium which contains 4.5 mg· L^{-1} of BAP in cv. Macçã (AAB group) for six subculture. Silayoi (2001) observed that semisolid MS medium supplemented with 5 ppm BA proliferated 2 shoots per explant at 45 days after culture of Klaui Khai cultivar. BA 5 ppm continuously used until the seventh subculturing produced 7.14 shoots per explant. Chinsuk et al. (2001) reported that MS solid medium with 5 ppm BA induced 0.45 shoots and 7 ppm BA produced 0.50 shoots at 6 weeks of culturing in initial culture of cv. Kluai Bep. Average number of shoots per explant was 5.57 when supplemented with 5 ppm BA and 5.71 shoots with 7 ppm BA at subculture

seven. Muhammad et al. (2004) indicated that 5.0 mg· L^{-1} of BAP proliferated 124 plants from each shoot tip after five subculturings.

Jafari et al. (2011) observed that high concentration of BAP (33 µM) treated to shoot tip explants simultaneously increased formation of abnormal shoots and then the transfer to proliferation media (BAP 22 µM with 2 µM IAA) gave the highest number of shoots per explants, 20.25± 2.5 on cv. Beragan. Elhory et al. (2009) reported that 100 µM BAP in combination with 1.0 µM IAA treated to shoot tip explants and 5.0 µM BAP at subculture two gave the highest number of shoots (29.08) on cv. Tanduk.

Low concentration of BAP 2.0 mg· L^{-1} with MS agar medium was used for initiation of shoot tip culture and then transferred to high concentration of BAP (5.0 mg· L^{-1}) at multiplication of Red banana shoot tips (Jaisy and Ghai 2011).

2.4.2 Influence of plant growth regulators on shoot proliferation of banana

Arinaitwe et al. (2000) reported that cultivars differed significantly in their shoot proliferation responses to different TDZ concentrations and that TDZ is more cost effective than BA. TDZ is one of the most active phenylureas having cytokinin-like activities. It is stable and more active at lower concentrations than the adenine-type cytokinins (Mok et al. 1987). Huettman and Preece (1993) described that TDZ less than 1 µM induced greater proliferation of axillary shoots than other cytokinin. TDZ also exhibited the effect of suppressing the elongation of shoots which could return to normal after transferring to an elongation medium containing a lower TDZ level or a different cytokinin. Lee (2001) indicated that TDZ at 0.2 mg· L^{-1} almost doubled the multiplication rate of Cavendish cultivars than BA at 4.0 mg· L^{-1}. TDZ at 0.2 mg· L^{-1} slightly suppressed the elongation of shoots and shoots became stunted and clumps of globular buds occurred at the base of large shoots at 2.0 mg· L^{-1}. Gübbük and Pekmezcl (2004) reported that BAP below 20 µM or TDZ below 1 µM did not increase shoot proliferation. Both BAP and TDZ with IAA increased in better shoot proliferation and elongation rather than using alone.

Makara et al. (2003) reported the carry-over effect of TDZ in banana *in vitro* propagation and 22.2 µM BAP as control treatment. TDZ had a carry-over effect that enabled shoots to continue proliferating on a hormone free medium as the culture cycles increased more than BAP. Accumulation of TDZ to high level (13.14 µM) resulted in suppression of shoot proliferation. Youmbi et al. (2006) reported that shoot proliferation and plant development were better at low concentrations of TDZ (0.05 to

0.8 µM) in all AAA group, AAB group and ABB group cultivars. Shirani et al. (2010) described that the highest ratio of scalp formation (8.89) was obtained at TDZ (7.5 µM) in cv. Rastali (AAB group) and he also mentioned that TDZ increased scalp induction rate 4.22 at 0.5 µM and 2.67 at 5.0 µM respectively for cv. Berangan Intan and cv. Berangan (AAA) before falling to 2.11 for both at 7.5 µM. In contrast, BAP at 11.1 µM was optimal for scalp induction and 22.2 µM was considered optimal for shoot proliferation as well as shoot elongation from excised scalps of all banana cultivars.

Jaisy and Ghai (2011) reported high multiplication rate (3.0) was observed at 5.0 mg· L^{-1} BAP with 20 mg· L^{-1} ascorbic acid in Red Banana (AAA). The maximum number of shoots (3.8) was obtained at 60 days after inoculation from 5.0 mg· L^{-1} BAP with 0.5 mg· L^{-1} NAA in cv. Makoke (AAB) (Demissie 2013).

2.4.3 *In vitro* rooting

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

Naphthalene acetic acid (NAA) was used frequently at lower concentrations for root induction of *in vitro* raised banana plants. Arinaitwe et al. (2000) achieved good rooting of Kibuzi, Bwara and Ndizwemiti banana cultivars on MS medium containing 1.2 µM NAA. Rahman et al. (2004) used different concentrations of NAA for root induction of *Musa sapientum* and found that 2.0 mg· L^{-1} was better. Viehmannová et al. (2007) reported that 14.79 number of average roots per plant formed in *in vitro* plants of *Musa acuminata* (AAA) on MS medium supplemented with 5 µM NAA but the longest roots were recorded on the basal MS and ½ MS medium. Srangsam and Kanchanapoom (2007) observed that MS medium without growth regulators facilitated better rooting, elongation and healthy growth of roots and MS with or without activated charcoal had no effect on root induction in cv. Kluai Sa and cv. Kluai Leb Mue Nang (AAA).

Jasrai et al. (1999) used 0.1 µM IAA with MS medium during the study of *ex vitro* survival of *in vitro* derived banana plants without green house facilities. Al-Amin et al. (2009) reported that the highest number of roots and root length were obtained by 0.5 mg· L^{-1} IAA combination with 0.5 mg· L^{-1} IBA.

Indolebutyric acid (IBA) was effective for root induction of *in vitro* raised banana plants and frequently used for this purpose. Habiba et al. (2002) described that root formation on half strength MS medium having 1.0 and 2.0 mg· L^{-1} IBA respectively. Molla et al. (2004) reported that a good number of healthy roots were produced on half MS medium containing 0.5 or 0.6 mg· L^{-1} IBA. Nandwani et al. (2000) also found that 1.0 mg· L^{-1} IBA was suitable in MS medium during rooting of cv. Basrai. Madhulatha et al. (2004, 2006) used IBA in combination with NAA during optimization of liquid pulse treatment for production of *in vitro* rooted plants of cv. Nendran (AAA). Tripathi and Tripathi (2008) stated that 1.0 mg· L^{-1} IBA developed 100% of roots in two weeks. Full strength MS medium supplemented with 5.0 µM IBA produced the highest number of roots, while the highest mean root length was obtained from hormone-free half strength MS medium at 4 weeks of culture (Elhory et al. 2009). Roy et al. (2010) observed that MS supplemented with 1.0 mg· L^{-1} IBA produced 6 roots per explant in cv. Malbhog (AAB) within 2 weeks. Jaisy and Ghai (2011) stated that addition of charcoal at 2% concentration instead of hormones (IAA, IBA) showed 95% success rate in Red Banana (AAA).

There were reports that roots can be induced without growth regulators (Silva et al.1998 and Albany el al. 2005) but most of the authors agreed with the inclusion of growth regulators for root induction.

2.5 Hardening of *In vitro* Raised Banana Plants

The transfer of rooted plantlets from aseptic culture conditions direct to the external environment resulted 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 was not fully active resulting in depletion of carbon source during hardening. In the greenhouse, and especially in the field, irradiance is much higher and air humidity much lower than the water potential of media with saccharose. Therefore, after *ex vitro* transplantation, plantlets usually need some weeks of acclimatization by gradual lowering in air humidity (Preece and Sutter 1991; Kadleček 1997 and Bolar et al. 1998).

The effects of triazoles on *in vitro* hardening and acclimatization of banana regenerated from floral apices were studied by Murali and Duncan (1995). Banana shoots were grown on culture media with 0.0, 1.0, 2.0 or 4.0 mg· L^{-1} of triazoles (triadimefon or uniconazole) for one month. The resulting plantlets were then transferred to a peat moss and sand (1: 1 v/v) potting mixture. *In vitro* triadimefon treatment (2.0 mg· L^{-1}) acted as a conditioning agent and obviated the need for hardening the plantlets. Triadimefon-treated plants were turgid and healthy as compared with control plants.

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

Jasrai et al. (1999) developed protocols for hardening of *in vitro* derived banana plants without greenhouse facilities. *In vitro* raised plants were transferred into polythene bags which were perforated 6 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 % of the plantlets were survived.

Scaranari et al. (2009) reported that plantlets of cv. Granded Naine maintained under black 50% shade cloth for nine weeks gave superior outcome in both summer and winter seasons. Shorter time of acclimatization was obtained with plantlets cultivated under red 70% shade cloth for six weeks in the summer. Bitar and Mohamed (2009) stated that the best transplant growth and chlorophyll content of cv. Grande Naine were achieved by using (1:1) Coco fiber + Vermiculite and 100% Coco fiber. Transplant grown in 100% sand showed the least performance, but their growth improved in Sand + Vermiculite, Sand + Coco fiber and Sand + Rice Hull. Substrate components were mixed in a 1: 1 volume to volume basis. Azam et al. (2010) reported that the *in vitro* raised plantlets cv. Bari-1 with ramified roots were transferred for primary hardening for seven days and then acclimatized into polythene bags containing garden soil and humus (1:1) and two weeks after transplantation, 98% of plants survived and flushed new leaves.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Site

The experiment were carried out at the Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University (YAU), from 2011 June to 2012 July.

3.2 Plant Materials

Musa acuminata (AAA) cv.'Red Dacca' was used as a test plant. For the sucker collection, a "Kyaukthanbat" farmer's field in Nay Pyi Taw area was selected.

3.3 Culture Media

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used as basal medium. The pH of the medium was adjusted to 5.8, and the medium was solidified with commercially available agar (3 g· L^{-1}) and added 30 g· L^{-1} of sucrose as carbon source. Liquid medium with cotton culture support was used for 2 weeks in initial culture.

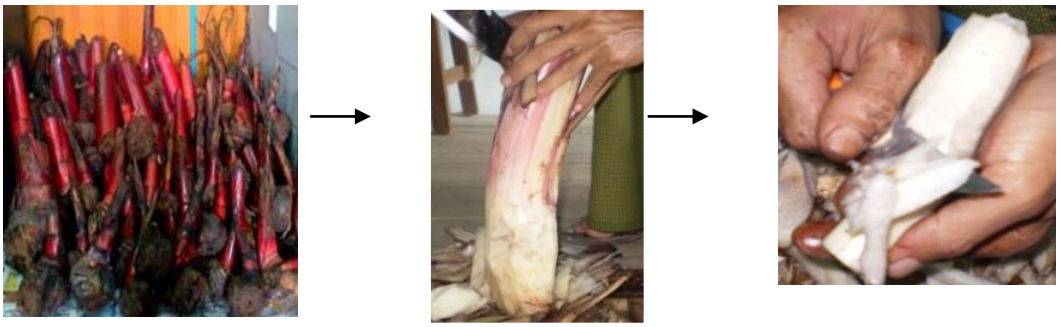
3.4 Experiment 1: Effects of BAP and TDZ on Shoot Initiation and Proliferation of Red Banana cv. "Red Dacca"

3.4.1 Shoot initiation and proliferation

Sword suckers were used as experimental materials. The outer layers of leaves and corm tissue of the sucker were removed to obtain approximately 10 cm long block containing shoot apex. This block was dipped in (1 g· L^{-1}) fungicide (Homai) for 15 minutes. Under aseptic conditions, the plant materials were surface sterilized by Clorox (NaOCl) 1% (v/v) for 15 minutes. The sheaths and bases of the leaves were removed to expose the shoot tip region. The shoot tip was decapitated and a block of tissue approximately 2 x 2 x 2 cm was excised and inoculated into initiation medium. Tissue blocks containing shoot buds were divided vertically into four parts and forty explants were used for one treatment (Plate 3.1). The excised explants were inoculated into medium supplemented with BAP (2.5, 5.0, 7.5 and 10.0 mg· L^{-1}) and TDZ (0.05, 0.15 and 0.25 mg· L^{-1}) (Table. 3.1). Cultures were maintained under white fluorescence lamp 20 µmol m^{-2} s^{-1} light intensity in 16 hours photoperiod at 25 ± 2˚C. Liquid medium with cotton culture support was used for 2 weeks in initial culture. The explants were transferred to fresh semi-solid medium and cultured for 8 weeks to initiate shoot formation.

Table 3.1 Initial culture medium with different PGRs concentration

Basal Medium	Plant Growth Regulators (mg· L^{-1})		Sucrose (g· L^{-1})	Agar (g· L^{-1})	pH
	BAP	TDZ			
MS	2.5	-	30	3	5.7
	5.0	-			
	7.5	-			
	10	-			
	-	0.05			
	-	0.15			
	-	0.25			



Donor plant selection

Removing leaf sheath and bases of shoot

Shoot tip were reduced in size 2.5 x 2.5 x 4 cm

Explants were inoculated in cotton culture

Approximately 2 x 2 x 2 cm explants were divided vertically into four parts

Shoot tip explant were prepared for surface sterilization

Plate 3.1 Explant preparation and inoculation of red banana

3.4.2 Shoot Multiplication

The multiple shoots induced from explant were excised into single shoot and subculture onto semi- solid multiplication medium. Based on the result of initial culture experiment, optimum level of TDZ 0.15 mg· L^{-1} was selected to use in multiplication of shoots from initial culture. The shoot clusters were subcultured every 6 weeks for up to 2 passages. Thirty five explants were cultured in each passage. Continuous dark condition was applied for both subculture 1 and subculture 2 at 25 ± 2°C. The explants were incubated for 6 weeks in each passage.

3.5 Experiment 2: Effect of Different Concentration and Combination of NAA and IBA on Rooting and Elongation of Shoots of Red Banana cv. "Red Dacca"

3.5.1 Rooting and elongation of shoots

When shoots were 4-5 cm high, single shoots were excised from shoot clumps and transferred on rooting medium. Half strength MS medium was solidified with 3 g· L^{-1} commercially available agar. NAA (0.5, 1.0 mg· L^{-1}) or IBA (0.5, 1.0 mg· L^{-1}) alone or NAA in combination with 0.5 mg· L^{-1} IBA were used. Basal medium without plant growth regulators was used as control (Table. 3.2). The culture condition was same as that of initial culture and culture duration was 8 weeks.

3.5.2 Hardening and acclimatization of *in vitro* raised banana plants

Rooted plantlets were taken out from the culture vessels and the roots were washed with water to remove the medium attached to the roots. The plants were transferred into wooden tray (2.5 x 3 x 0.6 ft) filled with compost and sand was mixed at 2:1 ratio. These plants were covered with plastic sheet under shade (27 ± 2°C) and humidity was maintained at 80 percent for primary hardening for 2 weeks. Secondary hardening was carried out in a net house (temperature 32 ± 2 °C and humidity 45 ± 2 percent) for 4 weeks.

Table 3.2 *In vitro* rooting medium with different PGRs combinations and concentrations

Treatment	Auxins (mg· L^{-1})		Remark
	NAA	IBA	
1	-	-	control
2	1.0	-	2 weeks in hormone free medium prior to transfer auxin containing medium (M1)
3	0.5	-	
4	-	1.0	
5	-	0.5	
6	1.0	0.5	
7	0.5	0.5	
8	1.0	-	Direct transfer to auxin containing medium (M2)
9	0.5	-	
10	-	1.0	
11	-	0.5	
12	1.0	0.5	
13	0.5	0.5	

3.6 Data Collection

The following data were collected:

1. Survival percent (%)
2. Number of days to induce first shoot
3. Number of shoots per explant
4. Shoot length (cm)
5. Number of roots per explant
6. Root length (cm)
7. Root diameter (cm)
8. Number of leaves per plant
9. Plant height (cm)
10. Fresh weight of shoot (g)

3.7 Statistical Analysis

A Randomized Complete block Design (RCB) was laid out at every stage of culture. The recorded data were subjected to Analysis of Variance (ANOVA) by using SAS version 9.0 and treatment means were compared by least significant difference (LSD) at 5 % level. Single degree of freedom pre-planned comparison was carried out by using the general linear model of SAS.

CHAPTER IV

RESULTS

4.1 Experiment 1: Effects of BAP and TDZ on Shoot Initiation and Proliferation of Red Banana cv. "Red Dacca"

4.1.1 Survival percent as affected by different levels of BAP and TDZ

Effects of BAP and TDZ on shoot initiation and proliferation of Red banana cv. "Red Dacca" are described in Figure 4.1. Survival percent was influenced by different levels of BAP and TDZ. Maximum survival percentage (100%) was obtained in TDZ 0.15 mg· L^{-1} and BAP 2.5 mg· L^{-1} containing medium gave 95 %. The lowest survival percent (75%) was obtained by BAP 5.0 mg· L^{-1} (Figure 4.1).

4.1.2 Shoot formation percent as affected by different levels of BAP and TDZ

Figure 4.1 presents the effects of different concentrations of BAP and TDZ on shoot formation percent. Among different levels of plant growth regulators, the maximum normal shoot formation (70.59 %) was observed in 0.15 mg· L^{-1} TDZ. While 0.05 mg· L^{-1} TDZ gave the minimum normal shoot formation at (28.57 %), 10.0 mg· L^{-1} BAP produced 63.63 % of shoot formation, which was the maximum normal shoot formation percent among different levels of BAP. Plate 4.1 shows difference between normal shoot and globular shoot formation during initial culture.

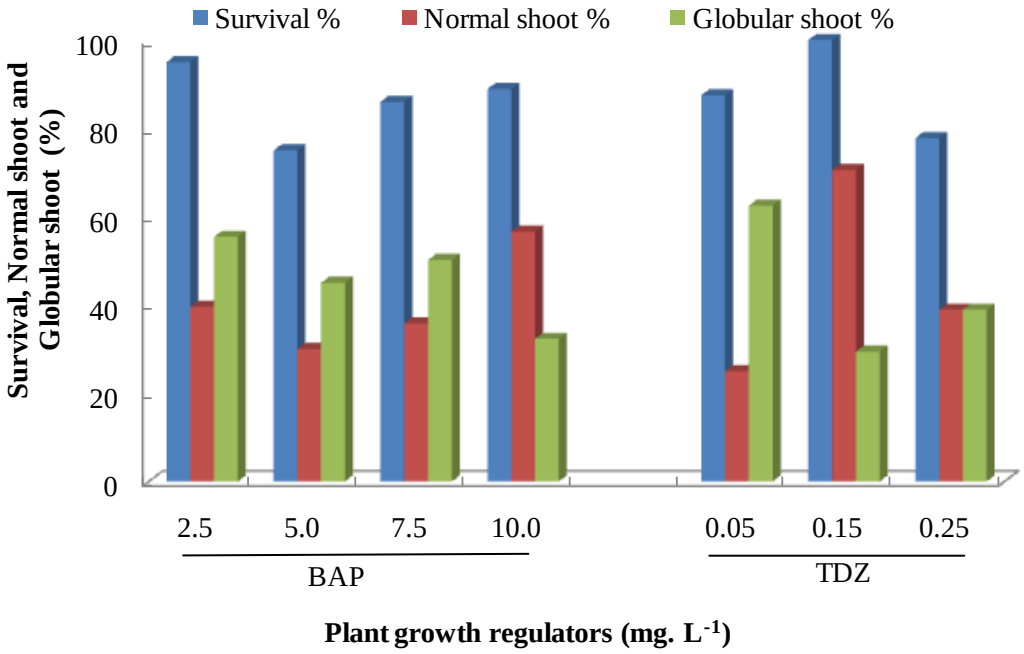


Figure 4.1 Survival (%), normal shoot formation (%) and globular shoot formation (%) as affected by BAP and TDZ at different levels at 10 weeks after inoculation of explants in *in vitro*

4.1.3 Days to induce new shoot as affected by different levels of BAP and TDZ

Table 4.1 describes the impact of BAP and TDZ significantly influenced days to induce new shoot. It was observed that the effect of plant growth regulators on days to induce shoot gave highly significant difference at 0.01 level. The minimum number of days (55.60) to induce new shoot was recorded in 2.5 mg· L^{-1} BAP and (57.80) in 0.15 mg· L^{-1}. TDZ The explants treated with 0.25 mg· L^{-1} TDZ showed the longest days (69.06) to induce new shoot.

Orthogonal contrast of the data indicated that comparison between minimum concentration of 2.5 mg· L^{-1} BAP and other levels of BAP was highly significant (P =0.0082). In addition, the single degree of freedom contrast showed highly significant difference at P = 0.0004 when comparing different levels of BAP and TDZ. When comparison was made between minimum concentration of 0.05 mg· L^{-1} TDZ and other higher concentrations, there was no significant difference (P= 0.3437) for number of days to induce new shoot. Low concentration of BAP was found to induce new shoot earlier than others.

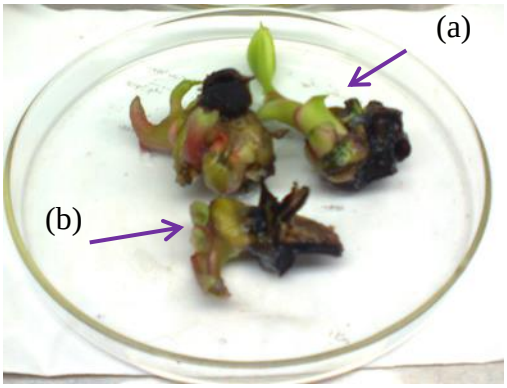


Plate 4.2 Difference between normal shoot (a) and globular shoot (b). Shoot formation pattern of red banana 8 weeks after in initial culture medium

4.1.4 Number of shoots per explant as affected by different levels of BAP and TDZ

Number of shoots per explant as affected by different levels of BAP and TDZ is described in Table 4.1. Significant difference at ($P \leq 0.05$) was observed in number of shoots per explant as the effect of plant growth regulators. It was found that 0.15 mg· L^{-1} TDZ and 5.0 mg·L^{-1} BAP gave the maximum number of shoots, 2.25 and 2.08 respectively, which was observed the highest. The minimum number of shoot was observed in TDZ 0.25 mg· L^{-1} with 1.25 shoots.

There was no significant difference in number of shoots per explant, when compared two types of plant growth regulators (contrast, $P = 0.9267$) between the different levels of BAP and TDZ. In addition, low concentration of BAP (2.5 mg· L^{-1}) was not significantly different from other levels of BAP in orthogonal contrast analysis. Moreover, orthogonal contrast indicated no significant difference between low concentration of TDZ and other levels. However, 5.0 mg· L^{-1} BAP and 0.15 mg· L^{-1} TDZ produced significantly greater number of shoots.

4.1.5 Shoot length as affected by different levels of BAP and TDZ

Table 4.1 describes the effect of different levels BAP and TDZ on shoot length. A highly significant difference at ($P \leq 0.01$) was observed in shoot length as affected by different plant growth regulators. The longest shoot length (6.65 cm) was obtained in TDZ 0.05 mg· L^{-1} treatment and the shortest shoot length (3.067 cm) was observed in 10.0 mg· L^{-1} BAP. Shoot length declined when treated with 0.25 mg· L^{-1}

TDZ. On the other hand, shoot length was slowly decreased in MS medium containing concentration of BAP 2.5 mg· L^{-1} to 10.0 mg· L^{-1}.

There was significant difference (contrast, P = 0.0295) between the different levels of BAP and TDZ. When the minimum level of BAP 2.5 mg· L^{-1} was compared to higher concentrations of BAP 5.0, 7.5 and 10.0 mg· L^{-1}, there was no significant difference (contrast, P = 0.1304). However, comparison between TDZ 0.05 versus 0.15 and 0.25 mg· L^{-1} showed highly significant difference at (P = 0.0009, contrast) in shoot length. It can be assumed that shoot lengths were greatly influenced by TDZ concentration.

4.1.6 Shoot fresh weight as affected by different levels of BAP and TDZ

Effect of different levels of BAP and TDZ on shoot fresh weight is presented in Table 4.1. Shoot fresh weight showed highly significant effect of plant growth regulators. BAP 7.5 mg· L^{-1} gave maximum shoot fresh weight of 1.32 g followed by 1.06 g in TDZ 0.15 mg· $L^{-1}$and minimum shoot fresh weight was observed as 0.34 g in TDZ 0.25 mg. L^{-1}.

According to orthogonal contrast, shoot fresh weight was significantly different between PGRs types and between the explants treated with different levels of plant growth regulators as shown in Table 4.1, except comparison between minimum concentration of BAP (2.5 mg· L^{-1}) and other higher concentrations of BAP. There was highly significant difference (contrast, P = 0.0049) between two cytokinin types, BAP and TDZ. Orthogonal contrast indicated that there was significant difference between the lower and higher concentrations of TDZ.

Table 4.1 Days to induce new shoot, number of shoots per explant, shoot length (cm) and shoot fresh weight as affected by different levels of BAP and TDZ at 8 weeks after initial culture in *in vitro*

Plant Growth Regulators (mg· L^{-1})		Days to induce	Number of shoots	Shoot length	Shoot fresh weight
BAP	TDZ	new shoot	per explant	(cm)	(g)
2.5	-	55.60d [z]	1.33c [z]	4.68b [z]	0.88b [z]
5	-	59.89bc	2.08ba	3.88bc	0.53c
7.5	-	59.70bc	1.58bc	4.13bc	1.32a
10	-	60.42bc	1.50c	3.067c	0.93b
-	0.05	61.80b	1.42c	6.65a	0.88b
-	0.15	57.80cd	2.25a	4.82b	1.06b
-	0.25	69.06a	1.25c	3.25bc	0.34d
	LSD (0.05)	3.94	0.58	1.60	0.19
	CV (%)	7.25	23.91	24.76	14.79
	Pr>F	<.0001**	0.0115*	0.0034**	<.0001**
	1,2,3,4 vs 5,6,7 [x]	0.0004** [y]	0.9267	0.0295*	0.0049**
	1 vs 2,3,4	0.0082**	0.1013	0.1304	0.4929
	5 vs 6,7	0.3437	0.1798	0.0009**	0.0323*

[z] Means within a column followed by the same letter are not significantly different 5 % LSD.

[y] Values for contrast represent P values from the F test (Pr>F). [x] Contrast for different levels of plant growth regulators.

4.2 Shoot Multiplication

4.2.1 Number of shoot during multiplication stage (subculture 1)

Effect of different levels of BAP and TDZ used in initial culture on number of shoot during multiplication stage (subculture 1) is presented in Table 4.2. The highest number of shoots 2.88 was obtained by TDZ 0.15 mg· L^{-1} followed by 2.37 at BAP 5.0 mg· L^{-1} (Plate 4.2). The minimum number of shoots (1.88) was obtained by BAP 10 mg· L^{-1} treated explants during initial culture. Furthermore, high-level TDZ-treated shoots enhanced shoot proliferation when transferred from initial culture to multiplication medium with TDZ 0.15 mg· L^{-1}. It was observed that highly significant difference (contrast, P = 0.0004) in number of shoots was observed in TDZ 0.15 mg· L^{-1}compared with other treatments. Therefore, TDZ 0.15 mg· L^{-1} was a suitable concentration for red banana cultivar multiplication.

4.2.2 Shoot length during multiplication stage (subculture 1)

There was no significant difference in the effect of multiplication medium on shoot length (Table 4.2). The longest shoot length (5.38 cm) was obtained by 10.0 mg· L^{-1} BAP treated explants in initial culture and followed by that of 2.5 mg· L^{-1} BAP while the shortest shoot length (3.25 cm) was observed with explants treated with 0.05 mg· L^{-1} TDZ. Although shoot length was highest in lower concentration of TDZ applied on initial medium, shoot length decreased in multiplication medium. There was no significant difference in shoot length according to single degree of contrast between 0.15 mg· L^{-1} TDZ and other plant growth regulators.

4.2.3 Shoot fresh weight during multiplication stage (subculture 1)

It was found that there was no significant difference in the effect of multiplication medium on shoots fresh weight of red banana in *in vitro* (Table 4.2). However, the highest fresh weight (2.51 g) was obtained by 0.05 mg· L^{-1} TDZ containing medium followed by (2.19 g) in 5.0 mg· L^{-1} BAP which was employed in initial culture. Single degree of freedom contrast was not significantly different in shoot fresh weight between TDZ and others plant growth regulators.

4.2.4 Number of leaves during multiplication stage (subculture 1)

There was no significant difference in effect of combination BAP and TDZ on number of leaves per plant (Table 4.2). The highest number of leaves (2.5) was obtained by using 0.15 mg· L^{-1} and 0.05 mg· L^{-1} TDZ. After that 2.5 mg· $L^{-1}$and 10.0 mg· L^{-1} BAP produced (2.25) leaves per explant. The minimum number of leaves

(1.75) was obtained by 5.0 mg· L^{-1} and 7.5 mg· L^{-1} BAP and 0.25 mg· L^{-1} TDZ. Single degree of freedom contrast was not significantly different in number of leaves.

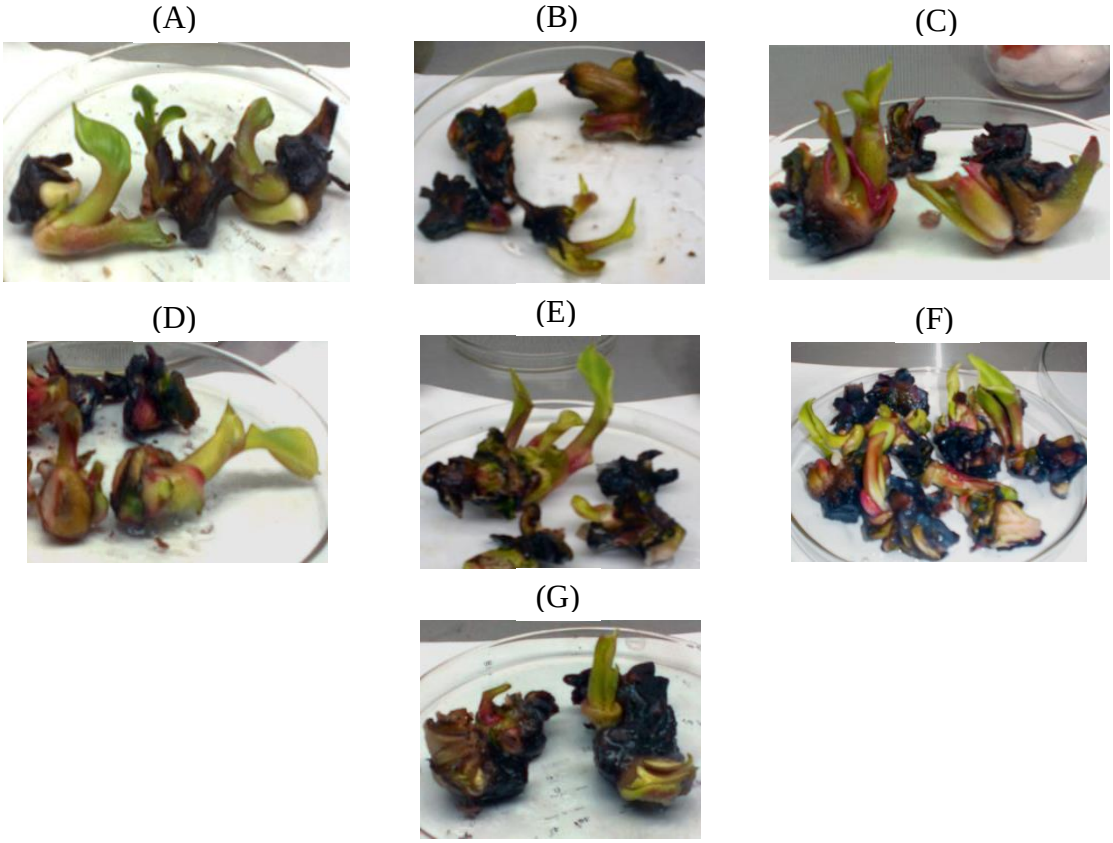


Plate 4.3 Subsequent multiplication of shoots at the end of subculture 1 developed from initial culture:

(A) BAP 2.5 mg· L^{-1}

(B) BAP 5.0 mg· L^{-1}

(C) BAP 7.5 mg· L^{-1}

(D) BAP 10.0 mg· L^{-1}

(E) TDZ 0.05 mg· L^{-1}

(F) TDZ 0.15 mg· L^{-1}

(G)TDZ 0.25 mg· L^{-1}

4.2.5 Number of shoots during multiplication stage (subculture 2)

Results on average number of shoots per explants as affected by TDZ are given in Table 4.2. The continuous treatment of 0.15 mg· L^{-1} TDZ resulted in average number of shoots (3.75) per explant. However, number of shoots was found to increase when 0.15 mg· L^{-1} TDZ was applied to each culture derived from different PGRs regime in initial culture. It was found that all BAP levels treated in initial culture showed increase in number of shoots per explant in subculture 2 (Plate 4.3). There was no significant difference in shoots per explant among the subculture 2 of multiplication stage whether it was cultured in BAP or TDZ in initial stage. It was found that there was no significant difference in single degree of freedom contrast in number of shoot per explant.

4.2.6 Shoot length during multiplication stage (subculture 2)

Effects of different levels of BAP and TDZ on shoot length during multiplication stage in subculture 2 are described in Table. 4.2. It was found that maximum shoot length (7.93 cm) was achieved from BAP 2.5 mg· L^{-1} while TDZ 0.25 mg· L^{-1} gave the minimum shoot length (3.60 cm). It can be clearly seen that different PGRs regime applied in initial culture was still affected to subculture 2. Results of shoot length as affected by TDZ are given in Table 4.2 and there was no significant difference on shoot length in subsequent use of BAP and TDZ. Single degree of freedom contrast among the treatments also revealed no significant difference among the tested treatments.

4.2.7 Shoot fresh weight during multiplication stage (subculture 2)

It was observed that maximum shoot fresh weight (1.87g) was achieved with 5.0 mg· L^{-1} BAP followed by 0.15 mg· L^{-1} TDZ (1.25 g) and minimum shoot fresh weight (0.49 g) was found on 7.5 mg· L^{-1} BAP which was employed in initial culture (Table. 4.2). There was not significant in shoot fresh weight in treatments by using single degree of freedom contrast.

4.2.8 Number of leaves during multiplication stage (subculture 2)

It was found that maximum number of leaves (3.67) was achieved by 7.5 mg· L^{-1} BAP followed by 5.0 mg· L^{-1} BAP with 3.33 and 0.15 mg· L^{-1} TDZ with 2.33. The number of leaves increased in subculture 2 by continuous subculturing on (0.15 mg· L^{-1}) TDZ. There was no significant difference in number of leaves in treatments by using single degree of freedom contrast (subculture 2).

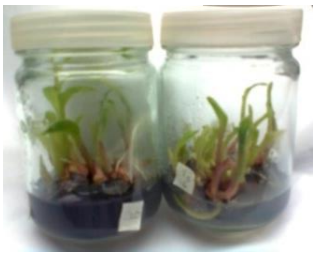
(A)

(B

(C)

(D)

(E)



(F)

(G)

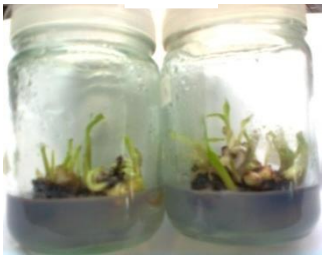


Plate 4.4 Subsequent multiplication of shoots at the end of subculture 2 developed from initial culture:

(A) BAP 2.5 mg· L^{-1}

(B) BAP 5.0 mg· L^{-1}

(C) BAP 7.5 mg· L^{-1}

(D) BAP 10.0 mg· L^{-1}

(E) TDZ 0.05 mg· L^{-1}

(F) TDZ 0.15 mg· L^{-1}

(G)TDZ 0.25 mg· L^{-1}

Table 4.2 Differences between subculture 1 and subculture 2 in 0.15 mg· L^{-1} TDZ containing medium

Initial PGRs (mg· L^{-1})	Multiplication							
	Number of shoots		Shoot length (cm)		Shoot fresh weight (g)		Number of leaves per shoot	
	Subculture 1	Subculture 2	Subculture 1	Subculture 2	Subculture 1	Subculture 2	Subculture 1	Subculture 2
BAP 2.5	2.31± 0.19bc [z]	4.00±0.14 [z]	4.88±1.76 [z]	7.93±2.63 [z]	2.00±0.30 [z]	1.12±0.11 [z]	2.25±0.25 [z]	2.67±0.33 [z]
BAP 5.0	2.37±0.16b	4.33±0.36	4.75±1.00	4.83±0.82	2.19±0.48	1.87±0.35	1.75±0.25	3.33±0.33
BAP 7.5	219±0.19bc	4.33±0.17	4.13±0.75	4.83±0.73	1.59±0.27	0.49±0.078	1.75±0.25	3.67±0.33
BAP 10.0	1.88±0.26 c	4.08± 0.46	5.38±2.55	4.67±0.88	1.86±0.46	1.04±0.17	2.25±0.25	2.67±0.67
TDZ 0.05	2.25± 0.18bc	4.33±0.33	3.25±0.25	4.43±0.72	2.51±0.30	1.069±0.12	2.5±0.29	3.67±0.33
TDZ 0.15	2.88 ±0.16a	3.75±0.29	4.63±0.99	4.27±0.62	1.86±0.35	1.25±0.178	2.5±0.50	2.33±0.33
TDZ 0.25	2.06±0.16bc	5.10±0.36	3.38±0.71	3.60±0.31	1.83±0.13	0.91±0.15	1.75±0.25	3.00±0.001
LSD(0.05)	0.44	1.0369	3.79	3.1948	1.01	1.49	0.95	1.19
CV (%)	13.02	13.63	58.75	36.37	34.36	66.22	30.34	21.88
Pr>F	0.0064**	0.2349	0.8778	0.1834	0.5971	0.291	0.3534	0.1702
TDZ 0.15 mg· L^{-1} vs others [x]	0.0004** [y]	0.1173[y]	0.8114[y]	0.4976[y]	0.6863 [y]	0.9682[y]	0.2009 [y]	0.0681[y]

[z]Means (± SE) within a column followed by the same letter are not significantly different 5 % LSD.

[y] Values for contrast represent P values from the F test (Pr ≥ F).[x] Contrast for different levels of plant growth regulators.

4.3 Experiment 2: Effect of Different Concentration and Combination of NAA and IBA on Rooting and Elongation of Shoots of Red Banana cv. "Red Dacca"

4.3.1 Number of root

Table 4.3 presents differences in 2 weeks hormone free medium prior to transfer to auxin containing medium (M1) and direct transfer to auxin containing medium (M2) on number of roots per shoot by using half strength (MS) medium in rooting stage. During *in vitro* multiplication, shoots were multiplied in the form of clusters. For *in vitro* rooting, shoots were separated from the cluster and transferred to rooting medium. The maximum number of roots (17.00) was obtained from NAA 1.0 mg· L^{-1} (M1) and 18.16 roots was achieved from combination of NAA 1 mg· L^{-1} and IBA 0.5 mg· L^{-1} (M2). According to this experiment, it was found that number of roots developed on auxin provided medium and PGRs free medium (control) are much different. Although root formation occurred on PGRs free medium, number of roots per shoot was two times less than PGRs-including medium and proper concentration of NAA in M2 medium provided maximum number of roots per shoot and it was two times of that found in PGRs free medium (control). In this study, using IBA 0.5 and 1.0 mg· L^{-1} showed no significant difference in root number when treated with IBA concentration in both medium. Although root number was increased in half strength MS medium supplemented with auxin, there was no difference in number of root in control treatment of both medium.

Although number of roots as affected by different concentration and combination of NAA and IBA was not significantly different in single degree of freedom contrast on M1, there was significant difference in root number in NAA and IBA treated shoots in M2 (Table 4.3). In M2 medium, differences between NAA and IBA, between NAA and NAA in combination with IBA were significantly different in number of roots per explant at 0.01 % probability level. Furthermore, control treatment showed significant difference in root numbers when compared to auxin supplemented medium.

4.3.2 Root length

Presence of auxins in the medium during root induction affected the root length significantly regardless of the medium (Table 4.3). Maximum root length (12.28 cm) was achieved from the control treatment and minimum root length (1.73 cm) was obtained from 0.5 mg· L^{-1} IBA on M1 medium. On the other hand, maximum root length (11.09 cm) was obtained from control and followed by 0.5 mg· L^{-1} IBA while minimum root length (2.06 cm) was resulted from the combination of 1.0 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA in M2 medium.

Root length as affected by different concentration and combination of NAA and IBA was significantly different in single degree of freedom contrast in (M1) medium (Table 4.3). Orthogonal contrast showed significant differences in root length in all comparison in M1 medium. On the other hand, hormone free shoots showed significant difference in root length when comparing to other auxins treated shoots in M2 medium.

4.3.3 Root diameter

Addition of auxins during rooting stage affected the root diameter significantly (P = 0.023) in M2 medium during root induction (Table 4.3), although there was no significant difference in M1 medium. The largest root diameter was observed in 1.0 mg· L^{-1} NAA followed by 1.0 mg· L^{-1} IBA with (1.72 mm) and (1.71 mm) respectively while control treatment gave the smallest root diameter (0.89 mm) in M1 medium. On the other hand, high concentration of both 1.0 mg· L^{-1} NAA and 1.0 mg· L^{-1} IBA reduced the root diameter (0.97 mm) and (1.15 mm) respectively in M2 medium. Control treatment showed the smallest root diameter (0.89 mm) and (0.51 mm) in both M1 and M2 medium respectively. Although higher concentration of NAA 1.0 mg· L^{-1} could increase in root diameter in M1 medium, it gave negative effect in root diameter in M2 medium.

Root diameters as affected by NAA and IBA were significantly different in single degree of freedom contrast in control treatment on both medium (Table 4.3). There was significant difference in root diameter when control treatment was compared to others in both M1 and M2 medium (contrast, P = 0.0361) and (contrast, P = 0.001) respectively.

4.3.4 Plant height

By supplementing auxin in rooting media, the plant heights were significantly different (P=0.004) in continuous auxin application medium (M2) (Table 4.3). The longest plant height (14.36 cm) was observed in M1 with medium containing 0.5 mg· L^{-1} NAA followed by combination of 0.5 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA which gave 13.67 cm. The shortest plant height (11.42 cm) was observed in the combination of 1.0 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA in M1. However, in M2 medium, the longest plant height (11.20 cm) was obtained from 1.0 mg· L^{-1} IBA. The shortest plant height (4.36 cm) was achieved from combination of 0.5 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA in M2 medium.

There was significant difference in orthogonal contrast comparison in plant height when comparing each NAA and IBA compared with combined effect of NAA and IBA in M2 medium. No significant difference in contrast was observed in plant height in M1 medium (Table 4.3).

Table 4.3 Differences between 2 weeks in hormone free medium prior to transfer to auxin containing medium (M1) and direct transfer to auxin containing medium (M2) at 8 weeks after rooting stage in *in vitro*

Plant growth regulators (mg· L^{-1})		Number of roots		Root length (cm)		Root diameter (mm)		Plant height (cm)	
		M1 medium	M2 medium	M1 medium	M2 medium	M1 medium	M2 medium	M1 medium	M2 medium
NAA	IBA								
0	0	9.17	9.76c[z]	12.28a[z]	11.09a[z]	0.89	0.51b[z]	13.00	9.36a[z]
0.5	-	11.67	11.83bc	8.71 ab	5.10bc	1.37	1.04a	14.36	10.81a
1.0	-	17.00	6.67d	6.37bc	4.52bc	1.72	0.97a	12.00	8.83a
-	0.5	12.50	14.08b	1.733d	7.27b	1.46	1.33a	11.49	10.18a
-	1.0	13.00	13.33b	6.26bc	3.09c	1.71	1.15a	12.91	11.20a
0.5	0.5	9.83	13.67b	5.54bc	5.00bc	1.02	1.11a	13.67	4.36b
1.0	0.5	10.17	18.16a	4.17cd	2.06c	1.69	1.07a	11.42	8.63a
LSD(0.05)		6.43	2.29	3.74	3.52	0.73	0.40	4.32	2.86
CV (%)		30.36	10.29	32.63	36.32	28.98	21.85	19.11	17.74
Pr>F		0.22	<.0001	0.0015	0.0025	0.14	0.023	0.70	0.004
Control vs others [x]		0.1819[y]	0.0018[y]	0.0018[y]	0.0002[y]	0.0361[y]	0.001[y]	0.8163[y]	0.7263[y]
NAA vs IBA		0.4627	<.0001	<.0001	0.7506	0.8899	0.0966	0.4979	0.3663
NAA vs NAA combined with IBA		0.0600	<.0001	<.0001	0.2842	0.4387	0.5219	0.6568	0.0038
IBA vs NAA combined with IBA		0.2122	0.0115	0.0115	0.1737	0.3646	0.2754	0.8118	0.0007

[z]Means within a column followed by the same letter are not significantly different 5 % LSD.

[y]Values for contrast represent P values from the F test (Pr ≥ F).[x] Contrast for different levels of plant growth regulators.

4.2 Hardening and acclimatization of *in vitro* raised banana plantlets

Acclimatization of red banana plantlet as affected by different concentration of NAA and IBA used in rooting medium is shown in Figure 4.2. Survival (100 %) was obtained from control treatment regardless of medium. Similarly, high concentration of both 1.0 mg· L^{-1} NAA and 1.0 mg· L^{-1} IBA resulted in 100% survival from M1 medium. Moreover, combination of 0.5 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA gave 100% survival from M2 medium.

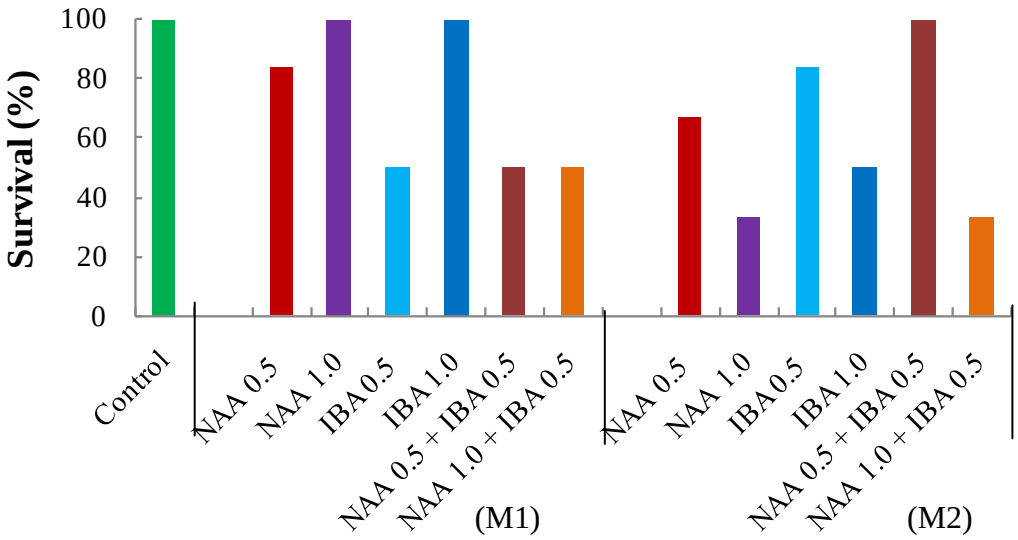


Figure 4.2 Survival percent of plantlets as affected by different concentration and combinations of NAA and IBA used in rooting medium.
(M1) 2 weeks in hormone free medium prior to transfer auxin containing medium, (M2) Direct transfer to auxin containing medium at 8 weeks after hardening

CHAPTER V

DISCUSSION

In this study, red banana micropropagation was affected by application of different levels of BAP and TDZ during initial culture. Survival percent was increased in lower concentration of BAP 2.5 mg· L^{-1} but high BAP concentration did not greatly affect survival percent. In contrast, higher TDZ concentration decreased survival percent. This finding is in accord with Strosse et al. (2004), who reported that there was decrease in survival percent when increasing plant growth regulator concentration. In addition, BAP 100.0 µM showed more survival percent than higher concentration of TDZ 100.0 µM in (AAA) cv. Williams banana. The effective dose range of TDZ is narrow, compared with other cytokinin such as BA and kinetin. Although increasing BAP concentration did not differ from other levels in survival percent, but higher TDZ concentration led to a decrease in this pattern. Thus, it can be assumed that survival percent was affected by TDZ concentrations.

Increasing BAP concentration has positive effect on the normal shoot formation percent and days to induce new shoot. Shirani et al. (2010) observed that lower level of BAP (2.5 mg· L^{-1}) produced not only more globular shoots, but also single shoots, thus leading to minimal shoot multiplication. It can be assumed that increasing BAP concentration has positive effect on the normal shoot formation percent. However, normal shoot formation percent was dependent on specific TDZ concentration in (AAA) cv. Pei-Chaio and (AAB) cv. Latundan (Lee 2001). Lalrinsanga et al. 2013 achieved 2.17 number of multiple shoots in shoot tip explant of cultivar Meitei-Hei, when treated with 5.0 mg· L^{-1} BAP in MS medium for 75 days of incubation. It might be due to the cultivar dependent phenomenon to induce new shoots when treated with plant growth regulators. But, normal shoot formation percent and days to induce new shoot seems to depend on specific TDZ concentration in red banana cultivar because only 0.15 mg· L^{-1} TDZ increased in normal shoot formation percent and days to induce new shoot in this study. Thus, certain dosage of TDZ will be required to induce new shoot for a particular period.

Both 5.0 mg· L^{-1} BAP and 0.15 mg· L^{-1} TDZ produced higher number of shoots per explant during initial culture. Rahman et al. (2013) observed that the highest average number of shoots was found in MS medium fortified with 4.0 mg· L^{-1} BAP. With lower level of BAP 2.5 mg· L^{-1}, most explants grew into a single shoot

(Shirani et al. 2010). Youmbi et al. (2006) reported that *Musa* spp. with weak multiplication potential cultivars- Gros Michel (AAA), Topala (AAB) and Fogamou (ABB)- were ameliorated for plant development when treated with low TDZ concentration (0.05 to 0.4 µM). But, cv. Ndiziwemiti (ABB) progressively increased proliferation with increasing TDZ (9.1 µM) up to 9.5 shoots per explant (Arinaitwe et al. 2000). Youmbi et al. (2006) stated that shoot heights were reduced when TDZ concentration is higher in *Musa spp*. High TDZ concentrations suppressed shoot elongation and that behaviour was attributed to the ability of TDZ to accumulate endogenous cytokinins in cultured tissues (Huetteman and Preece 1993). TDZ is resistant to all cytokinin oxidases and induces the accumulation of endogenous cytokinins (Kaminek 1992). TDZ could increase the biosynthesis of endogenous adenine-type cytokinins (Thomas and Katterman 1986; Huetteman and Preece 1993), thus, making it an effective cytokinin for stimulation of shoot bud proliferation in recalcitrant banana genotypes. Although 5.0 mg· L^{-1} BAP could increase the number of shoots per explant, 0.15 mg· L^{-1} TDZ produced greater number of shoot in this experiment. So, TDZ was to be more effective than adenine-based cytokinins for shoot proliferation.

Furthermore, shoot length and shoot fresh weight was decreased by higher TDZ concentration. Therefore, it is necessary to use TDZ with great care in shoot initiation and proliferation stage. Arinaitwe et al. (2000) observed that media supplemented with high TDZ concentrations induced to suppress shoot elongation. Farahani et al. (2008) reported that the presence of TDZ significantly reduced shoot elongation and shoot fresh weight. The effective dose range of TDZ is narrow, compared with BAP. TDZ is more efficient than adenine-based cytokinins and its application is narrow in dosage. It can be assumed that the activity of BAP and TDZ was different for shoot fresh weight of red banana cultivar according to their different concentrations. On the other hand, it can be noted that the small amount of TDZ concentration greatly influenced on shoot fresh weight of red banana.

Red banana cultivar highly responded to TDZ concentration changes in multiplication stage. Lower concentration of both BAP and TDZ that was applied in initial culture increased number of shoots per explant and shoot fresh weight in subculture 1 except higher concentration of 0.25 mg· L^{-1} TDZ used in initial culture. It can be assumed that low concentration of BAP and TDZ that were treated in initial culture increased shoot fresh weight when transferred to multiplication medium which contained 0.15 mg· L^{-1} TDZ. Shoot length was not affected by changing multiplication medium in both of TDZ 0.05 mg· L^{-1} and 0.25 mg· L^{-1} that was applied in initial culture. In contrast, all BAP concentration employed in initial culture increased shoot length. Both BAP and TDZ concentrations treated in initial culture increased shoot fresh weight when transferred to multiplication medium during subculture 1. Youmbi et al. (2006) reported that leaf numbers were not significantly different in MS medium containing 0.5 mg· L^{-1} TDZ in different banana cultivars. He also reported that there were different dosages in TDZ concentration for certain banana cultivars for highest shoot proliferation percent. Proliferation percent was dependent on TDZ concentration for a particular banana cultivar ranging from 0.05 µM to 0.8 µM. It is recommended that 0.15 mg· L^{-1} TDZ is a suitable concentration for red banana multiplication based on the finding in this study.

Number of shoots increased when applied with 0.15 mg· L^{-1} TDZ in subculture 1 and 2. Maximum number of shoots (5.10) was achieved when TDZ 0.25 mg· L^{-1} was employed in initial culture. Higher concentration of TDZ (0.25 mg· L^{-1}) tended to induce short shoots. Makara et al. (2003) reported that carry over effect of TDZ was still affective in hormone free medium with different subcultures. It was found that TDZ can be used to promote number of shoots in subsequent subcultures. Moreover, continuous culturing on medium supplemented with TDZ reduced shoot fresh weight when compared between subculture 1 and subculture 2. It can be assumed that although continuous culturing on medium supplemented with TDZ reduced shoot fresh weight, but increased leaf number. Therefore, based on the finding in this study, continuous culturing on TDZ containing medium should be done with great care. Further work should be done with subculture 3 and 4.

The highest number of roots was obtained from 1.0 mg· L^{-1} NAA in two weeks prior to transfer to hormone applied medium (M1) and combination of NAA 1.0 mg· L^{-1} and IBA 0.5 mg· L^{-1} in continuous applied hormone medium (M2) during rooting stage. There was no difference in root number when treated with IBA alone in

both media. Number of roots was not different in control treatment when compared between M1 and M2. Ali et al. (2011) reported that half MS medium with an increasing NAA or IBA concentration from 0.5 to 1.0 mg· L^{-1}, also increased number of roots. Furthermore, many researchers (Rahman et al. 2005; Elhory et al. 2009; Lalrinsanga et al. 2013 and Rahman et al. 2013) reported that medium supplemented with 1.0 mg· L^{-1} IBA produced the highest number of roots per shoot in banana. Moreover, Uddin et al. (2006) observed that Kanthali cultivar obtained maximum root frequency when treated with 0.5 mg· L^{-1} IBA in half MS medium. Elhory et al. (2009) reported that the highest root length was obtained from half strength hormone-free medium and number of roots decreased while root length increased. The present study revealed that increased number of roots obtained from M1 medium might be due to synergistic effects of NAA and TDZ. But, continuous auxin treated medium (M2) supplemented with higher concentration of NAA produced the lowest number of roots. Therefore, shoots obtained from continuous subculturing with TDZ should not be directly transferred to medium supplemented with high NAA concentration.

In this study, control treatment gave the highest root length and increased plant height regardless of the medium. Root length increased more in 1.0 mg· L^{-1} IBA than in IBA 0.5 mg· L^{-1} in M1 medium. In contrast, root length was decreased when IBA concentration in M2 was increased. In addition, increasing IBA concentration showed negative effect on root length in M2 medium. Viehmannová et al. (2007) reported that the longer root was obtained from control of half MS medium when compared to auxin treated medium It can be assumed that control treatment gave the highest root length regardless of medium. But, average root length increased in high concentration of IBA in half MS medium (Al-Almin et al. 2009). Uddin et al. (2006) stated that the longer root length was obtained from IBA 0.5 mg· L^{-1} than IBA 1.0 mg· L^{-1}. Elhory et al. (2009) reported that when the IBA concentration was increased, the root length was decreased and the shortest root length was obtained in MS medium with 2.0 mg· L^{-1} IBA in cv. Tanduk.

Increasing NAA concentration in combination with IBA reduced root length regardless of medium. There were no differences in root length in both M1 and M2 media when shoots were treated with NAA (1.0 mg· L^{-1}) alone and lower concentration of NAA (0.5 mg· L^{-1}) combined with IBA (0.5 mg· L^{-1}). Root length decreased in both M1 and M2 rooting media when NAA concentration was increased from 0.5 mg· L^{-1} to 1.0 mg· L^{-1}. Ali et al. (2011) reported that root length did not

increase in high concentration of NAA. It can be assumed that the lower concentration of IBA in both of the media showed synergistic effect of TDZ that was continuously treated in multiplication while lower concentration of NAA had only positive effect on M1 medium. Guo et al. (2011) reported that TDZ has shown both auxin- and cytokinin- like effects but it is chemically different from commonly used auxins and cytokinins. TDZ treated-tissues were maintained and enhanced the accumulation and transport of auxin by TDZ. Zaffari et al. (2000) observed that higher IAA levels were retained in basal portion of shoot apices in proliferation media in cv. Grande Naine (AAA).Therefore, great care should be taken to obtain satisfactory root length; in fact, hormone free medium should be applied to TDZ-treated shoots before transferring to rooting medium.

The control treatment showed decrease in root diameter in both of the media when compared to auxin-treated shoots during rooting stage. Root diameter was not affected by treating with auxin in this study. Plant height was reduced when increasing NAA concentration in both M1 and M2 medium. But, increasing IBA concentration had positive effect on plant height. The result from this experiment is consistent with the finding of Gübbük and Pekmezcl (2004). They reported that plant height was increased when MS medium was supplemented with 0.2 mg· L^{-1} IBA. It might be due to the carry over effect of TDZ that was successively used in multiplication medium. It is advisable that the shoots should be cultured on M1 medium rather than M2 medium.

Elhory et al. (2009) reported that the addition of IBA produced plantlets with vigorous root system that could ensure high survival rate during acclimatization. Kanchanapoom (2000) stated that auxin is not necessary for *in vitro* root formation in banana, but the root system produced was poor to sustain the plantlets in the outside environment. 100 % survival of plantlets was recorded on control treatment, 1.0 mg· L^{-1} NAA, 1.0 mg· L^{-1} IBA in M1 medium and combination of NAA 0.5 mg· L^{-1} and IBA 0.5 mg· L^{-1} in M2 medium 6 weeks after transferred to *ex vitro* condition. Based on the data, it can be assumed that it is possible to obtain 100 % survival plantlets in adaptation stage even without using PGRs in rooting stage. The plantlets of red banana developed in *in vitro* were found to be simple and easy to handle in this study. Parameters measured (number of roots, root length, root diameter and plant height) were different based on the PGRs used for rooting. Therefore, it is

advisable that subsequent growth and development after establishment of plantlets should be monitored.

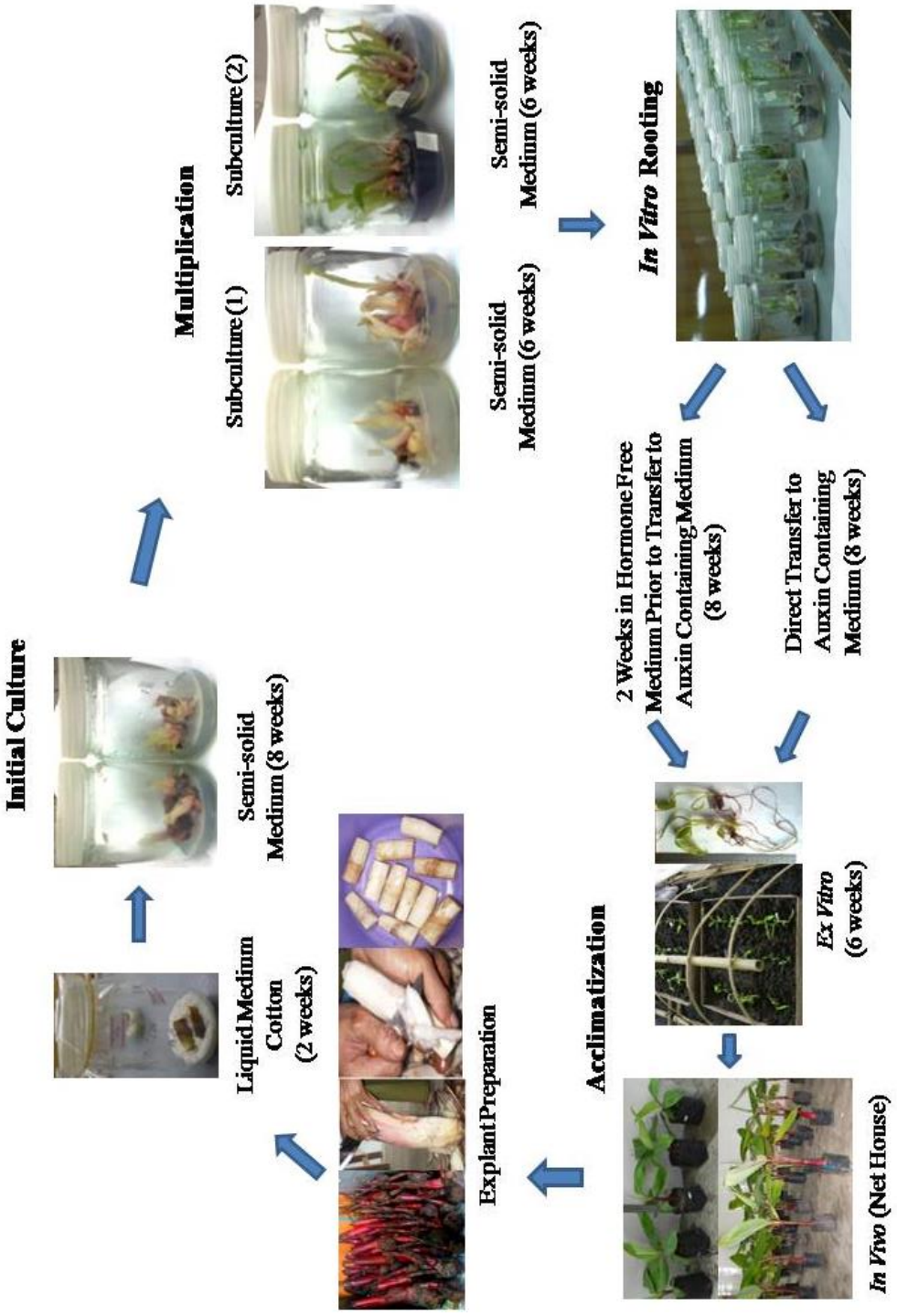


Plate 5.5 Flow diagram of red banana propagation in *in vitro*

CHAPTER VI

CONCLUSION

In initial culture, higher survival percent was obtained from 2.5 mg· L^{-1} BAP and increasing BAP concentration up to 10.0 mg· L^{-1} had positive effect on normal shoot formation percent and days to induce new shoot. Higher number of shoots per explants was obtained from 5.0 mg· L^{-1} BAP. Increasing BAP concentration did not affect shoot length.TDZ (0.15 mg· L^{-1}) showed maximum survival percent, number of shoots per explant, and higher normal shoot formation percent. Increasing TDZ concentration from 0.05 mg· L^{-1} to 0.25 mg· L^{-1} reduced in shoot length. In this study, it can be concluded that TDZ was superior to BAP in initial culture of red banana.

For subsequent multiplication, shoots developed from initial culture were treated with 0.15 mg· L^{-1} TDZ, which was suitable concentration for number of shoots per explant. In subculture 1, both BAP and TDZ concentration treated in initial culture increased number of shoots, shoot length and shoot fresh weight except higher concentration of BAP and TDZ. In contrast, both BAP and TDZ concentrations increased number of shoots, shoot length, but shoot fresh weight decreased in subculture 2. It can be concluded that specific dose of TDZ promoted the two subsequent multiplications. An area is still left open for further work in shoot multiplication on different combination of BAP and TDZ.

The highest number of roots was obtained from 1.0 mg· L^{-1} NAA in M1 medium and combination of 1.0 mg· L^{-1} NAA and 0.5 mg· L^{-1} IBA in M2 medium. Combination of NAA and IBA increased root number regardless of medium in this study. Hormone free medium was found to have maximum root length, but reduced root diameter. Root length and plant height decreased in both media when NAA concentration was increased from 0.5 mg· L^{-1} to 1.0 mg· L^{-1}. Root length increased more in 1.0 mg· L^{-1} IBA than in 0.5 mg· L^{-1} IBA in M1 medium. But, root length decreased when IBA concentration in M2 medium was increased. Increasing NAA and IBA concentration caused decrease in plant height in both of the media. Higher NAA concentration in combination with IBA resulted in negative effect on root length regardless of medium.

Although hormone free medium gave higher survival percent, the application of auxin was found to be required for *ex vitro* survival for red banana micropropagation. Further work should be done for the *ex vitro* growth and development of plantlets derived from different PGRs regimes in *in vitro* culture.

REFERENCES

Agustin, B. and J. Molina. 2002. Tissue culture in the banana industry. International training course on biotechnology for seed and seeding production, 2-6 December 2002, Los Bańos, Philippines.

Ahloowalia, B.S., J. Prakash, V. A. Savangikar, and C. Savangikar. 2004**.** PLANT TISSUE CULTURE. In: James, S.M. Low cost option for tissue culture technology for developing countries**.** Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Al-amin, M.D., M. R. Karim., M. R. Amin., S. Rahman and A. N. M. Mamun. 2009. *In vitro* micropropagation of banana (*Musa* spp.). Bangladesh J. Agri. Res. 34 (4): 645-659.

Ali, A., A. Sajid, N. H. Naveed, A. Majid, A. Saleem, U. A. Khan, F. L. Jafery and S. Naz. 2011. Initiation, proliferation and development of micropropagation system for mass scale production of banana through meristem culture. African Journal of Biotechnology 10 (70): 15731-15738.

Arinaitwe, G., P.R Rubaihayo and M.J.S Magambo. 2000. Proliferation rate effects of cytokinins on banana (*Musa* spp.*)* cultivars. Sci Hortic 86. pp. 13-21.

Azam, F. M. S., S. Islam, M. Rahmatulllah and A. Zaman. 2010 .Clonal propagation of banana (*Musa* spp.) cultivar 'BARI-1' (AAA Genome, *Sapientum* Subgroup). Acta Hort. 879.

Bakry, F., F. Carreel, C. Jenny and JP. Horry.2009. Genetic improvement of banana. In: Jain SM, Priyadarshan PM (eds) Breeding plantation tree crops: Tropical Species. Springer Science & Business Media. pp. 3-50.

Banerjee, N. and E. De Langhe.1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). Plant Cell Report. 4, pp. 351-354.

Berg, L. A. and M. Bustamante. 1974. Heat treatment and meristem culture for the production of virus free banana. Phytopathol. 64, pp. 320-322.

Bitar, A. D. and F. H. Mohamed. 2009. Effect of different substrate types on the growth of micropropagated banana transplants. Agricultural Research Journal. 9 (1): 75-80.

Bolar, J.P., J.L. Norelli, H.S. Aldwinckle and V. Hanke. 1998. An efficient method for rooting and acclimation of micropropagated apple cultivars. Hort Science 37, pp. 1251-1252.

Chang, S. and H. Shu. 2013. A method to suppress brownging in banana (*Musa*, AAA) embryogenic callus induced. Research Journal of Biotechnology. 8 (4).

Christil, J. R. and G. Deepali. 2011. Development of low cost methodology and optimization of multiplication and rooting hormones in the micropropagation of Red Banana *in vitro*. Plant Science Feed. pp. 2231-1971.

Cote, F., D. R. Alvard, L. Domergue, N. Mastache and C. Teission.1990. Micropropagation *in vitro* du bananier. Furits. 45. pp. 112-118.

Cote, F.X., Domergue, R., S. Monmarson, J. Schwendiman, C. Teisson, and J.V. Escalant, 1996. Embryogenic cell suspensions from the male flower of *Musa* AAA cv. Grand Nain. Physiologia Plantarum. 97. pp. 285–290.

Cronauer, S.S. and A.D. Krikorian. 1984. Multiplication of *Musa* from excised stem tips. Ann. Bet. 53. pp. 321-328.

Damasco, D. P. and R.C. Barba.1984. *In vitro* culture of Saba (*Musa* spp. cv. Saba [BBB]). The Pilippines Agriculutrualist. 67. pp. 351-358.

Dayarani, M. and M. S. Dhanarajan. 2013. Control of excessive browning during *in vitro* regeneration of *Musa* cv. laterita A. International Journal of Pharma and Bio Sciences. 4 (3): 471-476.

Demissie, A.G. 2013. Effects of different combination of BAP (6-benzylaminopurine) and NAA (naphthalene acetic acid) on multiple shoot proliferation of plantain (*Musa* spp.) cv. Makoke form meristem derived explant. Academia Journal of Biotechnology 1 (5): 071-080.

Dhed'a, D., F. Dumortier, B. Panis, D. Vuylsteke and E. De Langhe .1991. Plant regeneration in cell suspension cultures of cooking bananas cv. 'Bluggoe' (*Musa* spp. ABB group). Fruits. 465. pp. 125-135.

DOA. 2011. Annual report on fruit and nut crops. (Department of Agriculture).

Domigues, E.T., A. Thlnann-Neto and B. M. J. Medndes. 1995. Shoot tip culture of *Musa*, var. Maca. Scientia Agronomica. 52 (2): 387-394.

Drew, R. A. and M. K. Smith. 1990. Field evaluation of tissue cultured bananas in southeastern Queensland. Australian Journal of Experimental Agriculture. 30. pp. 569-574.

Elhory, S. M. A., M. A. Aziz, A. A. Rashid and A. G. Yunus. 2009. Prolific plant regeneration through organogenesis from scalps of (*Musa* spp.) cv. Tanduk. African Journal of Biotechnology. 8 (22): 6208-6213.

Escalant, J.V. and C. Teisson. 1989. Somatic embryogenesis and plants from immature zygotic embryos of the species *Musa acummata* and *Musa balbisiana*. Plant Cell Report.7, pp. 665–668.

FAO STAT Agriculture Data. 2001. http://apps.fao.org

Escalant, J.V., C. Teisson and F. Cote. 1994 Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* spp.). *In Vitro* Cellular and Developmental Biology.30. pp. 181–186.

Faisal, S. M., M. A. Hoque and A. Quasem. 1998. Field performance of *in vitro* plantlets against normal suckers of banana (*Musa sapientum*) cv. Chamopa. Plant Tissue Culture. 8 (2). pp. 125-129.

FAO. 1987. Tissue culture of selected tropical fruits. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO. 2003. Bulletin of statistics. Food and Agriculture Organization of the United Nations, Rome, Italy. 4 (2). pp. 132-133.

FAO STAT. 2011. Food and Agricultural Organization of the United Nations Statistical Database. From http://faostat.fao.org.

Farahani, F. and A. Majd. 2012. Comparison of liquid culture methods and effect of temporary immersion bioreactor on growth and multiplication of banana (*Musa*, cv. Dwarf Cavaendish). African Journal of Biotechnology. 11 (33): 8302-8308.

Farahani, F., H. Amiinpoor, M. Sheidai, Z. Noormonhammadi and M.H. Mainani. 2008. An improved system for *in vitro* propagation of banana (*Musa acuminata* L.) cultivars. Asian Journal of Plant Sciences. 7 (1): 116-118.

Ganapathi, T. R., J. S. S. Mohan, P. Suprasanna, V.A. Bapat and P.S. Rao. 1995. A low cost stratedy for *in vitro* propagation of banana. Current science (India). 68 (6): 646-650.

Ganapathi, T. R., N. S. Higgs, P. J. Balint-Kurti, C. J. Arntzen, G. D. May and J. M. Van Eck. 2001. Agrobacterium- mediated transformation of embryogenic cell suspensions of the banana cv. Rasthali (AAB). Plant Cell Report. 20. pp. 157-162.

Ganapathi, T.R., P. Suprasanna, V.A. Bapat, V.M. Kulkarni and P.S. Rao. 1999. Somatic embryogenesis and plant regeneration from male flower buds in banana. Current Science 76. pp. 1228–1231.

Goswami, N.K. and P.J. Handique. 2013. *In vitro* sterilization protocol for micropropagation of *Musa* (AAA group) 'Amritsagar' *Musa* (AAB group) 'Malbhog' and *Musa* (AAB group) 'Chenichampa' Banana. Indian Journal of Applied Research. 3 (6): 51-54.

Grapin, A., J. L. Ortiz, R. Domergue, J. Babeau, S. Monmarson, J. V. Escalant, C. Teisson and F. X. Côte. 1998. Establishment of embryogenic callus initiation and regeneration of embryogenic cell suspensions from female and male immature flowers of *Musa*. Info Musa. 17. pp. 13-15.

Grapin, A., J.L. Ortiz, T. Lescot, N. Ferriere and F. X. Cote. 2000. Recovery and regeneration of embryogenic cultures from female flowers of false horn plantain. Plant Cell, Tissue and Organ Culture. 61. pp. 237–244.

Gübbük, H. and M. Pekmezcl. 2004. *In vitro* propagation of some new banana types (*Musa* spp.). Turk J Agric. 28. pp. 355-361.

Guo, B., B. H. Abbasi, A. Zeb, L. L. Xu and Y. H. Wei. 2011. Thidiazuron: A multi-dimensional plant growth regulator. African Journal of Biotechnology. 10 (45): 8984-9000.

Gupta, P. P. 1986. Eradication of mosaic disease and rapid clonal multiplication of banana and plantains through meristem tip culture. Plant Cell Tissue and Organ Culture. 6 (1): 33-39.

Habiba, U., S. Reza, M. L. Saha, M. R. Khan and S. Hadiuzzaman. 2002. Endogenous bacterial contamination during *in vitro* culture of table banana identification and prevention. Plant Tissue Culture. 12 (2): 117-124.

Hamill, S. D., S. L. Shallock, and M. K. Smith. 1993. Comparison of decontamination methods used in initiation of banana tissue cultures from field collected suckers. Plant Cell Tissue and Organ Culture. 33. pp. 343-346.

Hutteman, C. S. and J. E. Preece. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture. 33. pp. 105-119.

Hwang, S. C., C. L. Chen, J. C. Line and H. L. Lin. 1984. Cultivation of banana using plantlets from meristem culture. Hort Sci. 19. pp. 231- 233.

Isaraeli,Y., E. Lahav and O. Reuveni. 1995. *In vitro* culture of bananas. Fruits. 43. pp. 219-223.

Jafari, N., R. Y. Othman and N. Khalid. 2011. Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. African Journal of Biotechnology. 10 (13): 2446-2450.

Jaisy, R.C. and Ghai. 2011. Development of low cost methodology and optimization of multiplication and rooting hormones in the micropropagation of Red banana in vitro. Plant Sciences Feed. 1 (7): 84-87.

Jasrai, Y. T., V. R. Kannan, A. Remakanthan and M. M. George. 1999. *Ex vitro* survival of *in vitro* derived banana plants without greenhouse facilities. Plant Tissue Culture. 9 (2): 127-132.

Kadleček, P. 1997. Effect of pretreatment by irradiance and exogenous saccharose under *in vitro* conditions on photosynthesis and growth of tobacco (*Nicotiana tabacum* L.) plants during acclimatization after transfer to soil.

Kanchanapoom, K. 2000. *In vitro* culture of the banana *Musa* (AAA group, 'Gros Michel') 'KluaiHom Thong' shoot tip. J ISSAAS. 6. pp. 43- 52.

Kanchanapoom, K. and N. Promsorn. 2011. The Influence of Explant Types and Orientation on *in Vitro* Culture of *Musa balbisiana* 'Kluai Hin' (BBB group). Not Sci Biol. 3(3): 89-92.

Kaminek, M. 1992. Progress in cytokinin research. Trends Biotechnol. 10: 159-164.

Ko, W. H., C. C. Su, C. L. Chen and C. P. Chao. 2009. Control of lethal browning of tissue culture plantlets of Cavendish banana cv. Formosana with ascorbic acid. Plant Cell Tissue and Organ Culture. 96. pp. 137-141.

Kumar, K. G., V. Krishna, V. Venkatesh and K. Pradeep. 2011. High frequency regeneration of plantlets from immature male floral explants of (*Musa paradisica*) cv. Puttabale- AB genome. Plant Tissue Cult.and Biotech. 21(2): 199-205.

Lalrinsanga, R., H. Vanlaldiki and W. I. Meltel. 2013. *In vitro* shoot tip culture of banana cv. Meitei Hei. The Bioscan. 8 (3): 839-844.S

Lee, K. S., F. J. Zapata-Arias, H. Brunner and R. Afza. 1997. Histology of somatic embryo initiation and organogenesis from rhizome explants of *Musa* spp. Plant Cell Tissue and Organ Culture. 51. pp. 1-8.

Lee, S. W. 2001. Thidiazuron in the improvement of banana micropropagation. The second international symposium of biotechnology of Tropical and Subtropical species.

Ma, S. S. and C. T. Shii. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. J. Chinese Society Hort. Sci. 18. pp. 135-142

Madhulatha, P., S. Anabalagan, S. Jayachandran and N. Sakthivel. 2004. Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana. Plant Cell Tissue and Organ. Culture. 76. pp. 189-191.

Magioli C., A.P.M. Rocha, D.E. Oliveira and E. Mansur. 1998. Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Report. 17. pp. 661-663.

Makara, A. M., P. R. Rubaihayo and M. J. S. Magambo. 2003. Carry-over effect of thidiazuron in banana *in vitro* propagation at different culture cycles and incubation conditions. African Crop Science Conference Proceedings, 6. pp. 113 -118.

Marroquin, C.G., C. Paduscheck, J.V. Escalant and C. Teisson. 1993. Somatic embryogenesis and plant regeneration through cell suspensions in *Musa acuminata*. *In Vitro* Cell. Dev. Biol. 29. 43-46.

Mbanaso, E. N. A., J. Crouch and F. Onofeghara. 2006. Response of cooking banana genotypes to fragmentation and incision during shoot tip culture. Info Musa. 15 (1-2).

Medes, B. M. J., S. B. Filippi and C. G. B. Demétrio. 1999. A statistical approach to study the dynamics of micropropagation rates, using banana (*Musa* spp.) as an example. Plant Cell Reprot. 18. pp. 967-971.

Mendes, B. M. J., S. B. Philippi, C. G. B. Demetrio and A. P. M. Redriguez. 1996. Efficacy of banana plantlet production by micropropagation. Pesqui Agropecu Bras. 31. pp. 863-867.

Mok, M.C., D. W. S. Mok, J. E. Turner and C. V. Mujer. 1987. Biological and biochemical effect of cytokinin-active phenylurea derivatives in tissue culture systems. Hort science. 22. pp. 1194-1197.

Molla, M. M. H., M. Khanam, Dilafroza, M. M. Khatun, M. A. Amin and M. A. Malek. 2004. *In vitro* rooting and *ex vitro* plantlet establishment of BARI Banana (*Musa spp*.) as influenced by different concentration of IBA (Indole 3-butyric Acid). Asian Journal of Plant Sciences. 3(2): 196-199.

Muhammad, A., I. Hussian, S. M. S. Naqvi and R. Hamid. 2004.Banana plantlet production through tissue culture. Agricultural Biotechnology Program (ABP) IABGR National Research Center (NARC). Pak. J. Bot. 36(3): 617-620.

Murali, T. P., and Duncan, E. J. 1995. The effects of *in vitro* hardening using triazoles on growth and acclimatization of banana. Scientia horicul. 64. pp. 243-251.

Nandwani, D., U. Zehr, B. E. Zehr and R. B Brwale. 2000. Mass propagation and *ex vitro* survival of Banana cv. Basrai trough tissue culture. Garten Bauwissen Chaft. 65 (6): 237-240.

Navarro, C., R. M. Escobedo and A. Mayo. 1997. *In vitro* plant regeneration for embryogenic cultures of a diploid and a triploid, Cavendish banana. Plant Cell Tissue and. Organ Culture. 51. pp. 17-25.

Nisyawati and Kariyana, K. 2013. Effect of ascorbic acid, activated charcoal and light duration on shoot regeneration of banana cultivar Barangan (*Musa acuminata* L.) *in vitro* culture. IJRRAS. 15 (1).

Novak, F.J., R. Afza, M. van Duren, M. Perea-Dallos, B.V. Conger and T. Xiaolang. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.). Biotech. 7. pp. 147-158.

Oglesby**,** R.P. and Griffis, J. L. 1986. Commercial *in vitro* propagation and plantation crops. In: Zimmerman, R.H., Griesbach, R.J., Hammerschlag, F.A. and Lawson, R.H. (eds.). Tissue Culture as a Plant Production System for Horticultural Crops. pp. 253-257.

Okole, B. N. and F. A. Schultz. 1996. Micro-cross sections of banana and plantains (*Musa spp*.): Morphogenesis and regeneration of callus and shoot buds. Plant Sci. 116. pp. 185–195.

Olivia, S. R., P. Bantawa, S. K. Ghosh, J. A. Teixeira da Silva, P. Debgosh and T. K. Monda. 2010. Micropropagation and field performance of “Malhog” (*Musa paradisiaca*, AAB group): A popular banana cultivar with high keeping quality of North East India. Tree and forestry science and biotechnology.

Preece, J. E. and E. G. Sutter. 1991. Acclimatization of micropropagated plants to the greenhouse and field. In: Debergh, P. C.; Zimmerman, R. H., eds. Micropropagation: technology and application. London: Kluwer Academic Publishers. pp.71–93.

Rachel, C. S. 2003. Conservation and management of *Musa* germplasm. Reginonal Training Workshop: Enhancing Capacity for Nematode Management in Musa, December 1-5, 2003 at the Institute of Plant Breeding, Philippines Los Bańos, College, Laguna, Philippines.

Rahman, M. Z., K. M. Nasiruddin, M. A. Amin and M. N. Islam. 2004. *In vitro* response and shoot multiplication of Banana with BAP and NAA. Asian J. Plant Sci. 3. pp. 406-409.

Rahman, M. Z., M.H. Rahman and M. Rezuanul Islam. 2006. High frequency plant regeneration of a dessert banana cv. Mehersagar for commercial exploitation. Biotechnology. 5 (3): 296-300.

Rhaman, S., N. Biswas, Md. M. Hassan, Md. G. Ahmed, ANK. Mamun, Md. R. Islam, Md. Moniruzzaman and Md. E. Haque. 2013. Micropropagation of banana (*Musa spp*.) cv. Agnishwar by *in vitro* shoot tip culture. International Research Journal of Biotechnology. 4 (4): 83-88.

Roy, O. S., P. Bantawa, S.K. Glosh, J.A. Teixeira da Silva, P. D. Ghosh and T. K. Mondal. 2010. Micropropagation and field performance of 'Malbhog' (*Musa paradisiaca*, AAB group): A popular banana cultivar with high keeping quality of North East India. Tree and Forestry Science and Biotechnology.

Scaranari, C., P. A. M. Leaf and P. Mazzafera. 2009. Shading and periods of acclimatization micropropagated banana plantlets cv. Grande Naine. Sci. Agric. 66. pp. 331-337.

Sein Hla Bo. 2003. Banana R & D in Myanmar. Advancing banana and plantain R&D in Asia and the Pacific.Vol. 12.Proceeding of the 2st BAPNET Steering Committee meeting. Jakarta (IDN), 2003/10/06-07. Los Banos (PHL): INIBAP-AP, 12. pp. 143-149.

Shii, C. T., S. S. Ma, I. C. Huang and W. H. Ching. 1992 Somatic embryogenesis and plantlet regeneration in suspension cell cultures of triploid bananas (*Musa* AAA) subgroup Cavendish.

Shirani, S., M. Sariah, W. Zakaria and M. Maziah. 2010. Scalp induction rate responses to cytokinins on proliferating shoot-tips of banana cultivars (*Musa spp*.). American Journal of Agricultural and Biological Sciences. 5 (2): 128-134.

Silayoi, B. 2001. Micropropagation of Kluai Khai (*Musa acuminata* ‘ Kluai Khai’) using sword suckers and inflorescences at various developmental stages. Kasetsart J (Nat. Sci). 35: 361-367.

Silva, S. O., R. P. Oliveira, A. P. de Matos, S. S. Neto, A. G. Praeres and C. A. P. Oliveira. 1998. Development of pre-acclimatized banana plantlets obtained from tissue culture. Acta Hort. 490. pp. 195-199.

Simonds, N. W. and K. Shepherd. 1955. The taxonomy and origins of the cultivated bananas. J. Linn. Soc. Lond. Bot. pp. 302-312.

Simonds, N. W. 1966. Bananas. Longman, London, 468 p.

Singh, H. P., G. Singh, J. C. Samuel and R. K. Pathak. 2002. Precision farming in horticulture. Proceedings of the National Seminar-cum-Workshop on Hi-Tech Horticulture and Precision Farming 2002.

Smith, M. K., S. D. Hamill, D. K. Becker and J. L. Dale. 2005. *Musa* spp. Banana and Plantain. Chapter 13.1. In R.E. Litz., ed. Biotechnology of Fruit and Nut Crops. CAB International Wallingford, UK. pp. 366-391

Srangsam, A. and K. Kanchanapoom. 2007. Establishment of *in vitro* culture of *Musa* AA group 'KluaiSa' and *Musa* AA group 'Kluai Leb Mue Nang' and the analysis of ploidy stability. Science Asia. 33. pp. 437-442.

Strosse, H., E Andre, L. Sági, R. Swennen and B. Panis. 2008. Adventitious shoot formation is not inherent to micropropagation of banana as it is in maize. Plant Cell Tissue and Organ Culture. 95. pp. 321-332.

Strosse, H., I. V. D. Houwe and B. Panis. 2004. Cell and tissue culture, and mutation induction. In: MOHAN JAIN, S.; SWENNEN, R. (Ed.). Banana improvement: cellular, molecular biology, and induced mutations. Enfield: Science Publishers. pp. 13-21.

Thomas, J. C. and F. R. Katterman. 1986. Cytokinin activity induced by thidiazuron. Plant Physiol. 81. pp. 681-683.

Titov, S., B. S. Kumar, M. Ajoy, A. Md. Sadurl and U.S. Nasir. 2006. Control of phenolic compound secretion and effect of growth regulators for organ formation of *Musa spp*. cv. Kanthali floral bud explants. American Journal of Biochemistry and Biotechnology. 2(3). pp. 97-104.

Trapthi, L. and J. N. Trapthi. 2008. High-frequency shoot regeneration of various cultivars of banana (*Musa* spp.).Journal of Crop Improvement. 22 (2): 171-179.

Tripathi, L. 2003. Genetic engineering for improvement of *Musa* production in Africa. Afr.J Biotechnol. 2(12): 503-508.

Uddin, S. N., S. Titov and A. Md. Wadud. 2006. *In vitro* propagation of banana *(Musa* spp. cv. Kanthali): An endogenous variety of bangladesh with controlling bacterial contaminations. American Journal of Plant Physiology.1 (2). pp. 169-176.

Viehmannová, I., C. E. Feraná, F. Hnilicka and C. D. Robles. 2007. The influence of growth regulators on root induction *in vitro* of the *Musa* genus. Agricultura Tropica Et Subropica. 40 (3).

Vuylsteke, D. 1998. Shoot tip culture for the propagation, conservation, and distribution of *Musa* germplasm. International Institute of Tropical Agriculture, Ibadan, Nigeria.82 p.

Withers, L. A. 1990. Cryopresrvation of plant cells. In: Pollar, J.W. and J.M. Walker (eds). Methods in molecular biology, Plant Cell and Tissue Culture.

Wong, W. C. 1986. *In vitro* propagation of banana (*Musa* spp.): initiation and development of shoot tip cultures on defined media. Plant Cell Tissue and Organ Culture. 6. pp. 159-166.

Youmbi, E., B. Ella and K. Tomekpe. 2006. Effect of thidiazuron on *in vitro* proliferation capacities of some banana (*Musa* spp.) cultivars with weak multiplication potential. Akdeniz Ünìversì Ziraat Fakoletesì Dergìsì.19 (2): 255-259.

Zaffari, G. R., G. B. Kerbauy, J. E. Kraus and E. C. Romano. 2000. Hormonal and histological studies related to *in vitro* bud formation. Plant Cell, Tissue and Organ Culture. 63. pp. 187-192.

APPENDIX

Appendix . 1 Composition of full and half strength of Murashige and Skoog's - 1962 (MS) medium used in red banana micropropagation

Components	Concentration in medium ($mg \cdot L^{-1}$)	
	FMS[a]	HMS[b]
NH_4NO_3	1650.00	825.00
KNO_3	1900.00	950.00
$CaC1_2.2H_2O$	440.00	220.00
$MgSO_4.7H_2O$	370.00	185.00
KH_2PO_4	170.00	85.00
$FeSO_4.7H_2O$	27.80	27.80
NA_2 EDTA	37.30	37.30
H_3BO_3	6.20	6.20
$MnSO_4.4H_2O$	22.30	22.30
$ZnSO_4.4H_2O$	8.60	8.60
KI	0.83	0.83
$Na_2MoO_4.2H_2O$	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025
Nicotinic acid	0.50	0.50
Pyridoxin.HCl	0.50	0.50
Thiamine.HCl	0.10	0.10
Glycine	2.00	2.00
Myoinositol	100.00	100.00

[a] Full strength MS medium, [b] Half strength MS medium